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A HYPOTHESIS FOR THE
STRUCTURE AND RHEOLOGY OF GLUTENIN

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It is suggested that because of the rapid lowering of the viscosity of its solutions by reducing agents the major, important fraction of glutenin consists of linear molecules made up of polypeptide chains linked to one another difunctionally by S.S bonds.   When dough is stretched the natural tendency of the polypeptide chains to return to a contracted state of low free energy accounts for the elasticity.   The inter-chain S.S bonds are essential for elasticity because inter-chain adhesion between individual polypeptide chains will not overcome the stronger intra-chain forces unless reinforced by the S.S bond.   If extension exceeds the elastic limit, viscous flow occurs because steric hindrance and molecular slip will prevent a return to the original conformation.   Disulphide interchange is believed to play an important part in stress relaxation.   Mechanical scission of S.S probably occurs when molecules at their elastic limit are subjected to too much stress; this may explain the work maximum in the Chorleywood Bread Process. Explanations are advanced for mechanical and activated dough development and evidence in favour of the hypothesis is discussed.

Introduction

Glutenin molecules show a wide variation in size, but their weight-average molecular weight is high, exceeding 1 million according to Jones et al.[1,2]   They are assemblages of polypeptide chains of mol. wt. 20,000, which are joined to one another by S.S bonds from evidence provided by Nielsen et al.[3]   It was concluded by these last workers that the elastic and cohesive properties of glutenin depended on the integrity of the S.S crosslinked structure.   The polypeptide chains, referred to throughout this paper as 'chains', though of similar mol. wt., do not appear to be identical because after reductive scission of S.S bonds they show a range of mobilities on starch-gel electrophoresis under reducing conditions as was first demonstrated by Woychik et al.[4] and confirmed by Elton & Ewart.[5]   From the amino acid analyses of Ewart[6] there would be on average about 180 amino acid residues in a chain, including 21 proline residues and 2·3 S.S bonds.   Since Beckwith & Wall[7] showed glutenin to have a negligible content of free SH groups, the content of S.S bonds per chain must be integral.   An average figure of 2·3 per chain implies perhaps that one third of the chains contain 3 S.S and two thirds contain only 2 S.S bonds.

The glutenin examined by Jones et al.[1,2] would therefore consist of aggregates of the order of 50 chains at least on average.   Although these chains appear to differ in their content of amino acids with ionic side-groups judging by electrophoretic evidence,[4,5] if they are randomly combined the average charge/mass ratio of a sample as large as 50 will not differ much from a mean value, and the large glutenin molecules will have an almost constant mobility in free solution, as was observed.[8]   It is only in small glutenin molecules that significant variations in mobility will become apparent: owing to the many ways of making selections from even a small group of chains, there will be numerous species of low mol. wt.   In starch-gel electrophoresis this low mol. wt. glutenin will give continuous tailing[5] owing to statistical variations in charge/mass ratio as the sample size diminishes and to size gradation by molecular sieving.   It is not easy to

reconcile the electrophoretic behaviour of glutenin in free solution with that observed in starch-gel electrophoresis under normal and reducing conditions except by postulating that it is a random copolymer of a relatively small number of types of polypeptide chain.

The chains will inevitably be linked in a linear configuration if only one S.S bond from each chain is involved, but there is the possibility that the remaining one or two S.S bonds are crosslinked between chains.   In the literature the term crosslink has been widely and correctly used for any inter-chain S.S bond, but in order to avoid confusion in this paper an S.S bond between chains which involves the opening of a 2nd or 3rd intra-chain S.S will be referred to as a branching crosslink or branching S.S bond.

In Fig. 1 only one of a, b or c could be described as a branching crosslink, e.g. if AB is the main chain c would be a branching crosslink.
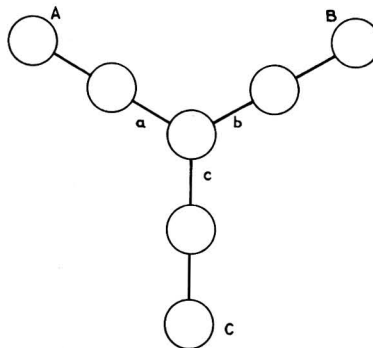

FIG. 1.   *Diagram of main chain and branching crosslink*

Influence of primary structure on secondary and tertiary structures

Recent research in molecular biology has established that during protein synthesis a linear code in the form of mRNA is translated into a linear polypeptide chain. Remarkable though this discovery was it did not explain how it was possible to specify, let alone to construct, the complex shapes involving secondary and tertiary structures, which many proteins adopt in their native states. As a result of studies on the enzyme ribonuclease Anfinsen and his co-workers[9-11] proposed an elegant solution to the problem: all the information for the secondary and tertiary structure is already incorporated in the primary sequence of the polypeptide chain. Following the lead of Anfinsen the principle has been established for a number of other single-chain proteins. Expressed in other words the native structure of a single-chain protein corresponds to the conformation with the lowest free energy. In such a conformation the sulphydryl groups are brought into juxtaposition, correctly paired, so that S.S bonds which stabilise the structure can readily be formed. If the number of S.S bonds is n it is easily shown that there are $\dfrac{(2n)!}{n!\,2n}$ different ways of pairing their component half-cystine residues; $n = 4$ for ribonuclease giving 105 ways. If all these were equally probable the yield of enzymic activity obtained on gently re-oxidising fully reduced ribonuclease in dilute solution would be less than 1%. In fact an average regenerated activity of 80% of the native specific activity was obtained.[10]

Re-oxidation of reduced gliadins and glutenins

Beckwith, Wall & Jordan[12] produced evidence that wheat gliadins after reduction could be re-oxidised at 0·1% concentration to regenerate proteins indistinguishable from native gliadin by all the tests applied including electrophoretic and immunochemical analysis. (Both Beckwith & Wall[7] and Beckwith *et al.*[12] determined the cystine contents of their materials by amperometric titration with silver nitrate claiming figures of 6·4 and 8·8 moles/10^5 g of protein respectively for glutenin and gliadin. Although these are low compared with 11·6 and 14·5 obtained by Ewart[6] from amino acid analysis after performate oxidation, it does not necessarily mean that the gliadin and glutenin had not been fully reduced, because no SH or S.S groups were discovered in the reduced products after cyanoethylation. If the amperometric method gave low values it should still have been able to distinguish between the presence or absence of residual SH or S.S groups.)

When Beckwith *et al.*[12] re-oxidised reduced gliadin in concentrated solution (5%) where there is a higher probability of inter-chain S.S being formed the product resembled glutenin in cohesiveness and starch-gel electrophoretic behaviour but was not as elastic. It had an intrinsic viscosity in aluminium lactate buffer, 3 M in urea, pH 3·1, of 0·38 dl g^{-1} whereas that of glutenin in a somewhat similar solvent, pH 4·2, was shown by Wu & Dimler[13] to be 0·58. (At the lower pH the greater positive charge would if anything give a more extended conformation, but even so the intrinsic viscosity is less.) In the ultracentrifuge the re-oxidised gliadin gave two peaks, one corresponding to native gliadin while the other had $s_{20} = 6$ S as against 2·4 S for glutenin. The authors suggested that re-oxidised gliadin may have a more compact and more highly branched structure than glutenin. The latter with greater intrinsic viscosity but

lower sedimentation coefficient than re-oxidised gliadin may be a more linear polymer because the extent of branching is limited. Beckwith & Wall[7] observed that in dilute solution the S.S bonds of glutenin did not re-form as quickly as those of reduced gliadin under similar conditions and suggested that the SH groups in reduced glutenin were unable to come together as readily as in the case of reduced gliadin. The last observation could suggest that there was some strain on some of the S.S bonds of glutenin and that consequently there are other conformations with nearly the same free energy level. Nevertheless the authors have not commented on the great significance of the fact that they do come together, in that it proves that these chains have a tendency to return to contracted conformations. It is most probably on this tendency that the elasticity of glutenin depends.

Evidence for linear nature of glutenin molecules

In the Appendix the effect of S.S scission on the average number of chains per molecule or number-average degree of polymerisation (D.P.) is considered for the simple case of linear glutenin. It is also deduced that the D.P. will fall less rapidly in the case of glutenin with branching. As the proportion of branching crosslinks rises the number-average D.P. will be less and less affected by S.S scission. The principle is illustrated in Fig. 2.

In Fig. 3 is plotted the number-average D.P. against fraction of bonds broken in a linear glutenin polymer with D.P. equal to 50 chains. The sharp drop closely resembles that in Fig. 4 of Beckwith & Wall's paper[7] for the viscosity of glutenin solution during reduction. They claimed this rapid fall was due to inter-chain S.S scission; the subsequent slow rise may have been due to the breakage of the more resistant intra-chain S.S. Olcott *et al.*[14] reported immediate and drastic decreases in the viscosity of gluten dispersions on addition of dilute solutions of cysteine, sodium bisulphite, potassium cyanide and mercaptoethanol. The curve of viscosity against time in their Fig. 4 is also like that of Fig. 3. This fall would not be due to the gliadin component of gluten because gliadin's viscosity increases slightly on reduction.[12]

(a)

(b)
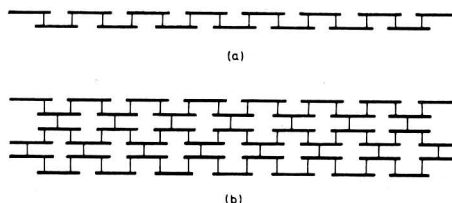
FIG. 2.   *Effect of breaking crosslinks on mol.-wt.*

Thick horizontal lines denote polypeptide chains of glutenin
Thin vertical lines represent S.S links
(a) Linear polymer of difunctional subunits. Scission of each S.S always produces one extra molecule, and the D.P. falls rapidly
(b) Polymer built up of trifunctional subunits. In only 2 out of 57 cases if one bond per molecule is broken would there by any change in the number of molecules. Hence number-average D.P. would (for a large number of molecules) be changed slightly to $\frac{57}{59}$ of its former value. Owing to the small fragments removed in this type of system, the viscosity-determined D.P. would be even less affected

FIG. 4. *Disulphide interchange of linear glutenin molecules*

The wavy line symbolises the polypeptide chains joined by S.S bonds. There are too many of these to be shown without complicating the diagram. The interchange reaction occurs as:

Although viscosity tends to be more sensitive to weight-average mol. wt., the number-average mol. wt. is correlated with the latter, the exact relationship depending on the mol. wt. distribution in the sample. In both cases a large excess of reducing agent was used which would lead to 1st order kinetics: therefore the 'fraction of bonds broken, is equivalent to 'time' in the abscissa.

This evidence strongly suggests that extensive branching is not present in the major part of glutenin and is to some extent supported by the opinion of the Peoria workers that glutenin has a more linear configuration than re-oxidised gliadin. It is not possible to decide definitely whether there is a small amount of branching or none at all on available evidence. Even a small proportion of branching crosslinks would have a big effect on the solubility of large molecules su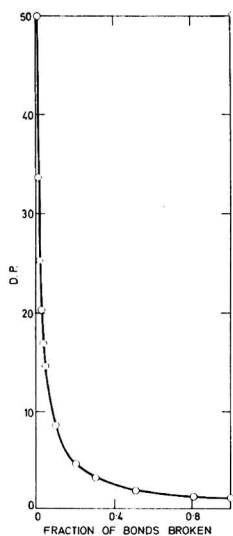ch as those of glutenin. Accordingly in this paper the hypothesis is put forward that among the major fraction of glutenin there is none.

Lability of S.S and presence of strain

Cecil & Wake[15] have concluded from a study of the reaction of sulphite with a number of proteins that although this reagent will usually sever inter-chain S.S, most of the intra-chain S.S are resistant unless a denaturing agent such as 3 M guanidine hydrochloride is present together with mercuric chloride. These workers believed that intra-chain S.S were not split by sulphite alone when the polypeptide ring closed by the S.S bond was stable. Trypsinogen and the oligopeptide oxytocin were quoted as containing strained intra-chain S.S bonds. Ryle & Sanger[16] proposed that the stability conferred on protein S.S by the configuration of the molecule was the probable reason why proteins in living tissues where SH-containing compounds of low mol. wt. such as GSH were present seem unlikely in general to take part in
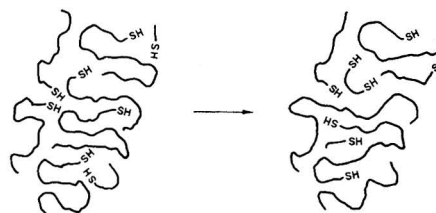
S.S interchange reactions. From this it might be concluded that gliadins with single-chain, stable configurations will not form part of the glutenin structure to any significant extent, but those with inter-chain S.S may.

Origin of glutelins

It would probably be disastrous for an organism if its enzymes underwent S.S interchange reactions *in vivo*, and a mutation which brought about severe strain in a peptide ring closed by an S.S bond such that the enzyme could take part in these reactions might well be lethal. In the case of cereal storage proteins, however, since their purpose seems to be merely that of a nitrogen store, such mutations would probably be unimportant and it may be that in the course of evolution of the Gramineae some of the storage proteins have suffered mutations such that one or more S.S bonds have become strained. As a result, for a given protein there were other conformations, besides the one in which the SH groups were juxtaposed, which had approximately the same free energy. During protein synthesis therefore a storage protein with branching crosslinks was created. This had poor solubility and hence was assigned to the glutelins under the old protein classification scheme.

Comparison of wheat with other cereals

The unusual properties of wheat, it is postulated in this paper, lie in the fortunate chance that in most of its glutelin only one S.S bond has become strained and as a result it forms in a linear configuration. With an average of 2·3 S.S per chain, $\frac{1}{2\cdot3}$ of the glutenin S.S bonds or ~ one fifth of the total flour S.S bonds are inter-chain. Rye has a lesser quantity of linear glutelin but nevertheless sufficient to give it rather inferior breadmaking properties. The quantity of linear hordenin in barley is enough to impart weak viscoelasticity to its dough, but the breadmaking properties are very poor. A glutelin in which branching crosslinks were present would tend to become a giant molecule and in consequence to be insoluble. It is significant that Ewart[17] found that the insoluble portions of total recovered protein after an exhaustive extraction of flour were respectively 11, 34, 35, 67 and 74% for wheat, rye, barley, oats and maize. The glutelins of oats and maize are so rich in branching crosslinks that they

are insoluble and have no dough-forming properties at all: the presence of hydrophobic amino acids is another factor.[18] The acetic-acid soluble fractions amounted to, in the same order, 24, 11, 7, 0·1, 0·3%,[17] and probably represent linear glutelins, which would be sufficiently swollen, if not actually soluble, under dough conditions to take part in S.S interchange (Fig. 4). It may be significant that the S.S interchange potentials of Redman & Ewart,[19] 1·6, 0·6, 0·3 for wheat, rye and barley, are very well correlated with the figures for acetic acid-soluble glutelin 24, 11, 7. An anomalous result was the high level of S.S interchange of 3% obtained for soluble proteins of maize. The maize flour was found to be appreciably contaminated by germ which would have raised its SH content. Only 10% of the maize proteins (presumably albumins and globulins) were soluble under conditions used, so unless these had a mol. wt. of > 150,000 (which is unlikely for most cereal albumins and globulins) they would not be able to form long chains, and hence could not form a visco-elastic dough. Perhaps some maize albumins and globulins contain either inter-chain or strained S.S bonds.

The fact that some insoluble material remains in wheat flour after exhaustive extraction or when glutenin has been reduced in 8 M urea could imply that branching crosslinks are present which are not due to S.S bonds, as has been suggested by Ewart.[17] Therefore in postulating that wheat glutenin has a linear structure it should be made clear that this refers to the major fraction which can be dispersed in dilute acids or which is convertible to soluble material by thiols in urea.

Explanation of viscoelasticity at the molecular level

When flour is wetted a rapid disulphide interchange reaction begins between the polypeptide chains of the soluble, linear glutenin, catalysed by the small fraction of the total SH groups which are in contact with the glutenins. These thiols may be of low mol. wt. or even of the size of proteins, such as the SH-containing enzyme β-amylase. It is significant that in ungerminated wheat Rowsell & Goad[20] found that β-amylase was bound only by S.S bonds to the glutenin but not the gliadin fraction. Whether precursors of mol. wt. 20,000 polymerise during dough formation to long strands is not known. It may be that the glutenin has already been formed in very long linear molecules and the S.S interchange reaction enables equilibrium to be approached by mechanisms as shown in Fig. 4, and at equilibrium:

$$D.P. = \frac{\text{total no. of glutenin chains}}{\text{no. of SH groups attached to glutenin}} \quad \ldots\ldots\ldots(1)$$

(This assumes that the glutenin molecules each contain an SH group left over from the hypothetical S.S interchange reaction which originally created them:
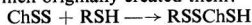
ChSS + RSH ⟶ RSSChSH

RSSChSH + ChSS ⟶ RSSChSSChSH etc., where Ch stands for a chain. If however glutenin was free from SH groups, being created by enzymic oxidation,

$$D.P. = \frac{\text{total no. of glutenin chains}}{(\text{no. of SH attached to glutenin}) + } \quad \ldots\ldots\ldots\ldots(2)$$
(original no. of glutenin molecules)

because each extraneous SH group which reacts with linear glutenin creates an extra glutenin molecule, ignoring cyclic configurations.)

Owing to the high ionic strength (~0·5) created by the presence of salt the gliadin and glutenin will not be in true solution. The glutenin seems to be sufficiently swollen to be able to take part in S.S interchange because Redman &

Ewart[21] found that protein-cystamine interchange occurred in dough where the cystamine hydrochloride produced an ionic strength of ~0·3 well above the level, ~0·03, at which glutenin precipitates.[22] As will be discussed more fully later, owing to the nature of glutenin the effective concentration of SH groups, i.e. those capable of diffusing into the swollen protein strands, will be a small fraction of that in solution. The mechanical action of mixing and the working of dough by gaseous fermentation may play a significant part in exposing glutenin to SH groups and perhaps rendering its S.S groups more active by straining them and by increasing their accessibility.

One of the main functions of kneading or mixing the dough is to draw the glutenin molecules from the clusters based on protein bodies,[23] in which they were laid down, into an extended conformation interlacing the dough in all directions. Glutenin only comprises about 3% of the weight of the dough and a linear concept enables the most efficient use of this small quantity to be made since it is the determinant of the dough's rheological properties. The glutenin has the power to extend because, owing to the high proline content, the polypeptide chains are kinked and folded. Force is needed to unfold these (Fig. 5) but once the internal adhesion is overcome great capacity for extension becomes available. The well known resistance of the dough measures the force needed to overcome the internal stresses in the fibrous glutenin with which it is threaded. The inter-chain S.S bond co-operates with secondary forces between contiguous portions of the
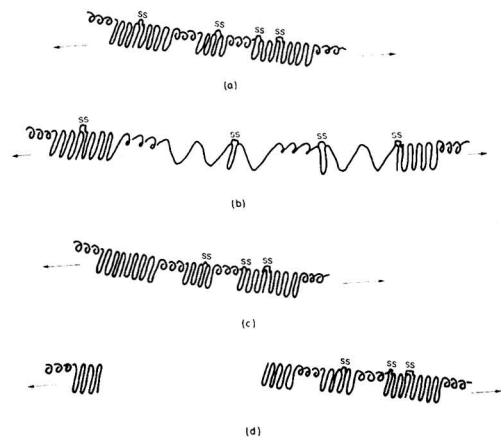
Fig. 5. *Explanation of how inter-chain S.S bonds impart elasticity to glutenin*

(a) Stylised diagram of a small part of a glutenin molecule lying between two adjacent shear planes in a dough. The α-helix is approximately proportional to the observed content. The number of bends in the chain is approximately equal to the number of proline residues
(b) Intermolecular forces between neighbouring chains are reinforced by the inter-chain S.S bonds, thus enabling the intra-chain secondary forces to be overcome, so elastically extending the chain. On release of stress the unextended conformation is resumed due to its lower free energy
(c & d) Where an inter-chain S.S is lacking the inter-chain secondary forces alone can no longer withstand the tensile stress and a discontinuity results. The greater the concentration of these (i.e. the shorter the glutelin molecules) the lower the resistance of the dough becomes.

two chains which it joins to enable them to unfold one another: the intra-chain secondary forces will be stronger than the less well organised inter-chain forces, but the presence of the strong covalent S.S bond is adequate to sway the balance in favour of the latter (Fig. 5). The intra-chain forces determine the tendency to return to the original conformation of lowest free energy upon release of stress i.e. they impart elasticity to the glutenin. If the extension is too great there may be molecular slip, or steric effects may come into play as the protein molecules become involved with other glutenin molecules, gliadin molecules, or starch granules, and the tendency to retract is partly or completely frustrated; the dough is then said to have undergone inelastic extension or viscous flow. Tension can be released if the S.S. groups forming part of a stressed sector of a glutenin molecule interchange with a nearby SH group as was pointed out by Bloksma;[24] this has been called stress relaxation.

This reaction is probably very important in dough behaviour. A minimum concentration of SH groups prevents stress from building up to high levels by increasing the probability that a stressed molecule will be severed by S.S interchange somewhere along its length before the molecule is mechanically broken. If the SH content drops below a certain level, e.g. by addition of a fast-acting improver, there is a tendency not only for the average D.P. to rise by equation (1) or (2) but for insufficient stress relaxation to occur. The result is that stress attains large values as high D.P. molecules become oriented and then stretched. Extensibility is low because the molecules are already close to their elastic limit. On further mixing, mechanical scission followed immediately by hydrogen abstraction from water will produce increasing quantities of SH which are attached to the glutenin molecules (Fig. 6). This causes a rapid fall in D.P. and consequently in resistance. The weak dough will approximate to a viscous fluid and exhibit an increase in extensibility.

Breadmaking processes; effect of excessive mixing

The 'ripening' of a dough during conventional breadmaking may therefore largely take place by orientation of the linear glutenin molecules in all directions by the gentle action of the growing gas bubbles as fermentation proceeds, so that a suitable molecular reinforcement is distributed more or less uniformly. In mechanical development as in the Chorley-wood Bread Process[25] (CBP) this extension is brought about rapidly by mixing. It is easy to see why there is a work maximum in this process. Once the glutenin molecules are unfolded into a linear conformation further work input extends them elastically, i.e. unfolds the constituent chains into linear conformations, and if mixing continues the molecules which are at their elastic limit, will be mechanically sundered at their weakest points, the S.S bonds (the thermo-chemical bond energies of C-S, C-C and C-N are considerably higher than those of S.S). In this case normal stress relaxation is inadequate to cope with the severe energy output. The observations of Heaps *et al.*[26] that extensibility decreases and that viscous and elastic work components reach maximal values as work input is increased are readily explained by the above account. It was also shown by these workers that the rate of work input was important and that viscous stress work reached a maximum value as the rate of work input increased. Angier *et al.*[27] demonstrated that C-C bonds can be broken during the milling of several polymers, and Axford & Elton[28] were the first to suggest in 1960 that mechanical scission of S.S bonds could occur during dough mixing. Though free
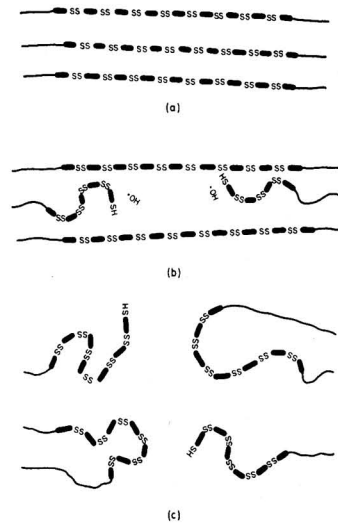
FIG. 6. *Explanation of how mechanical scission of S.S could lead to scission of neighbouring protein chains*

(a) Three glutenin molecules under tension

SS   denotes an interchain S.S bond
—   denotes a polypeptide chain of mol. wt. ~20,000
—   denotes remainder of molecule, of similar structure to the part shown in more detail

(b) Centre molecule has suffered mechanical scission of an S.S. bond causing the two fragments to retract. The two radicals have abstracted hydrogen from water molecules to form SH groups

(c) The newly created SH groups have broken two adjacent glutenin molecules by S.S interchange

radicals have been discovered in flour that has been ground *in vacuo* at 77°K by Redman, Axford, Elton & Brivati[29] they could not be detected in dough (Redman, Axford, Brivati & Ewart unpublished result). The reason is, very probably, that the sulphide radical would return to SH immediately in the presence of water by hydrogen abstraction. The result of excessive mixing should therefore be to produce extra SH groups as the long glutenin molecules are broken. Fig. 6 has already shown one possibility of how the retraction after scission would render the likelihood of rejoining the severed chain remote, and at the same time lead to the production of two extra SH which could endanger neighbouring chains so reducing the D.P. (equation (1) or (2)). Once the optimum energy input has been exceeded, deterioration begins to occur. With the loss of resistance due to the shorter molecules there is more opportunity for molecular slip and stress relaxation. The weak viscous dough can stand considerable extension perhaps because molecules are no longer being stretched: this avoids the destructive retraction which occurs in doughs of good resistance and which probably initiates the local flaws leading to rupture of the test-piece. The effect is qualitatively similar to that of over-improvement described in the previous section.

In the activated dough development process[30-32] the dough is mixed with a work consumption of approximately one fifth of that used in the CBP together with reducing agents such as cysteine or metabisulphite and oxidising agents such as

bromate and ascorbate. Since the reducing agents act rapidly during mixing they will lead to a decrease in number-average D.P. of glutenin and the shorter molecules would be relatively easy to orient by normal work inputs. In order to give the dough suitable resistance the average molecular size has to be increased through removal of SH groups by the slower acting oxidising agents.

Evidence for the hypothesis

If overworking a dough leads to mechanical scission of S.S, as explained earlier, it would be expected that this effect would be observable. It was mentioned that attempts to find free radicals in dough were unsuccessful, but it is likely that in an aqueous system they would revert almost instantaneously to SH groups by hydrogen abstraction, most probably from water:

$$-CH_2S\cdot \quad + H_2O \longrightarrow - CH_2SH \quad + HO\cdot$$
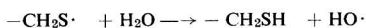
Sulphide or hydroxyl radicals could pick up electrons to revert respectively to sulphide or hydroxyl ions or the hydroxyl radicals may undergo a number of other reactions such as hydrogen abstraction until eventually two radicals could combine.

The lowered D.P. resulting from mechanical scission and the increased S.S interchange catalysed by new SH bonds would render the glutenin more soluble. Mecham et al.,[33] Godon et al.[34] and Tsen[35] have demonstrated that increased mixing causes more protein to be extracted from dough, which supports the hypothesis. Glutenin of low mol. wt. is even soluble in 70% ethanol.[5]

If mixing is carried out in air the free accessible SH will be re-oxidised to S.S at a rate that is greater than that at which they are created in glutenin. This would explain why a net decrease is observed on mixing in air.[36] If mixing is carried out under nitrogen then it would be expected that an increase in SH group concentration should be observed on prolonged mixing. Bloksma[37] and Tsen & Bushuk[38] did notice a small but definite increase in SH on dough mixing under nitrogen. More recently Mecham & Knapp[39] recorded increases ranging from 0·14 to 0·40 μ equiv. SH per g of flour or from 1·38–3·14 expressed in terms of μ equiv. per g of protein. Taking 2 as an intermediate value this corresponds to one 1 μ mole of S.S broken per g protein. Flour analysis[18] gives 130 μ moles of S.S per g of protein, so taking the earlier figure of one fifth these results could be explained if ~4% of the inter-chain S.S bonds in 1 g of flour protein had been broken.

Effective SH content

In a typical bread flour containing 11% protein, the content of glutenin chains in 1 g flour is ~2·5 μ moles whereas the SH content is ~1 μ mole. If all these SH groups reacted with glutenin the number-average D.P. by equation (1) or (2) would be ~2·5 which is known to be untrue. Bloksma[36] has pointed out that even on prolonged mixing in air or oxygen only about half the SH groups are oxidised. Large amounts of iodate are needed to oxidise these and even then it is far from complete. Evidently some of the SH groups are masked and being inaccessible even to small molecules they are unlikely to be accessible to large molecules such as glutenin. He has raised the possibility that the rheological properties may depend on the SH content of only a particular protein or peptide fraction of dough. It is relevant that Matsumoto & Hlynka[40] showed that less than a quarter of the total flour SH was associated with the acid-soluble protein. It is not improbable that the sources of SH groups such as GSH, thiotic acid, coenzyme A, cysteine, β-amylases and possibly other enzymes may be strongly adsorbed by the soluble proteins and therefore react only a small fraction of the total will be free to react with the glutenin. Another factor is that the reaction of SH groups with inter-chain S.S may be much more rapid when these are strained, for example during mixing.

Connection of D.P. with flour strength

The tensile strength of a dough will rise with increasing D.P. In this connexion it is interesting that low protein flours usually have high SH contents per g of protein, a factor which should cause a low D.P. This may help to explain why these flours are often known as weak. Strong flours conversely have higher protein contents, smaller proportions of water-soluble proteins, and hence higher glutenin contents. With lower SH contents per g of protein, their glutenins should be of higher D.P. It was shown as long ago as 1918 by Gortner & Doherty[41] that weak gluten changed from a gel to a sol at a much lower degree of hydration than a strong: this could well be due to differences in D.P. One of the actions of improvers could be to reduce the number of SH groups and hence raise the D.P. of the glutenin molecules (cf. equation (1) or (2)). Although improvers normally remove only a fraction of the available SH, it is probable that they reduce by a somewhat similar proportion the concentration of SH groups which have diffused into or which are associated with the glutenin. Frater & Hird[42] found that GSH reacted more rapidly with gluten if it had been broken down with proteases. When they added GSH equivalent to ~$\frac{1}{8}$ of the glutenin inter-chain S.S to dough (and probably only a fraction of this would have reacted with glutenin) the resistance as measured by the Brabender extensograph was lowered by a third. Similarly varietal differences in SH content or the effect of environment and storage conditions on this could play their part in affecting the resulting dough properties and introducing what have often been inexplicable variations in behaviour. The quantity and especially the D.P. of glutenin will markedly affect the viscosity of a flour suspension, and this may be one reason for the slower settling time of strong flours in the Zeleny test.[43] The well-known weakening effect of proteolytic enzymes on dough ties in with the concept of linear glutenin molecules. Proteolysis would have correspondingly less effect on crosslinked structures.

Conclusions

The evidence discussed renders plausible the hypothesis that the major fraction of glutenin which is responsible for the viscoelasticity of dough is a linear polymer of polypeptide chain sub-units and that there is no necessity to postulate the existence of branching crosslinks. It is hoped that this hypothesis will act as a stimulus to further research.

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APPENDIX

Linear glutenin

If n_0 linear glutenin molecules of number-average degree of polymerisation P_0 are subjected to inter-chain S.S fission such that a fraction α of the total inter-chain S.S bonds are broken, the number of molecules and number-average degree of polymerisation become respectively, n_t and P_t.

Fission of each inter-chain S.S creates a new molecule ∴ number of bonds broken $= n_t - n_0$.

The average number of bonds per molecule was $(P_0 - 1)$, therefore original number of bonds was $n_0 P_0 - n_0$

Hence
$$\alpha = \frac{n_t - n_0}{n_0 P_0 - n_0}$$

Dividing numerator and denominator by $n_0 P_0$:
$$\alpha = \frac{\dfrac{1}{P_t} - \dfrac{1}{P_0}}{1 - \dfrac{1}{P_0}} \quad \text{because } P_t = \frac{n_0 P_0}{n_t}$$

or $\alpha = \left(\dfrac{1}{P_t} - \dfrac{1}{P_0}\right)\dfrac{P_0}{(P_0 - 1)}$

Hence
$$P_t = \frac{P_0}{1 + \alpha(P_0 - 1)}$$

If $P_0 = 50$ and $\alpha = \dfrac{1}{49}$
$$P_t = 0 \cdot 5 \, P_0$$

Branched glutenin

For the general case of a macromolecular system with a probability γ that a given subunit is attached to a branching crosslink consider a linear strand of i subunits which is chosen at random. If this were part of a linear molecule not more than two bonds need be broken to cut this i-mer free. In the case of the branched system an extra γi bonds have to be broken. It is evident, therefore that to free a molecule requires more bond scission, and consequently the D.P. of the system will be less affected by this process.

FORMATION OF α-KETOGLUTARIC ACID BY WINE YEASTS AND ITS OENOLOGICAL SIGNIFICANCE

By B. C. RANKINE

α-Ketoglutaric acid was measured enzymically in wines made in the laboratory from three grape varieties by pure cultures of 12 wine yeasts of the genus *Saccharomyces*. The results were confirmed with the same juices and 4 yeasts on pilot-plant scale in replicated 30 gallon lots.

Mean values for the 12 yeasts ranged from 9 to 117 ppm (overall mean 53). In any one juice the yeasts differed by at least 10-fold in the amounts produced, and certain yeasts produced consistently high or low yields in all juices. The amounts of α-ketoglutaric acid produced depended somewhat on the grape juices used, even though these had comparable pH values, and a significant yeast–juice interaction occurred. The amount of α-ketoglutaric acid formed during fermentation at 15° was 60 per cent of that formed at 25°, and over twice as much was formed at pH 4·2 as at pH 3·0, using four yeast strains. Formation of α-ketoglutaric and pyruvic acids were not significantly correlated.

The α-ketoglutaric acid content of 18 white table wines made under comparable conditions on pilot-plant scale from different grape varieties using the same yeast strain ranged from 38 to 152 ppm (mean 90).

The significance of the results is discussed, particularly in relation to the binding of sulphur dioxide in wine, and recommendations are given on how to make wines which are low in α-ketoglutaric acid.

Formation of α-ketoglutaric acid by three yeasts in a chemically defined medium was lower with increased amounts of nitrogen as ammonium sulphate and higher in the presence of L-glutamic acid, both being used separately as sole nitrogen sources. These findings are discussed in relation to the rôle of α-ketoglutaric acid in nitrogen metabolism of yeasts.

Introduction

A knowledge of the α-ketoglutaric acid content of wines is important in evaluating the extent to which wines are able to bind sulphur dioxide and thus render it ineffective as an anti-oxidant and germicide. α-Ketoglutaric acid is similar to acetaldehyde and pyruvic acid in this regard, and its presence in wine has therefore an indirect effect on quality.

Interest at this Institute in α-ketoglutaric acid is part of a continuing investigation into the influence of wine yeasts on composition and quality of wines, and the work reported in this paper is concerned with the α-ketoglutaric acid content of wines and the influence of wine yeasts and other factors on the amounts produced during fermentation of grape juices.

Experimental

Determination of α-ketoglutaric and pyruvic acids

α-Ketoglutaric acid was determined by reductive amination to glutamic acid with glutamic dehydrogenase in phosphate buffer (pH 7·7), with the corresponding oxidation of reduced nicotinamide adenine dinucleotide (NADH₂). Oxidation of NADH₂, as measured by reduction in optical density at 340 nm, is proportional to the amount of α-ketoglutaric acid present. Sufficient ammonium ions for amination are present in the enzyme preparation, which is obtained from liver and suspended in ammonium sulphate. The method of analysis was similar to that described previously for pyruvic acid[1] (which was also used in this investigation), with the following differences.

The phosphate buffer was adjusted to pH 7·7 by dissolving 23·7 g Na₂HPO₄.2H₂O in 500 ml distilled water and titrating to pH 7·7 with a solution of 2·8 g KH₂PO₄ in 200 ml distilled water, and the solution was then made up to 1 litre with distilled water.

The optical density measurements were carried out at 37° since the reaction is too slow at room temperature.

Laboratory fermentations

These were carried out with a range of wine yeasts of the genus *Saccharomyces* selected from the yeast culture collection of the Australian Wine Research Institute, and included yeasts used in Australian and Californian wineries. A description of the yeasts is given elsewhere.[2]

The fermentations were carried out at 15° in 300 ml portions of sterile-filtered sulphited juices from crushed and pressed white grapes, as described previously.[2] All fermentations were carried out in duplicate, and the results of analysis of the filtered wines were treated statistically by analysis of variance to determine the significance of differences observed.

Pilot-plant fermentations

These were carried out with normal sulphited and pressed juice without sterile filtration in replicated 30 gallon lots in temperature-controlled fermenters at 15°, as described previously.[2] The wines were clarified, filtered and bottled, and stored at 15° until analysed. Each pure yeast culture was added to replicated samples of grape juices at a concentration of 3% by volume of an actively fermenting culture, which corresponded to a final concentration of approximately 3 million viable cells per ml. Previous work has shown that this concentration of inoculum dominates the naturally occurring microflora.

Results

Laboratory fermentations

The amounts of both α-ketoglutaric and pyruvic acids in wines made by 12 pure yeasts from three varieties of grape juices are shown in Table I.

The amounts of α-ketoglutaric acid differed considerably between yeast strains, and mean values for the three juices ranged from 9 to 117 ppm. The juices also differed significantly irrespective of the yeast strain; this indicates that the

TABLE I

Formation of α-ketoglutaric and pyruvic acids (ppm) by 12 yeast strains in 3 grape juices at 15°c
Means of duplicates

Yeasts		Grape juice							
		Pedro Ximenez		Tokay		Ugni Blanc		Mean	
		α-keto-glutaric	pyruvic acid	α-keto-glutaric	pyruvic acid	α-keto-glutaric	pyruvic acid	α-keto-glutaric	pyruvic acid
S. fructuum	No. 138	63	97	39	44	72	85	58	75
S. cerevisiae	No. 213	13	147	6	36	8	60	9	81
S. chevalieri	No. 317	105	85	46	31	116	68	89	61
S. ellipsoideus	No. 348	74	85	57	25	63	105	64	72
S. cerevisiae	No. 350	88	131	38	28	67	81	64	80
S. cerevisiae	No. 709	31	57	8	21	19	54	19	44
S. cerevisiae	No. 710	78	77	32	26	69	71	60	58
S. oviformis	No. 723	135	114	106	74	110	65	117	84
S. cerevisiae	No. 727	87	97	50	42	84	77	74	72
S. cerevisiae	No. 729	46	89	36	44	48	86	43	73
S. cerevisiae	No. 730	23	53	9	39	17	57	16	49
S. carlsbergensis	No. 731	25	71	19	21	23	41	22	44
Mean		64	92	37	36	58	71	53	66

L.S.D. (P < 0·05) between:

	means of duplicates	juice means	yeast means
α-ketoglutaric acid	13	10	20
pyruvic acid	16	15	31

Correlation coefficients between
α-ketoglutaric and pyruvic acids:
Pedro Ximenez 0·43 n.s.   Tokay 0·71**
Ugni Blanc   0·41 n.s.   Mean   0·50 n.s.

formation of α-ketoglutaric acid is influenced by the composition of the medium as well as by the strain of yeast. In addition, an interaction which existed between yeast strain and grape juice indicated that formation by certain yeasts was favoured more by one juice than another.

Measurements of pyruvic acid were made to find out whether a relationship existed between the amounts of pyruvic and α-ketoglutaric acids formed, since both are important in binding sulphur dioxide. Correlation coefficients were calculated and are shown in Table I. The mean value was 0·50 and was not significant.

The influence of pH on the formation of α-ketoglutaric acid in grape juice (Vitis vinifera cv. Ugni Blanc (syn. Trebbiano)). was examined using four yeasts over a pH range of 3·0 to 4·2, which corresponds to the range of values encountered in grapes used for making wine in Australia. The pH adjustments were made with hydrochloric acid or sodium hydroxide. The results are shown in Table II.

An increase in pH from 3·0 to 4·2 produced a more than two-fold increase in the yield of α-ketoglutaric acid.

The influence of temperature of fermentation was likewise examined using the same four yeasts, and the results are shown in Table III.

It can be seen that an increase in the temperature of fermentation from 15° to 25° produced an overall increase of 60% in the amounts of α-ketoglutaric acid formed.

Pilot-plant fermentations

An experiment was carried out in duplicate with the same four yeasts which were used for the laboratory fermentations, using the same Pedro Ximenez and Tokay juices which were used for the laboratory fermentations in Table I. The results are shown in Table IV from which it can be seen that the results obtained in laboratory fermentations were correlated with results obtained in the pilot-plant.

TABLE II

Influence of pH of grape juice (V. vinifera cv. Ugni Blanc) on formation of α-ketoglutaric acid (ppm) by 4 yeasts at 15°c
Means of duplicates

Yeast	pH				Mean
	3·0	3·4	3·8	4·2	
S. fructuum No. 138	48	65	63	87	65
S. cerevisiae No. 213	4	10	6	19	10
S. cerevisiae No. 350	46	38	23	97	51
S. cerevisiae No. 727	52	55	111	137	89
Mean	38	42	51	85	54

L.S.D. (P < 0·05) between:
means of duplicates   13
overall means   31

TABLE III

Influence of temperature (°c) of fermentation on formation of α-ketoglutaric acid (ppm) by 4 yeasts in Tokay grape juice, pH 3·3
Means of duplicates

Yeast	15°	20°	25°	Mean
S. fructuum No. 138	39	67	88	64
S. cerevisiae No. 213	6	15	19	13
S. cerevisiae No. 350	38	44	36	39
S. cerevisiae No. 727	50	61	76	62
Mean	33	47	55	45

L.S.D. (P < 0·05) between:
means of duplicates   11 ppm
overall yeast means   20 ,,
overall temperature means   17 ,,

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TABLE IV

Formation of α-ketoglutaric acid (ppm) by 4 yeast strains in 2 grape
juice at 15°c on pilot-plant scale
Means of duplicates

Yeast	Variety of grape juice		
	Pedro Ximenez pH 3·3	Tokay pH 3·3	Mean
S. fructuum No. 138	69	48	58
S. cerevisiae No. 213	21	15	18
S. cerevisiae No. 350	86	36	61
S. cerevisiae No. 727	86	34	60
Mean	65	33	49

L.S.D. (P < 0·05) between:
means of duplicates   10 ppm
means of yeasts   36 ,,
means of juices   26 ,,

TABLE V

Influence of initial nitrogen content on formation of α-ketoglutaric
acid (ppm) in synthetic medium at 15°c
Means of duplicates

Initial Nitrogen g/l (NH₄)₂ SO₄	Yeast		
	213	350	723
1	7	11	34
5	9	8	27
20	8	8	16
L.S.D. (P < 0·05)	2	3	5
L-glutamic acid 1 g/l	12	19	80

L.S.D. (P < 0·05) = 8

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In 1965 a range of 18 dry white wines were made in the pilot
winery from 9 grape varieties picked at various stages of ripe-
ness. The same yeast strain, *Saccharomyces oviformis* No.
723 which produces a high level of α-ketoglutaric acid, was
used to carry out the fermentations, and each wine was ferm-
ented at 15° and matured under comparable conditions.
The amounts of a α-ketoglutaric acid formed ranged from 38
to 152 ppm (mean 90) depending on the grape variety and
stage of ripeness. No correlation existed between pH and
α-ketoglutaric acid when all the wines were compared, but a
positive correlation existed for the grape varieties Clare
Riesling and Pedro Ximenez, for which four and three wines
respectively, were made from grapes picked from one vine-
yard at different stages of maturity. The total nitrogen
content of the wines was measured by the Micro-Kjeldahl
procedure and compared with the α-ketoglutaric acid content,
but the correlation was not significant.

Fermentations in a synthetic medium

Fermentations were carried out in a synthetic medium
formulated from Difco Yeast Carbon Base medium (nitrogen
free), using the concentration recommended by the manu-
facturer, with the addition of 10% dextrose. Three levels of
nitrogen were added as ammonium sulphate (1, 5 and 20 g/l)
and one level of L-glutamic acid (1 g/l), and fermentations
were carried out in replicated 50 ml quantities by three yeasts
selected for their ability to produce different amounts of
α-ketoglutaric acid in previous experiments. The results are
shown in Table V from which it can be seen that an increase in
ammonium sulphate concentration from 1 to 20 g/l had little
effect on the amount of α-ketoglutaric acid produced by two
yeasts, but brought about a reduction in the larger amount
produced by the third. Addition of L-glutamic acid as sole
nitrogen source resulted in more α-ketoglutaric acid being
produced by all three yeasts.

Discussion

The content of α-ketoglutaric acid in wines has not been
widely studied. Blouin[3] found 2 to 346 ppm in 200 Bordeaux
wines made in the period 1961–1963. The mean values
differed somewhat from one year to another, but averaged 80
ppm. Deibner & Cabibel-Hugues[4] found 0 to 82 ppm (mean

37) in 22 red and white wines from Southern France. Our
results ranged from 6 to 152 ppm and the mean value of 18
wines made on pilot-plant scale (Table V) was 90 ppm using a
yeast which produced a high level of α-ketoglutaric acid.
With the advent of a specific and rapid enzymic method more
data may be expected to be forthcoming.

The influence of yeast strains on formation of α-ketoglutaric
acid formation has been little studied. Apart from the work
of Peynaud & Lafon-Lafourcade[5,6] who found a range of 28 to
42 ppm for 6 species of *Saccharomyces*, no reports of any
further work were found. in the literature The results of the
present work show clearly the wide variation in the amounts
of α-ketoglutaric acid formed by strains of *Saccharomyces*.
The range of values for 12 strains tested under comparable
conditions in 3 grape juices was 9 to 117 ppm (mean 53),
which indicated the wide differences which existed between
different strains.

The amounts of α-ketoglutaric acid normally present in
wine are unlikely to have any direct effect on the aroma and
flavour of wine, but could significantly effect the binding of
sulphur dioxide. The dissociation constant of the α-keto-
glutaric bisulphite compound (a hydroxysulphonic acid) is
reported to be $8·8 \times 10^{-4}$ [8] and, more recently, 5×10^{-4}.[3] If
the latter value is used, in wine containing 30 ppm free
sulphur dioxide, 49% of the α-ketoglutaric acid is bound to
sulphur dioxide. For the range of α-ketoglutaric acid of
0–150 ppm this corresponds to 0–33 ppm sulphur dioxide
bound. Wines with 100 ppm α-ketoglutaric acid and 30
ppm free sulphur dioxide would have 22 ppm sulphur dioxide
bound to α-ketoglutaric acid. This is irrespective of other
binding compounds normally present, such as acetaldehyde
and pyruvic acid, as has been shown in both wine and model
systems.[9] The recommended content of free sulphur dioxide
in Australian wines depends on pH and ethanol content and
varies from < 10 to 50 ppm. It is usually low in red table
wines to encourage malo-lactic fermentation, and 30 ppm is
recommended for white table wines with pH values below
3·6. From the oenological viewpoint it is therefore import-
ant to keep the amount of α-ketoglutaric acid in wine as low as
possible, and from the results of this work certain recom-
mendations may be made on how this can be achieved:

The fermentation can be carried out with a yeast strain
which has been selected for low production of α-ketoglutaric
acid. Three strains of *S. cerevisiae* (Nos. 213, 709 and 730)
showed particular advantage in this regard, (Table I) and No.
213 is one of the strains used commercially in Australia.

It can be carried out at low temperature. At 15° the mean production by four yeasts was only 60% of that formed at 25°. (Table III).

The fermentation can be carried out at a low pH. At pH 3·0 the mean production by four yeasts was only 44% of that formed at pH 4·2.

Finally, it can be carried out in the presence of a low amount of sulphur dioxide. It has been found with experiments during the 1967 vintage that the high level of sulphur dioxide resulted in a somewhat higher overall level of α-ketoglutaric acid, although the amounts produced by the different yeast strains were in the same order as the results for 1966 vintage which are reported in the tables. Whiting & Coggins[7] have also shown with cider that addition of sulphur dioxide before fermentation resulted in a higher level of α-ketoglutaric acid.

From the biochemical viewpoint no significant overall correlation existed between the formation of α-ketoglutaric acid and that of pyruvic acid, which indicated that the pathways of formation of these two metabolites are not closely linked. α-Ketoglutaric acid is formed mainly as a de-amination product from glutamic acid in connexion with protein synthesis. It is one of the constituents of the tricarboxylic acid cycle in yeasts, but since this cycle does not occur in anaerobic fermentation, it would not function as a step in hydrogen transfer via the cytochrome system.

The results in Table V may be interpreted and explained in the light of recent work by Lewis & Rainbow[10] with a strain of *Saccharomyces cerevisiae*. Yeasts can grow with ammonium salts or many single amino acids as sole major sources of nitrogen, and these simple nitrogen compounds appear to be converted to glutamate as a first essential metabolic step in protein synthesis. When glutamate is supplied as the sole major source of nitrogen the α-amino group is mobilised in transamination reactions (which occur widely in yeast) resulting in the formation of other amino acids and α-ketoglutaric acid, which appears in the medium. However, with ammonia or certain other single amino acids as sole major sources of nitrogen, the yeast requires the endogenous α-ketoglutaric acid (formed from carbohydrate) in order to form glutamic acid, by the action of glutamic dehydrogenase or transaminases; consequently no surplus is left in the medium.

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ANALYSIS OF OILS AND FATS BY GAS CHROMATOGRAPHY

V.—Fatty acid composition of the leaf lipids of *Myosotis scorpioides*

By G. R. JAMIESON and E. H. REID

The fatty acids of the leaf lipids of *Myosotis scorpioides* have been shown to contain relatively large proportions of γ-linolenic and octadecatetraenoic acids as well as the more usual palmitic, linoleic and linolenic acids. Changes in the proportions of all the different classes of fatty acids have been found during the growing season of the plant, and also changes in fatty acid composition depending on the growth locality.

Introduction

In recent years there has been a great deal of investigation into the composition of leaf lipids,[1-7] and a close similarity in lipid composition of all deciduous tissues has been established.[8] The lipids of mosses have been studied by Gellerman & Schlenk,[9] the lipids of a moss and of green holly by Nichols,[8] and leaf protein lipids by Lima, Richardson & Stahmann.[10]

During a preliminary survey of the leaf lipids of various water and marsh plants it was found that the leaf lipids of *Myosotis scorpioides* contained relatively large amounts of γ-linolenic (18 : 3ω6)* and octadeca-6,9,12,15-tetraenoic (18 : 4ω3) acids. Neither of these acids is a normal component of seed oils although the 18 : 3ω6 acid has been found in the seed oils of *Oenothera biennis*,[11] *Humulus lupulus*[12] and from certain species of *Astelia* and *Collospermum*, family Liliaceae.[13] Both γ-linolenic and octadeca-6,9,12,15-tetraenoic acids have been found in the seed oils of some members of the Boraginaceae family[14-16] and in the leaf lipids of certain mosses and ferns.[17] It was also of interest to investigate the changes in fatty acid composition of leaf lipids during the growing season of a plant, since it has been indicated[18] that as a plant matures its leaf lipids become more saturated.

Experimental

Materials

The work was carried out between July and December, 1967, and in consequence the leaves used were from plants during the middle and latter parts of the growing season. The plants were gathered from the banks of a local pond which is situated in a sheltered locality. In September, November and December, samples were gathered from another pond about 200 ft higher and in an exposed locality.

Extraction of lipids

Leaves and stems were washed with cold water and air-dried for 24 hours. 20 g of the air-dried material were homogenised in a Waring blender with 400 ml chloroform-methanol (1 : 2 by vol) for 2 minutes. After filtration of the homogenate, the residue was washed with 3 × 100 ml chloroform. The combined filtrates were washed with saturated sodium chloride solution, and the chloroform layer was taken to dryness in a rotary film evaporator.[19]

Saponification and methylation

Samples of the lipid extract were saponified, the unsaponifiable material was removed, and the fatty acids were converted to methyl esters as described previously.[20]

Gas–liquid chromatography

The methyl esters from the leaf lipids were separated on a PE 800 gas chromatograph using both BDS (butanediol succinate) packed and DEGS (diethyleneglycol succinate) open tubular columns. The methyl esters were tentatively identified from the chromatograms by comparison with standard esters and by the use of separation factors.[21,22]

Silicic acid–silver nitrate column chromatography

Column chromatography using silicic acid–silver nitrate according to the technique of De Vries[23,24] was employed for the separation of the methyl esters into classes according to degree of unsaturation. The separation of 65 mg esters was carried out using 10 g adsorbent in a column 20 cm × 1·5 cm i.d. with a petroleum ether–benzene–diethyl ether solvent system, as used by Craig & Bhatty.[14] (B.p. of the petroleum ether, 40–60°.) The fractions collected from the column were monitored by gas–liquid chromatography. Saturated esters (15 mg) were eluted with 10% benzene in petroleum ether; mono-unsaturated esters (3 mg) with 25% benzene in petroleum ether; di-unsaturated esters (12 mg) with 50% benzene in petroleum ether; tri-unsaturated esters (23 mg) with 5% diethyl ether in benzene; and tetra-unsaturated esters (10 mg) with diethyl ether.

Permanganate–periodate oxidation

Each fraction containing unsaturated esters from the silicic acid column was oxidised with permanganate–periodate reagent in t-butanol using the procedure described by Kuemmel.[25] The methyl esters obtained from the permanganate–periodate oxidations were separated on the BDS column using programmed temperature operation.

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*i.e. the first double bond of the 3 is at the 6th carbon atom from the ω end of the chain

Results and Discussion

Gas–liquid chromatography of the fatty acid methyl esters of the leaf lipids of *Myosotis scorpioides* on both the weakly polar BDS and the strongly polar DEGS stationary phases indicated that there were six components in the C_{18} region, and that on hydrogenation all these components gave methyl stearate only. Using known methyl esters as standards, four of these components were identified as methyl stearate, methyl oleate, methyl linoleate and methyl linolenate, respectively. Separation factors were calculated for the remaining two components, X and Y (Table I). Type I separation factors apply to fatty acid esters of the same chain length, varying numbers of double bonds and the same carbon end-chain.

The factors calculated indicated that X is a $C_{18}\omega6$ ester and that Y is a $C_{18}\omega3$ ester. Type II separation factors apply to fatty acid esters of the same chain length, varying numbers of double bonds and different carbon end-chains. The Type II factors calculated indicate that the relative retention times of X and Y are in the same relationship as those of methyl linoleate and methyl linolenate, i.e. they are of the 3/6 type. Component X can therefore be tentatively identified as 18 : 3ω6 and component Y as 18 : 4ω3.

Separation of the fatty acid methyl esters into unsaturation classes by silicic acid–silver nitrate column chromatography gave in the monoene fraction one major component which had g.l.c. retention times identical with those of methyl oleate. However the products of permangate–periodate oxidation of this fraction indicate that the monoene was a mixture of oleic and vaccenic acids. The diene fraction contained one component which was identified as methyl linoleate by g.l.c. retention data and from the oxidation products. The triene fraction gave two peaks on the gas chromatograms with retention times identical with those of compound X and methyl linolenate respectively. The oxidation products were consistent with the triene fraction being a mixture of 18 : 3ω6. and 18 : 3ω3. The tetraene fraction gave one peak on the gas chromatograms with retention times identical with compound Y, and the oxidation products were consistent with compound Y being 18 : 4ω3 (Table II).

The fatty acid composition of the leaf lipids of *Myosotis scorpioides* is shown in Table III. The major constituents are palmitic, linoleic, γ-linolenic, linolenic and octadecatetraenoic acids. Although palmitic acid is the major saturated acid present, smaller amounts of all the n-even- and n-odd-numbered saturated acids from C_{12} to C_{24} were also present. Small amounts of the mono-unsaturated acids of all chain lengths between C_{15} to C_{24}, with the exception of C_{19}, were detected. Trace amounts of various branched chain acids were detected, and these amounts had increased in the August and December samples.

Table IV lists the proportions of various classes of acids. There is a decrease in the proportion of saturated acids during the latter part of the growing season. The proportions

TABLE I

G.l.c. retention data and separation factors of C_{14} methyl esters

Stationary phase	BDS		DEGS	
	standards	leaf lipids	standards	leaf lipids
18 : 0	1·00	1·00	1·00	1·00
Relative retention time				
18 : 1	1·09	1·09	1·18	1·18
18 : 2ω6	1·30	1·30	1·48	1·48
X	—	1·45	—	1·79
18 : 3ω3	1·62	1·62	2·03	2·03
Y	—	1·81	—	2·46
Separation factors				
Type I $\dfrac{X}{18 : 2\omega6}$		1·12		1·21
$\dfrac{Y}{18 : 3\omega3}$		1·12		1·21
Type II 3/6				
$\dfrac{18 : 3\omega3}{18 : 2\omega6}$		1·24		1·37
$\dfrac{Y}{X}$		1·25		1·37

TABLE II

Analysis of oxidation products from fractions by silicic acid column chromatography

	Monoene		Diene		Triene		Tetraene	
	Found	Expected	Found	Expected	Found	Expected	Found	Expected
Dicarboxylic								
6	—		1·3		36·1	38·3	100	100
7	—		2·5		—			
8	6·0		4·8		0·6			
9	59·6	100·0	89·8	100	62·5	61·7		
10	2·6		1·6		0·8			
11	31·8		—		—			
Monocarboxylic								
3	—		—		+[a]	55·6	100	100
4	—		1·2		—			
5	—		2·8		—			
6	0·3		96·0	100·0	+	44·4		
7	18·5		—		—			
8	0·6		—		—			
9	80·6	100·0	—		—			

[a] + detected but not determined quantitatively[25]

TABLE III

Fatty acid composition, weight % of total acids

Acid	July	August	September		October	November		December	
12 : 0	0·1	0·1	0·1	0·1[a]	0·1	0·1	tr[a]	0·2	0·1[a]
13 : 0	0·1	0·3	tr	0·1	tr	0·2	0·2	0·2	0·4
14 : 0	1·0	2·6	0·8	0·7	1·0	0·6	0·7	0·7	0·6
15 : 0	0·1	0·1	0·1	0·1	0·2	0·2	0·1	0·2	tr
15 : 1	0·1	0·2	0·1	0·2	0·3	tr	tr	0·2	0·1
16 : 0	21·3	19·5	17·3	14·2	17·6	14·2	16·0	15·0	14·7
16 : 1	1·8	1·6	1·7	2·2	0·8	0·5	1·5	0·7	0·5
17 : 0	0·6	0·5	0·3	0·2	0·4	0·2	0·1	0·2	0·1
17 : 1	n.d.	n.d.	n.d.	n.d.	0·3	0·2	0·2	0·1	0·1
18 : 0	2·1	2·2	1·8	1·7	1·8	1·5	1·5	2·1	1·5
18 : 1	4·0	3·7	4·0	3·7	3·4	2·7	2·7	4·1	2·7
18 : 2ω6	23·9	23·8	24·8	24·7	22·7	18·5	23·1	20·0	21·8
18 : 3ω6	12·8	13·4	14·3	14·7	13·8	14·5	15·9	16·0	16·4
18 : 3ω3	15·0	15·1	19·5	22·7	20·2	24·1	21·0	20·0	19·9
18 : 4ω3	5·6	6·1	6·2	8·4	8·7	13·9	9·8	9·2	11·9
19 : 0	0·1	0·1	0·1	0·1	0·1	0·1	0·1	0·1	0·1
20 : 0	0·5	2·3	1·5	1·5	1·1	1·6	1·6	0·8	1·2
21 : 0	0·2	0·2	0·2	0·1	0·1	tr	0·1	0·3	0·1
22 : 0	5·0	3·8	3·0	2·1	3·0	3·1	1·5	4·2	2·2
23 : 0	0·4	tr	0·2	0·1	0·3	0·2	0·1	tr	0·1
24 : 0	4·6	2·8	3·4	1·6	3·0	3·1	1·7	3·7	2·7
20 : 1 22 : 1 23 : 1 24 : 1	0·7	n.d.	0·4	0·6	0·9	0·5	1·9	1·0	1·7
Branched-chain	n.d.	1·6	0·2	0·2	0·2	0·1	0·2	1·0	1·1

[a] from exposed locality;
tr trace;
n.d. not determined.

TABLE IV

Amounts of fatty acids of different classes, %

	July	August	September		October	November		December	
Saturates	36·1	36·1	30·0	22·8[a]	28·8	24·9	23·8[a]	28·7	24·9[a]
Unsaturates									
mono	6·6	5·5	6·2	6·7	5·8	3·9	6·3	6·1	5·0
di	23·9	23·8	24·8	24·7	22·7	18·5	23·1	20·0	21·8
tri	27·8	28·5	33·8	37·4	34·0	38·6	36·9	36·0	36·3
tetra	5·6	6·1	6·2	8·4	8·7	13·9	9·8	9·2	11·9
ω3	20·6	20·2	25·7	31·1	28·9	38·0	30·8	27·2	31·8
ω6	36·7	37·2	39·1	39·4	36·5	33·0	36·9	38·0	38·2

[a] from exposed locality

of mono- and di-unsaturated acids remained fairly constant from July to October but there was a slight decrease in the di-unsaturated acids in the November and December samples. The tri- and tetra-unsaturated acid showed a large increase in November for the sample from the sheltered locality and in December for the sample from the exposed locality. If the acids are classified according to carbon end-chain, it is found that the proportion of ω6 acids remained fairly constant but the proportion of ω3 acids gradually increased during the period studied.

Recently it has been reported[18] that the sulpholipids and phospholipids of lucerne become more saturated during maturation and it was indicated that a general property of plants was that, as they matured, their leaf lipids became more saturated. This was not the finding in the present study of the fatty acids of *Myosotis scorpioides*. However it is noteworthy that during October and November 1967 there was very mild weather in the district from where the samples were taken. The sample taken during October, although it was mainly old growth, had some fresh shoots. The samples taken in November were fresh shoots and may indicate the fatty acid composition at an early stage in growth. The samples taken in December were the fresh shoots which had not grown much during the colder weather during this last month.

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VARIATION IN THE XANTHOPHYLL AND CAROTENE CONTENT OF LUCERNE, CLOVERS AND GRASSES

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The xanthophyll and carotene contents of eleven lucerne clones were found to be higher during September and November than during June and July. Both carotenoids reached a maximum at or before first flowering and then declined as blossoming progressed. Lutein decreased in November while violaxanthin increased. Six grasses and four legumes were analysed for the principal leaf xanthophylls, lutein, violaxanthin and neoxanthin, as well as total xanthophyll and carotene.

Introduction

A recent study[1] at this laboratory on the effects of dehydration on the carotenoids of lucerne showed a considerable variability in fresh lucerne both in total xanthophyll and in the relative amounts of the three principal xanthophylls: lutein, violaxanthin and neoxanthin. Subsequently a poultry pigmentation study[2] showed that while lutein caused strong skin pigmentation, the epoxide-containing xanthophylls, violaxanthin and neoxanthin, were relatively ineffective as broiler skin pigmenters. This latter study suggested that it would be an advantage for a feed formulator to have a lucerne meal not only rich in total xanthophyll but also high in lutein and low in violaxanthin and neoxanthin.

The present study was undertaken to ascertain the variation in total xanthophyll and carotene, and in the relative amounts of lutein, violaxanthin and neoxanthin at several stages of growth and in eleven lucerne clones during the growing season. It was noted that certain lucerne clones were darker green than others. Several species of grasses and legumes were also analysed to ascertain if a particular species might be high in lutein and total xanthophyll.

A previous study[3] described the development of a leaf and stem separation procedure which gave high xanthophyll, carotene and protein content in the leaf fraction. A leaf and stem separation was therefore made of the samples to reveal maximum differences in xanthophyll and carotene content between samples, and to permit comparisons uncomplicated by variations in leaf to stem ratios.

Experimental

Methods

The freshly harvested plant samples were quickly frozen between layers of dry ice and stored in this way until after freeze-drying. Following separation into leaf and stem fractions, the samples were ground in a Wiley mill so as to pass through a 40 mesh screen. All carotenoid analyses were carried out in duplicate. Total xanthophyll and carotene were determined by the method of Kohler et al.[4] Lutein, violaxanthin and neoxanthin were separated and analysed by the t.l.c. method of Nelson and Livingston.[5] Moisture content of samples was determined by drying them for 24 h in a forced-draught oven at 110°.

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Evaluation of the variation of xanthophyll and carotene content in the eleven lucerne clones was made by the Multiple Range Test method of Duncan.[6]

Materials

Lucerne stage of growth

Lucerne cv. Vernal, grown at a site near Madison, Wisconsin, was sampled at different stages of growth during the spring and early summer of 1966 at 7.30 a.m. Leaf samples contained only the green leaflets, while stem samples contained the stems, petioles and any flowers.

Grasses and legumes

The grasses and legumes were also grown at a site near Madison in 1966 and sampled at the same time of day as the lucerne. Grasses were sampled at early anthesis, and the legumes at first flowering. They were separated into leaf and stem fractions. The leaf fraction contained only the green blades of the grasses and the green leaflets of the legumes; the stem fraction contained the heads, stems, and sheaths of the grasses and the flowers, stems, and petioles of the legumes.

Lucerne—clonal and seasonal variation

The lucerne clones were grown near Fresno, California in 1967. The clones were sampled on each occasion at first flower and at approximately the same hour of the day. Colour of the plants was noted at each harvest.

Results and Discussion

Stage of growth

Both the xanthophyll and carotene contents in the leaves reached a maximum at first flower during the spring crop, and then decreased as blossoming progressed (Table I). Except for the first cut (10 in tall) the decrease in xanthophyll was correlated with plant maturity and was significant at the 10% level of probability. In the regrowth, the maximum was reached at an earlier stage of growth and declined to the stage of 45% flowering. Possibly because of the limited number of samplings of this crop the differences were not statistically significant at the 10% level of probability. The total carotenoid content of the spring crop and regrowth may be compared at the stage of first flowering. At this stage, the spring crop leaf fraction was slightly higher in both xanthophyll and carotene. The total carotene and xanthophyll in lucerne was greater in the regrowth than the spring crop owing to the greater percentage of leaf.

The relative percentage of the three principal xanthophylls in lucerne varied only slightly during the growth stages (Table II). In the spring growth, the lutein declined slightly at first flowering, with both neoxanthin and violaxanthin near maximum. In the regrowth lutein, violaxanthin and neoxanthin remained relatively constant.

The ratio of xanthophyll to carotene (X/C) was high in both leaf and stem fractions during the early stages of growth of the spring crop and then decreased as the plant matured (Table I). In the regrowth the X/C ratio was highest at first flowering and then decreased slightly at 45% flowering. The stem fraction had a consistently higher X/C ratio than the leaf in both the spring and second crops. However, the total carotenoid content of the stem fraction was only about 20% of that of the leaf in all samples analysed. The leaf fraction was therefore the main source of xanthophylls causing pigmentation.

Forages

Reed canarygrass had the highest xanthophyll and carotene content of the grass samples analysed (Table III). Since this plant contained a higher percentage of leaves than the other grasses, the total xanthophyll and carotene in the whole plant was about $1\frac{1}{2}$ times higher than in any of the other grasses. In all grasses analysed, the xanthophyll/carotene ratio (X/C) was greater than 2 : 1. In comparison, one of the legumes, birdsfoot trefoil cv. Empire, had a X/C ratio of only 1·71 in the leaf fraction. The total xanthophyll of this legume was much lower than in any of the other legumes sampled. As a source of xanthophyll for poultry feeds, this would be partly offset by the very high proportion of lutein which comprised 82% of the total xanthophyll in the leaf. This same sample had the lowest violaxanthin content, 7%, compared with yellow sweetclover which was high in violaxanthin and low in lutein.

TABLE I

Variation in xanthophyll and carotene content with stage of growth of Vernal lucerne

Stage of growth	Fraction	%	Date harvested	Carotene (C), mg/kg (dry basis)	Xanthophyll (X), mg/kg (dry basis)	Ratio X/C
Spring crop						
10 in tall	leaf	49	5/16	396	853	2·15
	stem	51		59	167	2·81
16 in tall	leaf	48	5/23	530	1128	2·13
	stem	52		64	183	2·86
21 in tall	leaf	38	5/30	515	1065	2·07
	stem	62		57	161	2·81
First flowering	leaf	34	6/13	614	1166	1·90
	stem	66		66	158	2·40
25% flowering	leaf	34	6/22	598	913	1·53
	stem	66		62	143	2·32
75% flowering	leaf	25	7/2	453	638	1·41
	stem	75		62	121	1·96
Regrowth						
12 in tall	leaf	53	7/11	680	1150	1·69
	stem	47		137	240	1·76
First flowering	leaf	50	7/20	596	1101	1·84
	stem	50		113	266	2·37
45% flowering	leaf	48	7/30	373	610	1·64
	stem	52		92	196	2·12

TABLE II

Variation in the content of the principal leaf xanthophylls at various stages of growth of Vernal lucerne

Stage of growth	Date harvested	% of total xanthophyll in leaf		
		Lutein*	Neoxanthin	Violaxanthin
Spring crop				
10 in tall	5/10	61	18	21
16 in tall	5/23	63	17	20
21 in tall	5/30	59	15	26
First flowering	6/13	57	18	25
25% flowering	6/22	60	17	23
75% flowering	7/2	60	18	22
Regrowth				
12 in tall	7/11	61	17	22
First flowering	7/20	62	17	21
45% flowering	7/30	61	20	19

*Includes 2–4% zeaxanthin

Carotenoid variation between lucerne clones

The mean values of the xanthophyll and carotene contents of the lucerne clones varied significantly at the 5% level between the two summer months and the two autumn months (Tables IV and V). The higher xanthophyll and carotene content in September and November may be due to a somewhat slower rate of plant growth resulting from less sunlight and cooler temperatures. Quackenbush *et al.*[7] found that the total carotenoid content of maize seedlings decreased under very intense light and increased at lower temperatures and moderate light intensity.

Although the total xanthophyll and carotene contents of the lucerne clones was significantly greater in September and November than in the warmer summer months, the percentage of lutein decreased in November (Table VI). Conversely, the percentage of violaxanthin was larger in November than in the other three months (Table VII). This suggests a photosynthetic relationship between lutein and violaxanthin. Blass *et al.*[8] and Sapozhinkov *et al.*[9] found that violaxanthin was converted to lutein in the light, and that the reverse reaction occurred in the dark. The lower light intensity of November apparently favoured the formation of violaxanthin. However, the increase in total xanthophyll in November, compared with June and July, offset the lower content of

TABLE III

Variation in xanthophyll and carotene content of six grasses at early anthesis and four legumes at first flowering

Plant	Date harvested	Fraction	%	Carotene, mg/kg (dry basis)	Xanthophyll, mg/kg (dry basis)	% of total xanthophyll		
						Lutein*	Neoxanthin	Violaxanthin
Grasses								
Tall fescue,	6/18	leaf	12	460	1122	65	23	12
(*Festuca arundinacea* Schreb.)		stem	88	101	268	61	16	23
Quackgrass	6/23	leaf	15	425	993	63	15	22
(*Agropyron repens* (L.) Beauv.)		stem	85	88	231	64	16	20
Timothy cv. Verdant	7/2	leaf	17	387	1010	71	17	12
(*Phleum pratense* L.)		stem	83	95	268	75	16	9
Cocksfoot cv. Potomac	6/20	leaf	21	521	1184	58	18	24
(*Dactylis glomerata* L.)		stem	79	62	166	57	19	24
Bromegrass cv. Sac	6/20	leaf	21	510	1131	58	25	17
(*Bromus inermis* Leyss.)		stem	79	77	207	63	25	12
Reed canarygrass	6/18	leaf	26	640	1492	60	16	24
(*Phalaris arundinacea* L.)		stem	74	90	235	67	14	19
Legumes								
Yellow sweetclover	6/8	leaf	33	594	1312	59	11	30
(*Melilotus officinalis* L.)		stem	67	59	152	66	17	17
Red clover cv. Lakeland	6/14	leaf	34	724	1724	61	17	22
(*Trifolium pratense* L.)		stem	66	66	176	67	18	15
Lucerne cv. Saranac	6/9	leaf	39	629	1352	58	13	29
(*Medicago sativa* L.)		stem	61	75	176	63	18	19
Birdfoot trefoil cv. Empire	6/1	leaf	42	449	758	82	11	7
(*Lotus corniculatus* L.)		stem	58	62	176	74	14	10

*Contains 2–4% zeaxanthin

TABLE IV

Seasonal variation of xanthophyll in lucerne clones

Clone	Colour	Xanthophyll, mg/kg (dry basis)				
		June	July	September	November	Mean*
1	Dark green	1006	1021	1131	962	1029·6
2	Dark green	948	923	1075	1135	1019·7
3	Dark green	933	785	1099	1077	974·2
4	Dark green	840	849	946	1153	947·1
5	Dark green	858	857	971	1066	938·3
6	Dark green	858	801	1021	968	911·5
7	Dark green	945	748	1003	882	894·3
8	Dark green	860	733	1082	894	892·8
9	Medium green	867	664	830	967	831·6
10	Light green	616	818	989	830	812·9
11	Light green	700	669	1094	643	776·2
Mean†		858	805	1021	962	

*Any two means scored by a solid line are not significantly different at the 5% level
†Any two means not scored by a solid line are significantly different at the 5% level

TABLE V

Seasonal variation of carotene in lucerne clones

Clone	Colour	Carotene, mg/kg (dry basis)				
		June	July	September	November	Mean*
1	Dark green	500	503	570	528	526·2
2	Dark green	459	517	489	592	514·0
3	Dark green	496	457	612	526	522·7
4	Dark green	480	464	550	566	515·9
5	Dark green	470	519	521	543	513·8
8	Dark green	483	455	578	543	515·9
7	Dark green	500	479	570	541	523·0
6	Dark green	450	438	464	469	455·1
9	Medium green	438	180	477	491	446·8
10	Light green	363	418	449	488	429·6
11	Light green	341	411	497	416	416·2
Mean†		453·7	459·0	526·9	518·0	

*Any two means scored by the same line are not significantly different at the 5% level
†Any two means not scored by the same line are significantly different at the 5% level

TABLE VI

Seasonal variation of lutein in lucerne clones

Clone	Colour	Lutein,* % of total xanthophyll				
		June	July	September	November	Mean†
10	Light green	74	82	76	67	74·8
8	Dark green	76	69	79	69	73·3
7	Dark green	77	72	72	71	73·0
3	Dark green	77	77	76	62	73·0
4	Dark green	76	72	73	70	72·8
1	Dark green	71	69	74	73	71·8
6	Dark green	72	76	71	67	71·5
11	Light green	70	70	77	67	71·0
9	Medium green	73	66	70	67	69·0
5	Dark green	67	75	69	64	68·8
2	Dark green	70	72	68	64	68·5
Mean**		73·0	72·7	73·2	67·4	

*Includes 2–4% zeaxanthin
†Any two means scored by a solid line are not significantly different at the 5% level
**Any two means not scored by a solid line are significantly different at the 5% level

TABLE VII

Seasonal variation of violaxanthin in lucerne clones

Clone	Colour	Violaxanthin, % of total xanthophyll				
		June	July	September	November	Mean*
1	Dark green	14	16	10	13	13·3
2	Dark green	16	13	18	22	17·3
3	Dark green	9	10	10	23	13·0
4	Dark green	11	14	11	20	14·0
5	Dark green	18	12	16	23	17·3
6	Dark green	13	10	14	23	15·0
7	Dark green	10	13	14	14	12·8
8	Dark green	10	17	8	17	13·0
9	Medium green	12	20	14	22	17·0
10	Light green	13	6	10	21	12·5
11	Light green	16	17	9	19	15·3
Mean†		12·9	13·4	12·2	19·7	

*Any two means scored by a solid line are not significantly different at the 5% level
†Any two means not scored by a solid line are significantly different at the 5% level

TABLE VIII

Seasonal variation of neoxanthin in lucerne clones

Clone	Colour	Neoxanthin, % of total xanthophyll				
		June	July	September	November	Mean*
1	Dark green	15	15	16	14	15·0
2	Dark green	14	15	14	14	14·3
3	Dark green	14	13	14	15	14·0
4	Dark green	13	14	16	10	13·3
5	Dark green	15	13	15	13	14·0
6	Dark green	15	14	15	10	13·5
7	Dark green	13	15	14	15	14·3
8	Dark green	14	14	13	14	13·8
9	Medium green	15	14	16	11	14·0
10	Light green	13	12	14	12	12·8
11	Light green	14	13	14	14	13·8
Mean*		14·1	13·8	14·6	12·9	

*Any two means scored by the same line are not significantly different at the 5% level

lutein so that the total amount of lutein was relatively constant during the three month period. There was no significant difference ($P = 0·05$) in the relative percentage of neoxanthin during the four months or between the eleven clones (Table VIII).

Although there was a significant ($P = 0·05$) difference in the percentage of lutein between several of the lucerne clones, the data could not be correlated with plant colour.

The light green lucerne (No. 11) had a significantly lower xanthophyll content than three of the dark green clones (nos. 1, 2, and 3). Although the lighter green clones 9 and 10 had a lower average xanthophyll content than the darker clones (nos. 1–8), this difference was not found to be significant at the 5% level.

A significant ($P = 0·01$) correlation of $r = 0·833$ was found between the mean values of the xanthophyll and carotene content of the lucerne clones presented in Tables IV and V.

The carotene content of the light coloured clones 10 and 11 was significantly lower than seven of the darker clones (nos. 1–7). The observed green colour of the plants would be dependent on the chlorophyll content as well as the thickness of the leaf and the proximity of the chlorophyll to the leaf surface. Although this study suggests that a relationship exists between intensity of green colour of the lucerne plant and carotenoid content, confirmation would require analysis of many more dark and light coloured clones over several seasons, and was beyond the scope of the present study.

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CHANGE IN CELL MEMBRANE PERMEABILITY IN TEA FLUSH ON STORAGE AFTER PLUCKING AND ITS EFFECT ON FERMENTATION IN TEA MANUFACTURE

By G. W. SANDERSON*

The permeability of cell membranes in tea flush increased on storage after plucking when the flush was allowed to desiccate as in orthodox tea manufacture. The increase in membrane permeability was roughly proportional to the degree of desiccation until the flush approached about 55% moisture content at which the permeability is a maximum.

At room temperature (about 22°) there was no appreciable change in membrane permeability in non-withering flush until after about 30 hours of storage. However, chilling temperatures (4° and 10°) markedly increased membrane permeability even in the absence of any desiccation, and killing temperatures ($-15°$ and 40°) were even more effective.

The importance of increase in cell membrane permeability to tea manufacture is discussed in relation to the ease with which the catechol oxidase can be mixed with the catechins in the cells, a process which is essential for the initiation of tea fermentation.

Introduction

Withering of the plucked flush of the tea plant, i.e. young shoot tips comprising the first two leaves, the bud, and the included stem, is the first step in orthodox tea manufacture.[1-3] In Ceylon the moisture content of the flush is reduced from about 78% to about 55% in 18 hours of withering under normal conditions.[2] This process conditions the flush for the next step in manufacture, namely the rolling process, which brings about the commencement of fermentation. It is well established that properly withered flush can be easily rolled into a well-twisted product which is commercially desirable and, it is this physical conditioning which has been alleged to be the principal benefit of the withering process.[2,4]

It was deduced many years ago that chemical changes also occur in tea flush from the effect that different withering conditions have on the organoleptic characteristics of the finished product, black tea.[5,6] The actual chemical changes taking place have only recently begun to be elucidated in any detail,[7-13] and their effect upon the quality to the made tea is still only poorly understood. This paper reports the results of an investigation which is part of a general study of the biochemistry of tea manufacture.

Over thirty years ago Evans[14] reported that the permeability of the cell membranes in tea flush increases during withering. This phenomena has been reinvestigated and the results of this investigation are presented in this paper together with a discussion of their importance in tea manufacture.

Experimental

Collection of fresh tea flush

The flush used in this investigation was collected from blocks of clonal tea growing in fields adjacent to the laboratory (1500 m elevation).

Measurement of membrane permeability

The permeability of cell membranes was measured as the decrease in dry weight of tissues after leaching with distilled water.[15-17] Two methods of leaching were used: Method A—60·0 g (fresh weight) of flush were placed in a 2 litre flask with 1·5 litres of distilled water. The flask was shaken continuously for 5 hours and the distilled water was changed once after the first hour. Method B—60·0 g (fresh weight) of flush were placed in 1·5 litres of distilled water and it was left to stand undisturbed for 24 hours.

All samples were air dried in an oven at 85° for 5 hours before determination of dry weight.

Results

Effect of moisture loss on membrane permeability in plucked flush

Duplicate samples of plucked flush were stored in two ways. One set of samples was spread evenly and approximately one leaf deep on table tops in a well ventilated laboratory. This set of samples withered (lost moisture) under conditions similar to those existing in commercial practice. A duplicate set of samples was placed in polythene bags on the table top alongside the withering flush. Under the latter conditions no moisture was lost by the flush. One set of samples which had been stored in polythene bags was spread exposed to wither after 20 hours. The temperature varied from 23° to 26° over the period of the experiment. Pairs of samples were periodically drawn from the different treatments; one of each pair was placed directly into the oven to dry and the other was leached according to Method A before drying.

The results are shown in Fig. 1. It was found that the permeability of the cell membranes of flush undergoing withering increased rapidly until the moisture content of the flush was reduced to about 55% when the permeability was maximal. The slight decrease in the amount of material leachable from the flush after this time is probably due to
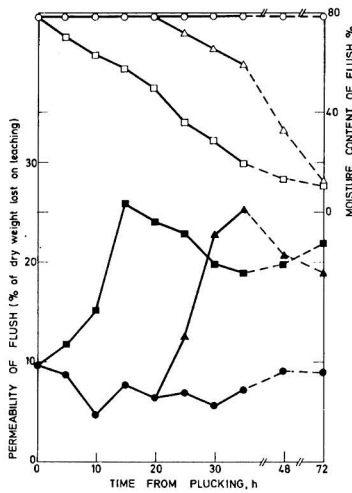
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FIG. 1. *Changes in the permeability of cellular membranes in tea flush on storage after plucking as shown by the leachability of cell contents and the effect of withering on these changes*

The storage period began 2 hours after plucking of the flush. All samples were stored at room temperature (23°C to 26°C). *A*. Record of withering: ○————○, flush stored in polythene bags (no withering); □————□, flush stored exposed (withering); △————△, flush stored in polythene bags until 20th hour (no withering) and stored exposed after 20th hour (withering). *B*. Record of dry weights of flush either leached or not leached: ●————●, flush stored in polythene bags (no withering); ■————■, flush stored exposed (withering); ▲————▲, flush stored in polythene bags until 20th hour (no withering) and stored exposed after 20th hour (withering)

the interaction of cellular constituents to form insoluble products when the tissues became highly desiccated. Flush stored so as to prevent withering showed no increase in cell membrane permeability until after the 30th hour when a slight increase in permeability began to take place. The relationship between withering and membrane permeability was further demonstrated using flush which was stored so as to prevent withering for 20 hours and then allowed to wither. As soon as withering began the permeability of the cell membranes began to increase in the same manner as in flush which had been allowed to wither from the start of the experiment.

Effect of temperature of storage on changes in membrane permeability in plucked flush

Samples of flush were stored in various ways as described in Table I. Leaching of tissues was carried out according to Method B in these experiments.

The results are shown in Table I. Storage of flush at the extreme temperatures studied, −15° and 40°, caused the permeability of the cell membranes to increase markedly even in the absence of any desiccation (Table I–A). At chilling temperatures, 4° and 10°, the permeability also increased appreciably. On the other hand, the permeability of cell membranes in flush stored at room temperature, 22° to 26°, or at a slightly higher temperature, 31°, did not change until after 21 hours of storage.

Flush stored in evacuated desiccators (Table I–B) showed the same pattern of membrane permeability changes as flush stored in polythene bags (Table I–A) except that the permeability of flush stored at room temperature was markedly reduced as compared with the initial conditions. Flush

TABLE I

Effect of temperature and method of storage on changes in the permeability of cellular membranes in plucked flush of tea plants

Storage temperature, °C	Length of storage, h*	Moisture content of flush, %	Dry weight after leaching, g/100 g unleached dry weight	Dry weight lost on leaching, %
		A. Stored in polythene bags		
−15	21	76·5	68·7	31·3
	45	76·6	72·5	27·5
4	21	76·1	90·5	9·5
	45	75·4	90·3	9·7
10	21	76·1	90·6	9·4
	45	75·6	90·4	9·6
24–26**	21	76·2	93·6	6·4
	45	76·2	91·5	8·5
31	21	76·2	93·1	6·9
40	21	74·2	88·5	11·5
	45	74·1	85·5	14·5
		B. Stored in evacuated desiccators		
4	21	72·6	91·2	8·8
24–26**	21	72·9	99·1	0·9
40	21	65·4	88·8	11·9
		C. Stored exposed on table tops		
24–26**	0**	76·7***	93·5***	6·5***
	21	35·0	87·6	12·4
	45	11·7	84·1	15·9

*   Storage period started 3 hours after plucking of the flush
** Uncontrolled room temperature determined by thermograph
*** This sample is the control for all the samples in this experiment

stored exposed and withering (Table I–C) showed the changes expected from the first set of experiments.

It is noteworthy that all flush stored at 40° had turned completely brown within 21 hours. This browning reaction is analogous to tea fermentation (the enzymatic oxidation of tea flavanols) which takes place only after mixing of the cell contents. In commercial practice this mixing is accomplished by mechanical means in the rolling process.[1-3] These results suggest that storage of flush at 40° allows fermentation to proceed because under these conditions the cell membranes deteriorate allowing the tea flavanols to come into contact with catechol oxidase by diffusion. It has previously been reported that treatment of these tissues with chloroform vapour has the same effect.[18]

Discussion

The changes in membrane permeability in tea flush during withering are believed to be of great importance in tea manufacture. To appreciate the importance of these changes one must bear in mind the requirements for tea fermentation which follows the withering stage in tea manufacture. Quantitatively speaking, the enzymatic oxidation of the flavanols present in the tea flush is the most important change taking place during fermentation.[19] However, the flavanols are located in the cell vacuoles[20] and the enzyme catechol oxidase which brings about this oxidation is located in the cell cytoplasm.[21] Therefore, fermentation is dependent on the mixing of the contents of the cells in the tea flush. This can be accomplished in flush which has been properly withered by relatively gentle means.

The rolling process of orthodox tea manufacture[1-3] known throughout the world today falls into the above category and it is known to be capable of producing well-fermented teas with excellent quality and appearance when well-withered flush is used (such as is the usual case in Ceylon and north India (Darjeeling)). On the other hand, it has been found that in areas where adequate withers are difficult to obtain because very wet atmospheric conditions are encountered over extended periods (such as is the case in north-east India, Indonesia, and East Africa) the orthodox rolling process often gives disappointing results. In these latter areas new and more drastic processing machines (such as C.T.C., Legg Cut, and Rotorvane machines) which cause a much higher percentage of cell damage have come into use. These new machines have been successful in improving the liquoring properties of teas in districts where poor withering conditions prevail. The results reported here indicate that new unorthodox processing methods have their benefit in promoting tea fermentation by bringing about mixing of the tea flavanols with catechol oxidase in tissues where the membrane permeability is too low to allow the less drastic orthodox methods to accomplish the same thing. This improvement of liquors in teas made from poorly-withered flush is, however, obtained at the expense of destroying the traditionally accepted appearance of the made tea.

It is important to note here that all other biochemical changes occurring in tea flush during withering which have to date been studied in any detail[9-13] take place more or less independently of moisture loss. These findings suggest that storage of tea flush after plucking in a non-desiccating environment can substitute for withering if alternative methods for orthodox rolling are adopted. The practical considerations of the findings reported here are discussed in more detail elsewhere.[10]

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MINERAL COMPOSITION OF APPLES

VIII.*—Further investigations into the relationship between composition and disorders of the fruit

By M. A. PERRING

In 1967, when an unusual number of disorders were present in apples grown in England, Cox's Orange Pippin apples with disorders at the time of picking or after storage were obtained from various sources and analysed for mineral constituents. The results were compared with those for sound apples, from similar or different samples.

Low levels of calcium were found to be associated with many of the disorders. At these low levels of calcium, high concentrations of other elements appeared to be a contributory factor in rendering apples liable to particular disorders.

Introduction

The quality and chemical composition of many apples grown in England in 1967 were unusually affected by the combination of two climatic factors. There were severe frosts and a period of cold weather at blossom time which together had a thinning effect on the fruit, and high values for hours of sunshine in June and July. Levels of many chemical constituents in the fruit at picking time were higher than usual but those of calcium were generally low. A number of disorders developed in the fruit at this time or a short time after picking. Some of these disorders are rarely seen in Cox's Orange Pippin apples, and the opportunity was taken to study them in relation to the chemical composition. The work was done with apples grown experimentally at the East Malling Research Station and with samples sent to the Ditton Laboratory by commercial growers and officers of the National Agricultural Advisory Service (N.A.A.S.).

Experimental and Results

The apples to be analysed were washed in distilled water and allowed to dry naturally before their stems and seeds were removed. Individuals and bulk samples were frozen at $-20°$ and grated before sub-samples were weighed for analysis.[1]

Duplicate sub-samples of about 1 g and 2 g respectively were digested for nitrogen determinations[2] and were ashed by the oxygen flask technique.[1] Dry matter was determined by drying sub-samples of about 5 g at 50° for 44 h. Nitrogen, potassium and phosphorus were determined by methods already described.[2,3] Calcium was determined with a Unicam SP900 flame spectrophotometer in the emission mode and with standard calcium solutions containing the proportions of the other major mineral elements found in the ash of an average Cox's Orange Pippin apple. Magnesium was determined with the same spectrophotometer in the absorption mode after the addition of strontium chloride to solutions of samples and magnesium standards. Sodium determinations were made with an EEL (Evans Electroselenium Ltd.) flame photometer after dry ashing.[4]

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* Part VII: *J. Sci. Fd Agric.*, 1968, **19**, 186

Orchard K4

When samples of 25 apples from an experimental plot at the East Malling Research Station were cut transversly for the removal of seeds before analysis, it was noticed that a few apples had watery patches internally. In some apples the water-logged tissue was confined to small regions at about 2 mm from the surface but in apples more severely affected the patches were as shown in a figure in the preceding paper in this series[5] although there were no external symptoms of pitting and distortion in the K4 apples. In all instances the core region was unaffected. All of the apples with the disorder were frozen separately for analysis and the numbers of the bulk samples were made up with sound apples. It was also possible to obtain a few extra apples with the disorder from some of the trees.

The results of analyses of these samples are shown in Table I. The calcium concentration in every apple affected by the disorder was lower than the mean for the treatment and most calcium levels were extremely low. The other constituents measured show no consistent trend although it is interesting to note that the two affected apples with relatively high calcium levels (3·0) had high nitrogen concentrations. One of these apples was recorded as having very slight damage in the form of one water-logged region about 10 by 0·5 mm near the surface in the median transverse section.

Samples of fruit from the same trial were stored at 2·8° until February and were inspected periodically for external damage. Samples with senescent breakdown were removed and frozen individually for analysis. These analytical results are shown in Table II together with results for bulk samples taken before storage. One apple of the 80 examined still had watery patches in a section cut near the calyx eye. This apple was affected by breakdown and it can be seen from Table II that its calcium concentration was similar to those of the affected apples in Table I. With two exceptions the apples with breakdown had lower concentrations of calcium than the treatment means and, if an allowance of 5% is assumed for water loss during storage, contained less than 3·0 mg of calcium per 100 g.

Orchard A

Two apples sent to the laboratory at picking time were found to have very severe water-core. All of the intercellular spaces from core to peel were saturated and the disorder was visible through the peel. These apples were analysed and the results are given in Table III. Although the calcium concentrations were low they were not exceptionally low. The nitrogen levels were high in both apples.

Orchard I

Ten sound apples and ten apples with disorders, taken from a sample picked late in September and barn-stored, were sent to the laboratory in November and were then frozen for analysis as two bulk samples. The sample with disorders included apples with lentical breakdown (all of these had bitter pit internally) and apples with severe bitter pit. The analytical results are shown in Table IV. Mean fruit size was

TABLE I

Analytical data for individual apples with 'watery patches' internally and comparable bulk samples of sound apples (*) from orchard K4, 1967

Mass as g, mineral concentrations as mg/100 g, and dry matter as % fresh weight

Treatment and trees	Mass per apple	Dry matter	K	N	P	Mg	Ca
DPG 11.5.9	131*	18·6	136	65	14·5	6·0	3·8
	139	17·4	164	57	14·0	4·8	1·8
DPG 27.5.9	120*	19·0	132	69	16·4	5·3	3·7
	108	18·3	135	65	14·1	5·4	2·2
	141	19·8	133	55	18·7	4·8	2·4
	171	18·9	153	91	16·2	5·8	3·0
SPC 15.13.17	124*	18·8	128	59	14·4	5·0	3·8
	115	18·3	101	69	13·4	3·9	2·2
SPC 31.5.9	119*	18·7	142	67	15·5	5·7	3·7
	141	18·5	151	56	14·9	4·8	2·0
	143	19·1	146	56	13·7	4·5	2·0
	164	19·0	157	88	17·4	5·6	3·0
	192	18·9	169	68	17·1	5·5	2·2
SRG 23.13.17	111*	18·4	155	74	15·4	5·8	3·5
	97	18·6	161	79	15·6	5·4	2·2
	185	17·8	161	102	12·4	5·5	1·4

TABLE II

Analytical data for individual apples with senescent breakdown after storage and bulk samples at picking time from orchard K4, 1967/68

Mass as g, mineral concentrations as mg/100 g, and dry matter as % fresh weight *at time of sampling.* Breakdown index: 0=sound, 3= severe

Treatment and trees	Date removed from store	Breakdown index	Mass per apple	Dry matter	K	N	P	Mg	Ca	Other disorders
DPG 11.5.9	Initial	0	131	18·6	136	65	14·5	6·0	3·8	
	18·1	3	123	17·9	145	84	15·7	6·4	1·9	Watery patches
	16·2	2	124	17·0	164	80	17·8	6·2	3·0	Bitter pit
DRG 11.13.17	Initial	0	127	18·5	149	62	14·7	5·3	3·5	
	18·1	2	163	16·1	172	90	19·8	6·5	2·8	Bitter pit
	16·2	1	109	20·0	205	46	17·9	6·2	2·8	Bitter pit
	16·2	1	135	19·1	180	56	17·8	6·3	3·7	Bitter pit
SPG 23.5.9	Initial	0	121	18·5	138	71	15·1	5·0	3·3	
	18·1	2	122	16·1	148	70	14·4	4·8	2·0	
SPG 3.13.17	Initial	0	120	18·4	135	78	16·1	5·3	4·3	
	12·2	1	148	15·6	147	67	17·0	5·9	2·8	
SPC 31.5.9	Initial	0	119	18·7	142	67	15·5	5·7	3·7	
	16·2	2	121	18·0	119	65	18·7	5·1	2·7	Bitter pit
SRC 31.13.17	Initial	0	160	18·9	157	67	15·1	5·3	3·3	
	26·1	2	136	16·0	141	69	14·4	5·6	3·0	
	12·2	2	116	16·5	164	70	16·2	6·5	3·6	
	16·2	1	129	17·6	172	80	18·4	5·9	2·5	Bitter pit
	16·2	1	131	17·8	143	59	14·9	6·0	3·1	Bitter pit

TABLE III

Analytical data for two apples with severe water-core from orchard A, 1967

Mass as g, mineral concentrations as mg/100 g, and dry matter, alcohol soluble and insoluble matter
as % fresh weight

Mass	K	N	P	Mg	Ca	Na	Dry matter	Alcohol soluble	Alcohol insoluble
92	155	86	14·5	5·8	3·8	1·7	17·0	13·7	3·3
109	164	97	14·7	5·5	3·5	1·3	14·6	11·8	2·8

TABLE IV

Analytical data for apples from orchard I

Mass as g, mineral concentrations as mg/100 g, and dry matter as % fresh weight in November 1967

Disorder	Number of apples analysed	Mass per apple	K	N	P	Mg	Ca	Dry matter
Sound	10	119	177	66	18·1	7·0	4·3	20·4
Lenticel breakdown and bitter pit	10	116	196	78	20·0	6·5	2·9	21·0

the same for both samples but the fruit with disorders had a lower level of calcium and higher levels of potassium, phosphorus and nitrogen than the sound apples.

Orchard J

Apples from a neighbouring orchard to orchard I began to develop lenticel breakdown a week after picking and samples of sound apples and apples with the disorder were sent to the laboratory about 3 weeks later. All the apples with lenticel breakdown were found to have bitter pit internally (the surface lesions and the internal pits were separated) and many of them were breaking down. The severity of lenticel breakdown was roughly assessed by counting the number of spots on the surface of the fruit. The size of the spots varied and in some instances they overlapped. All the apples were then cut and the severities of senescent breakdown and bitter pit were estimated. An index of breakdown has been described previously and bitter pit was assessed in the same way; 3 indicates an apple pitted through as far as the core and 0 indicates a sound apple. Scores of ½ were awarded to two apples, each of which had two slight pits near the surface. The eight sound apples were frozen as a bulk sample and the other apples were frozen separately.

The severity of disorders and the analytical results are shown together in Table V. It can be seen by comparing the results for sound apples with those for individuals with disorders that calcium was the only constituent measured that showed a consistent difference of concentration. All of the apples with disorders had lower calcium concentrations and some of these were exceptionally low.

Orchard H

Apples that had been kept in a scrubbed controlled atmosphere store were sent to the laboratory in January. They were found to be affected by lenticel breakdown, bitter pit, rotting by *Gloeosporium* and senescent breakdown. All of the apples with lenticel breakdown had bitter pit internally and one rotted apple was also breaking down. The fruit was

divided into 5 bulk samples for analysis as shown in Table VI. It was noticed that the sound apples looked redder than the apples with disorders and the area coloured red was estimated before the apples were frozen. The results of the analyses and other details are given in Table VI. The calcium concentration and % red colour were highest in the sound sample and lowest in the sample with the most disorders. The potassium, phosphorus, magnesium and nitrogen levels were all higher in the samples with lenticel breakdown.

Discussion

If it is assumed that a correct balance of constituents is necessary in an apple to ensure freedom from disorders, it is interesting to compare the results given here with those for average fruit. A considerable amount of information on fruit composition has been obtained in recent years[6,7] and the average concentrations for Cox's Orange Pippin apples grown in England are roughly as follows: K = 140, P = 15, Ca = 5·0, Mg = 5·0, N = 60 mg/100 g and dry matter = 17% fresh weight. It is evident that every calcium result shown here is below the mean and thus low calcium concentration is a common factor in all of the disorders. The predisposition of an apple to any one disorder is best considered by examining the disorders separately in relation to composition.

Watery patches

Watery patches of the type found in orchard K4 have been described previously but those apples (orchard P10, 1962) were also pitted and mis-shapen. They were found to have low levels of calcium and exceptionally high levels of potassium and magnesium. It was concluded that the 'patches' may have been due to an imbalance of calcium, and potassium and magnesium.[5] The present experiments indicate that low calcium only is associated with the 'patches' because potassium and magnesium levels were low in some of the K4 apples and none of the fruit had especially high levels of these elements (Table I). Thus the high levels of potassium and magnesium in the P10 fruit presumably resulted in the brown

lesions and possibly the distortion. It does seem, however, that a high level of nitrogen might increase the incidence of watery patches and the calcium : nitrogen ratios in the damaged apples were all lower than the means for the sound fruit.

The intercellular water was absorbed during storage apart from that in one apple already mentioned. The breakdown in this apple was distinct from the water-logged regions in contrast to the water-core breakdown described recently by Sharples.[8]

Water-core

If the two apples of orchard A are considered together it can be seen (Table III) that only calcium and nitrogen levels deviate significantly from the mean. The ratio of calcium to nitrogen in these apples was similar to those of the damaged apples from orchard K4.

In recent years water-core has been associated with excessive amounts of sorbitol in the fruit.[9] The incidence of water-core and the concentration of sorbitol in the fruit increase as harvesting is delayed[9] in contrast to a decrease in calcium concentraton.[10] Water-core has occasionally been observed in Cox's Orange Pippin apples when samples harvested late in 'time of picking' trials have been cut for analysis. It is possible that water-core results from an imbalance of sorbitol, calcium and nitrogen.

TABLE V

Details of disorders and composition of apples from orchard J

Mass as g, mineral concentrations as mg/100 g, and dry matter as % fresh weight in October 1967. Indices of breakdown and bitter pit: 0 = sound, 3 = severe. Lenticel breakdown as the approximate number of spots per apple

Number of apples analysed	Lenticel breakdown	Bitter pit index	Senescent breakdown index	Mass per apple	K	N	P	Mg	Ca	Dry matter
8	0	0	0	115	183	67	13·1	6·0	3·4	19·1
1	0	½	0	119	174	58	10·4	5·1	2·8	17·8
1	0	½	0	156	236	77	16·8	5·8	3·1	19·7
1	75	3	2	72	206	72	18·6	6·2	1·4	19·7
1	80	3	2	79	194	70	17·2	6·0	1·6	19·8
1	15	1	0	86	206	68	20·9	6·3	2·1	22·0
1	40	2	0	88	190	80	19·2	5·9	2·1	19·7
1	20	2	0	94	209	72	19·9	6·8	1·9	19·7
1	40	2	1	103	203	75	20·1	6·2	1·8	19·6
1	30	1	0	106	213	68	13·3	5·4	1·9	20·8
1	60	3	1	108	196	66	16·7	6·2	1·6	18·9
1	60	3	1	115	196	80	18·6	6·9	2·3	19·6
1	50	3	2	121	194	55	21·1	6·1	2·3	22·1
1	20	1	1	126	191	71	13·1	6·0	2·6	17·7
1	15	1	2	128	200	53	10·0	5·2	2·5	17·2
1	40	2	1	129	196	76	15·8	6·1	2·0	19·2
1	90	3	1	135	209	76	15·3	5·5	1·7	20·2
1	5	1	0	141	196	82	15·4	6·9	2·6	18·6
1	30	2	1	151	206	68	21·1	6·1	2·7	21·0
1	110	3	2	152	226	67	19·6	6·4	1·9	17·9
1	30	1	0	162	195	69	12·5	5·8	1·9	19·3
1	50	2	1	172	197	65	13·6	5·6	2·2	17·4
1	32	2	1	185	201	73	13·6	6·1	2·4	20·3

TABLE VI

Analytical data for apples of orchard H with different disorders that developed during storage in 1967/68

Mass as g, mineral concentrations as mg/100 g, and dry matter as % fresh weight after storage

Disorder	Number of apples analysed	Mass per apple	K	N	P	Mg	Ca	Na	Dry matter	% surface coloured red
Sound	20	147	148	73	14·7	5·6	3·8	3·3	19·4	37
Rotted	5	146	149	81	15·7	5·7	3·2	3·4	18·9	28
Bitter pit	6	131	152	78	15·8	5·1	2·6	3·3	19·2	28
Lenticel breakdown and bitter pit	10	158	174	89	18·1	6·6	2·7	3·6	18·6	22
Lenticel breakdown and bitter pit and rotted	8	163	177	97	17·4	6·4	2·3	2·5	18·8	7

Senescent breakdown

It has been suggested that individual apples with calcium concentrations of less than 3·0 and bulk samples with mean calcium concentrations of less than 4·5 mg/100 g are susceptible to senescent breakdown after a short time in store.[5] Apples from all of the orchards being considered should therefore have been liable to breakdown. The incidence of breakdown may be lessened by high concentrations of potassium, phosphorus, magnesium and dry matter[6] and in many instances these concentrations were exceptionally high (Tables II, IV, V, VI). The present results therefore emphasise the importance of calcium because many apples did break down.

There was a high incidence of breakdown in the J samples (Table V) and the *average* concentration of calcium was lowest in the most severely affected fruit and was highest in the sound fruit although there was a considerable overlap in the concentration in individuals affected to varying extents. Breakdown was probably delayed in the H samples by good storage conditions, but one apple was breaking down when it was received at the laboratory and it is possible that a high incidence of breakdown would have occurred a few days after removal from store.

Ten of the K4 apples which broke down had calcium concentrations lower than 3·0 mg/100 g. Two apples, however, had higher concentrations and an allowance for a loss in store as great as 10% does not bring the results below the mean for the treatment. No explanation for their breaking down can be deduced from the results for the other constituents measured.

Nitrogen concentrations in individual apples with senescent breakdown were not measured in earlier experiments.[5] The wide range of nitrogen in the K4 and J individuals (Tables II and V) indicate that, as with bulk samples, nitrogen level has little influence on breakdown.

Rotting by *Gloeosporium*

Although any conclusion must depend on the assumption of an equal spore load on each apple, and the apples of orchard H were not inoculated with spores of *Gloeosporium* under experimental conditions (the orchard was not sprayed against *Gloeosporium* incidentally), it is interesting to compare results in Table VI. If the sound and rotted, and the lenticel breakdown with and without rots pairs of samples are considered, the only consistent differences in the analytical results are the lower calcium and higher nitrogen levels in the rotted apples. This is in agreement with results of experiments with bulk samples of inoculated apples at the East Malling Research Station.[7]

Bitter pit

Bitter pit was recorded in all the samples with the exception of apples from orchard A which were not stored. Apples from orchards I and J were the most severely affected and these samples had very high levels of potassium and high levels of magnesium. There is a considerable overlap when severity of bitter pit in the individual J apples is compared with the calcium concentration or K:Ca or Mg:Ca ratios, but the mean analytical results for apples with the same severity of pitting show a marked decrease of severity of pitting as the calcium concentration increases or the Mg:Ca and K:Ca ratios decrease. A high K:Ca ratio is common to the fruit from all the orchards. There is no evidence that the concentrations of nitrogen in individual apples have any influence on the incidence or severity of bitter pit.

Lenticel breakdown

Lenticel breakdown is a disorder which is not frequently found on Cox's Orange Pippin apples grown in England though in some seasons there have been high incidences. A photograph of the disorder has recently been published by Ritter & Hansen who compared analytical data for the peel taken from apples with and without the disorder.[11] The concentration of calcium was lower and those of potassium, phosphorus, magnesium and nitrogen were all higher in the peel of apples with lenticel breakdown. They concluded that there was a close relationship between the disorder and bitter pit. This may easily be confirmed by comparing the number of lesions, or spots, with the severity of internal pitting in the J apples (Table V).

The mean concentration of calcium was low and those of potassium, nitrogen, phosphorus and magnesium were high in fruit of orchards H, I and J. The relation between high levels of nitrogen and phosphorus, and lenticel breakdown was most marked in the H and I samples.

Concentrations of the constituents measured in the J individuals were compared with the severity of lenticel breakdown which was roughly calculated on the basis of spots per unit of surface area of each apple. The surface area was calculated from the mass of the apple by assuming the apple to be spherical, and the application of the known mass/diameter relation for Cox's Orange Pippin apples. The reduction of the disorder with increasing concentrations of calcium is shown in Fig. 1. There was only a suggestion of increasing severity of lenticel breakdown with increasing magnesium, phosphorus and potassium concentrations in the J individuals and no evidence of any effect of nitrogen on the severity in these apples.

Nutrient ratios

It is well established that low levels of calcium and high levels of potassium and magnesium lead to increased in-
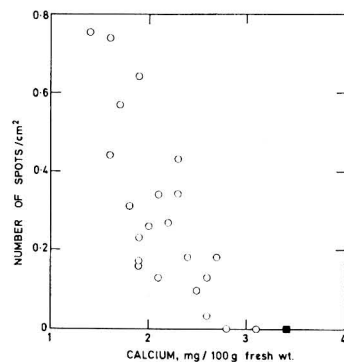

FIG. 1.   *The effect of the calcium concentration in apples of orchard J on the severity of lenticel breakdown expressed in terms of surface area of the fruit*

○ an individual apple; ■ a bulk sample of apples

cidences of bitter pit in apples and hence it has become fashionable to work with K : Ca or Mg : Ca ratios rather than with calcium concentrations. These have the advantages of being independent of changes in the fruit, such as water loss during storage, and of the basis of measurement, i.e. fresh weight or dry weight. The Mg : Ca ratio was better correlated with severity of lenticel breakdown (and bitter pit) in the J apples and so this ratio was also calculated for the fruit of the H and I samples. These results showed that if the ratio was less than 1·8, the apples were clear from bitter pit and if it was above 2·0 the apples had lenticel breakdown and increasingly severe bitter pit. Fruit with intermediate values had bitter pit.

Consideration of the K4 results, however, indicated that the application of the Mg : Ca ratio to the prediction of type of disorder was not possible. The ratios for many of the individual apples were between 2 and 4, yet no lenticel breakdown was found on the stored samples. About $\frac{1}{3}$ of the stored apples were pitted, but not severely. The main differences between the K4 fruit and the other fruit were that the mean levels of potassium and phosphorus were lower in the K4 apples.

Apparently very high levels of potassium and/or phosphorus are necessary in an individual apple for it to be susceptible to lenticel breakdown. The necessity of considering the deviation of the levels of a number of constituents from the normal, rather than limited ratios when attempting to predict the incidence of disorders is demonstrated in the example above.

Conclusions

It has been found that a deficiency of calcium in the fruit is related to a number of disorders and that the nature of the disorder depends on the balance of other constituents in the fruit. The question of whether the concentrations of the mineral elements have a direct effect on disorders in the apple, or are merely an indication of something that has occurred during the development of the fruit remains to be answered. In the case of calcium the effect appears to be direct because incidences of all of the disorders quoted above have been reduced by spraying with calcium solutions[12] and it is known that calcium sprays act directly through the fruit.[13] Also, reductions in the incidences of bitter pit and senescent breakdown have sometimes been achieved by post-harvest dips in calcium solutions.

When calcium concentrations in the fruit are low the presence of high concentrations of potassium, nitrogen, phosphorus or magnesium apparently lead to one or several disorders. An increase of these elements may be desirable to improve fruit quality[6] but the danger of attempting this without first raising the level of calcium in the fruit is emphasised by the storage results given here.

The samples with water-core and 'watery patches' have been considered separately because these may not be exactly the same disorder. The measurement of other constituents including sorbitol and boron would be of value in this respect. Watery patches might have been expected in some of the H, I and J apples and may have occurred. There was no information available about internal disorders in these apples when they were picked. The distribution pattern of the watery patches is similar to that of bitter pit and whether or not the patches become pits during storage is a matter of conjecture.

The brown surface pits on the mis-shapen P10 apples of 1962 were similar to lenticel breakdown. It is probable, because of their composition, that more lenticel breakdown would have developed in these apples if they had been stored.

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MINERAL COMPOSITION OF APPLES
IX.*—Uptake of calcium by the fruit

By B. G. WILKINSON

The amount of calcium in mature apples appears to be related to certain storage disorders. For Cox's Orange Pippin a difference of 2 mg in a total calcium content per fruit of about 5 mg can make the difference between good and bad keeping.

Most of the calcium moves into the fruit during the first few weeks of its development (approximating to the period during which the cell walls are being formed). Thereafter (during the period of cell expansion) the movement of calcium is no longer in one direction only, and as much as 1 mg may move out of the fruit. This may be of the same order of magnitude as that contributed by calcium sprays, which are now commonly applied to improve storage quality.

The factors affecting the movement of calcium into or out of apples are not known, but the limited evidence available suggests that movement of calcium out of the fruit may occur during periods of abnormally dry weather. These are the conditions which are usually associated with the occurrence of bitter pit.

Introduction

Evidence continues to accumulate that calcium is of vital importance in determining whether apples are likely to develop certain storage disorders such as bitter pit, senescent breakdown, lenticel breakdown, and possibly rotting.[1,2] The connexion between Ca content and bitter pit has been known for many years, and spraying with Ca salts is becoming an increasingly common control measure. Experiments with Ca sprays have confirmed that other disorders are affected by Ca, and the results of chemical analysis of fruit all point to the fact that Ca may be at a critical level in the fruit.

Storage potential is only one aspect of fruit quality, but from this aspect it would be advantageous if the Ca level in apples could be raised, and this may involve a greater knowledge of the way in which Ca accumulates in the fruit.

Experimental and Results

At various times since 1956 determinations have been made of Ca in Cox's Orange Pippin apples at different stages in development, and particularly near picking time. The fruit has been obtained from several experimental plots at the East Malling Research Station, and therefore, although of varied history, has been confined to one locality. All except one of these experiments have been referred to previously in other contexts, and in terms of concentration.[3,4] Here the results have been calculated as amounts of Ca per apple and are presented in Fig. 1. The diagram is given in two parts to avoid a confusion of points. Each point represents a determination from a bulk sample of 25 apples. The methods of sampling, preparation and analysis were as previously described.[3,4]

It will be seen that after a period of continuous uptake (until about the end of July) the amount of Ca per apple may either continue to increase at a reduced rate, may remain more or less constant, or may actually decline as shown markedly in the fruit from orchard P 10, 1961.

Discussion

Ca is unusual among the mineral elements in being in relatively low concentration in the fruit, and its uptake is on a comparatively small scale. It has been suggested that its major role is in the formation of the cell wall structure where it is fixed in insoluble form in what is in effect the skeleton of the fruit. All other major elements are active metabolically throughout the life of the fruit and are taken up continuously during growth. The movement of Ca can be considered as being in two stages. Stage 1 is the continuously rising uptake which takes place until about the end of July; stage 2 is the subsequent period during which Ca may rise or fall. When it does rise the rate is less than in stage 1. A fall in the amount of Ca indicates that Ca has moved back from the fruit to the tree.

If Ca is required mainly during cell division then it is to be expected that the uptake pattern would show a discontinuity
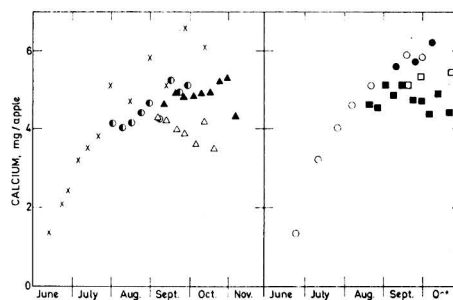
FIG. 1. *Changes in amounts of Ca in apples during development*

× D, 1956; △ P10, 1961; ▲ D3, 1962; ◐ D3, 1966
○ K3, 1956; ● K3, 1957; □ K3, 1958; ■ D3, 1964

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* Part VIII. Preceding paper

in midsummer. Little is known about the factors affecting stage 1. The comment has been made previously[3] that the amount of Ca available to the fruit early in the season could be important, the suggestion being that this might determine the ultimate level of Ca in the fruit. This is still a possibility, but the only data available are believed to be the two curves for orchards D, 1956 and K 3, 1956. These two curves are not dissimilar, but are for the same season.

However, the pattern of Ca movement between tree and fruit after the demands of the cell walls have been met (stage 2) could also be important. The curves in Fig. 1 were obtained from fruit of trees relying on soil Ca; there were no Ca spray programmes. The stage 2 patterns vary from continuing uptake (a further $1 \cdot 5$ mg in K 3, 1956), to return of Ca to the tree (about 1 mg in P 10, 1961). This suggests free mobility of some of the Ca at this stage.

Experiments with Ca sprays fall into a different category. Schumacher *et al.*[5] have claimed that Ca does not enter the fruit if the leaves alone are sprayed, but only if the Ca reaches the surface of the fruit. This suggests that Ca deposited on the leaf surface is not capable of joining the path of movement into the fruit in stage 2, as shown in K 3, 1956, for instance. The other possibility is that the Ca was applied when the fruit Ca was falling anyway, and it would be interesting to know whether the findings of Schumacher *et al.* would apply in all seasons and under all conditions.

Incidentally the increase in fruit Ca reported by Perring[6] as a result of one Ca spray programme was $1 \cdot 7$ mg. This was sufficient to affect storage behaviour and is of the same sort of order as that gained from the tree in stage 2 in some of our observations.

There is considerable evidence that Ca applied to the fruit surface can contribute to the Ca status of the fruit. For instance there are many reports of Ca dips being effective in reducing bitter pit. Using ^{45}Ca applied to the fruit, Martin[7] has shown qualitatively that Ca moves out of the fruit to adjacent leaves and shoots; and that ^{45}Ca applied to the leaves after harvest moves back into the tree and reappears in the new season's leaves and fruit—presumably to be utilised in cell formation. Martin concluded that soluble Ca is quite mobile in apple trees.

Reverting to the examples in Fig. 1 where the Ca was all supplied naturally from the tree, there is no clear evidence of the factors which influence whether there is a net gain or loss of Ca in stage 2, but since the changes are of a magnitude which could influence storage behaviour they are obviously important. Of the factors which may affect Ca movement the most likely is the weather. There are some seasonal differences. Comparing the same orchards in different seasons, D 3 showed a slight rise in 1962 and 1966, and a slight fall in 1964. K 3 showed less rise in 1958 than in 1956 and 1957. Inspection of the East Malling weather records suggest that rainfall may be a factor. For instance, the third quarters of 1961 and 1964 (when Ca seemed to be moving out of the fruit) were particularly dry—$4 \cdot 32$ and $2 \cdot 42$ in of rain respectively. Similar periods in 1956, 1957 and 1966 were wet—$9 \cdot 46$, $8 \cdot 78$ and $8 \cdot 11$ in respectively. In these periods Ca seemed to be gained. 1958 and 1962 were intermediate—$7 \cdot 48$ and $6 \cdot 31$ in respectively, and were close to the 35 year average of $6 \cdot 74$ in. Again, comparing the Ca pattern for fruit from the one orchard K 3, the rainfall in the third quarters of the years 1956, 1957 and 1958 decreased in that order. The three curves for D 3 also conform in that 1966, 1962 and 1964 are in order of decreasing rainfall for the third quarter. This limited evidence might suggest that rainfall increases late Ca uptake. There is some support for this view in results from irrigation trials. In two such trials in different parts of the country, in 1954 and 1959, the amount of Ca per apple was greater in fruit from irrigated trees than in controls. However irrigation sometimes limits the amounts of other constituents associated with good keeping properties,[8] and its effect on the level of Ca cannot be considered in isolation. It may be that high rainfall (or irrigation) when temperature and light are not restricting assimilation provides the most favourable conditions for effective Ca accumulation.

The observation that fruit Ca may either increase or decrease in stage 2 may account for the lack of effectiveness of Ca spraying under some conditions,[8] and for the fact that the generalisation that early picked apples are more liable to bitter pit is not always confirmed experimentally.[9] The suggestion that dry conditions may be associated with a loss of Ca by the fruit is also consistent with the fact that these conditions are usually considered to be a contributory factor in the occurrence of bitter pit.

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EFFECT OF SIMULATED RECLAMATION ON NITRIFICATION OF AMMONIUM SULPHATE APPLIED TO SALINE AND ALKALI SOILS FROM WEST PAKISTAN

By M. A. SINDHU and A. H. CORNFIELD

Nitrification of ammonium sulphate (100 ppm nitrogen) applied to alkali, saline–alkali, highly saline and moderately saline soils was studied during 12 weeks of incubation before and after simulated reclamation treatments, in which water plus calcium sulphate was used for the two alkali soils and water alone for the two saline soils. Before reclamation there were considerable differences in the pattern of nitrification due to soil type; this was accounted for mainly by the high loss of ammonia by volatilisation (61–96% of the ammonium–nitrogen applied) from the two alkali soils; from 0 to 80% of the nitrogen applied was recovered as nitrate after 12 weeks of incubation. After reclamation the pattern of nitrification was similar for all soils; 58–68% of the applied nitrogen was recovered as nitrate after 12 weeks. The moderately saline soil was the only one to show a lower recovery of applied nitrogen as nitrate after than before reclamation. There was some nitrite accumulation early in incubation in all the original soils, but after reclamation only in the two alkali soils.

Introduction

In a previous study[1] it was found that whilst reclamation of a moderately saline soil resulted in increased mineralisation of organic nitrogen originally present, reclamation of saline, saline–alkali and alkali soils resulted in reduced mineralisation during incubation. In all the original and reclaimed soils mineralised nitrogen accumulated as nitrate. Since nitrification rate was limited by ammonification rate it was not possible to assess the effect of reclamation on the nitrification process. The present paper reports on the nitrification rate of applied ammonium sulphate during incubation of the same original and reclaimed soils.

Experimental

Full details of the laboratory procedures used for reclamation of the soils are described in a previous paper.[1] They involved leaching (under suction) of the alkali and saline–alkali soils with water containing calcium sulphate, and of the moderately and highly saline soils with water alone, until soluble salts and exchangeable sodium were reduced to low levels. Characteristics of the soils before and after reclamation are presented in Table I. (Fuller details are given in a previous paper[1].)

Extractable mineral-N during incubation of soils treated with ammonium sulphate

The incubation procedure used involved treating 10 g portions of soil (after air-drying it and grinding it to pass a 2 mm sieve) in flat-bottomed tubes with sufficient water to raise the moisture content to 50% of the maximum water-holding capacity. The added water contained sufficient ammonium sulphate to supply 100 ppm N on the dry soil basis. Control samples containing no added ammonium sulphate were set up in the same way. The barium peroxide method[2] was used for aeration and absorption of carbon dioxide in the stoppered tubes. Sufficient samples were prepared to allow for analysis in duplicate after 3, 6, and 12 weeks of incubation at 30°. At the end of each incubation period two replicates of each treatment were extracted with N sodium chloride. Suitable aliquots of the extracts were analysed for (i) ammonium-N and nitrate- plus nitrite-N using the microdiffusion method[3] and (ii) nitrite-N using a colorimetric α-naphthylamine—sulphanilic acid method.

Measurement of volatilisation of ammonia during incubation

The above experiment showed very poor recovery of applied ammonium-N as extractable mineral-N from the original

TABLE I

Characteristics of the four soils studied before and after reclamation treatment

	Alkali		Saline-alkali		Highly saline		Moderately saline	
	Before	After	Before	After	Before	After	Before	After
pH	9·6	8·2	8·5	8·3	8·0	8·1	8·4	8·4
Total N%	0·088	0·074	0·096	0·083	0·068	0·051	0·084	0·075
Nitrate-N, ppm	110	0	112	0	109	0	46	0
Calcium and magnesium carbonates, me%*	81	80	114	112	110	109	80	79
Saturation Extract								
Sodium, me%	30·7	0·3	13·6	0·1	12·9	0·1	0·9	0·1
E.s.p.**	59·4	2·8	34·6	1·3	11·1	1·1	1·2	0·4
Electrical conductivity, mmho/cm³	80	2·6	50	2·3	86	2·1	7·5	1·0

* me%=mequiv. per 100 g soil
** E.s.p. = Exchangeable sodium percentage

alkali soils. Since it was suspected that this was due mainly to loss of ammonia by volatilisation, the above experiment was repeated except that in each incubation tube was placed a small vial containing 1 ml of $0 \cdot 1$ N sulphuric acid to absorb volatilised ammonia. After 12 weeks of incubation the ammonium-N content of the vials was determined by a colorimetric Nessler method.[1]

Results

Comparison of soil properties before and after reclamation

Exchangeable sodium and soluble salts were reduced to very low values by reclamation (Table I), but the contents of magnesium and calcium carbonates were not affected. The treatments reduced somewhat the total nitrogen content of the soils and removed nitrate completely. The soils contained no ammonium-N either before or after reclamation. The only soil in which reclamation resulted in much pH reduction was the alkali soil, which showed a pH reduction of $1 \cdot 4$. In general, the reclamation treatments had converted all the soils to 'calcareous' soils of similar pH, very low in soluble salts and sodium.

Extractable mineral-N during incubation

Mineral-N (ammonium-N, nitrate-N and nitrite-N) extracted by N sodium chloride (in ppm on the dry soil basis) as well as values for the three mineral forms are shown in Fig. 1. The results represent mineral-N due to applied ammonium sulphate, obtained by subtracting values for unamended soils from those for comparable soils receiving 100 ppm ammonium-N. The difference required for significance at $P < 0 \cdot 05$ was $2 \cdot 8$ ppm for mineral-N, $2 \cdot 7$ ppm for ammonium-N, $2 \cdot 6$ ppm for nitrate-N, and $1 \cdot 6$ ppm for nitrite-N.

Extractable mineral-N in the original moderately saline soil fell to a low value after 3 weeks of incubation, but increased with further incubation. In the original saline–alkali and highly saline soils, mineral-N declined with time of incubation up to 6 weeks, but remained unchanged thereafter. In the original alkali soil mineral-N declined with incubation time until none was found after 12 weeks. In the reclaimed soils mineral-N declined during 3 weeks of incubation but remained unchanged thereafter, except for a significant minimum level after 3 weeks in the moderately saline soil.

In the original soils, nitrate production from applied ammonium-N was most rapid in the moderately saline soil. In the original saline–alkali and highly saline soils, there was a lag period of 6 weeks before significant amounts of nitrate were formed. In the original alkali soil no nitrate was formed after any period of incubation. In the reclaimed soils the course of nitrate production from applied ammonium-N was similar in all soils except for the tendency for a lag period of 3 weeks in the reclaimed highly saline soil.

Nitrite accumulated in small but significant amounts in all the original soils during 3 weeks of incubation, but disappeared by 12 weeks. After reclamation there was slight initial accumulation of nitrite only in the alkali and saline–alkali soils.

In the original and reclaimed alkali soils no ammonium-N was found after any period of incubation. In the other soils applied ammonium-N persisted for a longer time in the original than in the reclaimed soils. After 12 weeks of incubation extractable soil mineral-N was accounted for entirely as nitrate in all the original and reclaimed soils.

Recovery of applied ammonium-N as extractable soil mineral-N and volatilised ammonia after 12 weeks of incubation

Table II shows the effects of reclamation on the percentage recovery, after 12 weeks of incubation, of applied ammonium-N as (a) extractable soil mineral-N (nitrate-N), (b) volatilised ammonia-N and (a) plus (b).

The extent of volatilisation of ammonia from the original soils ranged from virtually all the ammonium-N applied to the alkali soil to slight volatilisation from the moderately saline soil. In the reclaimed soils volatilisation was slight from the moderately saline soils and accounted for about one-quarter of the applied ammonium-N from the other soils.
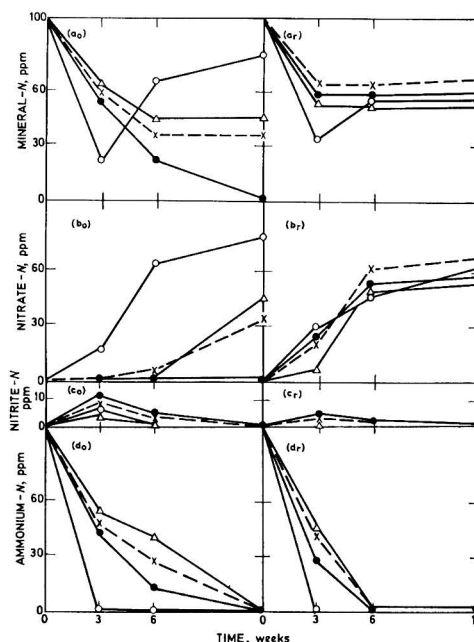

FIG. 1. *Extractable mineral-N in soils due to applied ammonium sulphate (100 ppm N) during incubation (30°c) for 3, 6 and 12 weeks of original and reclaimed soils*

● alkali soil; × saline–alkali soil; △ highly saline soil; ○ moderately saline soil
a_o (original), a_r (reclaimed) mineral-N
b_o (original), b_r (reclaimed) nitrate-N
c_o (original), c_r (reclaimed) nitrite-N
d_o (original), d_r (reclaimed) ammonium-N

TABLE II

Percentage recovery of added ammonium-N as (a) extractable soil mineral-N* and (b) volatilised ammonia after 12 weeks of incubation

	Before reclamation			After reclamation		
	(a)	(b)	(a) + (b)	(a)	(b)	(a) + (b)
Alkali	0	96	96	58	25	83
Saline-alkali	36	61	97	68	25	93
Highly saline	47	29	76	58	29	87
Moderately saline	80	3	83	60	6	66

*Accounted for entirely as nitrate.

In the original soils recovery of applied ammonium-N as soil mineral-N (nitrate) ranged from zero in the alkali soil to 80% in the moderately saline soil. After reclamation, recoveries as soil mineral-N ranged from 58% to 68% of the ammonium-N applied.

Virtually all the applied ammonium-N was accounted for as soil mineral-N plus volatilised ammonia in the two original alkali soils, but recovery was somewhat lower after reclamation. In the highly saline soil recovery was higher after than before reclamation, whilst the reverse was true in the moderately saline soil.

Discussion

The widely different recoveries of applied ammonium-N due to soil type after 12 weeks of incubation of the original soils was due largely to the high loss of ammonia by volatilisation from the two alkaline soils. Much or all of the applied ammonium-N was lost from these two soils before it had a chance to be nitrified. Jewitt[5] found that up to 87% of the ammonium-N applied to alkali soils of the Sudan was lost in this way. In the reclaimed soils, on the other hand, the recovery of applied ammonium-N as nitrate was similar for all soils after 12 weeks of incubation.

The moderately saline soil was the only one to show a similar pattern of nitrification, in relation to incubation periods, before and after reclamation. The other original soils (with the exception of the alkali soil, where no nitrate was found after any period of incubation) showed a lag phase of 6 weeks before significant amounts of nitrate were produced. This was accounted for only partly by the initial accumulation of nitrite, presumably because the ammonium-N temporarily inhibited the nitrification process beyond the nitrite stage.[6] With incubation beyond 6 weeks nitrate was formed at normal rates and this was correlated with disappearance of nitrite. A further study of ammonia volatilisation loss[7] showed that over 90% of the applied ammonium-N that volatilised did so during the first 6 weeks of incubation, indicating that during the 6–12 week period the inhibitory effect of ammonium-N on nitrification had disappeared.

In the original soils the losses of ammonia by volatilisation and the extent of nitrite accumulation were correlated better with exchangeable sodium percentage than with pH, indicating that the proportion of sodium on the exchange complex is the main factor determining these processes. Reclamation greatly reduced ammonia volatilisation from the two alkali soils, but had little effect on losses from the two saline soils. This confirms the importance of exchangeable sodium in determining volatilisation loss and also indicates that soluble salts content is of little significance with respect to this process. However, the moderate losses by volatilisation (about a quarter of the ammonium-N applied) during incubation even after reclamation of the two alkali soils and the highly saline soils indicates that in reclaimed soils for exchangeable sodium

percentage there is a critical value of about one, above which some loss of ammonia may occur by volatilisation.

In a previous study[1] when the same reclaimed soils were incubated without addition of ammonium sulphate there was a lag phase of at least 3 weeks before any nitrate accumulated. It was suggested that this may have been due to initial nitrogen immobilisation by profilerating organisms and/or to the fact that the reclamation treatments had removed or damaged a high proportion of the nitrifying organisms. The present study has shown that, except to a limited extent in the highly saline soil, the latter explanation is untenable, in that a fair proportion of the nitrate which accumulated did so during the first 3 weeks of incubation.

The inability to account for all the applied ammonium-N as soil mineral-N plus volatilised ammonia-N after 12 weeks of incubation indicates that other mechanisms of 'loss' of applied ammonium-N may be operative. One of these could be the loss of gaseous nitrogen through the reaction between ammonium and nitrite.[6] In the original alkali soil the absence of any nitrate by the end of incubation in spite of some nitrite accumulation early in incubation can only be explained in this way. In addition, microbial immobilisation[8] and ammonium fixation[9] may have occurred. Mineral-N immobilised initially as microbial protoplasm can be largely re-mineralised in time,[10] and fixed ammonium is also partly susceptible to nitrification.[9] Either or both of these have presumably occurred in the original and reclaimed moderately saline soils, as shown by the lower values for extractable mineral-N after 3 than after 12 weeks of incubation. The slopes of the nitrate-N curves of some of the soils indicate that further mineralisation and nitrification of immobilised and/or fixed ammonium-N might have occurred if incubation had been continued beyond 12 weeks.

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FRACTIONATION AND COMPARISON OF PUROTHIONIN AND GLOBULIN COMPONENTS OF WHEAT*

By D. G. REDMAN and N. FISHER

Purothionin, an unusual wheat protein and the 'fast-moving wheat globulins' have been separated into two components. Earlier evidence of the close similarity of purothionin and these 'globulins' is strengthened by amino acid analyses, 'fingerprints' (peptide mapping) and C-terminal end group determination of the individual components. A preparation similar to the crude 'lipoprotein' precursor of purothionin previously reported in the literature and stated to contain 'lecithin' on the basis of the presence of lipid phosphorus has been partially resolved into six components, and the presence of phosphatidyl choline, amino phospholipids and two glycolipids has been demonstrated in fractions containing purothionin.

Introduction

Purothionin, a crystallisable protein preparation derived after acid treatment from a fraction of wheat flour extractable in light petroleum,[1] has been shown in recent work[2,3] to bear a close resemblance to a wheat globulin fraction corresponding to the 'fast-moving globulins'.[4]† Each gave rise to a doublet of the same mobility on starch-gel[2] or polyacrylamide-gel electrophoresis[3] at acid pH, and a similar mobility on polyacrylamide-gel electrophoresis at pH 8·5 if the globulin had been pre-treated with acid.[3] The amino acid compositions of the globulin and purothionin doublet preparations were found by both groups of workers to be similar to each other, but not identical. The molecular weight of purothionin was shown to be approx. 10,000 by gel filtration[2] (Balls et al.[1] gave 10,200 by diffusion, 12,000 from amino acid composition); by this method the globulin pair had an elution volume similar to that of purothionin and was therefore probably similar in molecular weight.

In the present paper a report is given of the separation of the components giving rise to the doublet in purothionin and 'globulin' respectively and of some comparative analyses of the separate products. Preliminary studies of the separation of 'lipopurothionin' are also reported.

Experimental

Materials

An untreated flour ('Snowdown') of protein content (N × 5·7) 8·5% and moisture content 14·0% was used for all preparations.

Whatman microgranular pre-swollen carboxymethyl cellulose CM 52 was used.

Trypsin (crystalline), arginine and lysine monohydrochlorides were obtained from British Drug Houses Ltd., Poole, Dorset. Protamine sulphate (ex salmon roe) was obtained from Koch-Light Laboratories Ltd., Colnbrook, Bucks.

Hydrazine hydrate (99/100%) was obtained from Griffin & George Ltd., London and made anhydrous by the method of Kusama.[5] Benzaldehyde (analytical grade) was obtained from May & Baker Ltd., Dagenham, Essex.

Sephadex LH 20 was obtained from Pharmacia Ltd., Uppsala, Sweden.

Methods

Globulin preparation

Globulins were prepared substantially as before[2] prior to fractionation on carboxymethyl (CM) cellulose columns, except that one further preliminary stage was included:

The globulin residue from 1500 g flour, after removal of salt, was dispersed in water (200 ml). The pH of the suspension was adjusted to 3·0 with N acetic acid and held at this value for 5·5 h. After centrifugation at 59,000 g for 25 min the supernatant was freeze-dried to give approx. 1·6 g product. This was stirred in 0·02 M acetate buffer pH 5·2, 0·32 M in sodium chloride (Soln. A; 40 ml) for 2 h, and centrifuged at 145,000 g for 25 min, and the supernatant was loaded on a CM-cellulose column (74 × 2·4 cm) equilibrated in solution A. After loading, approximately 20 ml of solution A was added to the top of the column. The column was connected in series to two 2 l flasks. The first (proximal) contained solution A (1800 ml) and was magnetically stirred. The second contained solution A (1800 ml) made 2·19 M in sodium chloride. The top of the column and the neck of the proximal flask were fitted with bungs, so that only the level of the liquid in the distal flask dropped during elution. This system gives a slightly convex salt gradient. Flow-rates were approximately 1 ml/min. The eluate was monitored at 253·7 nm with an L.K.B. 'Uvicord', and fractions combined on the basis of the elution pattern were dialysed until free from chloride and freeze-dried. Columns were always unpacked between runs for regeneration.

Purothionin

Crude purothionin was prepared as described previously ('Crude purothionin II'[2]). It was found possible to purify the crude purothionin substantially by the following procedure (cf. Bligh & Dyer[6]). Purothionin (hydrochloride; 256 mg) was treated with methanol–chloroform 2 : 1 v/v (15 ml) and 4·5 ml water was added; a clear monophasic solution resulted. The solvent ratios were brought to 1:1:0·9 by volume (biphasic system) by addition of chloroform and water after

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† Nomenclature note: In view of the unsettled state of wheat protein nomenclature the terms 'globulin', 'globulin α' and 'globulin β' will be used to refer to the acid-treated water-soluble materials corresponding in electrophoretic mobility (starch gel, pH 3·3) to the doublet, and its component faster- and slower-moving bands, respectively. The terms purothionin α and purothionin β will denote the bands with corresponding mobility to these 'globulins' in acid conditions.

which a thick white emulsion formed. After being kept at 4° overnight the supernatant was pipetted off and 5 ml upper-phase solvent was added to the residual mixture which was centrifuged (1000 g; 1°) and separated. The combined supernatants were freeze-dried and yielded 180 mg of faintly buff product, giving an uncontaminated 'doublet' on starch-gel electrophoresis. The process could also be applied to the initial light petroleum extract of the flour (29·2 g lipid + chloroform–methanol–water 300:300:240 by volume) gave yields of 29·02 g oil (containing 'lipopurothionin') from the chloroform phase and 168 mg of white solid (mainly albumins as judged by electrophoretic mobilities) from the aqueous methanol phase).

Separation of purothionin components on CM-cellulose was carried out essentially as described for the 'globulins' except that smaller volumes were loaded ($\sim$ 50 mg in 15 ml).

Lipopurothionin

This term is used (pending an accepted systematic nomenclature) for the 'lipoprotein' precursor[1] of purothionin (a 'proteolipid'[7] by its solubility but not like other proteolipids previously reported to have been found in the interface of a chloroform–methanol–water system). The crude starting material was prepared by a modification of the method of Balls *et al*:[1] crude flour lipid (light petroleum extract 22 g) was ultracentrifuged (145,000 g, 3 h) to remove a product which was insoluble in methyl acetate and showed both ester and amide absorptions in the infra-red. The supernatant oil (20·6 g) was precipitated with methyl acetate (200 ml) instead of ethyl acetate used by the earlier workers. The precipitate was washed with methyl acetate (2 × 100 ml), and taken up in 20 ml light petroleum (40–60°) giving a clear deep brown solution, and precipitated as a buff sludge with methyl acetate (100 ml). The mixture was centrifuged and decanted, and the precipitation was repeated using 20 ml light petroleum and dropwise addition of methyl acetate till a faint turbidity was produced. On being kept at 4°, oiling out occurred; a further 80 ml methyl acetate was added, and the oil was scratched till it solidified. The liquor was centrifuged off and the solid was dried *in vacuo* over calcium chloride and phosphorus pentoxide to give 2·6 g of buff solid.

Fractionation of this product was carried out on Sephadex LH 20 columns (79 × 2·5 cm) pre-washed with chloroform (reagent grade, stabilised with 1% ethanol), chloroform–ethanol 1:1 v/v and finally chloroform. Chloroform was used as eluant and changed to chloroform–ethanol 1:1 v/v after elution of all chloroform-soluble products. Fraction volumes were 3 ml (chloroform) and 10 ml (chloroform–ethanol). Elution was monitored at 253·7 nm.

Starch-gel electrophoresis

This was carried out by the method of Elton & Ewart[4] in aluminium lactate buffer pH 3·3, $I=0·05$, at 5 V/cm for 4 h.

Amino acid analyses

Samples were hydrolysed in hydrochloric acid (2000 × wt. of sample) of constant b.p. under nitrogen in screwed polytetrafluoroethylene-capped culture tubes at 105° for 22 h. The acid was removed by rotary evaporation at room temperature, and the analyses were carried out on a 'Technicon' amino acid analyser using nor-leucine as internal standard. Correction factors for destruction or incomplete hydrolysis of amino acids were applied using values obtained for the

hydrolysis of purothionin α for additional times of 46 h and 75 h. Partial oxidation of cystine to cysteic acid was observed, and a correction was applied to the total CyS analyses.

C-terminal end groups

These were determined by the hydrazinolysis method of Akabori, Ohno & Narita[8] as applied by Press, Piggot & Porter[9] except that reaction times of 7 and 24 h were employed and additional experiments with 1·5 and 48 h were occasionally performed.

Performic acid oxidations

After oxidation by the method of Hirs[10] the solutions were immediately freeze-dried, suspended in water and again freeze-dried, the latter process being repeated three times.

Peptide maps ('fingerprints')

Proteins ($\sim$ 4 mg) oxidised by performic acid were placed over sodium hydroxide pellets *in vacuo* overnight, and then dissolved in water (0·5 ml). The pH was adjusted to 8·0 with a 5% v/v solution of triethylamine and then 0·1 ml of a trypsin solution (1 mg/ml in 0·001 N-HCl) and 1 drop of toluene were added and the mixture was incubated at 37° for 66 h. Clear solutions were obtained. These were taken to dryness over phosphorus pentoxide *in vacuo* and the residues were dissolved in 0·15 ml of the buffer used for electrophoresis. Half the solution was applied as a 2·5 cm streak to the papers (Whatman No. 3 MM; 40 × 35 cm) and electrophoresis was carried out in water–acetic acid–pyridine (100:10:1 by volume) for 2 h at 33 V/cm using the 'Pherograph' (L. Hormuth, Inh. W. E. Vetter, Wiesloch, Baden) high-voltage cooled electrophoresis unit. The papers were dried at 90° for 1·5 h. The chromatographic step (at right angles to the electrophoresis run) employed n-butanol–acetic acid–water (3:1:1 by volume). Ascending development was for 16 h (30 cm). After being dried for 1·5 h at 90° the papers were dipped in the cadmium–ninhydrin reagent of Heilmann, Barrollier & Watzke[11] before being heated at 90° for approximately 1 h to develop the spots.[12,13]

Results

The elution pattern obtained in the separation of the 'globulins' on CM-cellulose is given in Fig. 1. Yields from 1500 g flour were 'globulin α' 30 mg and 'globulin β' 20 mg. The 'overlap fraction' gave a yield of 10 mg.

The purothionins emerged at closely similar volumes to the 'globulins' when all other conditions were equal. The purothionin α peak was larger than the purothionin β peak as for the 'globulins', the yields from 2700 g flour being α 45 mg, β 25 mg and 'overlap fraction' 35 mg.

The electrophoretic behaviour of the fractionated purothionin and 'globulin' components is shown in Figs 2a and 2b.
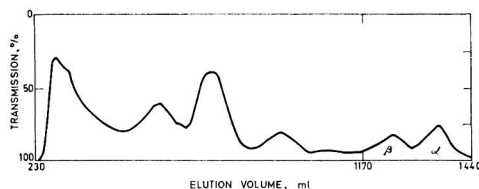

FIG. 1. *Gradient elution of 'globulins' from a carboxymethyl cellulose column*
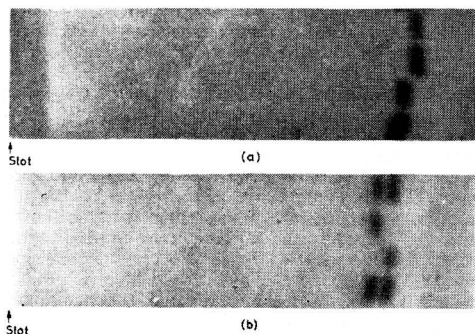
FIG. 2a. *Starch-gel electrophoretic migration of purothionin α (upper two channels) and purothionin β (lower two channels)*

FIG. 2b. *Starch-gel electrophoretic migration of 'globulins' α (higher mobility) and β respectively, compared with a doublet preparation of purothionin (outer two channels)*

The amino acid composition of these preparations is presented in Table I. It is relevant to note here that a purothionin 'doublet' preparation made with exclusion of oxygen and use of an antioxidant (butylated hydroxytoluene) contained a negligible amount of thiol groups as determined by the method of Ellman.[14]

A peptide map obtained after trypsin digestion of 'globulin α' is illustrated in Fig. 3. No differences in pattern were observed between peptide maps of corresponding purothionin and 'globulin α' components.

C-terminal amino acids

Purothionins α and β and 'globulins α' and 'β' were each found to contain C-terminal lysine (1·1 equiv./mole assuming a molecular weight of 10,000). Small amounts (0·1 equiv./mole) of arginine as ornithine were also obtained. For each

TABLE I

Comparison of the amino acid compositions of purothionin components with those of the 'globulins' of corresponding electrophoretic mobility
g anhydro amino acid/100 g anhydro amino acids recovered

	Purothionin α	Purothionin β	'Globulin α'	'Globulin β'
Asp*	4·91	7·81	4·79	8·61
Thr	5·13	4·66	5·52	4·32
Ser	8·89	7·53	8·51	7·59
Glu*	3·58	4·25	3·86	3·49
Pro	4·80	4·51	3·37	4·27
Gly	5·34	4·34	5·40	3·93
Ala	3·21	4·10	2·96	4·29
Val	1·78	1·99	1·82	1·72
CyS	14·76	15·02	15·34	15·17
Met	Trace	Trace	—	—
Ile	1·16	0·61	0·81	0·38
Leu	10·70	11·04	11·27	11·27
Tyr	2·53	2·94	2·34	2·62
Phe	3·52	3·46	3·86	3·61
Lys	13·29	13·84	12·43	14·97
His	Trace	Trace	1·01	0·27
Arg	16·40	13·91	16·71	13·49
Total	100·00	100·01	100·00	100·00

* Including asparagine and glutamine respectively.
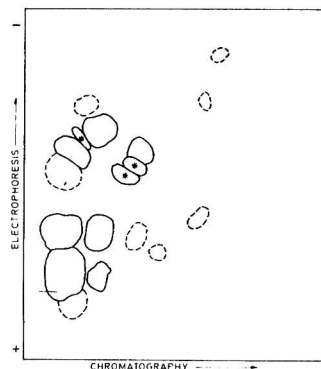
FIG. 3. *Peptide map of 'globulin α' after trypsin digestion of a sample oxidised by performic acid*

Orange spots are indicated by *. Spots with broken outlines were of low intensity. The origin is indicated by the horizontal line (bottom, left)

determination, loss corrections were applied based on the destruction of the corresponding free amino acids when subjected to the same treatment. A similar determination carried out using salmine sulphate gave the expected recovery of arginine as ornithine as was also observed by Niu & Fraenkel-Conrat.[15] A performic acid oxidised ('doublet') preparation of purothionin gave no cysteic acid after hydrazinolysis.

'Lipopurothionin'

A chloroform solution of the crude product obtained as described previously was fractionated on Sephadex LH 20. Preliminary experiments showed that part of the product was strongly adsorbed on the gel particles, and required ethanol (50% in chloroform) for elution. The elution pattern is shown in Fig. 4.

The fractions eluted with chloroform–ethanol gave the 'doublet' pattern of purothionin plus some streaking and a residue of material (? lipid) in the slot on starch-gel electrophoresis (the acid buffer presumably split any lipid-protein link). These fractions were subjected to thin-layer chromatography on kieselgel-G (Merck), using the solvent systems light petroleum (60/80°)–ether–acetic acid 70:30:2 by volume and
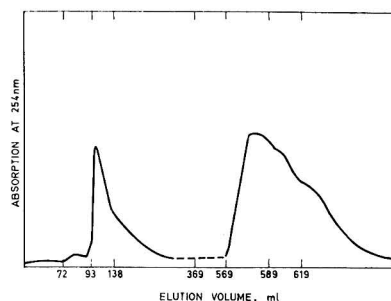

FIG. 4. *Fractionation of crude 'lipopurothionin' on Sephadex LH-20*
Elution with CHCl₃ changed at 369 ml to 1 : 1 v/v CHCl₃ : EtOH

di-isobutyl ketone–acetic acid–water 40:30:3 by volume,[16] respectively. Mono-, di- and tri-glycerides and sterols were absent; a trace of sterol esters was present. Phosphatidyl choline (shown by the R_f compared with a standard sample and by the modified Dawson spray reagent of Doizaki & Zieve[17] was the main phospholipid, but a small amount of phosphatidyl ethanolamine (identified by R_f value and ninhydrin reaction) was also present. Two glycolipids were present as major components as judged by the intensity of colour reaction given with a specific glycolipid reagent.[18]

Discussion

The great affinity of purothionin for protein dyes is of advantage in establishing the purity of the resolved preparations, since even minor contamination of one component with the other is readily demonstrable. Since a single band is obtained from each fraction on starch-gel electrophoresis even under overload conditions and also on the basis of the elution pattern obtained from the CM-cellulose column separation, the complete resolution of purothionin into two components may be regarded as established.

The acid treatment of the globulins before CM-cellulose chromatography is not essential to the separation of two components but improves the yields. This step was included after the observation of Nimmo *et al.*[3] that purothionin and globulin doublets had identical electrophoretic mobilities in alkaline media only, when the globulins had at some stage of their preparation been brought to pH 3.

The treatment of crude purothionin with the chloroform–methanol–water system, while again not essential to subsequent resolution, removes any residual lipid as well as some protein contamination.

The amino acid compositions of the corresponding purothionin and 'globulin' bands are very similar but still not identical (Table I). The differences between α and β purothionins are for the most part reflected by the corresponding differences between α and β 'globulins', especially in the case of aspartic acid. Histidine is still detected in the globulins but not in the purothionins, and other differences beyond experimental error ($\pm 5\%$) are evident. The amounts of isoleucine in the β components and of histidine in 'globulin β' are insufficient for the observed molecular weight of 10,000, and no explanation of the discrepancy has yet been found. It may be that the separated components still contain traces of impurities not detected by the methods used. (This is to some extent indicated by the finding of trace amounts of methionine and histidine in the purothionins.) Strong evidence that purothionin and 'globulin' components are very similar in amino acid sequence is derived from a study of the peptide maps obtained after trypsin digestion. This 'fingerprint' technique will often show minor differences in amino acid sequence in proteins, and it is therefore significant that no differences were observed in the peptide maps obtained from purothionin α and 'globulin α' respectively. The high arginine and lysine contents of these proteins makes trypsin digestion a very useful tool for analysis.

The demonstration that the purothionins and 'globulins' each have the same C-terminal end group (lysine) is additional evidence in favour of the probable identity of these compounds. The origin of the small quantity of arginine also found in the end group determinations is still obscure. It does not appear to be due to a slow release of this amino acid, as found by Wallén & Sjöholm in fibrinopeptide A,[19] on the basis of experiments using varying times of hydrazinolysis. Cystine

(cysteine), asparagine and glutamine would not be found using this method but the first two have been eliminated as described in the results section (55% recovery of cysteic acid has been reported by Locker[20]). The lysine recovery would give a molecular weight for these compounds of about 9000 which is of the same order of magnitude as determined by gel-filtration.[2]

The relationship of the respective precursors of purothionin and the acid-treated wheat globulins is still unresolved. Exhaustive extraction of flour with light petroleum (60/80°) (for 6 days) reduced the intensity of the fast-moving 'globulins' on starch gels but did not eliminate them entirely. While strongly pointing to the identity of lipopurothionins with globulins, the result is disappointingly inconclusive. Results obtained in the fractionation of crude lipopurothionin suggest that a direct comparison of these precursors will soon be possible. Acid treatment of these compounds is necessary to confer water-solubility on the products, a result attributable to dissociation of lipid from protein in the case of lipopurothionin and probably also in the case of the globulin, and there are indications that the lipid moieties may be different in the two cases.

Balls *et al.*[1] stated that the lipid combined with purothionin was 'lecithin' although only evidence of the presence of phosphorus in the crude product was given. The authors have indeed found phosphatidyl choline in their fractionated products but also two glycolipids, traces of amino-phospholipid and sterol ester. Further fractionation should establish the relationship between purothionin and the lipids present in the mixture.

Since purothionin has previously only been found in wheat, it was looked for in other cereals. When extracted by the same procedures used for wheat flour, barley flour was found to contain components with electrophoretic properties similar to purothionin and also components similar to globulin (confirmed in this case by amino acid analysis), whereas these have not been detected in the flours of rye, oats and maize. Work is in progress to characterise these materials.

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ENSILAGE OF WHOLE-CROP BARLEY
I.—Effects of variety and stage of growth

By R. A. EDWARDS, ELIZABETH DONALDSON and A. W. MacGREGOR*

The chemical composition and nutritive value of each of eight varieties of barley were examined at seven stages of growth. There were differences between some varieties at certain stages of growth but these were small. Dry matter content increased with increasing maturity while water-soluble carbohydrate content increased to the milky ripe stage and then fell sharply. *In vitro* organic matter digestibility was a maximum at the mealy ripe stages of growth. The optimum stage of growth for cutting barley for ensilage is discussed. Silage was made from barley cut at the early mealy ripe stage of growth, in small tower silos. There was no effluent, and fermentation losses were low. The *in vivo* digestibility of the silage organic matter was 65·5%.

Introduction

The introduction of feeding systems based on barley grain and barley straw leads naturally to a consideration of the desirability of feeding the aerial part of the barley plant as a whole since there is no justification for first separating the grain and straw only to recombine them at feeding.

Cereals have been used for making hay in many parts of the world and several workers have reported upon their composition and nutritive value.[1-4] In this country green cereals have been used for many years for making silage but usually at an immature stage of growth when dry matter contents are about 15 to 16%. During 1965 attempts were made to ensile barley at a relatively mature stage in tower silos, for which a high dry matter content, was required. At this time relatively little work had been done on the composition of different barley varieties at different growth stages. Sotola[3] reported on the composition of Horsford barley at stages from 55 to 97 days after sowing and on the nutritive value and composition of hays made at the milk, dough and ripe stages of growth. Sosulski & Larter[5] examined the composition of Canadian diploid and tetraploid barleys at the five-leaf and mature stages and Dougall[6] the composition of barley at different stages of growth under East African conditions. Washko[7] reports on the composition of barley grazed in the spring and autumn in America. An investigation was carried out in 1966 of the changes in the composition and nutritive value of eight varieties of barley at seven stages of growth to provide the information on which the making of satisfactory barley silage could be based. From a study of the work of Sotola[3] and preliminary results of this investigation it was decided to make silage from barley cut at the early mealy-ripe stage.

Composition of barley

Experimental

Eight varieties of barley randomised in four blocks were sown on 23 March, 1966. The varieties were Cambrinus, Ymer, Zephyr, Europa, Sultan, Tern, Impala and Tellus. Seed was sown at the rate of 140 lb/acre and fertiliser (23–11½–11½) was broadcast at the rate of 2½ cwt/acre. Cuts of barley were made by hand at about 5 cm above ground level from each plot at seven stages of growth as shown in Table I.

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TABLE I

Stages of growth at which cuts of barley were made

Stage		Stage of growth*	Date of cutting	Days after sowing
1	10.5	Heading completed	1 July	99
2	10.53	Flowering	6 July	105
3	10.54	Kernels watery ripe	11 July	110
4	11.1	Milky ripe	18 July	117
5	11.2 (a)	Mealy ripe-early	3 Aug.	133
6	11.2 (b)	Mealy ripe-late	15 Aug.	145
7	11.4	Ripe for cutting	13 Sept.	174

* Large, E. C., *Pl. Path.*, 1954, **3**, 128

Analytical methods

Samples were chopped to about 1 cm length and dried overnight in a forced-draught oven at 100°. It was assumed that loss in weight was due to moisture loss.

For analysis the dried material was ground to pass a 1 mm screen. Proximate analysis was carried out by routine procedures except for crude fibre which was determined by digestion with a mixture of trichloracetic and nitric acids.[8] Calcium and phosphorus were determined in a hydrochloric acid–nitric acid extract of the ashed herbage,[9] calcium by flame spectrophotometry and phosphorus by the vanadium phosphomolybdate method. *In vitro* organic matter digestibility (OMD) was determined by the method of Alexander & McGowan.[10] The determination of water-soluble carbohydrates (WSC)[11] was carried out on finely chopped fresh material within one hour of harvest.

All samples were anlysed for dry matter, ash, crude protein, crude fibre, calcium, phosphorus and OMD. Water-soluble carbohydrate determinations were carried out on samples from four varieties, Cambrinus, Europa, Ymer and Zephyr at each stage of growth.

Results

For the purpose of statistical analysis the experiment was considered as a split-plot randomised block and subjected to an analysis of variance. Significant interactions between

variety and stage of growth were demonstrated for % dry matter (P = 0·001), % OMD (P = 0·001), % WSC (P = 0·01). No significant interactions were demonstrated for the other constituents examined. The results for the constituents with no significant interactions are presented together and those for constituents with significant interactions are presented individually.

Table II gives the composition of the dry matter of barley plants according to variety. The figures represent means of seven stages of growth.

Differences between varieties are small for all constituents and there is no significant difference (P = 0·05) in ether extract, crude protein or ash. There was evidence for differences in contents of crude fibre, nitrogen-free extractives, calcium and phosphorus. Europa and Sultan had significantly higher crude fibre and lower nitrogen-free extractives than Ymer. All varieties except Cambrinus had a significantly higher calcium content than Ymer but, except for significantly lower figures for Impala and Europa, there were no differences from Ymer in phosphorus content.

Table III gives the composition of the dry matter of the barley plant at seven stages of growth. The figures are means of the eight varieties.

Analysis of variance showed significant differences (P = 0·001) between stages of growth for all constituents. There was a significant fall in ash content (P = 0·05) with each stage of growth except between stages 6 and 7. The calcium content at stage 2 is significantly higher (P = 0·05) than at any other stage and there is a significant fall with each stage to stage 7. Stages 1 and 3 do not differ significantly. The phosphorus content falls significantly with each stage from 1 to 4 and then rises significantly with each stage thereafter to stage 7. The falls in content of ether extract from stage

1 to 2, 2 to 3 and 6 and 7 were statistically significant (P = 0·05) as was the rise between stages 5 and 6. The crude fibre content at stage 2 was significantly higher than at stage 1 (P = 0·05) and there was a significant fall with each stage to stage 6 when there was a significant rise to stage 7. The content of nitrogen-free extractives rose with each stage to stage 5 which did not differ significantly from stage 6 and there was then a fall to stage 7. The crude protein content showed a significant fall (P = 0·05) with each stage of growth from 1 to 5 with a small but significant increase between stages 6 and 7.

The significant interaction between variety and stage of growth in dry matter content render the use of varietal or stage means invalid for comparison. Table IV gives the interaction means for the dry matter contents of the different varieties at different stages of growth. Impala had a significantly higher dry matter content than Ymer at stage 4 as did all varieties, except Cambrinus, at stage 5. Impala was again higher at stage 6 and all others, except Europa, at stage 7. There was an increase in dry matter content with stage of growth for all varieties. Samples taken at the second stage of growth carried significant quantities of extraneous water and values for dry matter contents for this stage are biased. This factor was not a significant one at any of the other sampling stages.

There is a significant interaction between variety and stage of growth in the case of WSC content. The interaction means are given in Table V. Europa had a significantly lower WSC content than Ymer (P = 0·05) at stages 1, 3, 4, 5 and 6 while both Zephyr and Cambrinus showed significantly lower levels at stage 3. The effect of stage of growth is well defined, there being a rise in WSC content for each of the four varieties to a maximum value at stage 4 and then a decline to a very low value at stage 7.

TABLE II

Percentage composition of the dry matter of different barley varieties
(Figures represent means of seven stages of growth)

| | Variety | | | | | | | | S.E. of differences between varieties |
	Impala	Tern	Cambrinus	Ymer	Tellus	Europa	Zephyr	Sultan	
Ash	4·9	5·0	5·1	4·8	5·1	5·1	4·9	5·1	0·16
Crude protein	7·2	7·4	7·1	7·3	7·3	7·3	7·3	7·4	0·17
Crude fibre	26·4	25·2	26·2	25·6	26·0	27·0	25·9	26·5	0·43
Ether extract	1·4	1·4	1·5	1·5	1·4	1·5	1·4	1·5	0·06
Nitrogen-free extractives	60·1	61·2	60·1	60·8	60·1	59·1	60·5	59·5	0·56
Calcium	0·41	0·40	0·37	0·35	0·41	0·46	0·38	0·39	0·011
Phosphorus	0·18	0·19	0·19	0·20	0·19	0·18	0·20	0·20	0·005

TABLE III

Percentage composition of the dry matter of barley at different stages of growth
(Figures are means of eight varieties)

| | Stage of growth | | | | | | | S.E. of differences between stages |
	1	2	3	4	5	6	7	
Ash	7·5	6·5	5·6	4·7	3·8	3·5	3·5	0·09
Crude protein	10·3	8·7	7·2	6·7	5·6	6·0	6·6	0·14
Crude fibre	31·3	32·1	28·6	25·3	20·4	19·5	25·4	0·30
Ether extract	1·9	1·4	1·3	1·3	1·4	1·6	1·3	0·06
Nitrogen-free extractives	49·0	51·3	57·4	62·1	68·8	69·5	63·2	0·36
Calcium	0·51	0·55	0·52	0·41	0·35	0·23	0·19	0·014
Phosphorus	0·23	0·20	0·18	0·17	0·16	0·17	0·21	0·003

There was also a significant interaction between variety and stage of growth in the case of OMD. Interaction means for the different varieties at various stages of growth are given in Table VI. Impala had a significantly lower OMD than Ymer (P = 0·05) at stages 2 and 7, Tellus at stages 2 and 4, Europa at stages 6 and 7, and Sultan at stages 3 and 4. Although Zephyr and Europa showed considerable differences from the other varieties in their response to increasing maturity all varieties showed a significant fall in OMD (P = 0·05) between stages 1 and 2. There was then a rise to stage 4 and a period of relatively constant digestibility to stage 6 and a significant fall to stage 7.

TABLE IV

Mean dry matter contents for varieties at seven stages of growth

Variety	Stage of growth						
	1	2	3	4	5	6	7
Impala	20·0	20·8	26·2	31·1	37·4	40·7	44·6
Tern	20·1	20·3	25·6	29·5	35·8	38·8	44·6
Cambrinus	18·9	19·8	24·9	28·8	34·3	37·2	43·1
Ymer	19·1	20·5	25·5	28·9	33·3	37·5	39·2
Tellus	18·4	19·5	26·0	29·6	35·8	38·6	43·3
Europa	18·8	19·5	26·2	28·6	35·1	38·8	39·0
Zephyr	19·1	20·3	26·2	29·0	35·1	38·7	41·3
Sultan	18·3	19·5	25·7	29·0	35·8	38·9	45·0

SE of differences between stages = 0·78
SE of differences between varieties = 0·80

TABLE V

Mean water-soluble carbohydrate contents for varieties at seven stages of growth

Variety	Stage of growth						
	1	2	3	4	5	6	7
Cambrinus	16·4	19·3	25·0	32·7	25·5	15·6	3·9
Europa	14·4	16·8	21·7	30·1	22·3	12·0	4·2
Ymer	18·3	18·8	27·9	32·9	25·0	14·6	4·5
Zephyr	18·6	17·0	24·9	31·6	23·8	16·7	5·7

SE of differences between stages = 1·06
SE of differences between varieties = 1·12

TABLE VI

Mean *in vitro* organic matter digestibilities of varieties at seven stages of growth

Variety	Stage of growth						
	1	2	3	4	5	6	7
Impala	60	53	57	61	63	61	52
Tern	65	55	58	61	64	61	57
Cambrinus	63	54	58	62	63	62	58
Ymer	62	55	59	62	63	62	57
Tellus	62	53	58	59	63	62	56
Europa	61	54	58	60	60	59	47
Zephyr	62	55	58	61	62	63	56
Sultan	61	54	56	59	63	61	56

SE of differences between stages = 1·24
SE of differences between varieties = 1·21

By the time stage 7 was cut a considerable amount of grain had been lost from the heads.

Discussion

Sotola[3] examined the composition of Horsford barley over a period from 55 to 97 days after sowing during which the crop progressed from the stage 'bloom on some heads' to that of being 'dead ripe'. Dry matter figures are given for the early part of the period only and were 15·7% at the 55th day and 25·3% at the 76th day. The figures are similar to those obtained in the present investigation at comparable stages of development. Washko[7] confirmed the low dry matter values for barley in the early stages of growth but showed barley cut in the spring to have a higher dry matter than comparable material cut in the autumn. Sosulski[5] showed that the dry matter content of diploid barley plants changed from 9·0% at the five-leaf stage to 39·7% at maturity and that of tetraploids from 8·9 to 35·4%.

Sotola[3] found that the protein content of the dry matter of Horsford barley fell very sharply from 17·0% at the 'full bloom' stage to 9·94 at the 'watery kernels' stage and then declined gradually to 7·29% at the 'dead ripe' stage. Sosulski[5] showed a decline from about 38% for the five-leaf stage to 4·5 to 5·0% at maturity. Dougall[6] working under East African conditions found a decrease from about 34% crude protein one week after to 15·6% eleven weeks after sowing, and that the level remained relatively constant thereafter. The crude protein levels in the present investigation are generally lower than those quoted and differ in that at maturity there is a slight recovery of protein content. The difference of crude protein level may be due to different treatments with nitrogenous fertilisers. N.A.A.S. workers (personal communication) have shown a decline in the crude protein content of barley from early July to August and confirm the rise in the later stages noted here. Their levels of crude protein were similar for a rate of nitrogen fertilisation comparable with that in this trial and crude protein content increased with increased applications of fertiliser nitrogen.

The ether extract contents of the barleys were considerably lower than those quoted by Sotola,[3] particularly in the early stages of growth, but the variation during the growth period is similar.

The level of nitrogen-free extractives in the early growth stages of the present data agree well with those of Sotola but the increase thereafter, obtained by Sotola, is slower and continues to the dead ripe stage at which a value of 64·41% was recorded. Dougall[6] found the nitrogen-free extractive content to increase from 50% at week 1 to 80% at week 11. The fall in the content of nitrogen-free extractives between stages 6 and 7 in the present data is probably due to the loss of grain previously recorded. It is of interest to note that extrapolation of the results from stages 5 and 6 would have given a value at stage 7 of about 71%.

Sosulski & Larter[5] showed a drop in ash content from about 16% at the five-leaf stage to about 9 to 9·5% at maturity. The present investigation also records a fall in ash content with increasing maturity but the levels are lower being only 3·5% at maturity and 7·5% at stage 1. The calcium and phosphorus figures quoted by Sosulski & Larter showed a considerable drop from the five-leaf to the mature stage which confirms the trend shown here. Their levels of calcium are higher, being 0·82 and 0·74 in the young material and 0·47 and 0·36 in the mature. Phosphorus content was higher in the young material but was lower by

the mature stage. The figures for calcium and phosphorus quoted by Sotola[3] confirm the trend with stage of growth, but while the calcium contents are comparable, his phosphorus levels are generally higher than those of the present investigation, the lowest levels reached being 0·25%.

Sotola[3] records a rise in crude fibre from 22·8% at the stage of 'bloom on some heads' to 32·3% at the milk stage and a decline to 19·3% at the dead ripe stage. This is different from the pattern shown in the present investigation which showed a decline in crude fibre content from flowering to mealy ripe-late with a rise to the last stage. A part, at least, of this late rise in crude fibre content is attributable to loss of grain from the heads. The change in crude fibre content in Sotola's[3] data was paralleled by changes in digestibility (*in vivo*) from 51% at the milk stage, to 60% at the dough stage to 62% at the dead ripe stage. No digestibility figures for the earlier stages are recorded. Similar confirmation of the crude fibre changes recorded here is given by the *in vitro* digestibility figures shown in Table VI where there is a rise in digestibility as crude fibre falls and *vice versa*. N.A.A.S. workers (personal communication) recorded very similar changes in digestibility with increasing maturity, and it would appear that some of the changes recorded in the present work between stages 6 and 7 are only partly due to the loss of grain from the head. The *in vitro* digestibility figures for the mealy stages of growth agree well with the *in vivo* figure given by Sotola[3] but at the milky ripe stage they are considerably higher.

Several varieties showed significant differences from Ymer in their content of certain constituents but no variety was consistently superior to the others. Intervarietal differences were of no practical significance.

In assessing the suitability of a material for ensilage two groups of factors are important. Firstly there are those which govern its suitability for preservation and secondly there are those which govern its nutritive value. The suitability of a crop for preservation depends primarily upon its dry matter and its content of readily available carbohydrate. Preservation depends upon the development of acidity by the lactic acid bacteria in order to control the activities of the undesirable *Clostridia*. These activities are inhibited by the combined effect of osmotic pressure and pH. At high osmotic pressure a higher pH gives satisfactory preservation and at lower osmotic pressure a lower pH is necessary. A high dry matter content in the herbage will give a higher osmotic pressure and preservation at a higher pH and the activities of the lactic acid bacteria need not be so extensive. Ideally a material for ensilage should have a dry matter content of at least 30%. The herbage preserving acid is produced by bacterial action on the available carbohydrate in the material to be ensiled. The extent and speed of acid development will depend upon the amount of available carbohydrate present. For satisfactory preservation a level of at least 10% is desirable although it will vary with dry matter content. From the figures obtained in the present investigation, the ideal stage for ensiling would be later than the milky ripe stage particularly if a tower silo is to be used. For the trench type of silo material at an earlier stage but not before the watery kernels stage could be used. The available carbohydrate as represented by the WSC is adequate for preservation purposes except at the 'ripe for cutting' stage. It would appear that barley should be cut before the late mealy stage.

The major determinant of nutritive value is the energy

content of the food, which in turn depends upon the organic matter content and its digestibility. The organic matter content of the dry matter varies little with stage of growth while digestibility is a maximum at the mealy stages of growth. Protein, the other major determinant, falls with stage of growth and is at a minimum at the early mealy ripe stage. The higher protein levels are unfortunately at those growth stages at which the material has a low dry matter content. The contents of both calcium and phosphorus fall with increasing maturity and at the mealy stages are low. Although dry matter does not affect nutritive value, dry matter intake is higher with the silage of high dry matter content, and so material cut after the milky ripe stage is to be preferred.

It is suggested that from the point of view of preservation and nutritive value, barley for silage should be cut between the milky and late mealy ripe stages of growth and made in tower silos. Where a trench type of silo has to be used it would be more realistic to cut at the watery kernels stage to facilitate compaction. Material cut at the early mealy ripe stage would have a dry matter of about 35% and an estimated metabolisable energy of about 2·2 Mcal/kg dry matter, an estimated digestible crude protein of about 3%, and a calcium content of 0·35 and phosphorus content of 0·16%. In most cases supplementation of a diet high in barley silage with protein, calcium and phosphorus would be necessary. Twelve pounds of dry matter would be required to provide the energy for maintenance for an 1100 lb dairy cow. This would require the provision of an extra 0·38 lb digestible crude protein and almost 20 g phosphorus to provide for maintenance.

Silage studies

Experimental and Results

In a separate experiment barley (cv. Ymer) was cut at the early mealy ripe stage on 20 July 1966 with a flail-type forage harvester and ensiled in two metal silos (153 cm diameter × 182 cm high). The silos were each filled with 1049 kg fresh material and sealed with plastic sheeting. Effluents were not produced during the period of storage. Silo A was opened on 31 January 1967 and the silage was fed to 6 sheep (Scottish Blackface) in an attempted digestibility trial but this was abandoned because of extremely low intakes of dry matter which averaged only 20 g $W^{0.73}$ over the last 6 days of the experiment. The composition of the original barley and the silage is given in Table VII. Silo B was opened on 16 March 1967 and the silage was fed at the maintenance level to 3 dry Ayrshire cows in a digestibility trial. The results of the trial are given in Table VIII.

Discussion

The silages were well preserved (pH 3·9–4·0) in spite of the low concentrations of fermentation acids present. The residual WSC content (excluding starch) was about 12%, which is high compared with that normally found in fresh grass silages (usually 1–2%) but not abnormal when compared with silages of high dry matter content.

Total losses of dry matter during ensilage were 4·0% (silo A) and 9·6% (silo B) including 2·6% and 5·2% of waste material, respectively. The fermentation losses were therefore very low and confirm previous findings with wilted grass of similar dry matter content. Provided that anaerobic conditions are rapidly achieved and maintained, losses during

Percentage digestibility, percentage of digestible nutrients, and energy values of true dry matter
(*in vivo* results with Ayrshire cows)

	% Digestibility				Digestible nutrients			
	1	2	3	Mean	1	2	3	Mean
Dry matter	50·6	54·9	49·2	51·6	—	—	—	—
Organic matter	64·9	69·8	61·8	65·5	56·9	61·2	54·1	57·4
Crude protein	46·2	45·9	40·8	44·3	3·2	3·1	2·8	3·0
Ether extract	57·7	63·5	49·7	57·0	0·9	1·0	0·8	0·9
Crude fibre	52·5	64·3	50·0	55·6	13·8	16·9	13·1	14·6
Nitrogen-free extractives	73·7	76·0	71·0	73·6	39·0	40·2	37·6	39·0
*ME (Mcal/kg)	—	—	—	—	2·07	2·23	1·97	2·09
†Starch equivalent	—	—	—	—	49·9	54·4	47·3	50·5

* Metabolisable energy (calculated from total digestible nutrients)
† Using 0·29 factor in fibre correction

TABLE VII

Composition of whole barley and silages
(% of true dry matter)

	Barley	Silage A	Silage B
Dry matter	32·4	32·4	31·6
Ash	12·8	15·3	12·4
Inorganic matter	87·2	84·7	87·6
Crude protein	6·3	6·4	6·3
Ether extract	1·3	1·5	1·6
Crude fibre	24·3	24·8	26·2
Total N	1·01	1·02	1·00
Protein N	0·77	0·39	—
Non-protein N	0·24	0·63	—
Volatile N	—	0·07	—
Water-soluble carbohydrates	25·3	11·7	12·2
Cellulose	25·6	24·6	25·4
Lignin	6·0	6·2	6·5
Acetic acid	—	1·59	1·62
Propionic acid	—	0·16	0·14
Butyric acid	—	Abs.	Abs.
Succinic acid	—	0·09	0·10
Lactic acid	—	3·35	3·08
Ethanol	—	0·53	0·46
pH	5·8	3·9	4·0

ensilage will be low. The added advantage with crops of this dry matter level is that there is no effluent problem. However, the dangers of oxidation must be stressed, as cereal crops are more difficult to consolidate than grass and it is important with material of this type to ensure that it is well chopped before it is ensiled.

The digestibility of the organic matter of silage B, determined using dry cows, averaged 65·5%, a value which is similar to that obtained by the *in vitro* method for barley cut at a similar stage of growth.

Acknowledgments

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ENSILAGE OF WHOLE-CROP BARLEY

II.*—Composition of barley and barley silage at different stages of growth

A. W. MacGREGOR and R. A. EDWARDS

Samples of whole barley cv. Zephyr were cut at 7 stages of growth and after being chopped were ensiled in small laboratory silos. Changes in individual carbohydrates and organic acids during ensilage were studied. The water-soluble carbohydrates, which rose to a maximum (32·6% of the dry matter) at the milky kernels stage of growth, consisted mainly of fructosans with smaller amounts of fructose, glucose and sucrose. The main organic acid of the plant, malic acid, disappeared during ensilage. The practical significance of the changes is discussed.

Introduction

The ease with which a forage crop is conserved in the ensilage process by natural fermentation is largely dependent on the contents of moisture and water-soluble carbohydrate (WSC) of the fresh material. There is abundant evidence that wilting the crop to a dry matter (DM) content of 30% prior to ensiling is advantageous because not only are effluent losses greatly reduced but undesirable clostridial fermentation is inhibited.[1] In addition, the crop must contain a supply of readily available carbohydrates which can be fermented during ensilage to lactic and acetic acids by the lactic acid bacteria present on the herbage.[2,3]

Grass crops must be wilted before they attain a DM content of 30% but the DM content of whole barley increases naturally from less than 20% in the very young plant to more than 40% in the mature plant. Wilting of the whole barley before ensiling is unnecessary therefore, if the crop is cut at a late stage of growth.

Although there are very few reports in the literature on the carbohydrate content of whole barley there is some evidence that immature plants do contain sufficient soluble carbohydrates to support adequate fermentation in the ensilage process.[4]

An extensive investigation was carried out to determine the composition of several barley varieties at various stages of growth so that the best stage at which to cut the barley for silage could be determined. The results showing the changes in the organic acid and carbohydrate fractions for one variety of barley are given in this paper. In addition, small laboratory-scale silos were filled with whole barley samples cut during the first 5 stages of growth, and the resulting silages were analysed.

Experimental

Replicated plots of whole barley cv. Zephyr were grown on one of the School farms. Samples were cut 5 cm above the ground at 7 stages of growth (see Table I) and immediately brought into the laboratory where the whole barley was chopped into small lengths (0·5–1·0 cm). The replicates were thoroughly mixed together.

Determination of carbohydrates

All determinations were carried out in triplicate and included appropriate blanks and standards. A 25 g sample of the fresh material was macerated with 250 ml chilled distilled water for 5 min and then centrifuged (11,000 g; 10 min). The supernatant liquid was retained and its pH was determined. The starch-containing residue was washed twice with water, treated with boiling ethanol and stored at $-18°$ until subsequently analysed. The washings and supernatant were combined and, where necessary, brought to pH 6 by the addition of dilute alkali, boiled for 2 min, filtered through glass-fibre paper using Hyflosupercel filter-aid and de-ionised on a mixed-bed resin column containing equal wet volumes of Amberlite resins IRC-50(H) and IR-45(OH). The de-ionised extract was reduced in volume using a rotary film evaporator and made up to a standard volume of 50 ml.

A 1 ml sample of the extract was applied to Whatman 3MM chromatography paper and eluted with one of the following solvent systems: ethyl acetate–pyridine–water (10 : 4 : 3); ethyl acetate–acetic acid–formic acid–water (18 : 3 : 1 : 4). The latter solvent was not used when oligosaccharides containing sucrose were present in the extracts because these carbohydrates were hydrolysed by the acid in it.

The individual carbohydrates were eluted from the chromatograph paper, and their concentrations were determined by one of the following techniques.

Reducing carbohydrates were determined by the ceric sulphate–ferricyanide method of Lampitt et al.[6]

Sucrose, fructosans and fructose-containing oligosaccharides were hydrolysed with dilute acid[7] and the resulting carbohydrates were determined by the method of Lampitt et al.[6]

Mannitol was oxidised by sodium meta-periodate to formic acid[8] which was then determined by the procedure of Kolthoff & Belcher.[9]

The starch-containing residue from the aqueous extract was filtered to remove the ethanol and steeped in 60 ml boiling water for 5 min to gelatinise the starch. After the extract had been cooled in ice, 60 ml of cold perchloric acid (72%) were slowly added with stirring. The perchloric acid was removed on the centrifuge (11,000 g; 10 min) after 30 min and the extraction was repeated with a further aliquot of perchloric acid. The acid was again removed on the centrifuge and the residue was washed 4 times with water.

These washings were combined with the perchloric acid extracts and made up to a standard volume of 500 ml.

Starch was isolated from these solutions by the method of MacWilliam *et al.*[10] and determined, after hydrolysis to glucose with $1 \cdot 5$N sulphuric acid for 2 h at 100°, by the ceric sulphate–ferricyanide procedure of Lampitt *et al.*[6]

The WSC determinations were carried out in the following way. A 3 ml sample of the 50 ml standard extract was diluted to 25 ml and a $0 \cdot 5$ ml portion of this solution was hydrolysed with dilute acid[7] before its carbohydrate content was determined by the procedure of Lampitt *et al.*[6]

Determination of organic acids

A 25 g sample of the fresh material was steeped in 50 ml of $0 \cdot 6$N sulphuric acid at 4°. A crystal of thymol was added to inhibit mould and bacterial growth. After one week the acid extract was quickly filtered through muslin and a 10 ml portion was neutralised with alkali, reduced to small volume and carefully re-acidified with sulphuric acid. The organic acids in a known amount of this concentrated extract were separated by silicic acid column chromatography.[11] The concentrations of the individual organic acids eluted from the column were determined with an automatic titrator[12] (V. A. Howe & Co. Ltd., London).

Determination of buffering capacities

The buffering capacities of the whole barley silages were determined by the method of Playne & McDonald.[13]

Determination of dry matter

Fresh plant material was dried in a forced-draught oven at 100° for 16 h (or until constant weight). The dry matter contents of the silages were not determined, and the results

of silage analyses were expressed on the basis of ensiled plant dry matter.

Preparation of laboratory silos

Small laboratory silos[14] were filled with whole barley samples which were harvested at the first 5 stages of growth investigated in an earlier experiment. Samples (70 g) of the finely chopped whole barley were packed into boiling tubes (32 × 200 mm) and sealed with mercury seals. These were stored in the dark at room temperature and opened after 8 weeks.

The pH, carbohydrate and organic acid contents of the silages were determined by the procedures already described.

Results

Carbohydrates

The changes which occurred in the carbohydrate fraction of the whole barley over the 7 stages of growth investigated are shown in Table I. Initially, glucose and fructose were present in the highest concentration but as the barley matured the relative amounts of these decreased and the fructose-containing oligosaccharides and fructosans increased in concentration until the milky kernels stage was reached. Thereafter, the concentrations of these carbohydrates also decreased.

Chromatographic examination of the oligosaccharide fraction showed that it was a complex mixture containing at least 5 carbohydrates. None of these was positively identified but after dilute acid hydrolysis they all yielded fructose indicating that they were all fructose-containing oligosaccharides. The predominant component of this mixture was tentatively identified as raffinose on the basis of its chromatographic mobility.

TABLE I

Composition of whole barley at different stages of growth
(% of dry matter)

	Heading completed	Flowering	Watery kernels	Milky kernels	Mealy ripe–early	Mealy ripe–late	Ripe for cutting
Dry matter	19·1	20·3	26·2	29·0	35·1	38·7	42·2
Fructose	6·01	5·02	4·12	3·10	2·90	3·10	2·21
Glucose	6·00	6·00	4·22	2·93	2·75	1·98	1·07
Galactose	0	0	0	0·16	0·44	0·46	0·58
Xylose	0	0	0	0	0	0	0
Arabinose	0	0	0	0	0	0	0
Sucrose	1·90	1·46	2·28	3·30	2·13	1·97	0·44
*Oligosaccharides	1·61	3·35	6·88	7·60	3·21	3·45	1·51
Fructosans	3·08	3·25	7·20	12·80	12·21	6·60	2·30
Water-soluble carbohydrates (calculated)	19·3	20·0	26·5	32·6	25·5	18·5	8·6
Water-soluble carbohydrates (found)	19·2	20·2	26·7	30·6	25·5	18·1	8·4
Mannitol	0	0	0	0	0	0	0
Starch	0·28	0·25	0·38	1·02	18·50	34·80	41·30
Formic acid	0·09	0·06	0·10	0·06	0	0	0
Acetic acid	0·43	0·71	0·33	0·65	0·64	0·64	0·51
Succinic acid	Trace	Trace	Trace	Trace	Trace	Trace	Trace
Malonic acid	0	0	0·09	0·08	0·07	0·06	0·07
Lactic acid	0	0	0	0	0	0	0
Malic acid	5·63	2·82	1·73	1·31	0·71	0·32	0·15
Citric acid	0·48	0·40	0·54	0·43	0·24	0·09	0·06
pH	5·9	5·9	5·9	5·9	5·9	6·0	6·0

* excluding sucrose but including short-chain fructosans

Xylose and arabinose were not detected in the barley samples but small amounts of galactose became apparent at the milky kernels stage and later stages of growth.

The gross changes in the water soluble carbohydrate fraction of the barley at the different stages of growth are given by the WSC (found) figures—starch is not included in these results. These agree very well with the WSC (calculated) figures which were obtained by adding the concentrations of each soluble carbohydrate found in the barley. The concentrations of the fructosans and of all oligosaccharides were expressed as fructose for this calculation. The WSC content of the barley rose to a maximum (32·6%) at the milky kernels stage of growth and thereafter it steadily decreased until only 8·6% remained at maturity.

The amount of starch present in the barley during the first 3 stages of growth was very small but it increased rapidly after the milky kernels stage. From the late mealy ripe stage of growth starch was quantititively the most important carbohydrate determined.

The carbohydrate compositions of the various barley silages are given in Table II. Glucose was not found in silages made at the 3 earliest stages of growth and fructose was absent from those made at the 2 earliest stages.

Sucrose was not found in any of the silages and there was considerable breakdown of the fructosan and oligosaccharide fractions except in the silage made from barley at the early mealy ripe stage when an increase in the oligosaccharide content was found.

Appreciable amounts of xylose, and trace amounts of galactose and arabinose were found in all silages.

The starch content of the silages was very similar to that of the original barley except for the silage made at the early mealy ripe stage. Here, the results indicate that about 2·5% of the starch had been broken down in the silage fermentation process.

The mannitol content of the silages rose to a maximum in the watery kernels silage and then decreased slightly in the silages made from material at subsequent stages of growth.

Organic acids

A detailed study of the organic acids present in the whole barley samples (Table I) showed that the concentration of malic acid was very much higher than that of any other acid except during the last 2 stages of growth when acetic acid predominated. At the flowering stage the high initial malic acid concentration had fallen by half and thereafter it slowly decreased until in mature barley its relative concentration was less than 3% of its initial value.

Formic and acetic acids were the only volatile acids detected in the barley samples. The concentration of formic acid was extremely low and this acid could not be detected after the milky ripe stage. Acetic and citric acids were present initially in similar amounts but whereas the latter decreased in concentration during growth the concentration of the former remained much more constant.

Lactic acid was not detected at any stage of growth, and only small amounts of malonic acid were found.

The organic acid analyses of the silages are shown in Table II. Although acetic and lactic acids predominated there were also substantial amounts of succinic acid present in all silages. Large amounts of butyric acid were found in the silages made from barley cut during the first two stages of growth.

Discussion

Composition of whole barley

The primary carbohydrate products of photosynthesis are glucose and fructose.[15,16] These may be utilised by the green plant in one of two ways depending on the stage of growth of the plant. During their initial stages of growth young plants rapidly increase in size and so require for their immediate use most of the energy which they obtain through photosynthesis. At this stage the plant metabolises most of the glucose and fructose to form some of the other materials which a growing plant requires. As plants mature, however, their rate of growth decreases and so they do not require all

TABLE II

Composition of whole barley silages
(results expressed as percentage of ensiled plant dry matter)

	Heading completed	Flowering	Watery kernels	Milky kernels	Mealy ripe-early
Fructose	0	0	3·51	6·00	2·65
Glucose	0	0	0	1·08	3·71
Galactose	Trace	Trace	Trace	Trace	Trace
Xylose	1·11	1·83	0·59	0·37	0·39
Arabinose	Trace	Trace	Trace	Trace	Trace
Sucrose	0	0	0	0	0
Oligosaccharides	1·31	2·51	3·08	3·21	5·20
Fructosans	1·12	1·25	1·08	1·17	1·22
Water-soluble carbohydrates (calculated)	3·8	6·0	8·7	12·5	14·1
Water-soluble carbohydrates (found)	4·2	5·9	8·3	12·2	14·0
Mannitol	1·36	3·18	6·51	6·30	6·20
Starch	0·27	0·27	0·40	1·01	16·00
Acetic acid	3·00	3·01	1·95	1·92	2·05
Succinic acid	1·65	1·58	1·49	0·97	1·15
Lactic acid	3·72	4·52	6·86	6·18	3·35
Butyric acid	1·95	1·12	0·40	Trace	0·02
pH	4·61	4·63	3·95	4·10	4·52
Buffering capacity (mequiv./100 g DM)	97·4	85·2	75·5	65·2	50·1

of the energy made available to them through photosynthesis for their immediate use. They begin to store their energy in the form of carbohydrates of high molecular weight.

This general scheme can be used to explain the changing carbohydrate pattern in growing whole barley (Table I). The carbohydrates present in highest concentration during the initial periods of growth studied were glucose and fructose. These accounted for more than 50% of the total WSC content of the barley samples thus forming a large, readily available source of energy and material for the immediate requirements of the young plant. The presence of small amounts of fructosan at this stage indicated that limited storage of excess photosynthetic products had already taken place.

The glucose and fructose concentrations steadily decreased as the barley matured. This shows that the plant has a decreasing requirement for readily available carbohydrate metabolites and, therefore, stores its excess carbohydrates in a more permanent form. This change in the general metabolic scheme is also indicated by the increased amounts of fructosan and fructose-containing oligosaccharides found during the watery kernels and milky ripe stages of growth.

The chemical natures of the oligosaccharides, which were found in relatively high concentrations during the intermediate stages of growth studied, were not elucidated, but all of these oligosaccharides contained fructose which was liberated on hydrolysis with dilute acid. They probably represent the first stages in the transfructosidation of sucrose, i.e. they are intermediate compounds which are formed during the synthesis of fructosans from sucrose and fructose. Schlubach et al.[17] examined the oligosaccharides present in young barley plants and showed that some had a straight-chain and others a branched-chain structure. These workers suggested that the oligosaccharides were the precursors of the straight- and branched-chain fructosans found in barley.

The sucrose content of the barley samples was surprisingly low. The maximum value found ($3 \cdot 30\%$) is very much lower than the values of 5–6% frequently found in immature grass although the variation of the sucrose content of the barley with stage of growth is similar to that found in grass.[20] (Sucrose is usually one of the most readily detected photosynthetic products because it tends to accumulate in the leaves of plants and act as a temporary energy store.[18,19])

It may be noted that the highest sucrose concentration was found at the milky ripe stage when the synthesis of fructosan was at a maximum. This indicates an increase in the activity of carbohydrate transfer via sucrose within the plant. This increased sucrose concentration was also associated with a decreased glucose and fructose concentration showing that these carbohydrates were rapidly used to synthesise sucrose and subsequently fructosan.

The starch content of the barley samples during the initial periods of growth was very low and it was not until the milky ripe stage had been reached that appreciable starch synthesis took place. At this stage the plant requires only a small portion of the photosynthetic energy which it receives for its own use, and so the excess is used to synthesise starch which accumulates in the already formed ear. The starch concentration continued to increase as the barley matured.

The reason for the accumulation of both fructosan and starch in barley is not clear. Starch is found only in the grain but fructosan has been found not only in the grain, but also in the leaves and stems of barley where it tends to accumulate prior to the onset of rapid starch synthesis.[21,22]

At one time, it was proposed[4] that the fructose-containing oligosaccharides and fructosan were starch precursors—these carbohydrates, formed in the leaves of barley plants, moved into the stems and were then translocated to the site of starch synthesis. This hypothesis certainly explains why the onset of starch synthesis might be accompanied by a lowered fructosan concentration in the whole barley samples. But the high rate of starch synthesis found in barley kernels would require an extremely fast transfer of precursors from their site of synthesis in the leaves through the stem to the developing grain. In addition, careful investigation of the developing barley grain has revealed that it itself supports a high rate of starch synthesis.[23,24] From these observations it is now concluded that most of the barley starch is synthesised in situ in the grain.

The precise function of fructosans, and their associated low molecular weight oligosaccharides, in barley during the period of rapid starch development is not completely understood. Some of the fructosan is almost certainly transferred to the grain and the remainder may well be utilised by the plant to satisfy its decreasing immediate energy requirements.

The results in Table I suggest that as the plant matures the rate of starch synthesis accelerates but that of fructosan synthesis is lowered. Although the reasons for this change in emphasis in the carbohydrate metabolism of the maturing plant are known the factors controlling the change-over are not understood.

There is very little information available concerning the accumulation of organic acids in barley plants but malic and citric acids have been shown to be predominant in mature barley grain.[25,26] In this present investigation malic acid was found to be the most important acid in whole barley during the initial stages of growth. Its function is not known but it may act as a temporary energy store for the young plant.

Composition of whole barley silage

The WSC and moisture content of fresh material are extremely important factors in determining the extent of fermentation during ensilage. In low DM silages extensive fermentation must take place before a sufficiently low pH value is obtained to preserve the material.[27] Under these conditions a low WSC concentration in the ensiled material may limit the fermentation process.

However, the ensiled whole barley samples all contained sufficient amounts of WSC to support adequate lactic acid fermentations during ensilage. Nevertheless, in the wettest samples, all the glucose, fructose and sucrose as well as most of the fructosan and oligosaccharides were broken down in the fermentation process leaving only small amounts of residual WSC in the silages. As the DM of the barley samples increased the amount of WSC found in the silages also increased.

The relatively large amounts of xylose and much smaller amounts of arabinose and galactose found in the barley silages were probably produced by acid hydrolysis of the hemicellulose fraction of the barley. Previous studies[28] have shown that there is considerable hemicellulose breakdown during the ensilage of low DM grass and there is evidence that barley straw contains hemicelluloses which liberate large amounts of xylose on hydrolysis.[29]

Two types of organisms are mainly responsible for the fermentation of carbohydrates in silage.[30] Homolactic

bacteria metabolise hexoses to form only lactic acid, but heterolactic bacteria produce, in addition, mannitol, acetic acid and carbon dioxide from fructose, and ethanol and carbon dioxide from glucose. Silage-preserving acid, therefore, is more efficiently produced by the homolactic bacteria.

The presence of mannitol in the barley silages indicates that heterolactic bacteria have been active during ensilage. The smaller amounts of mannitol found in the wet silages may suggest that these bacteria were less active in the wet silage but this is not necessarily so. There is evidence that mannitol may be broken down in low DM silage. It appears unlikely that bacteria would cause such breakdown because of the reduced nature of mannitol, but the high acid concentration found in wet silage may be responsible.

Amylases, especially α-amylase, are the most important starch-degrading enzymes in plants. These enzymes convert starch into a mixture of glucose, maltose, and dextrins of low molecular weight. Because barley contains only small quantities of these enzymes[31] it is not surprising that very little starch breakdown occurred during the ensilage of the whole barley samples (cf. Tables I and II). It is unlikely that sufficient glucose was formed during this limited starch degradation to make an appreciable contribution to the silage fermentation process.

Some workers[32,33] have studied the effect of adding starch-rich products such as barley and barley malt meals to silage and found that, whereas the barley meal had no effect on the resulting silage, the addition of malt yielded a better quality product presumably because the increased amylase activity in the malt degraded the starch to simpler carbohydrates which the bacteria present in the silage were then able to utilise.

An adequate supply of lactic acid bacteria on fresh herbage is necessary to ensure that the pH of the subsequent silage falls sufficiently fast to inhibit excessive clostridial activity. This does not present a problem with large-scale silages because the machinery used to harvest the forage usually contains large populations of the necessary bacteria[34] and such machinery inoculates the forage. In the preparation of laboratory silos, however, the material to be ensiled is unlikely to come into contact with a source of lactobacilli and so may not contain sufficiently high numbers of these bacteria to promote the desired vigorous fermentation. Under these conditions fermentation of the WSC to forage-preserving organic acids would be slow, and clostridial activity might be allowed to develop.

The presence of butyric acid and the high pH values found in some of the barley silages, especially in those made from low DM material, suggest that some clostridial development had taken place. This undesirable type of fermentation was more pronounced in the silages made from barley which contained a high moisture content because not only do clostridia multiply rapidly under wet conditions but a more acid pH must be achieved in the silage before they are inactivated.

A feature of these barley silages is the very low levels of lactic acid which were found. Silages prepared from samples of Italian ryegrass having similar moisture and WSC contents contained much higher amounts of lactic acid and had high lactic/acetic acid ratios indicating that there had been extensive fermentation by homolactic bacteria during ensilage.[27] In the wet barley silages some of the lactic acid produced would have been metabolised by the clostridia to form butyric acid but this reaction would not have occurred in the silages made from barley at later stages of growth.

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The results from the silage made from barley at the early mealy ripe stage of growth, in which the concentrations of acetic and lactic acids were very similar (34·2 mequiv. and 37·2 mequiv. respectively), suggest that a mainly heterolactic fermentation had taken place. This conclusion is supported by the high concentration of mannitol found in the silage. The relatively high pH value found is quite normal for a high DM silage and is not indicative of extensive clostridial activity.

The buffering capacity of this silage was rather low (50·1) compared with the values usually found for silages made from forage crops such as Italian ryegrass[13] but this is not surprising because it contains such a low organic acid concentration.

Citric and malic acids were not found in the silages although they were both present in the original barley samples. There is evidence that malic acid in the presence of carbohydrates and an atmosphere containing a high carbon dioxide content can be used by plant enzymes to form succinic acid.[35] Malic acid, therefore, may be the precursor for some if not for all of the rather large amounts of succinic acid which were synthesised during ensilage of the whole barley samples.

Acknowledgments

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STRUCTURES AND PESTICIDAL ACTIVITIES OF DERIVATIVES OF DINITROPHENOLS

IX.*—Effects of substitution of dinitrophenols with C_4 to C_{13} α-branched alkyl groups and of esterification on the eradication of apple mildew

By M. PIANKA and P. J. J. SWEET

The eradication of apple mildew caused by *Podosphaera leucotricha* (Ell. and Everh.) Salm. with 2-(C_4 to C_{13} α-branched alkyl)-4,6-dinitrophenols and 4-(C_4 to C_{13} α-branched alkyl)-2,6-dinitrophenols was examined. 4-(1-Ethylbutyl)- and most 4-(C_7 to C_{12} α-branched alkyl)-2,6-dinitrophenols were significantly more active than their 2-alkyl-4,6-dinitrophenol analogues. Compounds containing 4-C_{13} alkyls did not show significant activity. Activity generally increased as the α-alkyl branch lengthened.

Methyl carbonates did not show lower activity than the parent phenols, but esterification of 4-(C_{12} or C_{13} α-branched alkyl)-2,6-dinitrophenols to ethyl carbonates or crotonates gave compounds with reduced activity. Methyl-, ethyl- or isopropyl-carbonates and crotonates of 4-(1-ethylhexyl)-2,6-dinitrophenol had much higher activity than the corresponding esters of the 4-(1-methylheptyl) isomer or of 2-(1-ethylhexyl)-4,6-dinitrophenol. Compounds containing long ester chains (C_7 or C_8) had less activity than 4-(1-ethylhexyl)-2,6-dinitrophenol.

O-Methylation produced compounds with less activity than the parent 4-(1-ethylhexyl)- and 4-(1-propylpentyl)-2,6-dinitrophenols.

Introduction

In 1961 several batches of the compound designated MC1143 were prepared by nitration of '*o*-caprylphenol' from various sources by the method of Pianka & Edwards[1] and by condensing 'dinitro-*o*-caprylphenol' thus obtained with methyl thiolochloroformate according to Pianka.[2] The various samples of MC1143 were tested against powdery mildew of cucumber caused by *Sphaerotheca fuliginea* (Schlecht. ex Fr.) Poll. (previously known as *Erysiphe cichoracearum* D.C.), powdery mildew of barley caused by *Erysiphe graminis* Mérat, and powdery mildew of apple caused by *Podosphaera leucotricha* (Ell. & Everh.) Salm. The samples of MC1143 varied greatly in their efficiency and therefore in their composition.

Kirby[3] found differences in phytotoxicity to barley between dinitro-octylphenol obtained on hydrolysing a sample of MC1143, and dinitro-octylphenol obtained on hydrolysing Karathane.

Preliminary investigations showed that 2-(α-branched alkyl)-4,6-dinitrophenols were generally more active than 4-(α-branched alkyl)-2,6-dinitrophenols against spider mites and cucumber mildew, whereas 4-(α-branched alkyl)-2,6-dinitrophenols were generally more active than 2-(α-branched alkyl)-4,6-dinitrophenols against apple and barley mildews.[4,†] The effects of substitution with C_3 to C_{13} α-branched alkyl groups and of esterification on the acaricidal activity of dinitrophenols was investigated and reported.[2,5,6] In Parts IX–XI these effects on the eradication of apple, barley and cucumber mildew will be described.

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* Part VIII, *J. Sci. Fd Agric.*, 1968, **19**, 60

† Parts of these investigations were reported in 1966 in papers presented to the Polish Academy of Sciences and the Polish Institute of Industrial Organic Chemistry and to the Pesticides Group of the Society of Chemical Industry.

Experimental

Preparative methods

The compounds referred to in this section were used in the tests described in Parts IX–XII.

Esters

A number of alkyldinitrophenyl esters were prepared as described earlier.[2,6,7] Several new esters were synthesised by the method of Pianka & Edwards[6] from the appropriate alkyldinitrophenols and chloroformates or acyl chlorides. When appropriate the residue was crystallised from suitable solvents. The oils were not distilled. The physical data of the new carbonates are shown in Table I and of the new acid esters in Table II.

Phenols

2-(1-Ethylpropyl)-, 2-(C_6 to C_{13} α-branched alkyl)-4,6-dinitrophenols and 4-(C_5 to C_{13} α-branched alkyl)-2,6-dinitrophenols were prepared according to Pianka & Edwards.[1] The preparation of 2-isopropyl-, 2-s-butyl-, 2-(1-methylbutyl)-4,6-dinitrophenols and 4-t-butyl-2,6-dinitrophenol has been described in literature.

4-s-Butyl-2,6-dinitrophenol.—This compound was prepared by the general method described by Pianka & Edwards[1] by heating under reflux 4-s-butylphenol, in carbon tetrachloride, with nitric acid for 3 h; the cyclohexylamine salt, m.p. 197°, formed in di-isopropyl ether, was acidified in solution in methanol, and the phenol was extracted with petroleum. The compound was obtained as an oil which solidified to yellow crystals, m.p. 44° (depressed by 2-s-butyl-4,6-dinitrophenol) (Found: N, 11·4. $C_{10}H_{12}N_2O_5$ requires N, 11·7%).

TABLE I

Physical data of new dinitrophenyl carbonates

Alkyl	Carbonate	Appearance and n_D^{20} of oil or appearance and m.p. of solid	Formula	Found: N, %	Required: N, %
2-Alkyl-4,6-dinitrophenyl carbonates					
Isopropyl (3*)	Methyl[a]	Light brown prisms, m.p. 77	$C_{11}H_{12}N_2O_7$	9·5	9·9
1-Methyloctyl (9)	Methyl	Light red, 1·5139	$C_{17}H_{24}N_2O_7$	8·0	7·6
4-Alkyl-2,6-dinitrophenyl carbonates					
t-Butyl (4)	Methyl[a]	Cream coloured prisms, m.p. 93–95	$C_{12}H_{14}N_2O_7$	9·4	9·4
1-Methylbutyl (5)	Methyl	Yellow, 1·5233	$C_{13}H_{16}N_2O_7$	9·4	9·0
1-Methylbutyl (5)	Ethyl	Orange, 1·5170	$C_{14}H_{18}N_2O_7$	8·2	8·6
1-Methylpentyl (6)	Methyl	Yellow, 1·5208	$C_{14}H_{18}N_2O_7$	8·9	8·6
1-Methylpentyl (6)	Ethyl	Yellow, 1·5152	$C_{15}H_{20}N_2O_7$	8·1	8·2
1-Ethylpentyl (7)	Methyl	Yellow, 1·5188	$C_{15}H_{20}N_2O_7$	8·2	8·2
1-Ethylpentyl (7)	Ethyl	Orange, 1·5148	$C_{16}H_{22}N_2O_7$	8·1	7·9
1-Propylbutyl (7)	Methyl	Yellow, 1·5179	$C_{15}H_{20}N_2O_7$	7·7	8·2
1-Propylbutyl (7)	Ethyl	Yellow, 1·5127	$C_{16}H_{22}N_2O_7$	7·5	7·9
1-Propylpentyl (8)	s-Butyl	Orange, 1·5033	$C_{19}H_{28}N_2O_7$	7·0	7·1
't-Octyl'† (8)	Methyl	Brown, 1·5221 ($n_D^{22.8}$)	$C_{16}H_{22}N_2O_7$	7·9	7·9
't-Octyl'† (8)	Ethyl	Pale yellow crystals, m.p. 54–55°	$C_{17}H_{24}N_2O_7$	7·6	7·6
1-Butylpentyl (9)	Ethyl	Pale brown, 1·5085	$C_{18}H_{26}N_2O_7$	7·6	7·3
1-Methylnonyl (10)	Methyl	Red brown, 1·5096	$C_{18}H_{26}N_2O_7$	7·7	7·3
1-Methyldecyl (11)	Methyl	Red brown, 1·5072	$C_{19}H_{28}N_2O_7$	6·9	7·1
1-Ethylnonyl (11)	Methyl	Yellow, 1·5081	$C_{19}H_{28}N_2O_7$	7·6	7·1
1-Propyloctyl (11)	Methyl	Pale yellow, 1·5085	$C_{19}H_{28}N_2O_7$	7·0	7·1
1-Propyloctyl (11)	Ethyl	Pale yellow, 1·5048	$C_{20}H_{30}N_2O_7$	7·0	6·8
1-Pentylhexyl (11)	Methyl	Orange, 1·5072	$C_{19}H_{28}N_2O_7$	7·4	7·1
1-Pentylhexyl (11)	Ethyl	Orange, 1·5040	$C_{20}H_{30}N_2O_7$	7·4	6·8
1-Ethyldecyl (12)	Methyl	Red brown, 1·5058	$C_{20}H_{30}N_2O_7$	7·0	6·8
1-Propylnonyl (12)	Methyl	Red brown, 1·5058	$C_{20}H_{30}N_2O_7$	7·0	6·8
1-Butyloctyl (12)	Methyl	Pale yellow, 1·5068	$C_{20}H_{30}N_2O_7$	6·7	6·8
1-Butyloctyl (12)	Ethyl	Pale yellow, 1·5031	$C_{21}H_{32}N_2O_7$	7·1	6·6
1-Pentylheptyl (12)	Methyl	Pale yellow, 1·5060	$C_{20}H_{30}N_2O_7$	6·8	6·8
1-Pentylheptyl (12)	Ethyl	Pale yellow, 1·5020	$C_{21}H_{32}N_2O_7$	6·7	6·6
1-Propyldecyl (13)	Methyl	Red brown, 1·5041	$C_{21}H_{32}N_2O_7$	7·0	6·6
1-Butylnonyl (13)	Methyl	Yellow, 1·5050	$C_{21}H_{32}N_2O_7$	7·1	6·6
1-Pentyloctyl (13)	Methyl	Yellow, 1·5048	$C_{21}H_{32}N_2O_7$	6·8	6·6

* Total number of carbon atoms of the nuclear alkyl substituent.
Solvent for crystallisation: [a] propan-2-ol
† Substantially 1,1,3,3,-tetramethylbutyl

TABLE II

Physical data of new dinitrophenyl esters

Alkyl	Ester	Appearance and n_D^{20} of oil or appearance and m.p. of solid	Formula	Found: N, %	Required: N, %
2-Alkyl-4,6-dinitrophenyl esters					
Isopropyl (3)	Crotonate[a]	White needles, m.p. 57–59°	$C_{13}H_{14}N_2O_6$	9·2	9·5
s-Butyl (4)	Benzoate[b]	White rhombs, m.p. 72·5–74°	$C_{17}H_{16}N_2O_6$	8·1	8·1
1-Propylpentyl (8)	Acrylate	Pale yellow, 1·5260	$C_{17}H_{22}N_2O_6$	8·4	8·0
4-Alkyl-2,6-dinitrophenyl esters					
t-Butyl (4)	Crotonate[a]	Light brown prisms, m.p. 93–94°	$C_{14}H_{16}N_2O_6$	8·9	9·1
1-Methylpentyl (6)	Crotonate	Pale yellow, 1·5340	$C_{16}H_{20}N_2O_6$	7·9	8·3
1-Methylhexyl (7)	Crotonate	Red brown, 1·5257	$C_{17}H_{22}N_2O_6$	8·3	8·0
1-Methylheptyl (8)	Acetate	White crystals, m.p. 57–59°	$C_{16}H_{22}N_2O_6$	8·5	8·3
1-Propylbutyl (7)	Crotonate	Yellow, 1·5310	$C_{17}H_{22}N_2O_6$	7·4	8·0
1-Propylpentyl (8)	Acetate	Pale yellow crystals, m.p. 60–62°	$C_{16}H_{22}N_2O_6$	8·9	8·3
1-Propylpentyl (8)	Isobutyrate	Orange, 1·5098	$C_{18}H_{26}N_2O_6$	7·7	7·7
1-Propylpentyl (8)	Acrylate	White crystals, m.p. 58–60°	$C_{17}H_{22}N_2O_6$	8·1	8·0
1-Propylpentyl (8)	Methacrylate	Orange, 1·5242	$C_{18}H_{24}N_2O_6$	7·3	7·7
't-Octyl'† (8)	Crotonate	Brown, 1·5140 (n_D^{25})	$C_{18}H_{24}N_2O_6$	7·6	7·7
1-Butylpentyl (9)	Crotonate	Pale brown, 1·5210	$C_{19}H_{26}N_2O_6$	7·3	7·4
1-Propyloctyl (11)	Crotonate	Pale yellow, 1·5208	$C_{21}H_{30}N_2O_6$	6·7	6·9
1-Pentylhexyl (11)	Crotonate	Orange, 1·5198	$C_{21}H_{30}N_2O_6$	7·3	6·9
1-Butyloctyl (12)	Crotonate	Pale yellow, 1·5182	$C_{22}H_{32}N_2O_6$	6·7	6·7
1-Pentylheptyl (12)	Crotonate	Pale yellow, 1·5175	$C_{22}H_{32}N_2O_6$	7·0	6·7

Solvents for crystallisation: [a] propan-2-ol; [b] methanol
† Substantially 1,1,3,3-tetramethylbutyl

Eradicant tests

Kirby & Frick[8] described curative tests with apple mildew on potted apple rootstocks. In these tests the rootstocks were dipped in the diluted test compound two days after inoculation, with the objective of preventing visible mildew lesions occurring on the leaves.

In the eradicant tests used in the present experiments eradication of established lesions on the leaves was taken to be indicated by the absence of tufts of spore chains 4–5 days after treatment with the diluted test compound.

Test plants

Malling Merton 106 rootstocks were kept at 4° until required. Weekly batches were potted up in pure peat in 7 in. clay pots. They were then cut back to 2–3 in. above the rooting medium level and allowed to break to give one to four shoots. When about 12 in. of growth had occurred the rootstocks were transferred to the testing glasshouse containing infected rootstocks. During winter months additional u.v. lighting was provided by means of 400 W lamps to give a 16 h daylength.

Infection

The test batches were inoculated with *Podosphaera leuco-tricha* (Ell. & Everh.) Salm. by blowing spores from heavily infected rootstocks over them. Within 7–10 days sporing lesions were clearly visible.

Assessments

Prior to treatment an assessment was made of the mildew present on each leaf of each rootstock in the batch. A watch-maker's tag was fixed above the topmost leaf assessed so that the same leaves could be re-assessed after treatment and in such a position as to exclude leaves carrying an infection of less than grade 3. Four replicate rootstocks per treatment were then drawn from the batch in such a manner that differences in disease incidence were spread as evenly as possible over the treatments in a test. Treatments were applied on the day of the first assessment. A second assess-ment was carried out 4–5 days after treatment when regrowth of spore tufts could be clearly seen. On glasshouse-grown rootstocks apple mildew occurs on both leaf surfaces. For ease of application and assessments only the dorsal leaf surfaces were treated and assessed. The infected area of each leaf was assessed as closely as possible to the values indicated below and given the appropriate grade.

Grade	% Leaf area infected
0	No mildew lesions
1	1
2	4
3	11
4	27
5	63 or greater

Application of test compounds

The test compounds were formulated as emulsifiable con-centrates using naphtha and an emulsifying agent and diluted with tapwater to 10 or 20 ppm of the test compound. In order to ensure good wetting of the mycelium within lesions a standard quantity of an anionic wetting agent was added to the spray dilutions. The dilutions were applied to the in-fected test plants by a paint spray gun to point of run-off. Previous tests indicated that the blank formulations including the wetting agent had negligible mildew activity.

Evaluation of eradicant action

At the second assessment eradication was assumed if no regrowth of spore tufts could be detected.

Degree of eradication due to a test compound was calcu-lated using the expression:

$$\left(1 - \frac{X_0 Y_1}{Y_0 X_1}\right) \times 100$$

In this expression X_0 and X_1 were indices calculated from mean grades of infection of leaves of untreated control plants before and after the test plants were treated with the test compounds; Y_0 and Y_1 were indices calculated on the same basis for plants treated with each test compound.

Statistical analysis of results

Since a very great number of tests were involved in this work and each test usually included one or more standard or reference compound, it was possible to use results for these compounds to establish whether or not the severity of in-fection before treatment influenced the degree of eradication given by a treatment.

Regression analysis showed a negative relationship, and hence it was possible to confine the analysis of variance to the figures for the assessment after treatment in each test, the pre-treatment figures being used only to calculate the degree of eradication.

Results

The results of the tests are presented in Tables III–IX (Tests A1–A21). They were analysed statistically and arranged in order of percentage degree of eradication. The figures that did not differ significantly at P = 0·05 are joined by an unbroken line.

TABLE III

Degree of eradication of *Podosphaera leucotricha* with alkyldinitrophenols at 20 ppm

Test No.	Alkyl group	Eradication, %
A1	4-(1-Ethylhexyl) (8)	96
	4-(1-Methylpentyl) (6)	25
	2-(1-Methylbutyl) (5)	11
	2-(1-Methylpentyl) (6)	7
	4-(1-Ethylpropyl) (5)	5
	2-(1-Ethylpropyl) (5)	3
	4-(1-Methylbutyl) (5)	0
	Untreated	0
A2	4-(1-Propylpentyl) (8)	99·2
	4-(1-Ethylhexyl) (8)	97·5
	4-(1-Methylheptyl) (8)	67
	2-(1-Methylheptyl) (8)	33
	2-(1-Propylpentyl) (8)	27
	2-s-Butyl (4)	9
	2-(1-Ethylhexyl) (8)	7
	4-s-Butyl (4)	0
	Untreated	0
A3	4-(1-Ethylhexyl) (8)	98·7
	4-(1-Propylbutyl) (7)	91
	4-(1-Ethylpentyl) (7)	84
	4-(1-Methylhexyl) (7)	78
	2-(1-Methylhexyl) (7)	20
	2-(1-Propylbutyl) (7)	19
	2-(1-Ethylhexyl) (8)	19
	2-(1-Ethylpentyl) (7)	15
	Untreated	
A4	4-(1-Ethylhexyl) (8)	99
	4-(1-Propylhexyl) (9)	99
	4-(1-Ethylheptyl) (9)	95
	4-(1-Ethylbutyl) (6)	61
	2-(1-Propylhexyl) (9)	50
	4-(1-Methyloctyl) (9)	44
	2-(1-Methyloctyl) (9)	20
	2-(1-Ethylbutyl) (6)	15
	2-(1-Ethylheptyl) (9)	14
	Untreated	
A5	4-(1-Butylpentyl) (9)	99·7
	4-(1-Butylhexyl) (10)	99·7
	4-(1-Ethylhexyl) (8)	98
	4-(1-Propylheptyl) (10)	95
	2-(1-Butylpentyl) (9)	75
	4-(1-Ethyloctyl) (10)	65
	2-(1-Propylheptyl) (10)	33
	4-(1-Methylnonyl) (10)	13
	2-(1-Ethyloctyl) (10)	13
	2-(1-Butylhexyl) (10)	3
	Untreated	
A6	4-(1-Pentylhexyl) (11)	99
	4-(1-Ethylhexyl) (8)	98
	4-(1-Butylheptyl) (11)	97
	4-(1-Propyloctyl) (11)	81
	4-(1-Ethylnonyl) (11)	75
	2-(1-Butylheptyl) (11)	60
	2-(1-Propyloctyl) (11)	48
	2-(1-Pentylhexyl) (11)	11
	4-(1-Methyldecyl) (11)	3
	Untreated	
A7	4-(1-Ethylhexyl) (8)	98
	4-(1-Butyloctyl) (12)	92
	4-(1-Pentylheptyl) (12)	91
	2-(1-Pentylheptyl) (12)	69
	4-(1-Propylnonyl) (12)	59
	4-(1-Ethyldecyl) (12)	56
	4-(1-Hexylheptyl) (13)	56
	4-(1-Butylnonyl) (13)	54
	4-(1-Pentyloctyl) (13)	41
	4-(1-Propyldecyl) (13)	29
	2-(1-Hexylheptyl) (13)	24
	Untreated	

TABLE IV

Degree of eradication of *Podosphaera leucotricha* with 4-(1-ethyl-hexyl)- and 4-(1-propylpentyl)-2,6-dinitrophenols and anisoles at 20 ppm

(Test A8)

Name of compound	Eradication, %
4-(1-Ethylhexyl)-2,6-dinitrophenol (8)	100
4-(1-Propylpentyl)-2,6-dinitrophenol (8)	99·8
4-(1-Ethylhexyl)-2,6-dinitroanisole (8)	76
4-(1-Propylpentyl)-2,6-dinitroanisole (8)	74
Untreated	

TABLE V

Degree of eradication of *Podosphaera leucotricha* with 4-alkyl-2,6-dinitrophenyl methyl carbonates at 10 ppm

Test No.	Alkyl group	Eradication, %
A9	1-Ethylhexyl (8)	99
	1-Methylpentyl (6)	90
	1-Methylbutyl (5)	60
	1-Methylpropyl (5)	58
	s-Butyl (4)	18
	Untreated	
A10	1-Propylhexyl (9)	98
	1-Butylpentyl (9)	95
	1-Ethylhexyl (8)	88
	1-Propylpentyl (8)	85
	1-Ethylheptyl (9)	68
	1-Methylheptyl (8)	43
	1-Ethyloctyl (10)	39
	1-Methyloctyl (9)	24
	Untreated	
A11	1-Butylhexyl (10)	99
	1-Propylheptyl (10)	97
	1-Ethylhexyl (8)	85
	1-Ethyloctyl (10)	54
	1-Methyldecyl (11)	40
	1-Methylnonyl (10)	9
	Untreated	
A12	1-Ethylhexyl (8)	86
	1-Butylnonyl (13)	34
	1-Pentyloctyl (13)	31
	1-Ethylnonyl (11)	26
	1-Propylnonyl (12)	22
	1-Ethyldecyl (12)	19
	1-Propyldecyl (13)	16
	Untreated	

TABLE VI

Degree of eradication of *Podosphaera leucotricha* with 4-alkyl-2,6-dinitrophenyl ethyl carbonates at 10 ppm (Test A13)

Alkyl group	Eradication, %
1-Ethylhexyl (8)	99·4
1-Pentylhexyl (11)	98
1-Butylheptyl (11)	94
1-Butyloctyl (12)	79
1-Pentylheptyl (12)	78
1-Propyloctyl (11)	48
1-Hexylheptyl (13)	30
Untreated	

In Table III the prefix 4- indicates that the alkyl group occurs in a 4-alkyl-2,6-dinitrophenol and the prefix 2- indicates that the alkyl group occurs in a 2-alkyl-4,6-dinitrophenol. 4-(1-Ethylhexyl)-2,6-dinitrophenol or its methyl- or ethyl-carbonate was used as the reference compound in the tests.

Table III (Tests A1–A7) reports results of eradicant tests with 4-(C_4 to C_{13} α-branched alkyl)-2,6- and 2-(C_4 to C_{13} α-branched alkyl)-4,6-dinitrophenols at 20 ppm. Table IV (Test A8) reports results with two methyl ethers of the very active 4-(1-ethylhexyl)- and 4-(1-propylpentyl)-2,6-dinitro-

TABLE VII

Degree of eradication of *Podosphaera leucotricha* with 4-alkyl-2,6-dinitrophenols and their esters

Test No.	Name of compound	Eradication, %
A14 (20 ppm)	4-(1-Butylhexyl)-2,6-dinitrophenyl methyl carbonate (10)	100
	4-(1-Ethylhexyl)-2,6-dinitrophenyl methyl carbonate (8)	99·9
	4-(1-Butylhexyl)-2,6-dinitrophenol (10)	99·5
	4-(1-Butylhexyl)-2,6-dinitrophenyl crotonate (10)	99·5
	4-(1-Butylhexyl)-2,6-dinitrophenyl ethyl carbonate (10)	99
	4-s-Butyl-2,6-dinitrophenol (4)	37
	4-s-Butyl-2,6-dinitrophenyl isopropyl carbonate (4)	32
	Untreated	
A15 (10 ppm)	4-(1-Pentylhexyl)-2,6,dinitrophenol (11)	100
	4-(1-Ethylhexyl)-2,6-dinitrophenyl methyl carbonate (8)	99·5
	4-(1-Pentylhexyl)-2,6-dinitrophenyl methyl carbonate (11)	99·3
	4-(1-Butylpentyl)-2,6-dinitrophenol (9)	99
	4-(1-Butylpentyl)-2,6-dinitrophenyl methyl carbonate (9)	99
	4-(1-Butylpentyl)-2,6-dinitrophenyl ethyl carbonate (9)	98
	4-(1-Pentylhexyl)-2,6-dinitrophenyl ethyl carbonate (11)	98
	4-(1-Butylpentyl)-2,6-dinitrophenyl crotonate (9)	97
	4-(1-Pentylhexyl)-2,6-dinitrophenyl crotonate (11)	97
	4-(1-Propylbutyl)-2,6-dinitrophenyl ethyl carbonate (7)	94
	4-(1-Propylbutyl)-2,6-dinitrophenyl crotonate (7)	91
	4-(1-Propylbutyl)-2,6-dinitrophenyl methyl carbonate (7)	90
	4-(1-Propylbutyl)-2,6-dinitrophenol (7)	88
	Untreated	
A16 (10 ppm)	4-(1-Ethylhexyl)-2,6-dinitrophenyl methyl carbonate (8)	99
	4-(1-Pentylhexyl)-2,6-dinitrophenyl methyl carbonate (11)	99
	4-(1-Pentylhexyl)-2,6-dinitrophenyl crotonate (11)	99
	4-(1-Pentylhexyl)-2,6-dinitrophenyl methyl carbonate (11)	95
	4-(1-Butyloctyl)-2,6-dinitrophenyl methyl carbonate (12)	94
	4-(1-Pentylheptyl)-2,6-dinitrophenyl methyl carbonate (12)	93
	4-(1-Pentylheptyl)-2,6-dinitrophenyl crotonate (12)	84
	4-(1-Butylheptyl)-2,6-dinitrophenyl crotonate (11)	83
	4-(1-Propyloctyl)-2,6-dinitrophenyl methyl carbonate (11)	78
	4-(1-Hexylheptyl)-2,6-dinitrophenyl methyl carbonate (13)	75
	4-(1-Propyloctyl)-2,6-dinitrophenyl crotonate (11)	68
	4-(1-Butyloctyl)-2,6-dinitrophenyl crotonate (12)	63
	4-(1-Hexylheptyl)-2,6-dinitrophenyl crotonate (13)	25
	Untreated	

TABLE VIII

Degree of eradication of *Podosphaera leucotricha* with C_8 alkyl dinitrophenyl esters at 20 ppm

Test No.	Name of compound	Eradication, %
A17	4-(1-Ethylhexyl)-2,6-dinitrophenyl methyl carbonate	99
	4-(1-Ethylhexyl)-2,6-dinitrophenyl ethyl carbonate	99
	4-(1-Ethylhexyl)-2,6-dinitrophenyl crotonate	99
	2-(1-Ethylhexyl)-4,6-dinitrophenyl crotonate	31
	2-(1-Ethylhexyl)-4,6-dinitrophenyl methyl carbonate	25
	2-(1-Ethylhexyl)-4,6-dinitrophenyl ethyl carbonate	8
	Untreated	
A18	4-(1-Ethylhexyl)-2,6-dinitrophenyl methyl carbonate	90
	4-(1-Ethylhexyl)-2,6-dinitrophenyl isopropyl carbonate	88
	4-(1-Methylheptyl)-2,6-dinitrophenyl methyl carbonate	36
	4-(1-Methylheptyl)-2,6-dinitrophenyl isopropyl carbonate	13
	Untreated	
A19	4-(1-Ethylhexyl)-2,6-dinitrophenyl methyl carbonate	99·9
	4-(1-Propylpentyl)-2,6-dinitrophenol	99·8
	4-(1-Propylpentyl)-2,6-dinitrophenyl methyl carbonate	99·3
	4-(1-Propylpentyl)-2,6-dinitrophenyl ethyl carbonate	99·2
	4-(1-Propylpentyl)-2,6-dinitrophenyl crotonate	99
	Untreated	

TABLE IX

Degree of eradication of *Podosphaera leucotricha* with 4-(1-ethyl-hexyl)-2,6-dinitrophenyl esters at 20 ppm

Test No.	Carbonate	Eradication, %
A20	Methyl	99·8
	Butyl	99·5
	Isopropyl	99
	s-Butyl	98
	Hexyl	98
	1-Methylheptyl	86
	Untreated	
A21	Ester:	
	Methyl carbonate	99·7
	Isobutyrate	99
	Methacrylate	99
	Acetate	98
	Chloroacetate	97
	Octanoate	95
	Untreated	

phenols. Table V (Tests A9–A12) reports results with 4-(C_4 to C_{13} α-branched alkyl)-2,6-dinitrophenyl methyl carbonates and Table VI (Test A13) with 4-(C_{11} to C_{13} α-branched alkyl)-2,6-dinitrophenyl ethyl carbonates. Table VII (Tests A14–A16) lists results obtained with various esters of 4-(C_4 to C_{13} α-branched alkyl)-2,6-dinitrophenols and Tables VIII & IX (Tests A17–A21) list the results obtained with C_8 α-branched alkyl dinitrophenyl esters.

Discussion

Structure of alkyldinitrophenols and their activity

Pianka[4] reported results of eradicant tests with 2-(C_4 to C_8 α-branched alkyl)-4,6-dinitrophenols and 4-(C_4 to C_{12} α-branched alkyl)-2,6-dinitrophenols at 10 ppm. Tests A1 to A7 reported in this paper were carried out with C_4 to C_{13} α-branched alkyldinitrophenols at 20 ppm. The results confirm the validity of the general inferences drawn earlier.[4]

Statistical analysis of the present results shows that at C_4 (s-butyl), C_5 (1-methylbutyl) and (1-ethylpropyl), and C_6 (1-methylpentyl) there were no significant differences between the activities of the 4-alkyl-2,6-dinitro- and 2-alkyl-4,6-dinitrophenols (Tests A1, A2). However, 4-C_6 (1-ethylbutyl)- (Test A4), 4-C_7 (1-methylhexyl)-, (1-ethylpentyl)- and (1-propylbutyl)- (Test A3), 4-C_8 (1-methylheptyl)-, (1-ethylhexyl) and (1-propylpentyl)- (Test A2), 4-C_9 (1-ethylheptyl)-, (1-propylhexyl)- (Test A4) and (1-butylpentyl)-, 4-C_{10} (1-ethyloctyl)-, (1-propylheptyl)-, and (1-butylhexyl)- (Test A5), 4-C_{11} (1-propyloctyl)-, (1-butylheptyl)- and (1-pentylhexyl)- (Test A6), and 4-C_{12} (1-pentylheptyl)- 2,6-dinitrophenols (Test A7) were significantly more active than their 2-alkyl-4,6-dinitrophenol analogues. There was no significant difference between the activities of the 4-C_9 (1-methyloctyl)- or 4-C_{13} (1-hexylheptyl)-2,6-dinitrophenols and their 2-alkyl-4,6-dinitrophenol analogues (Tests A4, A7).

The observation that generally in 4-(α-branched alkyl)-2,6-dinitrophenols with alkyl groups possessing the same total number of carbon atoms, activity increased as the α-alkyl branch lengthened[4] was confirmed in the present series of tests. Thus, with C_9-alkyl, activity rose from (1-methyloctyl) to (1-ethylheptyl) to (1-propylhexyl) (Test A4); with C_{10}-alkyl, activity rose from (1-methylnonyl) to (1-ethyloctyl) to (1-propylheptyl) to (1-butylhexyl) (Test A5); with C_{11}-alkyl, from (1-methyldecyl) to (1-ethylnonyl) or (1-propyloctyl) to (1-butylheptyl) to (1-pentylhexyl) (Test A6); and with C_{12}-alkyl, activity rose from (1-ethyldecyl) or (1-propylnonyl) to (1-butyloctyl) or (1-pentylheptyl) (Test A7).

It is reasonable to assume that 4-(1-methyldodecyl)- and 4-(1-ethylundecyl)-, the two possible isomers of 4-(C_{13} α-branched alkyl)-2,6-dinitrophenols which were not tested, would not be more active than those with the longer α-alkyl branch, i.e. the (1-propyldecyl)-, (1-butylnonyl)-, (1-pentyloctyl)- or (1-hexylheptyl) isomers, which themselves gave no significant degree of eradication (Test A7), and that therefore none of the 4-(C_{13} α-branched alkyl)-2,6-dinitrophenols would be active. A significant degree of eradication of *Podosphaera leucotricha* was achieved, therefore, with 4-(α-branched alkyl)-2,6-dinitrophenols with an alkyl group of not more than 12 carbon atoms.

Effect of esterification on activity

Esterification to methyl carbonates did not reduce the activity of the compounds compared with that of the parent phenols. However, esterification to ethyl carbonates or to crotonates gave reduced activity compared with the parent 4-C_{12} (1-pentylheptyl)- and (1-butyloctyl)-2,6-dinitrophenols.

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Thus, the phenols were almost as active as 4-(1-ethylhexyl)-2,6-dinitrophenol (Test A7), whereas their ethyl carbonates were considerably less active than 4-(1-ethylhexyl)-2,6-dinitrophenyl ethyl carbonate (Test A13). The crotonates of these C_{12}-alkylphenols were significantly less active than their methyl carbonates. Also the crotonate of the C_{13} 4-(1-hexylheptyl)-2,6-dinitrophenol was less active than the methyl carbonate (Test A16). Perhaps the crotonates of these C_{12} and C_{13} alkyldinitrophenols do not penetrate as readily as the methyl carbonates to the vital sites of action. Reduction in activity with longer ester chains was demonstrated also with esters of 4-(1-ethylhexyl)-2,6-dinitrophenol: thus the 1-methylheptyl carbonate was less active than the methyl carbonate (Test A20) and the octanoate than the acetate (Test A21).

However, the activity of certain 4-(α-branched alkyl)-2,6-dinitrophenols with C_{11} or lower alkyl groups did not differ from that of their crotonates. The crotonates of 4-C_{11} (1-pentylhexyl)- (Tests A15, A16), 4-C_{10} (1-butylhexyl) (Test A14) and 4-C_9 (1-butylpentyl)- (Test A15) 2,6-dinitrophenols were as active as the parent phenols or their methyl carbonates.

Activity of C_8 alkyl dinitrophenols and their esters

Kirby, Frick & Gratwick[9] demonstrated the greater control of *Podosphaera leucotricha* with 4-(1-ethylhexyl)- and 4-(1-propylpentyl)- than with 4-(1-methylheptyl)-2,6-dinitro- or 2-(1-ethylhexyl)-4,6-dinitrophenol, and Pianka[4] gave a tentative explanation of the relationships between the structure of these phenols and the eradication of this organism. These results showed that esters of 4-(1-ethylhexyl)-2,6-dinitrophenol had much higher eradicant activity than those of 2-(1-ethylhexyl)-4,6-dinitrophenol (Test A17), or those of 4-(1-methylheptyl)-2,6-dinitrophenol (Test A18). Although the difference between the percentage degree of eradication was small we found, with Byrde, Clifford & Woodcock,[10] that the crotonate of 4-(1-propylpentyl)-2,6-dinitrophenol was significantly inferior in activity to the phenol (Test A19).

Possible mode of action of esters

It has been demonstrated that etherification of alkyldinitrophenols results in compounds with lowered acaricidal[2,6] and herbicidal[7] activities. Etherification also produced compounds with reduced mildew-eradicant properties compared with 4-(1-ethylhexyl)- and 4-(1-propylpentyl)-2,6-dinitrophenols (Test A8). This effect may be related to the stability of the ethers. It may be assumed that, as with the esters of certain alkyldinitrophenols,[2,7] the anti-mildew activity of the esters examined was due to the hydrolytic products, i.e. the alkyldinitrophenols, since the structure–activity relationships of the esters seems to follow the general pattern observed with the alkyldinitrophenols.

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STRUCTURES AND PESTICIDAL ACTIVITIES OF DERIVATIVES OF DINITROPHENOLS

X.*—Effects of substitution of dinitrophenols with C_3 to C_{13} α-branched alkyl groups and of esterification on the eradication of barley mildew

By M. PIANKA and P. J. J. SWEET

The studies of the eradication of barley mildew due to *Erysiphe graminis* Mérat with alkyldinitrophenols reported earlier[1] were continued. The degree of eradication was highest with 4-(C_9 α-branched alkyl)-2,6-dinitrophenols. 4-Alkyl-2,6-dinitrophenols containing a heptyl or higher alkyl branch were significantly less active, and compounds containing the C_{12} or C_{13} alkyls were not active since the most compact of the C_{12} α-branched alkyls is 1-pentylheptyl. 2-(1-Methylheptyl)- and 2-(1-propylpentyl)-4,6-dinitrophenols had high activity, but their esters showed reduced activity. Esterification of 4-(α-branched alkyl)-2,6-dinitrophenols to methyl carbonates did not affect the activity shown by the compounds, but when certain 4-C_{10} and C_{11} alkyl-2,6-dinitrophenols were esterified to ethyl carbonates the activity of the products was reduced. Also activity was diminished by conversion of some 4-C_9 and C_{10} alkyl-2,6-dinitrophenols to crotonates. Whereas the methyl- and ethyl- carbonates of 4-(1-ethylhexyl)-2,6-dinitrophenol gave consistently high degrees of eradication of barley mildew, the performance of the crotonate of this phenol was not consistent. Lower aliphatic esters of the active C_8-alkyl phenols were themselves active. Esterification of 4-(1-ethylhexyl)-2,6-dinitrophenol to the benzoate, isopropyl carbonate or *S*-methyl thiolocarbonate gave compounds with substantially reduced activity. 2-t-Butyl-4,6-dinitrophenyl or 4-t-butyl- or t-octyl-2,6-dinitrophenyl esters gave little or no significant eradication.

Introduction

Kirby *et al.*[2] observed that *Podosphaera leucotricha* (Ell. and Everh.) Salm. and *Erysiphe graminis* Mérat, the causative organisms of mildew of apple and barley differed in their susceptibility to alkyldinitrophenols.

In preliminary investigations of the eradication of barley mildew, 4-(1-ethylhexyl)-2,6-dinitrophenol which gave 99–100% eradication of apple mildew at 10 ppm showed only 68–78% eradication of barley mildew at 20 ppm. The degree of eradication of barley mildew with 2-(C_4 to C_8 α-branched alkyl)-4,6-dinitrophenols and 4-(C_4 to C_{12} α-branched alkyl)-2,6-dinitrophenols was therefore studied at 20 ppm.[1,†] This paper describes the effects of substitution of dinitrophenols with C_3 to C_{13} α-branched alkyl groups and of esterification on the degree of eradication of barley mildew.

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* Part IX: Preceding paper

† These investigations were reported in 1966 in papers presented to the Polish Academy of Sciences and the Polish Institute of Industrial Organic Chemistry and to the Pesticides Group of the Society of Chemical Industry

Experimental

Materials

These were prepared as described in Part IX.

Eradicant tests

Kirby & Frick[3] described curative tests carried out on barley plants to prevent visible mildew lesions occurring on the leaves. As with apple mildew[4] it was considered desirable to eradicate established lesions due to *Erysiphe graminis* Mérat from the leaves of barley seedlings as indicated by the absence of tufts of spore chains 4-5 days after treatment with the diluted test compound.

Test plants

Proctor barley seedlings were used growing in seed compost contained in 3 in plastic pots. The 'stand' was reduced to ten seedlings in a pot and the centre shoots were excised for ease of handling. Treatment and assessments were confined to the first leaf. Four to six replicate pots were used for each treatment.

Infection

The seedlings were infected by blowing over them spores from infected barley seedlings grown in boxes. When infection was clearly visible a preliminary assessment was made, each leaf being graded on a scale similar to that used for apple mildew tests.[4] The replicates were so selected that differences in degree of infection were spread as evenly as possible over the treatments in a test.

Application of test compounds

The test compounds were formulated as emulsifiable concentrates with naphtha and an emulsifying agent and diluted with tapwater to 10, 20, or 40 ppm. In each case a standard quantity of an anionic wetting agent was added. The plants were dipped in the test dilutions for 15 sec. Previous tests showed no eradication of mildew when infected plants were dipped in the blank formulations containing the wetting agent.

The evaluation of the eradicant action of the test compounds and the statistical analysis of the results were carried out as described in Part IX.[4]

Results

The results of the tests are presented in Tables I–IX. They were analysed statistically and arranged in order of percentage degree of eradication. The figures that did not differ significantly at $P = 0.05$ are joined by an unbroken line.

Table I (Test B1) contains results of eradicant tests with certain 4-(α-branched alkyl)-2,6-dinitrophenols at 20 ppm not reported previously.[1] Tables II & III (Tests B2–B7) report results with 2- or 4- (C_4 to C_{13} α-branched alkyl)-dinitrophenols and their methyl- and ethyl-carbonates and crotonates. Tables IV–VIII (Tests B8–B16) present results of tests with 2- or 4- (α-branched alkyl)- and two t-alkyl-dinitrophenyl carbonates and other esters. Table IX (Tests B17–B21) gives results of tests with (C_8 α-branched alkyl)-dinitrophenols and their esters.

Discussion

Structure of alkyldinitrophenols and their activity

The 4-C_{13} (1-hexylheptyl)- (Test B1) and (1-propyldecyl)-(Test B5) 2,6-dinitrophenols were considerably less active than 4-C_8 (1-ethylhexyl)-2,6-dinitrophenol. The present results (Test B1) confirmed the very high activity of the 4-C_9 isomers with a pentyl chain (1-butylpentyl) or hexyl chain (1-propylhexyl) and the high activity of the 4-C_{10} isomer with a hexyl chain (1-butylhexyl).[1] 4-(α-Branched alkyl)-2,6-dinitrophenols where one of the alkyl branches was heptyl or a higher alkyl, showed reduced activity: 1-propylheptyl (Tests B1, B4) and all 4-(α-branched alkyl)-dinitrophenols reported by Pianka[1] in which one of the alkyl branches was heptyl, octyl or nonyl. It was therefore not surprising to find that there was no activity against barley mildew by compounds with more than the 4-(C_{11} α-branched alkyl),[1] since one of the branches in the most compact of the C_{12} (α-branched alkyl) compounds is heptyl (in pentylheptyl). Activity against barley mildew was highest at 4-C_9 alkyl there being a significant rise of activity from C_8 (1-ethylhexyl) to C_9 (1-propylhexyl)- or from C_8 (1-propylpentyl) to C_9 (1-butylpentyl)-2,6-dinitrophenols (Test B1).

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TABLE I

Degree of eradication of *Erysiphe graminis* with 4-alkyl-2,6-dinitrophenols at 20 ppm (Test B1)

Alkyl	Eradication, %
1-Butylpentyl (9*)	94
1-Propylhexyl (9)	92
1-Butylhexyl (10)	84
1-Propylpentyl (8)	82
1-Ethylhexyl (8)	74
1-Propylheptyl (10)	48
1-Methylhexyl (7)	42
1-Hexylheptyl (13)	41
Untreated	

* Total number of carbon atoms of the nuclear alkyl substituent

TABLE II

Degree of eradication of *Erysiphe graminis* with 4-alkyl-2,6-dinitrophenols and esters at 20 ppm

Test No.	Name of compound	Eradication, %
B2	4-(1-Propylbutyl)-2,6-dinitrophenyl ethyl carbonate (7)	86
	4-(1-Propylbutyl)-2,6-dinitrophenyl methyl carbonate (7)	85
	4-(1-Propylbutyl)-2,6-dinitrophenol (7)	76
	4-(1-Propylbutyl)-2,6-dinitrophenyl crotonate (7)	67
	4-(1-Ethylbutyl)-2,6-dinitrophenyl ethyl carbonate (6)	57
	4-(1-Ethylbutyl)-2,6-dinitrophenyl methyl carbonate (6)	57
	4-(1-Ethylbutyl)-2,6-dinitrophenol (6)	56
	4-(1-Ethylbutyl)-2,6-dinitrophenyl crotonate (6)	47
	Untreated	
B3	4-(1-Butylpentyl)-2,6-dinitrophenyl methyl carbonate (9)	99
	4-(1-Butylpentyl)-2,6-dinitrophenyl ethyl carbonate (9)	98
	4-(1-Butylpentyl)-2,6-dinitrophenyl crotonate (9)	92
	4-(1-Ethylhexyl)-2,6-dinitrophenyl ethyl carbonate (8)	82
	4-(1-Pentylhexyl)-2,6-dinitrophenyl methyl carbonate (11)	75
	4-(1-Pentylhexyl)-2,6-dinitrophenol (11)	70
	4-(1-Pentylhexyl)-2,6-dinitrophenyl ethyl carbonate (11)	45
	Untreated	
B4	4-(1-Ethylhexyl)-2,6-dinitrophenol (8)	82
	4-(1-Propylheptyl)-2,6-dinitrophenol (10)	48
	4-(1-Propylheptyl)-2,6-dinitrophenyl methyl carbonate (10)	47
	4-(1-Pentylhexyl)-2,6-dinitrophenyl ethyl carbonate (11)	44
	4-(1-Propylheptyl)-2,6-dinitrophenyl ethyl carbonate (10)	34
	4-(1-Propylheptyl)-2,6-dinitrophenyl crotonate (10)	27
	Untreated	
B5	4-(1-Ethylhexyl)-2,6-dinitrophenyl methyl carbonate (8)	93
	4-(1-Ethylhexyl)-2,6-dinitrophenol (8)	92
	4-(1-Ethyldecyl)-2,6-dinitrophenyl methyl carbonate (12)	50
	4-(1-Propyldecyl)-2,6-dinitrophenol (13)	49
	4-(1-Methylheptyl)-2,6-dinitrophenyl crotonate (8)	45
	4-(1-Propyldecyl)-2,6-dinitrophenyl methyl carbonate (13)	42
	4-(1-Methylnonyl)-2,6-dinitrophenyl methyl carbonate (10)	39
	4-(1-Methyldecyl)-2,6-dinitrophenol (11)	32
	4-(1-Methyldecyl)-2,6-dinitrophenyl methyl carbonate (11)	32
	4-(1-Propylnonyl)-2,6-dinitrophenyl methyl carbonate (12)	30
	Untreated	

TABLE III

Degree of eradication of *Erysiphe graminis* with 4-alkyl-2,6-dinitro- and 2-alkyl-4,6-dinitro-phenols and phenyl esters at 20 ppm

Test No.	Name of compound	Eradication, %
B6	4-(1-Ethylhexyl)-2,6-dinitrophenyl ethyl carbonate (8)	85
	2-Isopropyl-4,6-dinitrophenyl ethyl carbonate (3)	28
	2-(1-Ethylhexyl)-4,6-dinitrophenyl ethyl carbonate (8)	23
	2-(1-Butylheptyl)-4,6-dinitrophenol (11)	22
	2-(1-Pentylhexyl)-4,6-dinitrophenol (12)	18
	2-(1-Propyloctyl)-4,6-dinitrophenyl methyl carbonate (11)	14
	2-(1-Ethylheptyl)-4,6-dinitrophenyl ethyl carbonate (9)	12
	2-(1-Propylhexyl)-4,6-dinitrophenyl ethyl carbonate (9)	1
	Untreated	
B7	2-(1-Methylheptyl)-4,6-dinitrophenol (8)	71
	4-(1-Methylheptyl)-2,6-dinitrophenyl ethyl carbonate (8)	39
	4-(1-Methylheptyl)-2,6-dinitrophenyl crotonate (8)	34
	2-(1-Methylheptyl)-4,6-dinitrophenyl methyl carbonate (8)	33
	4-(1-Methylheptyl)-2,6-dinitrophenyl methyl carbonate (8)	33
	2-(1-Methylheptyl)-4,6-dinitrophenyl ethyl carbonate (8)	32
	2-(1-Hexylheptyl)-4,6-dinitrophenyl crotonate (13)	32
	4-(1-Methylheptyl)-2,6-dinitrophenol (8)	27
	Untreated	

Dinitrophenols with 1-methylalkyl substituents, with the exception of 4-C_5 (1-methylbutyl)-2,6-dinitrophenol and 2-C_8 (1-methylheptyl)-4,6-dinitrophenol,[1] were not very active. The present results confirm the high activity of 2-(1-methylheptyl)-4,6-dinitrophenol; it was significantly more active than 4-(1-methylheptyl)-2,6-dinitrophenol (Tests B7, B17, B19). Of the other 2-(α-branched alkyl)-4,6-dinitrophenols the (1-propylpentyl)-compound was highly active.[1]

Activity of esters

The 2-(C_3 to C_{13} α-branched alkyl)-4,6-dinitrophenyl carbonates (Tests B6–B8, B13, B14, B17–B19) and crotonates (B7, B13, B14, B17, B19) or benzoate (Test B15) that were tested and 2-t-butyl-4,6-dinitrophenyl methyl carbonate (Test B8) had either little or no significant activity. The activity of 2-(1-methylheptyl)- and 2-(1-propylpentyl)-4,6-dinitrophenols was substantially reduced after esterification (Tests B7, B8, B17–B19, B14).

Esterification of 4-(α-branched alkyl)-2,6-dinitrophenols to methyl carbonates did not reduce the activity; the order of activity of the methyl carbonates generally followed that of the parent phenols. Esterification of certain 4-(α-branched alkyl)-2,6-dinitrophenols (1-ethylbutyl, 1-propylbutyl—Test B2; 1-methylheptyl—Test B7; 1-butylpentyl—Test B3) to ethyl carbonates had no significant effect on the activity, but with the longer-chain 4-C_{10} (1-propylheptyl)- and 4-C_{11} (1-pentylhexyl)-2,6-dinitrophenols (Tests B4, B3) it resulted in compounds with significantly reduced activity. As with the phenols, the 4-C_9 (1-butylpentyl)- and (1-propylhexyl)-2,6-dinitrophenyl methyl carbonates had very high activity, followed by those of 4-C_8 (1-ethylhexyl)- and (1-propylpentyl)-compounds (Test B9).

TABLE IV

Degree of eradication of *Erysiphe graminis* with 4-alkyl-2,6-dinitro- and 2-alkyl-4,6-dinitro-phenyl methyl carbonates at 20 ppm (Test B8)

Alkyldinitrophenyl	Eradication, %
4-(1-Propylhexyl)-2,6-dinitrophenyl (9)	97
4-(1-Ethylhexyl)-2,6-dinitrophenyl (8)	94
2-t-Butyl-4,6-dinitrophenyl (4)	37
2-(1-Ethylhexyl)-4,6-dinitrophenyl (8)	19
4-t-Butyl-2,6-dinitrophenyl (4)	11
2-s-Butyl-4,6-dinitrophenyl (4)	11
2-(1-Methylheptyl)-4,6-dinitrophenyl (8)	9
2-(1-Methylbutyl)-4,6-dinitrophenyl (5)	9
4-(1-Methylheptyl)-2,6-dinitrophenyl (8)	6
4-'t-Octyl'-2,6-dinitrophenyl (8)	6
Untreated	

TABLE V

Degree of eradication of *Erysiphe graminis* with 4-alkyl-2,6-dinitrophenyl methyl carbonates at 20 ppm

Test No.	Alkyl group	Eradication, %
B9	1-Butylpentyl (9)	98
	1-Propylhexyl (9)	97
	1-Ethylhexyl (8)	96
	1-Propylpentyl (8)	96
	1-Ethylpentyl (7)	92
	1-Propylbutyl (7)	88
	1-Methyloctyl (9)	70
	1-Methylheptyl (8)	49
	1-Butylheptyl (11)	48
	1-Ethyloctyl (10)	44
	1-Hexylheptyl (13)	43
	1-Pentylheptyl (12)	38
	Untreated	
B10	1-Ethylhexyl (8)	88
	1-Ethylnonyl (11)	38
	1-Butylnonyl (13)	34
	1-Pentyloctyl (13)	32
	Untreated	

TABLE VI

Degree of eradication of *Erysiphe graminis* with 4-alkyl-2,6-dinitrophenyl ethyl carbonates at 20 ppm

Test No.	Alkyl group	Eradication, %
B11	1-Ethylhexyl (8)	94
	1-Propylpentyl (8)	83
	1-Propylhexyl (9)	83
	1-Butylhexyl (10)	65
	't-Octyl' (8)	47
	1-Methylheptyl (8)	47
	t-Butyl (4)	22
	Untreated	
B12	1-Butylpentyl (9)	91
	1-Ethylhexyl (8)	86
	1-Ethylheptyl (9)	68
	1-Propylheptyl (10)	52
	1-Methyloctyl (9)	30
	1-Ethyloctyl (10)	24
	Untreated	

TABLE VII

Degree of eradication of *Erysiphe graminis* with 4-alkyl-2,6-dinitro- and 2-alkyl-4,6-dinitro-phenyl esters

Test No.	Name of compound	Eradication, %
B13 (20 pm)	4-(1-Ethylhexyl)-2,6-dinitrophenyl methyl carbonate (8)	82
	4-(1-Ethylhexyl)-2,6-dinitrophenyl crotonate (8)	57
	4-s-Butyl-2,6-dinitrophenyl crotonate (4)	25
	2-(1-Ethyloctyl)-4,6-dinitrophenyl methyl carbonate (10)	18
	2-Isopropyl-4,6-dinitrophenyl crotonate (3)	17
	2-(1-Methyloctyl)-4,6-dinitrophenyl methyl carbonate (9)	16
	Untreated	
B14 (20 ppm)	4-(1-Ethylhexyl)-2,6-dinitrophenyl ethyl carbonate (8)	98
	4-(1-Ethylpropyl)-2,6-dinitrophenyl crotonate (5)	55
	4-(1-Ethylhexyl)-2,6-dinitrophenyl crotonate (8)	44
	2-(1-Methylheptyl)-4,6-dinitrophenyl crotonate (8)	39
	2-(1-Methyloctyl)-4,6-dinitrophenyl ethyl carbonate (9)	37
	2-(1-Propylpentyl)-4,6-dinitrophenyl crotonate (8)	36
	2-(1-Ethylhexyl)-4,6-dinitrophenyl crotonate (8)	31
	2-(1-Ethyloctyl)-4,6-dinitrophenyl ethyl carbonate (10)	31
	2-(1-Ethylheptyl)-4,6-dinitrophenyl crotonate (9)	31
	2-(1-Propylhexyl)-4,6-dinitrophenyl crotonate (9)	23
	Untreated	
B15 (40 ppm)	4-(1-Ethylhexyl)-2,6-dinitrophenyl crotonate (8)	97
	4-s-Butyl-2,6-dinitrophenyl isopropyl carbonate (4)	35
	2-s-Butyl-4,6-dinitrophenyl benzoate (4)	26
	4-'t-Octyl'-2,6-dinitrophenyl crotonate (8)	24
	2-s-Butyl-4,6-dinitrophenyl isopropyl carbonate (4)	22
	Untreated	

TABLE VIII

Degree of eradication of *Erysiphe graminis* with 4-alkyl-2,6-dinitrophenyl esters at 20 ppm (Test B16)

Name of compound	Eradication, %
4-(1-Ethylpentyl)-2,6-dinitrophenyl methyl carbonate (7)	99
4-(1-Ethylhexyl)-2,6-dinitrophenyl ethyl carbonate (8)	99
4-(1-Ethylhexyl)-2,6-dinitrophenyl methyl carbonate (8)	99
4-(1-Ethylpentyl)-2,6-dinitrophenyl crotonate (7)	98
4-(1-Ethylpentyl)-2,6-dinitrophenyl ethyl carbonate (7)	96
4-(1-Methylheptyl)-2,6-dinitrophenyl methyl carbonate (7)	90
4-(1-Ethylheptyl)-2,6-dinitrophenyl methyl carbonate (9)	87
4-(1-Methylhexyl)-2,6-dinitrophenyl ethyl carbonate (7)	85
4-(1-Ethylheptyl)-2,6-dinitrophenyl ethyl carbonate (9)	77
4-(1-Ethylheptyl)-2,6-dinitrophenyl crotonate (9)	77
4-(1-Methylhexyl)-2,6-dinitrophenyl crotonate (7)	69
Untreated	

TABLE IX

Degree of eradication of *Erysiphe graminis* with C₈ alkyl dinitrophenols and esters at 20 ppm

Test No.	Name of compound	Eradication, %
B17	4-(1-Ethylhexyl)-2,6-dinitrophenol	100
	4-(1-Ethylhexyl)-2,6-dinitrophenyl methyl carbonate	99
	4-(1-Ethylhexyl)-2,6-dinitrophenyl ethyl carbonate	99
	4-(1-Ethylhexyl)-2,6-dinitrophenyl crotonate	99
	2-(1-Methylheptyl)-4,6-dinitrophenol	78
	4-(1-Methylheptyl)-2,6-dinitrophenol	59
	4-(1-Methylheptyl)-2,6-dinitrophenyl methyl carbonate	54
	4-(1-Methylheptyl)-2,6-dinitrophenyl ethyl carbonate	47
	2-(1-Methylheptyl)-4,6-dinitrophenyl ethyl carbonate	44
	4-(1-Methylheptyl)-2,6-dinitrophenyl crotonate	44
	2-(1-Methylheptyl)-4,6-dinitrophenyl crotonate	40
	2-(1-Methylheptyl)-4,6-dinitrophenyl methyl carbonate	39
	Untreated	
B18	4-(1-Ethylhexyl)-2,6-dinitrophenyl methyl carbonate	99
	4-(1-Ethylhexyl)-2,6-dinitrophenyl ethyl carbonate	96
	4-(1-Methylheptyl)-2,6-dinitrophenyl ethyl carbonate	39
	4-(1-Methylheptyl)-2,6-dinitrophenyl methyl carbonate	37
	2-(1-Methylheptyl)-4,6-dinitrophenyl methyl carbonate	32
	2-(1-Methylheptyl)-4,6-dinitrophenyl ethyl carbonate	26
	Untreated	
B19	4-(1-Ethylhexyl)-2,6-dinitrophenol	99
	4-(1-Ethylhexyl)-2,6-dinitrophenyl methyl carbonate	98
	4-(1-Ethylhexyl)-2,6-dinitrophenyl ethyl carbonate	97
	4-(1-Ethylhexyl)-2,6-dinitrophenyl crotonate	91
	2-(1-Methylheptyl)-4,6-dinitrophenol	89
	4-(1-Ethylhexyl)-2,6-dinitrophenyl *S*-methyl thiolocarbonate	42
	4-(1-Methylheptyl)-2,6-dinitrophenyl ethyl carbonate	29
	2-(1-Methylheptyl)-4,6-dinitrophenyl crotonate	29
	4-(1-Methylheptyl)-2,6-dinitrophenol	27
	4-(1-Methylheptyl)-2,6-dinitrophenyl *S*-methyl thiolocarbonate	27
	4-(1-Methylheptyl)-2,6-dinitrophenyl crotonate	26
	2-(1-Methylheptyl)-4,6-dinitrophenyl *S*-methyl thiolocarbonate	17
	Untreated	

Degree of eradication of *Erysiphe graminis* with 4-(1-ethylhexyl)-2,6-dinitrophenyl esters at 20 ppm (Test B20)

Ester	Eradication, %
Crotonate	99
Benzoate	29
Isopropyl carbonate	28
Untreated	

Degree of eradication of *Erysiphe graminis* with 4-(1-propylpentyl)-2,6-dinitrophenyl esters at 10 ppm (Test B21)

Ester	Eradication, %
Acetate	79
Isobutyrate	79
Methacrylate	70
Methyl carbonate	67
s-Butyl carbonate	36
Untreated	

Esterification of the higher 4-(α-branched alkyl)-2,6-dinitrophenols to crotonates caused reduction in activity. Thus, whilst there was no significant reduction in activity with 4-C₆ (1-ethylbutyl) and 4-C₇ (1-propylbutyl) (Test B2) or (1-ethylpentyl) (Test B16), there was reduction in activity with the 4-C₉ (1-butylpentyl) (Test B3) or 4-C₁₀ (1-propylheptyl) (Test B4) isomers.

Whereas the methyl carbonate of 4-(1-ethylhexyl)-2,6-dinitrophenol gave a consistently high degree of eradication (93%—Test B5; 94%—Test B8; 96%—Test B9; 88%—Test B10; 82%—Test B13; 99+%—Tests B16, B17; 99%—Test B18; 98%—Test B19) as did the ethyl carbonate (82%—Test B3; 85%—Test B6; 94%—Test B11; 86%—Test B12; 98%—Test B14; 99+%—Tests B16, B17; 96%—Test B18; 97%—Test B19), the crotonate was, surprisingly, less consistent. Thus, in Tests B17, B19, B20 the crotonate gave degrees of eradication of 99+, 91, 99% respectively, whereas in Tests B13 & B14 it gave 57 & 44%. In Test B17 the crotonate was not significantly different in activity from that of the methyl- or ethyl-carbonate, but in Tests B13, B14, B19 it was significantly inferior.

The acetate, isobutyrate and methacrylate of 4-(1-propylpentyl)-2,6-dinitrophenol were not significantly different in activity from the methyl carbonate (Test B21). The aromatic benzoate of 4-(1-ethylhexyl)-2,6-dinitrophenol was significantly less active than the crotonate (Test B20). Aromatic carbonates proved less acaricidal than aliphatic ones,[5] and benzoates of dinoterb and medinoterb had low pre-emergence herbicidal activity.[6] The branched isopropyl carbonate (Test B20) and the *S*-methyl thiolocarbonate of 4-(1-ethylhexyl)-2,6-dinitrophenol (Test B19) were significantly less active than the crotonates. Reduction of acaricidal activity was observed when 2-(1-methylheptyl)-4,6-dinitrophenol was converted to the isopropyl carbonate or *S*-methyl thiolocarbonate.[7]

Activity of t-alkyl compounds

Kirby *et al.*[2] observed that in curative tests the 4-C₅ (1-methylbutyl)- and (1,1-dimethylpropyl)-2,6-dinitrophenols had very similar activities (97 and 99% disease control at 150 ppm). In eradicant tests 4-C₅ (1-methylbutyl)- and (1-ethylpropyl)-2,6-dinitrophenols had high activity (75 and 68% at 20 ppm).[1] No significant degree of eradication was found with 4-t-alkyl compounds. 4-C₄ t-Butyl-2,6-dinitrophenol at 40 ppm[1] or its methyl- or ethyl-carbonate at 20 ppm (Tests B8, B11), 4-C₈-(1,1,3,3-tetramethylbutyl)-2,6-dinitrophenol at 40 ppm[1] or its methyl- or ethyl-carbonate at 20 ppm (Tests B8, B11) or crotonate at 40 ppm (Test B15) gave little or no significant eradication of barley mildew.

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STRUCTURES AND PESTICIDAL ACTIVITIES OF DERIVATIVES OF DINITROPHENOLS

XI.*—Effects of substitution of dinitrophenols with C_3 to C_{13} α-branched alkyl groups and of esterification on the eradication of cucumber mildew

By M. PIANKA and P. J. J. SWEET

The eradication of cucumber mildew caused by *Sphaerotheca fuliginea* (Schlecht. ex Fr.) Poll. with 2-(C_4 to C_{13} α-branched alkyl)-4,6-dinitrophenols and 4-(C_4 to C_{13} α-branched alkyl)-2,6-dinitrophenols was examined. 2-(C_8 to C_{13} α-Branched alkyl)-4,6-dinitrophenols were highly active, but *O*-methylation produced compounds with no activity. 4-(C_7 to C_{10} and several C_{11} to C_{13} α-branched alkyl)-2,6-dinitrophenols gave a significant degree of eradication. 2-(C_6 to C_{13} α-Branched alkyl)-4,6-dinitrophenols were significantly more active than their 4-alkyl-2,6-dinitrophenol analogues. Esterification of C_4 to C_7 α-branched alkyl dinitrophenols to methyl- or ethyl- carbonates or crotonates gave compounds with enhanced activity. Generally, the methyl- or ethyl- carbonates of 2-C_8-alkyl-4,6-dinitrophenols were as active as the parent phenols, but the higher alkyl carbonates or crotonates had lower activity. Esterification of 2-(C_9 to C_{13} α-branched alkyl)-4,6-dinitrophenols to ethyl carbonates and crotonates gave compounds with reduced activity.

Introduction

Preliminary investigations were carried out into the eradicant activity of 2-(C_4 to C_8 α-branched alkyl)-4,6- and 4-(C_4 to C_{12} α-branched alkyl)-2,6-dinitrophenols against *Sphaerotheca fuliginea* (Schlecht. ex Fr.) Poll., previously known as *Erysiphe cichoracearum* D.C. (the causative organism of cucumber mildew).[1,†] Generally the 2-alkyl phenols proved more active than the 4-alkyl phenols. The effects of substitution of 2,4- or 2,6-dinitrophenols with a 6- or 4-(C_3 to C_{13} α-branched alkyl group) and of esterification of the phenols on eradication of cucumber mildew are presented in this paper.

Experimental

Materials

The compounds were prepared as described in Part IX.[2]

Eradicant tests

The objective of the tests reported here was to obtain eradication of established lesions on cucumber leaves as indicated by the absence of tufts of spore chains 4–5 days after treatment with the diluted test compound.

Test plants

Young cucumber plants cv. Butcher's Disease Resister, at the two-leaf stage were used. Four replicate plants were used for each treatment.

Infection

The test plants were infected with *Sphaerotheca fuliginea* (Schlecht. ex Fr.) Poll. by blowing spores over them from infected stock plants.

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* Part X: Preceding paper.

† These investigations were reported in 1966 in papers presented to the Polish Academy of Sciences and the Polish Institute of Industrial Organic Chemistry and to the Pesticides Group of the Society of Chemical Industry.

Assessments

When infection was clearly visible a preliminary assessment was made using a grading system based on the percentage leaf area infected with mildew. The large leaves made it possible to use more grades than with apple or barley mildew. The infected area of each leaf was assessed as near as possible to the values indicated below:

Grade	% Leaf area infected
0	No mildew lesions
1	0·5
2	1
3	2
4	4
5	8
6	16
7	32
8	64 or greater

Replicate plants were selected in such a manner that differences in degree of infection were spread as evenly as possible over the treatments in a test.

Application of test compounds

The test compounds were formulated as emulsifiable concentrates using naphtha and an emulsifying agent and diluted with tapwater to 20, 25 or 50 ppm of the test compound. No wetting agent was used in these tests. The dilutions were applied to the infected test plants by a paint spray gun to the point of run-off. Tests with the blank formulations showed no activity.

The evaluation of the eradicant action of the test compounds and the statistical analysis of the results were carried out as described in Part IX.[2]

Results

The results of the tests are presented in Tables I–X (Tests C1–C32). They were analysed statistically and arranged in order of percentage degree of eradication. The figures that did not differ significantly at $P = 0·05$ are joined by an unbroken line.

In Tables I, IV & V the prefix 4- indicates that the alkyl group occurs in a 4-alkyl-2,6-dinitrophenol and the prefix 2- indicates that the alkyl group occurs in a 2-alkyl-4,6-dinitro-phenol or phenyl ester.

Table I (Tests C1–C8) presents results of eradicant tests with 2-(C_4 to C_{13} α-branched alkyl)-4,6- and 4-(C_4 to C_{13} α-branched alkyl)-2,6-dinitrophenols. Table II (Tests C9, C10) reports results with anisoles of highly active 2-(C_8, C_9 & C_{13} α-branched alkyl)-4,6-dinitrophenols. Table III (Tests C11–C13) lists results obtained with 2-alkyl-4,6-dinitrophenols

TABLE I

Degree of eradication of *Sphaerotheca fuliginea* with alkyldinitrophenols

Test No.	Alkyl group	Eradication, %
C1 (25 ppm)	2-(1-Ethylhexyl) (8)	95
	2-(1-Methylpentyl) (6)	58
	2-(1-Ethylpropyl) (5)	47
	2-(1-Methylbutyl) (5)	22
	2-s-Butyl (4)	19
	4-(1-Ethylpropyl) (5)	14
	4-s-Butyl (4)	12
	4-(1-Methylpentyl) (6)	11
	Untreated	
C2 (50 ppm)	2-(1-Methylpentyl) (6)	98
	2-(1-Ethylpropyl) (5)	51
	4-(1-Methylpentyl) (6)	46
	4-(1-Ethylpropyl) (5)	36
	4-(1-Methylbutyl) (5)	36
	2-(1-Methylbutyl) (5)	29
	Untreated	
C3 (25 ppm)	2-(1-Propylheptyl) (10)	100
	2-(1-Ethylhexyl) (8)	99
	2-(1-Ethyloctyl) (10)	98
	2-(1-Methylhexyl) (7)	78
	4-(1-Propylheptyl) (10)	76
	2-(1-Ethylbutyl) (6)	68
	4-(1-Ethyloctyl) (10)	65
	4-(1-Methylhexyl) (7)	50
	4-(1-Ethylbutyl) (6)	36
	Untreated	
C4 (25 ppm)	2-(1-Propylpentyl) (8)	98·5
	2-(1-Ethylhexyl) (8)	96·5
	2-(1-Methylheptyl) (8)	94
	2-(1-Ethylpentyl) (7)	87
	4-(1-Propylpentyl) (8)	85
	2-(1-Propylbutyl) (7)	84
	4-(1-Propylbutyl) (7)	81
	4-(1-Methylheptyl) (8)	81
	4-(1-Ethylhexyl) (8)	81
	4-(1-Ethylpentyl) (7)	79
	Untreated	
C5 (25 ppm)	2-(1-Butylpentyl) (9)	100
	2-(1-Propylhexyl) (9)	99·8
	2-(1-Ethylheptyl) (9)	97·5
	2-(1-Methyloctyl) (9)	94
	2-(1-Ethylhexyl) (8)	91
	4-(1-Butylpentyl) (9)	86
	4-(1-Propylhexyl) (9)	81
	4-(1-Methyloctyl) (9)	67
	4-(1-Ethylheptyl) (9)	68
	4-(1-Methylnonyl) (10)	62
	Untreated	
C6 (25 ppm)	2-(1-Butylheptyl) (11)	98
	2-(1-Propyloctyl) (11)	96
	4-(1-Propyloctyl) (11)	64
	4-(1-Butylheptyl) (11)	44
	Untreated	
C7 (50 ppm)	2-(1-Pentylheptyl) (12)	99·9
	2-(1-Hexylheptyl) (13)	99·7
	2-(1-Pentylhexyl) (11)	99
	2-(1-Ethylhexyl) (8)	91
	4-(1-Pentylhexyl) (11)	71
	4-(1-Pentylheptyl) (12)	50
	4-(1-Hexylheptyl) (13)	15
	4-s-Butyl (4)	8
	Untreated	
C8 (25 ppm)	2-(1-Pentylheptyl) (12)	96
	2-(1-Ethylhexyl) (8)	87
	4-(1-Propylnonyl) (12)	46
	4-(1-Pentyloctyl) (13)	45
	4-(1-Butyloctyl) (12)	41
	4-(1-Butylnonyl) (13)	41
	4-(1-Pentylheptyl) (12)	35
	4-(1-Propyldecyl) (13)	26
	Untreated	

TABLE II

Degree of eradication of *Sphaerotheca fuliginea* with 2-alkyl-4,6-dinitro-phenols and anisoles at 50 ppm

Test No.	Name of compound	Eradication, %
C9	2-(1-Methylheptyl)-4,6-dinitrophenol (8)	100
	2-(1-Ethylhexyl)-4,6-dinitrophenol (8)	96
	2-(1-Ethylhexyl)-4,6-dinitroanisole (8)	14
	2-(1-Methylheptyl)-4,6-dinitroanisole (8)	14
	Untreated	
C10	2-(1-Propylhexyl)-4,6-dinitrophenol (9)	100
	2-(1-Butylpentyl)-4,6-dinitrophenol (9)	100
	2-(1-Propylpentyl)-4,6-dinitrophenol (8)	99·8
	2-(1-Ethylheptyl)-4,6-dinitrophenol (9)	99·1
	2-(1-Ethylhexyl)-4,6-dinitrophenol (8)	99·1
	2-(1-Hexylheptyl)-4,6-dinitrophenol (13)	96
	2-(1-Ethylheptyl)-4,6-dinitroanisole (9)	16
	2-(1-Butylpentyl)-4,6-dinitroanisole (9)	16
	2-(1-Hexylheptyl)-4,6-dinitroanisole (13)	8
	2-(1-Propylpentyl)-4,6-dinitroanisole (8)	5
	2-(1-Propylhexyl)-4,6-dinitroanisole (9)	0
	Untreated	

TABLE III

Degree of eradication of *Sphaerotheca fuliginea* with 2-alkyl-4,6-dinitrophenols and their esters at 25 ppm

Test No.	Name of compound	Eradication, %
C11	2-(1-Ethylhexyl)-4,6-dinitrophenol (8)	97
	2-(1-Ethylhexyl)-4,6-dinitrophenyl methyl carbonate (8)	89
	2-(1-Ethylhexyl)-4,6-dinitrophenyl crotonate (8)	29
	2-(1-Hexylheptyl)-4,6-dinitrophenyl crotonate (13)	16
	Untreated	
C12	2-(1-Methyloctyl)-4,6-dinitrophenol (9)	94
	2-(1-Methyloctyl)-4,6-dinitrophenyl ethyl carbonate (9)	53
	Untreated	
C13	2-(1-Methyloctyl)-4,6-dinitrophenol (9)	99·9
	2-(1-Ethylhexyl)-4,6-dinitrophenol (8)	98
	2-(1-Ethylhexyl)-4,6-dinitrophenyl ethyl carbonate (8)	97
	2-(1-Methyloctyl)-4,6-dinitrophenyl ethyl carbonate (9)	62
	Untreated	

TABLE IV

Degree of eradication of *Sphaerotheca fuliginea* with alkyldinitro-phenyl methyl carbonates at 25 ppm

Test No.	Alkyl group	Eradication, %
C14	2-(1-Ethylhexyl) (8)	100
	2-(1-Methylbutyl) (5)	99·9
	2-Isopropyl (3)	93
	4-(1-Methylbutyl) (5)	91
	4-(1-Methylpentyl) (6)	91
	2-(1-Butylhexyl) (10)	90
	2-(1-Propyloctyl) (11)	83
	2-(1-Butylheptyl) (11)	79
	4-(1-Propyloctyl) (11)	77
	4-(1-Butyloctyl) (12)	75
	Untreated	
C15	2-(1-Methylpentyl) (6)	100
	2-(1-Propylpentyl) (8)	100
	2-(1-Methylhexyl) (7)	100
	2-(1-Ethylhexyl) (8)	99+
	4-(1-Propylpentyl) (8)	99
	2-(1-Butylhexyl) (10)	97
	2-s-Butyl (4)	93
	4-(1-Methylhexyl) (7)	93
	2-(1-Pentylhexyl) (11)	58
	Untreated	
C16	2-(1-Ethylbutyl) (6)	99+
	2-(1-Ethylhexyl) (8)	99+
	2-(1-Butylpentyl) (9)	99+
	2-(1-Ethylpentyl) (7)	96
	2-(1-Propylheptyl) (10)	87
	Untreated	
C17	2-(1-Methylpentyl) (6)	100
	2-(1-Propylbutyl) (7)	100
	2-(1-Ethylhexyl) (8)	99+
	2-(1-Ethylpropyl) (5)	89
	Untreated	

and esters. Tables IV–VI (Tests C14–C24) report results of tests with methyl carbonates, ethyl carbonates and crotonates. Tables VII–X (Tests C25–C32) list results obtained with 2-(C_8 α-branched alkyl)-4,6-dinitro- and 4-(C_8 α-branched

alkyl)-2,6-dinitro- phenols and their various esters.

The total number of carbon atoms of the nuclear alkyl substituent is given in all Tables in the headings or in parentheses.

TABLE V

Degree of eradication of *Sphaerotheca fuliginea* with alkyldinitro-phenyl ethyl carbonates at 25 ppm

Test No.	Alkyl group	Eradication, %
C18	2-(1-Ethylhexyl) (8)	97
	4-(1-Propylbutyl) (7)	96
	4-(1-Ethylbutyl) (6)	87
	4-(1-Ethylpropyl) (5)	81
	4-(1-Methylhexyl) (7)	77
	4-(1-Methylbutyl) (5)	72
	4-(1-Methylpentyl) (6)	71
	4-s-Butyl (4)	62
	4-(1-Methylheptyl) (8)	61
	4-(1-Ethylpentyl) (7)	55
	Untreated	
C19	2-(1-Methylhexyl) (7)	99
	2-(1-Ethylbutyl) (6)	97
	2-(1-Propylbutyl) (7)	97
	2-(1-Propylhexyl) (9)	97
	2-(1-Methylbutyl) (5)	95
	2-(1-Ethylhexyl) (8)	94
	2-(1-Ethylpentyl) (7)	93
	2-(1-Butylpentyl) (9)	79
	2-(1-Propylheptyl) (10)	54
	Untreated	

TABLE VI

Degree of eradication of *Sphaerotheca fuliginea* with alkyldinitro-phenyl esters

Test No.	Name of compound	Eradication %
C20 (50 ppm)	2-(1-Ethylhexyl)-4,6-dinitrophenyl methyl carbonate (8)	99
	4-(1-Propylhexyl)-2,6-dinitrophenyl methyl carbonate (9)	87
	4-'t-Octyl'-2,6-dinitrophenyl methyl carbonate (8)	72
	4-(1-Propylhexyl)-2,6-dinitrophenyl ethyl carbonate (9)	50
	4-(1-Butylhexyl)-2,6-dinitrophenyl methyl carbonate (10)	49
	4-t-Butyl-2,6-dinitrophenyl methyl carbonate (4)	22
	4-(1-Butylhexyl)-2,6-dinitrophenyl ethyl carbonate (10)	20
	4-'t-Octyl'-2,6-dinitrophenyl ethyl carbonate (8)	14
	Untreated	
C21 (25 ppm)	2-(1-Ethylhexyl)-4,6-dinitrophenyl methyl carbonate (8)	100
	2-(1-Ethylhexyl)-4,6-dinitrophenyl ethyl carbonate (8)	98·5
	2-(1-Ethylheptyl)-4,6-dinitrophenyl methyl carbonate (9)	93
	2-(1-Pentylheptyl)-4,6-dinitrophenyl methyl carbonate (12)	85
	2-(1-Ethylheptyl)-4,6-dinitrophenyl ethyl carbonate (9)	84
	2-(1-Ethyloctyl)-4,6-dinitrophenyl ethyl carbonate (10)	81
	2-(1-Ethyloctyl)-4,6-dinitrophenyl methyl carbonate (10)	80
	2-(1-Pentylheptyl)-4,6-dinitrophenyl ethyl carbonate (12)	75
	Untreated	
C22 (25 ppm)	2-s-Butyl-4,6-dinitrophenyl ethyl carbonate (4)	100
	2-(1-Methylbutyl)-4,6-dinitrophenyl crotonate (5)	100
	2-(1-Ethylpropyl)-4,6-dinitrophenyl crotonate (5)	100
	2-Isopropyl-4,6-dinitrophenyl ethyl carbonate (3)	98
	2-(1-Ethylhexyl)-4,6-dinitrophenyl ethyl carbonate (8)	98
	2-(1-Butylhexyl)-4,6-dinitrophenyl ethyl carbonate (10)	77
	2-(1-Propyloctyl)-4,6-dinitrophenyl ethyl carbonate (11)	71
	2-(1-Pentylhexyl)-4,6-dinitrophenyl ethyl carbonate (11)	62
	2-(1-Butylheptyl)-4,6-dinitrophenyl ethyl carbonate (11)	58
	Untreated	
C23 (25 ppm)	2-(1-Methylhexyl)-4,6-dinitrophenyl methyl carbonate (7)	99·8
	2-(1-Propylpentyl)-4,6-dinitrophenyl methyl carbonate (8)	99·7
	2-(1-Methylpentyl)-4,6-dinitrophenyl methyl carbonate (6)	99·4
	2-(1-Methylpentyl)-4,6-dinitrophenyl crotonate (6)	98
	2-(1-Methylhexyl)-4,6-dinitrophenyl methyl carbonate (7)	98
	2-(1-Propylpentyl)-4,6-dinitrophenyl crotonate (8)	98
	2-(1-Ethylhexyl)-4,6-dinitrophenyl methyl carbonate (8)	92
	2-(1-Methylhexyl)-4,6-dinitrophenyl crotonate (7)	78
	2-(1-Ethylhexyl)-4,6-dinitrophenyl crotonate (8)	46
	Untreated	
C24 (25 ppm)	2-(1-Methylpentyl)-4,6-dinitrophenyl crotonate (6)	97
	2-(1-Ethylbutyl)-4,6-dinitrophenyl crotonate (6)	94
	2-(1-Ethylhexyl)-4,6-dinitrophenyl crotonate (8)	58
	2-(1-Butylpentyl)-4,6-dinitrophenyl crotonate (9)	54
	2-(1-Propylheptyl)-4,6-dinitrophenyl crotonate (10)	50
	2-(1-Butylhexyl)-4,6-dinitrophenyl crotonate (10)	41
	2-(1-Butylheptyl)-4,6-dinitrophenyl crotonate (11)	41
	2-(1-Propyloctyl)-4,6-dinitrophenyl crotonate (11)	35
	Untreated	

TABLE VII

Degree of eradication of *Sphaerotheca fuliginea* with C_8 alkyldinitro-phenols and esters at 50 ppm

Test No.	Name of compound	Eradication, %
C25	2-(1-Methylheptyl)-4,6-dinitrophenol	99
	2-(1-Methylheptyl)-4,6-dinitrophenyl methyl carbonate	98
	4-(1-Ethylhexyl)-2,6-dinitrophenyl methyl carbonate	94
	2-(1-Methylheptyl)-4,6-dinitrophenyl S-methyl thiolocarbonate	91
	4-(1-Ethylhexyl)-2,6-dinitrophenyl crotonate	89
	4-(1-Methylheptyl)-2,6-dinitrophenyl methyl carbonate	87
	4-(1-Methylheptyl)-2,6-dinitrophenyl crotonate	84
	4-(1-Methylheptyl)-2,6-dinitrophenol	80
	4-(1-Ethylhexyl)-2,6-dinitrophenol	79
	4-(1-Ethylhexyl)-2,6-dinitrophenyl S-methyl thiolocarbonate	77
	Untreated	
C26	2-(1-Methylheptyl)-4,6-dinitrophenol	99
	2-(1-Methylheptyl)-4,6-dinitrophenyl methyl carbonate	98
	4-(1-Ethylhexyl)-2,6-dinitrophenol	90
	4-(1-Methylheptyl)-2,6-dinitrophenol	87
	4-(1-Methylheptyl)-2,6-dinitrophenyl methyl carbonate	81
	Untreated	
C27	2-(1-Propylpentyl)-4,6-dinitrophenyl methyl carbonate	100
	2-(1-Propylpentyl)-4,6-dinitrophenyl ethyl carbonate	99·7
	2-(1-Propylpentyl)-4,6-dinitrophenyl crotonate	99
	2-(1-Propylpentyl)-4,6-dinitrophenol	97
	2-(1-Ethylhexyl)-4,6-dinitrophenol	94
	2-(1-Ethylhexyl)-4,6-dinitrophenyl methyl carbonate	91
	2-(1-Ethylhexyl)-4,6-dinitrophenyl decyl carbonate	8
	Untreated	
C28	2-(1-Methylheptyl)-4,6-dinitrophenyl ethyl carbonate	95
	2-(1-Methylheptyl)-4,6-dinitrophenyl crotonate	91
	2-(1-Methylheptyl)-4,6-dinitrophenyl S-methyl thiolocarbonate	89
	4-(1-Ethylhexyl)-2,6-dinitrophenyl crotonate	83
	4-(1-Ethylhexyl)-2,6-dinitrophenyl ethyl carbonate	81
	4-(1-Methylheptyl)-2,6-dinitrophenyl crotonate	76
	4-(1-Methylheptyl)-2,6-dinitrophenyl ethyl carbonate	71
	4-(1-Ethylhexyl)-2,6-dinitrophenyl S-methyl thiolocarbonate	60
	4-(1-Methylheptyl)-2,6-dinitrophenyl S-methyl thiolocarbonate	59
	Untreated	

TABLE VIII

Degree of eradication of *Sphaerotheca fuliginea* with 2-(1-methyl heptyl)-4,6-dinitrophenol and its esters at 50 ppm (Test C29)

Compound	Eradication, %
Methyl carbonate	96
Ethyl carbonate	95·5
Phenol	95
Crotonate	80
Untreated	

TABLE IX

Degree of eradication of *Sphaerotheca fuliginea* with 2-(1-ethyl hexyl)-4,6-dinitrophenol and its esters

Test No.	Compound	Eradication, %
C30 (25 ppm)	Phenol	100
	Acetate	100
	Butyrate	99
	Propyl carbonate	80
	Hexyl carbonate	76
	Isopropyl carbonate	68
	Untreated	
C31 (20 ppm)	Methyl carbonate	99
	Phenol	99
	Ethyl carbonate	95
	Crotonate	85
	Untreated	

TABLE X

Degree of eradication of *Sphaerotheca fuliginea* with 4-(1-ethyl-hexyl)-2,6-dinitrophenyl esters at 50 ppm (Test C32)

Name of compound	Eradication, %
2-(1-Ethylhexyl)-4,6-dinitrophenyl methyl carbonate (standard)	99
Acetate	95
Acrylate	87
Hexyl carbonate	55
1-Methylheptyl carbonate	55
Chloroacetate	50
Butyl carbonate	46
Methacrylate	46
Benzoate	41
Untreated	

Discussion
Structure of alkyldinitrophenols and their activity
Activity of 2-(α-branched alkyl)-4,6-dinitrophenols

With 2-alkyl-4,6-dinitrophenols there was no significant degree of eradication at C_4 (s-butyl) (Test C1) or at C_5 (1-methylbutyl) and (1-ethylpropyl) (Tests C1, C2). All the 2-(C_6 to C_{13} α-branched alkyl)-4,6-dinitrophenols that were tested showed significant eradication (Tests C1–C8) and activity increased from C_6 to C_8 alkyl. 2-(C_8 to C_{13} α-Branched alkyl)-4,6-dinitrophenols were highly active. Several 2-C_9- (Tests C5, C13), C_{11}- (Test C7), C_{12}- (Tests C_7, C_8) and C_{13}- alkyl (Test C7) -4,6-dinitrophenols were more active than C_8 2-(1-ethylhexyl)-4,6-dinitrophenol which was used as the standard in most tests.

Activity of 4-(α-branched alkyl)-2,6-dinitrophenols

With 4-alkyl-2,6-dinitrophenols there was no significant degree of eradication at C_4 (s-butyl) (Tests C1, C7), at C_5 (1-methylbutyl) (Test C2) and 4-(1-ethylpropyl) (Tests C1, C2) or at C_6 (1-methylpentyl) (Tests C1, C2) and 1-ethyl-butyl) (Test C3). 4-C_7 (1-Methylhexyl) (Test C3), (1-ethyl-pentyl), and (1-propylbutyl) (Test C4), 4-C_8 (1-methylheptyl) and (1-propylpentyl) (Test C4), 4-C_9 (1-methyloctyl), (1-ethyl-heptyl), (1-propylhexyl) and (1-butylpentyl) (Test C5), and 4-C_{10} (1-methylnonyl) (Test C5), (1-ethyloctyl) and (1-propyl-heptyl) (Test C3)-2,6-dinitrophenols had significant activity. At 4-C_{11} to C_{13} the structure-activity relationship was less regular. 4-C_{11} (1-Propyloctyl) and (1-butylheptyl) (Test C6), 4-C_{12} (1-butyloctyl) and (1-pentylheptyl), 4-C_{13} (1-propyl-decyl), (1-butylnonyl) (Test C8) and (1-hexylheptyl) (Test C7) -2,6-dinitrophenols showed no significant degree of eradica-tion, whereas 4-C_{12} (1-propylnonyl) and 4-C_{13} (1-pentyloctyl) (Test C8) -2,6-dinitrophenols showed a significant degree of eradication.

Comparison of activity of 2-(α-branched alkyl)-4,6-dinitro-phenols and 4-(α-branched alkyl)-2,6-dinitrophenols

There were no significant differences between the activity of 2-C_4 (s-butyl) (Test C1) or 2-C_5 (1-methylbutyl) (Test C2) and (1-ethylpropyl) (Tests C1, C2)-4,6-dinitrophenols and their 4-alkyl-2,6-dinitrophenol analogues. The 2-(C_6 to C_{13} α-branched alkyl)-4,6-dinitrophenols were significantly more active than their 4-alkyl-2,6-dinitrophenol analogues: at C_6 (1-methylpentyl) (Tests C1, C2) and (1-ethylbutyl) (Test C3); at C_7 (1-methylhexyl) (Test C3) and (1-ethylpentyl) (Test C4)

(but not the (1-propylbutyl) isomer—Test C4); at C_8 (1-methyl heptyl), (1-ethylhexyl) and (1-propylpentyl) (Test C4); at C_9 (1-methyloctyl), (1-ethylheptyl), (1-propylhexyl), and (1-butyl-pentyl) (Test C5); at C_{10} (1-ethyloctyl) and (1-propylheptyl) (Test C3); at C_{11} (1-propyloctyl), (1-butylheptyl) (Test C6), and (1-pentylhexyl) (Test C7); at C_{12} (1-pentylheptyl) (Tests C7, C8); at C_{13} (1-hexylheptyl) (Test C7).

Thus the observation that 2-(α-branched alkyl)-4,6-dinitro-phenols were more active than their 4-alkyl-2,6-dinitrophenol analogues was confirmed by the present series of tests. Pianka[3,4] and Pianka & Edwards[5] observed that 2-(α-branched alkyl)-4,6-dinitrophenols were also more toxic to spider mites than their 4-alkyl-2,6-dinitrophenol analogues.

Effect of esterification on activity
Esterification to methyl- and ethyl- carbonates or crotonates of 2-(C_4 to C_7 α-branched alkyl)-4,6-dinitrophenols and 4-(C_4 to C_7 α-branched alkyl)-2,6-dinitrophenols

Esterification of 2-(C_4 to C_7 α-branched alkyl)-4,6-dinitro-phenols to methyl- or ethyl- carbonates or crotonates gave compounds with enhanced acaricidal activity.[5] A similar effect on the eradication of cucumber mildew was observed when 2-(C^4 to C^7 α-branched alkyl)-4,6-dinitrophenols and 4-(C^4 to C^7 α-branched alkyl)-2,6-dinitrophenols were esteri-fied to methyl- or ethyl-carbonates or crotonates.

Thus, the C_4 2-s-butyl-4,6-dinitrophenyl methyl carbonate (Test C15) or ethyl carbonate (Test C22) had high activity and 4-s-butyl-2,6-dinitrophenyl ethyl carbonate (Test C18) had significant eradicant activity whereas the parent phenols showed no significant degree of eradication (Tests C1, C7). The C_5 2-(1-methylbutyl)-4,6-dinitrophenyl methyl- (Test C14) and ethyl- carbonates (Test C19) and crotonate (Test C22), and 2-(1-ethylpropyl)-4,6-dinitrophenyl methyl carbon-ate (Test C17) and crotonate (Test C22) were very active, and 4-(1-methylbutyl)-2,6-dinitrophenyl methyl- (Test C14) and ethyl- carbonates (Test C18) had significant eradicant activity whereas the parent C^5 alkyl phenols had no significant activity (Tests C1, C2).

The C_6 2-(1-methylpentyl)-4,6-dinitrophenyl methyl carbon-ate and crotonate (Tests C15, C17, C23, C24), were as active as, or more active than the C_8 2-(1-ethylhexyl)-4,6-dinitro-phenyl methyl carbonate or crotonate, and 2-(1-ethylbutyl)-4,6-dinitrophenyl methyl- (Test C16) and ethyl-carbonates (Test C19) or crotonate (Test C24) were highly active, whereas the parent C_6 alkyl phenols were significantly less active than the C_8 alkyl phenol (Tests C1, C3). 4-(1-Methylpentyl)-2,6-dinitrophenyl methyl- (Test C14) and ethyl- carbonates (Test C18) and 4-(1-ethylbutyl)-2,6-dinitrophenyl ethyl car-bonate (Test C18) showed significant eradicant activity where-as the parent phenols had no significant activity (Tests C1–C3). The C_7 2-(1-methylpentyl)-4,6-dinitrophenyl methyl- (Tests C15, C23) and ethyl-carbonates (Tests C19, C23) were highly active and the crotonate was significantly more active than that of 2-(1-ethylhexyl)-4,6-dinitrophenol (Test C23). 2-(1-Ethylpentyl)-4,6-dinitrophenyl methyl- (Test C16) and ethyl-carbonates (Test C19) and 2-(1-propylbutyl)-4,6-dinitro-phenyl methyl- (Test C17) and ethyl- carbonates (Test C19) also showed high activity; the 2-C_7 alkyl phenols showed lower activity than the standard 2-(1-ethylhexyl)-4,6-dinitro-phenol (Tests C3, C4). 4-(1-Methylhexyl)-2,6-dinitrophenyl methyl- (Test C15) and ethyl-carbonates (Test C18), 4-(1-ethylpentyl)-2,6-dinitrophenyl ethyl carbonate (Test C18) had significant eradicant activity and 4-(1-propylbutyl)-2,6-dinitro-phenyl ethyl carbonate (Test C18) was highly active.

Esterification to methyl- or ethyl- carbonates or crotonates of 2-(C_8 to C_{13} α-branched alkyl)-4,6-dinitrophenols and 4-(C_8 to C_{13} α-branched alkyl)-2,6-dinitrophenols

The 2-C_8 (1-methylheptyl)-4,6-dinitrophenyl methyl carbonate (Tests C25, C26, C29) or ethyl carbonate (Test C29) was as active as the parent phenol, but the crotonate had significantly lower activity (Tests C28, C29). 2-(1-Ethylhexyl)-4,6-dinitrophenyl methyl carbonate was in one test (C11) inferior to the phenol, in other tests (C27, C31) it was as active as the phenol. Generally, the methyl- and ethyl-carbonates of this phenol (Tests C11, C13–C22, C27, C31, C32) showed a high degree of eradication. The crotonate was significantly less active than the phenol (Tests C11, C31) or its methyl- (Tests C11, C23, C31) or ethyl-carbonate (Test C31). 2-(1-Propylpentyl)-4,6-dinitrophenyl methyl carbonate (Tests C15, C23, C27) or ethyl carbonate or crotonate (Test C27) were highly active. The methyl- and ethyl- carbonates (Test C27) were more active than the phenol. Esterification of the 4-(1-methylheptyl)-2,6-dinitrophenol (Tests C25, C26) to the methyl carbonates had no effect on the activity of the compounds, but the activity of the methyl carbonates of the (1-ethylhexyl) (Test C25) and (1-propylpentyl) (Test C15) phenols was enhanced. Esterification of the first two phenols (Test C25) to the crotonates had no significant effect on activity.

The 2-C_9 (1-methyloctyl)-4,6-dinitrophenol gave a compound with considerably reduced activity on esterification to the ethyl carbonate (Tests C12, C13). Bearing in mind that 2-(1-ethylheptyl)-4,6-dinitrophenol was significantly more active than 2-(1-ethylhexyl)-4,6-dinitrophenol (Test C5) and that the methyl carbonate of the C_8 alkyl phenol was generally as active as the phenol, it may be assumed that activity of the compounds was reduced on esterification of the C_9 alkyl phenol to the methyl- and ethyl- carbonates since these were significantly less active than the corresponding carbonates of the C_8 alkyl phenol (Test C21). The activity of 2-(1-butylpentyl)-4,6-dinitrophenyl methyl carbonate was as high as that of the C_8 alkyl phenol (Test C16), the ethyl carbonate of the C_9 alkyl phenol was significantly lower than that of the C_8 alkyl phenol (Test C19) and the crotonate had no significant activity (Test C24). Thus activity was lowered on esterification of 2-(1-butylpentyl)-4,6-dinitrophenol to the ethyl carbonate or crotonate. However, activity was not affected by esterification of 2-(1-propylhexyl)-4,6-dinitrophenol to the ethyl carbonate (Test C19). The activity of the methyl carbonate of 4-(1-propylhexyl)-2,6-dinitrophenol was significantly higher than that of the ethyl carbonate (Test C20).

On the basis of a comparison of the activities of the 2-C_{10} (1-ethyloctyl)- and (1-propylheptyl)-4,6-dinitrophenols with that of 2-C_8 (1-ethylhexyl)-2,6-dinitrophenol (Test C3) and of their carbonates, it may be argued that activity is reduced on esterification of the (1-ethyloctyl) phenol to the methyl- or ethyl- carbonate (Test C21), and that the reduction of activity was particularly severe on esterification of 2-(1-propylheptyl)-4,6-dinitrophenol to the ethyl carbonate (Test C19) or crotonate (Test C24) since these esters showed no significant degree of eradication. 2-(1-Butylhexyl)-4,6-dinitrophenyl methyl- and ethyl- carbonates and crotonate were significantly less active than the corresponding esters of 2-(1-ethylhexyl)-4,6-dinitrophenol (Tests C14, C15, C22, C24). 4-(1-Butylhexyl)-2,6-dinitrophenyl ethyl carbonate had no significant activity and the methyl carbonate had slight activity (Test C20).

The 2-C_{11} (1-propyloctyl)- and (1-butylheptyl)-2,6-dinitrophenols had high activity (Test C6) and the (1-pentylhexyl)

isomer had significantly higher activity than 2-(1-ethylhexyl)-4,6-dinitrophenol (Test C7). The methyl carbonates of these phenols showed reduced activity (Tests C14, C15). The ethyl carbonates of the (1-butylheptyl) and (1-pentylhexyl) phenols (Test C22) and the crotonates of the (1-propyloctyl) and (1-butylheptyl) phenols showed no significant degree of eradication (Test C24). The activity of the compounds was lowered therefore on esterification of 2-C_{11} alkyl-4,6-dinitrophenols.

On the basis of a comparison of the activities of the 2-C_{12} (1-pentylheptyl)- and C_{13} (1-hexylheptyl)-4,6-dinitrophenols with that of the 2-C_8 (1-ethylhexyl)-4,6-dinitrophenol (Tests C7, C8) and of their esters one may consider that the activity of the compounds was reduced significantly on esterification of the C_{12} alkyl phenols to the methyl- or ethyl- carbonate (Test C21) and activity was severely reduced on conversion of the C_{13} alkyl phenol to the crotonate (Test C11).

Esterification of C_8 alkyl dinitrophenols to carbonates other than methyl or ethyl, or to esters other than crotonate

As observed earlier[5] the *S*-methyl thiolocarbonate of 2-(1-methylheptyl)-4,6-dinitrophenol had lower acaricidal activity than the parent phenol or its methyl- or ethyl- carbonate or crotonate. Similarly, *S*-methyl thiolocarbonate of 2-(1-methylheptyl)-4,6-dinitrophenol showed a lower degree of eradication of cucumber mildew than did the parent phenol or its methyl- or ethyl-carbonate (Tests C25, C28) or crotonate (Test C28). *S*-Methyl thiolocarbonate of 4-(1-methylheptyl)-2,6-dinitrophenol was less active than the ethyl carbonate or crotonate (Test C28) and *S*-methyl thiolocarbonate of 4-(1-ethylhexyl)-2,6-dinitrophenol was less active than its methyl carbonate (Test C25) or ethyl carbonate or crotonate (Test C28).

Esterification of 2-(1-ethylhexyl)-4,6-dinitrophenol to C_3 to C_{10} alkyl carbonates reduced the activity of the compounds (Tests C27, C30). The acetate and butyrate were as active as the phenol (Test C30).

The acetate and acrylate of 4-(1-ethylhexyl)-2,6-dinitrophenol had considerable eradicant activity against cucumber mildew, but other esters (chloroacetate, methacrylate, benzoate) and C_4 to C_8 alkyl carbonates showed no significant degree of eradication (Test C32).

Activity of t-alkyl compounds

At 50 ppm 4-t-butyl-2,6-dinitrophenyl methyl carbonate and 4-(1,1,3,3-tetramethylbutyl)-2,6-dinitrophenyl ethyl carbonate showed no significant degree of eradication of cucumber mildew. 4-(1,1,3,3-Tetramethylbutyl)-2,6-dinitrophenyl methyl carbonate gave 72% eradication at 50 ppm and was significantly more active than the ethyl carbonate (Test C20).

Possible mode of action of esters

Methylation of several highly active 2-(C_8 to C_{13} α-branched alkyl)-4,6-dinitrophenols (Tests C9, C10) resulted in anisoles which gave no significant degree of eradication of cucumber mildew. Reduction of acaridical,[4,5] herbidical[6] and apple mildew eradication[2] also resulted from etherification of active alkyldinitrophenols. As discussed earlier,[4] this effect may be related to the stability of the ethers and their inability to undergo ready hydrolysis to the active parent phenols.

The degree of eradication of cucumber mildew with (C_4 to C_7 α-branched alkyl) dinitrophenols was generally increased by esterification. Perhaps the high polarity of these phenols

hinders their penetration to the vital sites of action and the ester chain assists it and brings about a build-up of a higher concentration of the toxicants in the organism. The esters would then hydrolyse to the fungitoxic parent phenols. A similar postulate was advanced[4] to explain the higher acaricidal activity of dinoseb and dinosam carbonates than that of the phenolic dinoseb and dinosam.

The more lipophilic character contributed by the higher C_8 to C_{13} alkyl group may facilitate penetration of the phenol. Sometimes esterification of the higher alkyl phenols proved detrimental to activity. This was generally so with the 2-(C_{10} to C_{13} α-branched alkyl)-4,6-dinitrophenols. This reduction in activity may be associated with the steric effect of the α-branched alkyl group hindering the hydrolysis of the esters to the phenols, or the increase in bulk of the molecule, or both.

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STRUCTURES AND PESTICIDAL ACTIVITIES OF DERIVATIVES OF DINITROPHENOLS

XII.*—*In vitro* activity of C_3 to C_{13} α-branched alkyldinitrophenols and their esters and ethers against the spores of *Venturia inaequalis* and other fungi

By M. PIANKA

The activity *in vitro* of 2-(C_3 to C_{13} α-branched alkyl)-4,6-dinitrophenols and 4-(C_4 to C_{13} α-branched alkyl)-2,6-dinitrophenols and of their various esters and ethers was determined against the spores of *Venturia inaequalis* (Cooke) Wint., *Botrytis cinerea* Pers., *Fusarium bulbigenum*, Cooke & Massee, var. *lycopersici* (Brushi) Wollenw. and *Cercospora melonis* Cooke. Several alkyldinitrophenols were highly active against *Venturia* and the 2-alkylphenols were generally more active than the 4-alkylphenols. The alkyldinitrophenols were not effective against the spores of *Botrytis*, *Fusarium* and *Cercospora*.

Esterification of several lower alkyldinitrophenols to methyl carbonates gave compounds with enhanced activity, but methyl carbonates of C_7 to C_{11} alkyl dinitrophenols showed considerably reduced activity. Esterification to ethyl- or other alkyl-carbonates gave compounds with considerably reduced activity against *Venturia*. Esterification of 2-isopropyl- and 2-t-butyl-4,6-dinitrophenols to crotonates gave compounds with enhanced activity against *Venturia*, but crotonates of other phenols showed reduced activity. The acrylates of several C_6 to C_8 alkyl dinitrophenols proved highly active. Ethers of active phenols showed considerably reduced activity.

Introduction

Kirby *et al.*[1] examined the inhibition *in vitro* of the spore germination of *Venturia inaequalis* (Cooke) Wint. with 4-n-alkyl- and 4-(1-methylalkyl)-2,6-dinitrophenols and 2-n-alkyl- and 2-(1-methylalkyl)-4,6-dinitrophenols containing a total number of ten carbon atoms in the alkyl side chain, and reported a peak of activity lying at C_7. It was decided to examine the effects of substitution of 2,4- or 2,6-dinitrophenols with 6-C_3 to C_{13} or 4- C_4 to C_{13} α-branched alkyl groups, and of esterification and etherification of these phenols on the *in vitro* inhibition of the germination of spores of *Venturia inaequalis*, *Botrytis cinerea*, *Fusarium bulbigenum*, and *Cercospora melonis*.

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* Part XI: Preceding paper.

Experimental

Materials

These were prepared as described in Part IX.[2]

Methods

All the tests were carried out by the Montgomery-Moore[3] slide germination technique using *Venturia inaequalis* (Cooke) Wint., *Botrytis cinerea* Pers., *Fusarium bulbigenum*, Cooke & Massee, var. *lycopersici* (Brushi) Wollenw. and *Cercospora melonis* Cooke. The values of LD_{95} were determined as described by Pianka & Hall.[4] Acetone solutions were used instead of methanol solutions.

Results

Table I summarises the LD_{95} values obtained with 2- (C_3 to C_{13} α-branched alkyl)-4,6-dinitrophenols, 4-(C_4 to C_{13} α-branched alkyl)-2,6-dinitrophenols, and with some of their acetates, acrylates and methyl ethers against *Venturia*. The LD_{95} values of these compounds were also determined against *Botrytis*, *Fusarium* and *Cercospora*.

In addition, the LD_{95} values against these four organisms were obtained with the methyl carbonates of the phenols of Table I [except for 4-(1-ethylpentyl)-2,6-dinitrophenyl methyl carbonate]; the ethyl carbonates and crotonates of the 2-(C_3 to C_9 alkyl)-4,6-dinitrophenols and 4-(C_4 to C_9 alkyl)-2,6-dinitrophenols [except for 4-(1-methylbutyl)-2,6-dinitrophenyl crotonate]; the ethyl carbonates and crotonates of 2-C_{10} (1-ethyloctyl)-, (1-propylheptyl)-, (1-butylhexyl)-, C_{11} (1-propyloctyl)-, (1-butylheptyl)-, (1-pentylhexyl)-, C_{12} (1-pentylheptyl)- and C_{13} (1-hexylheptyl)-4,6-dinitrophenols, of their 4-alkyl-2,6-dinitrophenol analogues and of 4-C_{12} (1-butyloctyl)-2,6-dinitrophenol; the isopropyl carbonate and S-methyl thiolocarbonate of 4-C_8 (1-methylheptyl)-2,6-dinitrophenol; the propyl-, isopropyl-, butyl-, s-butyl-, isobutyl-, hexyl-, octyl-, 1-methylheptyl- and decyl- carbonates, S-methyl thiolocarbonate and butyrate, isobutyrate, octanoate, chloroacetate, 2- and 3-chloropropionates, α-methacrylate, 3-methyl crotonate, benzoate and p-chlorobenzoate of 4-C_8 (1-ethylhexyl)-2,6-dinitrophenol; the s-butyl carbonate, isobutyrate and α-methacrylate of 4-C_8 (1-propylpentyl)-2,6-dinitrophenol; the isopropyl carbonate of 4-C_8 't-octyl'-2,6-dinitrophenol; the isopropyl carbonate and S-methyl thiolocarbonate of 2-C_8 (1-methylheptyl)-4,6-dinitrophenol; and the propyl-, isopropyl-, hexyl-, and decyl-carbonates and butyrate of 2-C_8 (1-ethylhexyl)-4,6-dinitrophenol.

Activity against *Venturia inaequalis*

Some of the results are recorded in Table I. The following compounds were found to be highly active: the methyl carbonates of 2-isopropyl- and s-butyl-4,6-dinitrophenols (LD_{95} of 35 & 55 ppm); 4-s-butyl-, (1-methylbutyl)-, (1-ethylpropyl)-, (1-ethylbutyl)- and (1-methylhexyl)-2,6-dinitrophenols (LD_{95} of 45, 40, 50, 100, 100 ppm respectively); and the crotonates of 2-isopropyl- and t-butyl-4,6-dinitrophenols (LD_{95} of 30 & 80 ppm). The following esters had LD_{95} values > 100 ppm; the methyl carbonates of 2-(1-methylbutyl)- and (1-ethylpropyl)-4,6-dinitrophenols and of 4-t-butyl-, (1-methylpentyl)-, (1-propylbutyl)-, (1-propylpentyl)-, (1-propylhexyl)-2,6-dinitrophenols; the ethyl carbonates of 2-isopropyl-, (1-methylpropyl)-, (1-methylbutyl)-4,6-dinitrophenols and of 4-s-butyl-, t-butyl-, (1-methylheptyl)-, and (1-propylpentyl)-2,6-dinitrophenols; the crotonates of 2-(1-methylbutyl)-, (1-propylbutyl)- and (1-methylheptyl)-4,6-dinitrophenols and of 4-(1-propylbutyl)-2,6-dinitrophenol; the S-methyl thiolocarbonate of 4-(1-methylheptyl)-2,6-dinitrophenol; the chloroacetate and 2- and 3- chloropropionates of 4-(1-ethylhexyl)-2,6-dinitrophenol. All the other compounds showed LD_{95} values > 1000 ppm.

Activity against *Botrytis cinerea*

4-(1-Ethylpropyl)-2,6-dinitrophenyl methyl carbonate had high activity (LD_{95} of 35 ppm). The other compounds had LD_{95} values > 100 ppm or > 1000 ppm.

Activity against *Fusarium bulbigenum*

The methyl carbonates of 4-s-butyl- and 4-t-butyl-2,6-dinitrophenols and 2-s-butyl-4,6-dinitrophenols had LD_{95} values of 80, 35 & 100 ppm respectively; 2-(1-methylpentyl)-

TABLE I

Inhibition of spore germination of *Venturia inaequalis* with 2-(C_3 to C_{13} α-branched alkyl)-4,6-dinitrophenols and 4-(C_4 to C_{13} α-branched alkyl)-2,6-dinitrophenols and with their acetates, acrylates and methyl ethers, LD_{95} values, ppm

Alkyl	Number of carbon atoms in alkyl group	Phenols		Acetates		Acrylates		Anisoles	
		2-Alkyl	4-Alkyl	2-Alkyl	4-Alkyl	2-Alkyl	4-Alkyl	2-Alkyl	4-Alkyl
Isopropyl	3	> 100	—*	—	—	—	—	—	—
s-Butyl	4	100	> 100	—	—	—	—	—	—
t-Butyl		> 100	> 100	—	—	—	—	—	—
1-Methylbutyl	5	> 100	> 100	—	—	—	—	—	—
1-Ethylpropyl		> 100	> 100	—	—	—	—	—	—
1-Methylpentyl	6	70	55	> 100	—	12	—	—	—
1-Ethylbutyl		40	> 100	—	—	—	—	—	—
1-Methylhexyl	7	25	40	> 1000	—	20	—	—	—
1-Ethylpentyl		25	95	—	—	—	—	—	> 1000
1-Propylbutyl		15	70	—	> 1000	—	25	—	—
1-Methylheptyl	8	28	60	> 1000	> 1000	> 100	> 1000	> 1000	> 1000
1-Ethylhexyl		45	45	> 1000	50	> 1000	25	> 1000	> 1000
1-Propylpentyl		20	> 100	—	> 1000	> 1000	> 100	> 1000	> 1000
't-Octyl'†		—	> 100	—	—	—	—	—	—
1-Methyloctyl	9	30	> 100	—	—	—	—	—	> 1000
1-Ethylheptyl	7	75	—	—	—	—	—	> 1000	—
1-Propylhexyl		20	85	—	—	—	—	> 1000	> 1000
1-Butylpentyl		8	75	—	—	—	—	> 1000	> 1000
1-Methylnonyl	10	—	> 100	—	—	—	—	—	—
1-Ethyloctyl		10	> 100	—	—	—	> 1000	—	—
1-Propylheptyl		20	55	—	—	—	> 1000	—	—
1-Butylhexyl		15	> 100	—	—	—	> 1000	—	—
1-Methyldecyl	11	—	> 1000	—	—	—	—	—	—
1-Ethylnonyl		—	> 1000	—	—	—	—	—	—
1-Propyloctyl		> 100	> 1000	—	—	—	—	—	—
1-Butylheptyl		> 100	> 1000	—	—	—	—	—	—
1-Pentylhexyl		50	> 100	—	—	—	—	—	—
1-Ethyldecyl	12	—	> 1000	—	—	—	—	—	—
1-Propylnonyl		—	> 1000	—	—	—	—	—	—
1-Butyloctyl		—	> 1000	—	—	—	—	—	> 1000
1-Pentylheptyl		> 100	> 1000	—	—	—	—	—	—
1-Propyldecyl	13	—	> 1000	—	—	—	—	—	—
1-Butylnonyl		—	> 1000	—	—	—	—	—	—
1-Pentyloctyl		—	> 1000	—	—	—	—	—	—
1-Hexylheptyl		> 1000	> 1000	—	—	—	—	> 1000	—

* Not tested   † Substantially 1,1,3,3-tetramethylbutyl

4,6-dinitrophenyl acrylate had an LD_{95} of 70 ppm, the acrylate and acetate of 4-(1-ethylhexyl)-2,6-dinitrophenol had LD_{95} of 50 & 45 ppm. The other compounds had LD_{95} values > 100 ppm or > 1000 ppm.

Activity against *Cercospora melonis*

4-t-Butyl-2,6-dinitrophenyl methyl carbonate had an LD_{95} value of 90 ppm. All the other compounds had LD_{95} > 100 ppm or > 1000 ppm.

Discussion

Effect of alkyldinitrophenols on spore germination

As with cucumber mildew[5,6] and with spider mites,[5,7,8,9] 2-alkyl-4,6-dinitrophenols were generally more active than 4-alkyl-2,6-dinitrophenols in inhibiting *in vitro* the germination of spores of *Venturia inaequalis*. High activity began at C_6 and remained high for 2-alkylphenols at C_{11} and for 4-alkylphenols at C_{10} (Table I).

The peak of activity for 2-(1-methylalkyl)-4,6-dinitrophenols and 4-(1-methylalkyl)-2,6-dinitrophenols lay at C_7 (Fig. 1) (cf. Kirby *et al.*).[1] 2-(1-Ethylalkyl)-4,6-dinitrophenols had a maximum at C_9 and 2-(1-propylalkyl) phenols at C_7 and a plateau at C_8 to C_{10}. The activity maximum of 4-(1-ethylalkyl)-2,6-dinitrophenols lay at C_8 and of (1-propylalkyl)-2,6-dinitrophenols at C_{10}.

The alkyldinitrophenols were not effective in inhibiting *in vitro* the germination of spores of *Botrytis cinerea*, *Fusarium bulbigenum* and *Cercospora melonis*.

Effect of esterification and etherification

Esterification of 4-(C_4 to C_{13} α-branched alkyl)-2,6-dinitrophenols to methyl carbonates did not reduce the eradicant activities of the compounds against apple mildew[2] or barley mildew,[10] but esterification of 2-(C_{10} to C_{13} α-branched alkyl)-4,6-dinitrophenols reduced activity against cucumber mildew[6]

and esterification of 2-(C_8 to C_{13} α-branched alkyl)-4,6-dinitrophenols reduced acaricidal activity.[9] Although the activity of these phenols was high, that of the methyl ethers was much lower, and it was assumed that this was due to the inability of the ethers to undergo ready hydrolysis to the parent phenols. The un-ionised esters, when not too bulky, would penetrate more readily to the sites of action than the highly polar parent phenols, allowing a build-up of the toxicants in the organism. The esters would then readily hydrolyse to the parent phenols leading to a lethal effect.[2,6,8,9,10]

Conversion to methyl carbonates enhanced the inhibition of spore germination of *Venturia* with 2-C_3 & C_4 alkyl-4,6-dinitrophenols, of 4-C_4 & C_5 alkyl- and C_6 (1-ethylbutyl)-2,6-dinitrophenols, and thus may have aided their penetration into the spores. It generally lowered activity of the higher alkyldinitrophenols. The special effect due to esterification to methyl carbonates of the C_3 to C_5 alkyl dinitrophenols was also evident in the enhanced inhibition of spore germination of *Botrytis*, *Fusarium* and *Cercospora*. Thus, 4-(1-ethylpropyl)-2,6-dinitrophenol had an LD_{95} value > 100 ppm against *Botrytis*, its methyl carbonate an LD_{95} value of 35 ppm; 4-s-butyl- and 4-t-butyl- 2,6-dinitrophenols had LD_{95} values > 100 ppm against *Fusarium*, their methyl carbonates had LD_{95} values of 80 & 35 ppm; 4-t-butyl-2,6-dinitrophenol had an LD_{95} value > 100 ppm against *Cercospora* and its methyl carbonate an LD_{95} value of 90 ppm.

Esterification to ethyl carbonates reduced the inhibition of spore germination even of those C_3 to C_6 alkyl phenols whose methyl carbonates proved active. All the carbonates of the C_8 alkyl dinitrophenols that were tested had LD_{95} values > 100 ppm or > 1000 ppm against *Venturia*.

Commercial dinocap was reported as having some effect against apple scab due to *Venturia inaequalis* (Cooke) Wint. (Kirby *et al.*).[1] The six C_8 α-branched alkyl dinitrophenyl crotonates shown by Kirby & Hunter[11] and Clifford *et al.*[12] to be present in commercial dinocap had negligible inhibition of spore germination of *Venturia* [the 2-(1-methylheptyl)
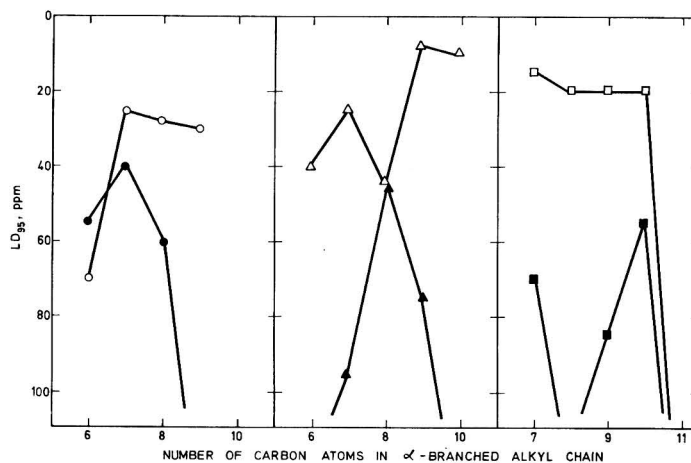
FIG. 1. *Inhibition of spore germination of* Venturia inaequalis *with 2-α-branched alkyl-4,6-dinitrophenols and 4-α-branched alkyl-2,6-dinitrophenols*

○ 2-(1-methylalkyl)	● 4-(1-methylalkyl)	△ 2-(1-ethylalkyl)
▲ 4-(1-ethylalkyl)	□ 2-(1-propylalkyl)	■ 4-(1-propylalkyl)

somer had an $LD_{95} > 100$ ppm; the 2-(1-ethylhexyl) and (1-propylpentyl), 4-(1-methylheptyl), (1-ethylhexyl) and (1-propylpentyl) isomers had $LD_{95} > 1000$ ppm]. However, the acrylate and acetate of 4-(1-ethylhexyl)-2,6-dinitrophenol were highly active against spores of *Venturia* (Table I).

The activity against spores of *Fusarium* was also greatly enhanced on esterification of this C_8 alkylphenol ($LD_{95} > 1000$ ppm) to the acrylate (LD_{95} of 45 ppm) or acetate (LD_{95} of 50 ppm). Esterification of dinitrophenols with short (C_3 & C_4) alkyl side chains to crotonates enhanced the activity of the compounds against spores of *Venturia*. Thus, the LD_{95} values of 2-isopropyl and 2-t-butyl-4,6-dinitrophenols were > 100 ppm, whereas their crotonates had LD_{95} values of 30 & 80 ppm. Esterification of the dinitrophenols with longer (C_6 & C_7) alkyl side chains (and with the C_8 alkyl side chain)

to acrylates enhanced the activity against spores of *Venturia* (Table I). Increase in activity against the spores of *Fusarium* on esterification to the acrylate was also evident with 2-(1-methylpentyl)-4,6-dinitrophenol (LD_{95} of phenol > 100 ppm; of acrylate 70 ppm).

Etherification of highly active alkyldinitrophenols with LD_{95} values as low as 8 ppm against *Venturia* resulted in anisoles with LD_{95} values > 1000 ppm (Table I). This reduction may be due to the hydrolytic stability of the anisoles.

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ERRATA

In the paper by Girgis & Said *J. Sci. Fd Agric.*, 1968, **19**
    Page 615, Table II subheading: *for* 'Florida[4]'   *read* 'Florida[3]'

In the paper by Pianka, *J. Sci. Fd Agric.*, 1966, **17**
    Page 54, Title of Table XIV: *for* '6-R·$CH_2(NO_2)_2$·O·CX·YR'   *read* '6-R·$C_6H_2(NO_2)_2$·O·CX·YR''
    Compound 96 *for* 'CHMeBu' *read* 'CHMePr'

In the paper by Pianka & Edwards, *J. Sci. Fd Agric.*, 1967, **18**
    Page 358, Title of Table IV: *for* 'Contact acaricidal activity of dinitroalkylphenols and of certain salts' *read* 'Contact acaricidal activity of dinitroalkylphenols'

In the paper by Pianka and Browne, *J. Sci. Fd Agric.*, 1967, **18**
    Page 452, Table VIII: *for* 'Ethyl thiolcarbonate' *read* 'Ethyl thiolocarbonate'

In the paper by Pianka and Edwards, *J. Sci. Fd Agric.*, 1968, **19**
    Page 63, paragraph 3, line 14: *insert* '(No. 38)' *before* '(Table VII)' right hand column.

JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

NOVEMBER, 1968

1.—AGRICULTURE AND HORTICULTURE

General: Soils and Fertilisers

Concentration of toxicants in the rice soil profile. J. P. Hollis (*Phytopathology*, 1967, **57**, 461).—Established theory of the interrelationship of S^- and Fe^{2+} in rice soils (profile formation described) suggests that H_2S can exist in toxic proportions in rice soils only at $pH < 6·5$ with $[Fe^{2+}] \not> 10$ ppm. In three rice fields examined the max. $[H_2S]$ were 4, 8, and 12 ppm. Half of the total sol. S^- in one field was present as H_2S. In other fields sol. S^- occurred in much higher concn. (10- or 100-fold) than could be predicted by chemical theory. Sorption of H_2S on soil colloids may restrict its reaction with sol. Fe^{2+} and may explain the lack of odour in rice fields in spite of high concn. of H_2S found.   A. G. POLLARD.

Chloride exclusion by clay mineral surfaces. D. G. Edwards (*J. Aust. Inst. agric. Sci.*, 1967, **33**, 107).—Exclusion of Cl^- from both Fithian illite and Wyoming bentonite decreased as the hydration of the saturating alkali metal cation diminished. Results also confirmed the work of Aylmore and Quirk that double layers do not develop in Ca-clay systems.   E. G. BRICKELL.

Formation and swelling of complexes between montmorillonite and some organic compounds. B. K. G. Theng (*J. Aust. Inst. agric. Sci.*, 1967, **33**, 106).—Polyvinyl alcohols and some alkylammonium cations are adsorbed around clay crystals or clay domains within which swelling can still occur although adsorption of water vapour between domains is restricted.   E. G. BRICKELL.

Adsorption of amino-acids and peptides by montmorillonite and illite. R. H. Laby (*J. Aust. Inst. agric. Sci.*, 1967, **33**, 112).—Results suggest that any increase in the ionic strength of the soil solution will promote the desorption of org. cations held by cation exchange or proton transfer. In addition, uncharged intermediates which have positive dielectric increments (e.g. amino-acid dipolar ions) will tend to be salted out of the adsorbed phase while compounds such as plant phenols and sugars will tend to be salted on to the mineral surfaces.   E. G. BRICKELL.

Chemistry of soil minerals. IV. Synthetic alkylammonium montmorillonites and hectorites. R. M. Barrer and L. W. R. Dicks (*J. chem. Soc.* A, 1967, 1523–1529).—The hydrothermal synthesis of a number of aluminosilicate and gallosilicate montmorillonites at 200° and of hectorites at 100° having tetraethyl-, tetrapropyl- and tetrabutyl-ammonium ions in place of alkali metal is described. The products have been studied by electron-microscopy, X-ray diffraction, differential thermal analysis and thermogravimetry. In the montmorillonites there is considerable hydrolysis of the quaternary ions. The difference between the synthetic organo-clays and those prepared by ion-exchange, in basal spacing and swelling in org. solvents is described. In the synthetic minerals the quaternary ions were difficult to exchange. The effect of different cations on the gas-adsorbing area of the clays is studied. (10 references.)   E. J. H. BIRCH.

Effects of soil buffering capacity for phosphate on the relation between uptake of phosphorus and the phosphorus extracted by sodium bicarbonate. N. J. Barrow (*J. Aust. Inst. agric. Sci.*, 1967, **33**, 119–121).—Results of glass-house experiments with bromegrass suggest that early in the growth of annual plants the bicarbonate extraction procedure may overestimate the availability of P on soils of high buffering capacity, and may under-estimate availability on soils of low buffering capacity. For a given value of extractable P, uptake was greatest on soils of lowest buffering capacity.   E. G. BRICKELL.

Reactions of soil with condensed phosphates. I. Comparable insolubilisation of mono- and poly-phosphoric ions by Al, Fe and Ca

compounds. A. Malquori and L. Radaelli (*Industrie chim. belge, Chimie et Civilisation*, 1967, **32**, (III), 879).—Preliminary results are presented of a study of fixation, mobility and penetrability of ortho-, pyro-, trimeta- and tripoly-phosphates in soil. The systems $Al_2O_3·nH_2O$, $Fe_2O_3·nH_2O$, $CaCl_2$ and $CaCO_3$ together with mono- and poly-phosphoric ions were studied. (In French.)   M. SULZBACHER.

Inorganic transformation of water-soluble phosphates in some Indian soils of varying pH. B. K. Dhar and S. K. Saxena (*Technology, Q. Bull. Fertil. Corp. India*, 1966, **3**, 192–194).—Phosphorus fractionations of native and applied PO_4^{3-} carried out at specified time intervals are reported for four Indian soils (pH $5·3$ to $8·4$). Native P was present only as Fe phosphates (predominating in acid soils) or Ca phosphates (predominating in alkaline soils). Added water-sol. phosphates were converted to Al, Fe and Ca phosphates, the proportion of Fe phosphates increasing with time after application. Recovery of P also fell with time (up to 105 days) due to formation of 'occluded' phosphates or to some biological fixation. (17 references.)   E. C. APLING.

Influence of iron oxides on the physical properties of soils. T. L. Deshpande (*J. Aust. Inst. agric. Sci.*, 1967, **33**, 110–111).—Aggregates were separated from the soils, and iron oxides removed by dithionite treatment; changes in permeability of the aggregates and stability to wet sieving and mechanical dispersion were studied. There was no significant increase in swelling due to removal of iron oxides; sp. surface areas mainly decreased significantly. In several soils the adsorption of chloride was not reduced by removal of the oxides. It would appear that lack of effectiveness of iron oxides as aggregate stabilisers is due to adsorption of anions such as phosphate or silicate which render the particles negatively charged.   E. G. BRICKELL.

Changes in some physical and chemical properties of the surface of an impoverished red-brown earth soil under pasture. A. L. Clarke (*J. Aust. Inst. agric. Sci.*, 1967, **33**, 108).—Stability of aggregates in water increased mainly in the surface 2 in. and was greatest under *Lolium rigidum* and least under *Trifolium subterraneum*. Aggregation in the 0–3 in. layer, apart from seasonal variations, generally increased linearly with herbage yield. Infiltration capacity increased linearly with yield of herbage in preceding years. N accumulated in the top, 1 in. surface layer under all treatments (addition of urea and mulching) but not below this depth.   E. G. BRICKELL.

Effects of exotic softwood crops on the chemical fertility of a tropical soil. J. B. D. Robinson (*E. Afr. agric. For. J.*, 1967, **33**, 175–189).—Replacement of indigenous forest cover of a Humic Ferrisol with coniferous trees resulted in a decline in soil org. matter, reduced C/N ratio, decrease in exchangeable Mn and available P, and increase in pH. There were no consistent changes in soil N, exchangeable bases or % base saturation. Exchangeable H^+ and cation exchange capacity were not affected.   A. H. CORNFIELD.

Penetration of surface water and movement of subterranean water in arid zones. M. A. Tamers (*Acta cient. venez., Supl. No.* 3, 1967, **18**, 358–371).—The application of radiocarbon dating of carbonate species in groundwater to the study of water penetration and movement is described, and methods which are proposed for correction of results to eliminate the effect of dissolution of limestone (essentially radiocarbon-free) are applied to several aquifers in Venezuela. Ages ranging from modern to 35,000 years were obtained, and generally the older dates correlated well with expectation derived from climatic considerations. The practical importance of such studies for the management of available water supplies in arid zones is discussed. (20 references.)   E. C. APLING.

Instrument for soil moisture determination. M. Salaruddin and B. V. Khasbardar (*Indian J. Technol.*, 1967, **5**, 296–299).—The

portable moisture meter described is based on electrical resistivity, the two moisture-sensitive elements (concentric monel-metal mesh electrodes) containing, respectively, plaster of Paris/fibre glass (for H_2O contents from saturation to wilting point) and plaster of Paris alone (for contents below wilting point). The $\%$ of available and actual H_2O are obtained by referring the meter readings to a standard and to a separate calibration graph, respectively. The max. error is $\sim 2\%$ and no temp. correction is necessary for readings taken during a particular season. W. J. BAKER.

Exchangeable aluminium. I. Efficiency of various electrolytes for extracting aluminium from acid soils. J. B. Skeen and M. E. Sumner (*S. Afr. J. agric. Sci.*, 1967, **10**, 3–10).—The method described for the determination of exchangeable Al comprises a long series of successive extractions under identical conditions. The efficiency of cations (as chlorides) in 0.2 N solution is in the (descending) order NH_4, K, Ca, Mg, and Na. The amounts of Al released by each extraction are plotted in summation against the rotation no. of the extraction samples. Assuming the extracted amounts of exchangeable Al to be decreasing, and those of the non-exchangeable Al to be constant, the slope of the curve, after it has become rectilinear, will give the amount of non-exchangeable Al per extraction. The difference between the total and the non-exchangeable Al will give the exchangeable Al. (18 references.) P. S. ARUP.

Exchangeable aluminium. II. Effect of concentration and pH value of extractant on extraction of aluminium from acid soils. J. B. Skeen and M. E. Sumner (*S. Afr. J. agric. Sci.*, 1967, **10**, 303–309).— Further to Part I (cf. *ibid.*, 1967, **10**, 3) increases in the concn. of NH_4Cl from 0.1N to 0.5N caused increases in the apparent exchangeable Al removed, owing to the inclusion of some of the non-exchangeable Al. At higher concn. of NH_4Cl, a decrease was observed. The total amount of Al removed by 0.2 N-NH_4Cl increased with decreases in the pH from 4.2 to 3.8. The results are discussed. P. S. ARUP.

Decomposition of *Pinus radiata* litter on the forest floor. I. Changes in dry matter and nutrient content. G. M. Will. **II. Microfauna population.** J. H. Styles (*N.Z. Jl Sci.*, 1967, **10**, 1030– 1044; 1045–1060).—I. During 6 years under a stand of 33-year pines growing in silty volcanic sand, the dry-matter of freshly-fallen litter (in 1 mm mesh nylon bags) decreased steadily up to 3 years exposure (litter-wt. decreased 50% in 2 years), but subsequent decomposition was severely restricted, probably by exclusion of earthworms, etc. Concn. of P, Ca, Mg and K, but not of N, decreased greatly (K by $\sim 80\%$ during the first 3 months, P almost as much); loss of Ca was slow. Concn. of Mg increased during the first year and that of N during the second. Final chemical composition of natural litter around the bags was similar to that of the enclosed litter. (20 references.)

II. Microfauna in the bagged litter were examined periodically during $4\frac{1}{2}$ years. No. of specimens was max. during second and third years but then decreased rapidly; the pattern of species reflected progressive litter decomposition; the omnivorous mites (especially Oribatei) increasing greatly in the third year. When the bags were well covered with natural litter, etc. the no. of insects and arthropods decreased greatly. Because the nylon-mesh excluded macrofauna, the final litter-breakdown was less than that of natural litter. (17 references.) W. J. BAKER.

Soil organic phosphorus. IV. Fractionation of organic phosphorus in alkaline soil extracts and the identification of inositol phosphates. J. K. Martin and A. J. Wicken (*N.Z. Jl agric. Res.*, 1966, **9**, 529–535).—Fractions, isolated from five N.Z. soils on ion-exchange resin columns by a method previously described (Martin, *ibid.*, 1964, **7**, 750) were further subdivided. T.l.c. showed that for each soil, fraction no. 4 contained inositol mono- and di-phosphates (range 1 to 7% of total soil org. P), and fraction no. 5 contained inositol penta-/hexa-phosphates (range 4 to 42% of total soil org. P). No other P esters were detected in these fractions. Inositols isolated after hydrolysis of the phosphate deriv. were identified by paper chromatography and electrophoresis. Myo- and *dl*-inositols were present in the five soils in a nearly constant ratio (myo-: $dl = 1.8$ to $4.0 : 1$). (21 references.) E. G. BRICKELL.

Factors affecting solubility and possible fractionation of organic colloids extracted from soil and leonardite with an acetone–water– hydrochloric acid solvent. L. K. Porter (*J. agric. Fd Chem.*, 1967, **15**, 807–811).—Colloids were easily extractable from a soil and from leonardite (an oxidised lignite coal) with aq. HCl mixtures containing $COMe_2$ (optimum concn. $\sim 80\%$) or nine other org. solvents: they were recovered from the solvent by dialysis and

evaporation of the dialysate, and their solubility characteristics were investigated in a large no. of solvents, and solvent combinations. Fractionation of the colloids from the org. solvents on lipophilic Sephadex Gel LH-20 is proposed. (29 references.) P. S. ARUP.

Air oxidation of prepared humic acids. L. M. Du Plessis and F. W. Pauli (*S. Afr. J. agric. Sci.*, 1967, **10**, 101–112).—Five humic acids were prepared by the biological decomposition of maize stalks and leaves, two with additions of glycine, two with NH_4NO_3 and one without added N. Samples of the acids extracted with aq. NaOH $+ SnCl_2$ were submitted to atm. oxidation at $170°$ for 1500 h. Yields of humic acids were inversely proportional to the C/N ratios of the composts. As regards functional groups and C, H, and N composition, the five acids were almost identical, but they differed considerably from a commercial acid. (21 references.) P. S. ARUP.

Combined effect of temperature and desiccation on *Beijerinckia* species in soil. B. W. Strijdom and C. J. Steenkamp (*S. Afr. J. agric. Sci.*, 1967, **10**, 197–202).—At a high level of desiccation three out of four strains were eliminated from soil kept at $30°$ during 114 days. The effects were less severe at a lower level of desiccation, or when the soils were kept at $10°$. P. S. ARUP.

Response of soil bacteria to high temperatures and diurnal freezing and thawing. R. S. Young, P. H. Deal and O. Whitfield (*Nature, Lond.*, 1967, **216**, 355–356).—After exposure of soil to dry heat at 135–$200°$ for 72–96 h very few micro-organisms survived, but some were still viable when the soil was heated for 16–24 h and then submitted to freeze–thaw cycles. Even very low concn. of unsterile soil (0.01 mg per ml) in glucose-broth resulted in growth of micro-organisms under freeze–thaw conditions, after only ~ 48 h total growth-time. Micro-organisms continued to grow when incubated in soil impregnated with mineral salts–sugar media, even after initial heating for 2–24 h at $135°$, but survival was greatly diminished during freeze–thaw cycles after heating at $135°$ for 4–8 h or more. In general, freeze–thaw conditions following heat treatment impede bacterial growth in an environment where they would otherwise grow. These results and those reported previously (*ibid.*, 1963, **199**, 1078) are discussed briefly in respect of the ability of possible space-craft contaminants to survive and grow in a freeze-thaw atm., e.g. as on Mars. W. J. BAKER.

Three-channel flame photometer for soil-analysis. O. E. Clinton (*N.Z. Jl Sci.*, 1967, **10**, 1069–1075).—The apparatus is designed for the determination of K, Ca and Mg in the NH_4 acetate extracts; flame emission of K and Ca and atomic absorption for Mg are measured simultaneously. Calibration permits direct readings of K and Ca concn. in units convenient for soil tests. About 35,000 analyses during a year have been made, mainly for advisory purposes. W. J. BAKER.

Dynamics of minor elements in soil. A. Cottenie and R. Gabriels (*Industrie chim. belge, Chimie et Civilisation*, 1967, **32**, (III), 864– 867).—A method for study of the dynamics of trace elements such as Fe, Al, Mn, Zn, Cu in soil is discussed. Different fractions are analysed. Tables of content of trace elements in soil samples from Belgium are given and agronomic aspects are considered. (In French.) M. SULZBACHER.

Sulphur in soil and plant relationships. S. Roberts (*Diss. Abstr.* B, 1967, **27**, 2962).—The available S in soil was evaluated by a rapid routine method using the X-ray spectrograph for determining the S extracted by 0.005 M-$CaCl_2$. To intensify the X-emission from low-S extracts, cations present having high X-ray absorption coeff. were exchanged for H^+ in a filter cation-exchange unit after which a definite amount of LiCl was added before evaporating to dryness and prep. for mineral analysis. The precision of the method varied from ± 2 to $\pm 10\%$ of the amount of S present in extracts containing 0.5–14 ppm of S. In a comparison of extractable S and plant-available S in soil, samples were taken at depths down to 6 ft. in 11 different soils. In nine of the soils the total S extracted by 0.005 M-$MgCl_2$ determined by X-ray spectrograph, and the reducible S extracted by 0.1 M-LiCl (determined by the methylene blue method) were significantly correlated with the S accumulated in the tops of wheat plants grown for 3 weeks in combined sand–soil cultures. Under fallow conditions an appreciable amount of N but no S was mineralised during June–Aug. (Washington U.S.A.) whereas under wheat both exchangeable NO_3^--N and extractable S declined during this period. Mutual effects of N and S and of org. matter in soils on immobilisation processes are examined. A. G. POLLARD.

Some aspects of the relation between labile soil phosphorus and plant response. I. F. Fergus (*J. Aust. Inst. agric. Sci.*, 1967, **33**,

111).—For any one soil of the six tested, the capacity values of the labile P pool for three plant varieties were similar, although there were small significant differences between the species in total P uptake. Irregularities in the curves relating to isotopically exchangeable P, were suppressed by addition of sodium azide, suggesting microbial interference.　　　　E. G. BRICKELL.

Evaluation of agricultural soils by enzymic methods. J. R. Ramírez Martínez (*Acta cient. venez., Supl. No.* 3, 1967, **18**, 93–97).—Problems of soil amelioration in developing countries are briefly discussed. The validity of the practice of liming acid soils in order to increase the rate of mineralisation is indicated by correlations found between grass yield and content of organic P in soils with pH > 5·5, and by the observation that phosphatase activity in soil is generally insignificant at pH < 5·0 and attains a max. at pH 7–8.　　　　E. C. APLING.

Phosphate sorption by soils as a measure of the phosphate requirement for pasture growth. P. G. Ozanne and T. C. Shaw (*Aust. J. agric. Res.,* 1967, **18**, 601–612).—A good correlation between phosphate sorbed, and phosphate required was found; the relationship was linear over the range 0–500 ppm of sorbed P. Although affected by different sampling depths, equilibrium concn., and degree of soil mixing, the relationship remained linear with a correlation coeff. ~0·9. (16 references.)　　　　E. G. BRICKELL.

Fate of labelled mineral nitrogen after addition to three pasture soils of different organic matter contents. J. R. Simpson and J. R. Freney (*Aust. J. agric. Res.,* 1967, **18**, 613–623).—Recoveries of ^{15}N within the soil–plant system were high (90–100%) except when nitrate was added to a high-N soil (69%). Labelled ammonium was immobilised rapidly, particularly in low-N soils, but nitrate reacted much more slowly. However, the total mineral N (labelled and indigenous) in unplanted soils and the total plant uptake of N were not appreciably affected by the chemical form of the added N. (29 references.)　　　　E. G. BRICKELL.

Influence of some soil and fertilising factors on manganese content of grass. B. van Luit (*Landbouwvoorlichting,* 1967, **24**, 302–304).—The availability of Mn to grass is notably decreased by applications of alkaline fertilisers e.g. Nitro-chalk, but may be increased by the acid fertiliser $(NH_4)_2SO_4$. Satisfactory uptake of Mn occurs on sandy or clay soils of pH ≯5·5. The soil-C/N ratio had no effect on the uptake.　　　　P. S. ARUP.

Uptake of zinc and phosphorus by plants from a sandy soil. M. C. Laker (*S. Afr. J. agric. Sci.,* 1967, **10**, 323–330).—Applications of P (60 ppm as superphosphate) increased the citric acid-sol. P much more than the water-sol. P; Zn uptake by maize was also increased. Plant yields increased linearly with P applications up to 20 ppm but those at 60 ppm were less effective. (14 references.)　　　　P. S. ARUP.

Effect of previous applications of lime and zinc on subsequent uptake of phosphorus and fertiliser zinc by rye plants in a pot experiment. M. C. Laker (*S. Afr. J. agric. Sci.,* 1967, **10**, 11–18).—In the soils previously used in a field experiment (cf. *ibid.,* 1966, **9**, 107) the applied lime stimulated P-uptake without, however, increasing plant growth. The lime reduced P-absorption by the soil; it also reduced Zn-uptake from the topsoil, but not from the subsoil. Zinc stimulated P-uptake when applied without lime. (19 references.)　　　　P. S. ARUP.

Potassium nitrate. I. Y. Araten, A. Baniel and R. Blumberg (*Fertil. Soc.,* 1967, 16 pp).—An account is given of the uses and advantages of KNO_3 as a fertiliser for various crops and of the economic aspects of its manufacture. Details are given of a process developed by Haifa Chemicals Ltd. for the economical manufacture of KNO_3 by the treatment of solid KCl with 60–70% HNO_3 in the presence of C_5 alcohols at 5°–10°. The org. solvent extracts HCl in preference to HNO_3 leaving solid KNO_3 as the main product. The HCl, obtained by countercurrent extraction, is evaporated to azeotropic composition, and can be used for the treatment of phosphate rock. (34 references.)　　　　P. S. ARUP.

Lime acid soils for better crops. K. N. Synghal (*Fertil. News,* 1967, **12**, No. 12, 15–20).—A review of the literature regarding use of agricultural limestone in India. Methods, frequency and cost of lime application are also discussed. (15 references.)　　　　I. DICKINSON.

Fragmented rock gypsum as a sulphur fertiliser. J. K. Powrie (*J. Aust. Inst. agric. Sci.,* 1967, **33**, 127–129).—A single dressing of coarsely-crushed rock gypsum has value as a S fertiliser for light soils in high-rainfall areas where fine gypsum is of little use unless more than one application is made during the season.　　　　E. G. BRICKELL.

Caking in fertilisers: pulverised materials as anti-caking agents. S. Varma and N. P. Misra (*Technology, Q. Bull. Fertil. Corp.*

India, 1966, **3**, 121–125).—Retention of anti-caking agents (limestone and soapstone) on $CaNH_4$ nitrate was studied. Retention of soapstone was small although the anti-caking effect was significant; retention of limestone generally increased with reduction in particle size, but varied greatly between samples, and anti-caking effect was directly related to retention. A generalised simple expression is derived for the determination of the amount of anti-caking agent required to give monolayer coverage of the spheroidised fertiliser granules, useful to indicate the min. requirement of anti-caking agent. Soaptsone appears to prevent direct contact between granules, while limestone action is related to its ability to absorb the saturated solution of the fertiliser. Use of anti-caking agents is not a substitute for efficient drying or proper packaging.　　　　J.A.C. ABSTRACT.

Cobaltic biuret complex. P. K. Pal, R. M. Sanyal and B. K. Bannerjee (*Technology, Q. Bull. Fertil. Corp. India,* 1966, **3**, 217–218).—Spectrophotometric and conductometric studies of the interaction of $[Co(NH_3)_6]Cl_3$ and biuret at pH 1·6 are reported, showing that the biuret-Co^{3+} complex is formed in the ratio of 3 : 1.　　　　E. C. APLING.

Rapid ammonium citrate method for determination of available phosphorus [in fertilisers]. G. O. Guerrant, J. D. Hunter and C. H. McBride (*J. Ass. off. analyt. Chem.,* 1967, **50**, 1273–1279).—Available P is extracted from a fertiliser sample on a glass filter paper in a Gooch crucible in 10–20 min. by repeated spraying with neutral NH_4 citrate solution at 65° from a hypodermic syringe. The volumetric quimociac method (suitably modified), gave satisfactory results for the determination of P.　　　　A. A. ELDRIDGE.

Comprehensive method for [determination of] nitrogen [in fertilisers]. C. W. Gehrke, J. P. Ussary, C. H. Perrin, P. R. Rexroad and W. L. Spangler (*J. Ass. off. analyt. Chem.,* 1967, **50**, 965–975).—N as NO_3^-, NO_2^- in presence of org. matter, and NO_3^- in presence of Cl⁻ is reduced by Cr powder in boiling aq. HCl; after addition of K_2SO_4, HgO and H_2SO_4 the solution is heated to fuming. The use of antiforming additives is desirable. The method is applicable to all fertilisers and all forms of fertiliser-N. (16 references.)　　　　A. A. ELDRIDGE.

Determination of molybdenum in fertilisers by atomic absorption spectrophotometry. W. L. Hoover and S. C. Duren (*J. Ass. off. analyt. Chem.,* 1967, **50**, 1269–1273).—After removal of NO_3^- with H_2SO_4, KCNS is used as complexing agent, Mo being extracted into isoamyl alcohol and thus separated from interfering substances, particularly Mn, Ca and Sr. The separated org. phase is aspirated through the atomic absorption burner and the extinction is compared with that of standards. The effect of Fe up to 0·5% is negligible; from 0·5 to 2% the interference can be overcome by measuring the amounts of KCNS and $SnCl_2$ used in preparing the sample solution for extraction. 2·5 ppm of Mo in fertilisers can be accurately determined.　　　　A. A. ELDRIDGE.

Fly ash in agriculture. J. P. Capp and C. F. Engle (*Inf. Circ. U.S. Bur. Mines,* 1967, No. 8348, 210–220).—Merits of the ash are: high pH to neutralise acid soils, higher content of trace elements (especially B) than normal soils, higher water retention, and loosening effect on compact or heavy soils. Soil modification or conditioning is more effective if sintered ash is used, the raw ash having less desirable features. Recent experiences are summarised. Red clover and rye grew in sintered fly-ash/soil (1 : 1; 3 : 1) and oats in raw fly-ash/peat (1 : 3) without serious toxicity. Fescue grass and lucerne were successfully established with, good root-growth, on fly ash/soil mixtures and also on reclaimed mining-spoil (pH 3·5) when 600 tons of fly-ash per acre were mixed in to a depth of ~ 1 ft. Rate of release of B during leaching of either raw or sintered fly-ash is almost constant during the growing season.　　　　W. J. BAKER.

Physical improvement of soil by means of Styromull. R. Eder (*Mitt. Klosterneuburg Rebe u. Wein Obstb. u. Früchteverwert.,* 1967, **17**, 259–263).—Pot experiments were made on the growth of vines in heavy soils mixed with Styromull, a byproduct of the manufacture of insulating material. The most satisfactory results as regards improvement in soil structure and plant growth were obtained with additions of 40% of Styromull. (10 references.)　　　　P. S. ARUP.

Use of fluorocarbon resins on plough mouldboards to reduce adherence of clay. I. M. W. Wood (*J. Aust. Inst. agric. Sci.,* 1967, **33**, 124–126).—Provided wear characteristics are satisfactory then fluorocarbon resin-coated mouldboards offer considerable promise for ploughing on colloidal soils. They reduce draught requirements (9%), enable ploughing to be commenced at a higher level of soil moisture, and also greatly improve the efficiency of the ploughing operation.　　　　E. G. BRICKELL.

Plant Physiology, Nutrition and Biochemistry

Delayed damage to *Phaseolus vulgaris* L. seeds by water trapped during soaking. W. Heydecker and P. J. Orphanos (*Nature, Lond.,* 1967, **216**, 388–389).—Short soaks are favourable to germination but soaking for > 4 h causes progressive damage through entry of excess water into the intercotyledonary cavity, drainage then being prevented by the swollen cotyledons. Damage, which suggests a detrimental imbalance in O_2 supply to the embryo (high outside, low inside), is completely prevented by (1) stripping the testae off the soaked seeds to release trapped water, (2) a 1% H_2O_2 soak so that enzymatically released O_2 can slowly expel water from the intact seeds, which will then germinate immediately on removal from the solution, (3) allowing the intact seeds, before soaking for 24 h, to imbibe aerobically $\sim 33\%$ of the water needed for radicle emergence. Germination is probably initiated early during such imbibition and then continues despite the subsequent restriction of O_2 supply.   W. J. BAKER.

Structure of the cuticle wax of prune plums and its influence as a water barrier. J. M. Bain and D. McG. McBean (*Aust. J. biol. Sci.,* 1967, **20**, 895–900).—Electron microscopy showed an inner layer of a matrix of thin platelets and an outer layer composed of fragile projections, many of which appeared tubular. The incidence and complexity of the projections increased as the fruit matured. Deposits of wax remained uniform at about 300 $\mu g/cm^2$ and thickness 3–5 μ. Wax acts as a water barrier in drying but the effect may be minimised by raising the drying temp. to 65°.   E. G. BRICKELL.

Influence of root temperature and nutrient applications on root growth and mineral nutrient of *Taxus media* and *Forsythia intermedia* during the dormant season. M. M. Meyer, jun. and H. B. Tukey, jun. (*Proc. Am. Soc. hort. Sci.,* 1967, **90**, 440–446).—The roots of both *Taxus* and *Forsythia* continued to grow whilst the above-ground parts were dormant. Application of N, P, and K during the dormant season increased the nutrient content of the above-ground parts. Growth in the following spring was increased by fertiliser application during the dormant season, the effect increasing with earliness of application.   A. H. CORNFIELD.

Absorption of radioactive phosphate from tap and nodal roots of white clover *Trifolium repens* L. M. Uengo and R. Dorrington Williams (*J. Br. Grassld Soc.,* 1967, **22**, 165–169).—In nutrient solutions containing ^{32}P-labelled phosphate the P absorbed by the tap-root was distributed evenly within the whole plant. The ability of nodal roots to compensate for loss of the tap-root depended on their position and size. When translocation from the 2nd and 7th nodal roots was studied, similar distribution to that from the tap-root occurred only from the nodal root closer to the centre of the plant. The backward movement of ^{32}P absorbed from nodal roots at the base of the plant increased with root size. Removal of tap-roots resulted in a temporary depression of translocation which disappeared within 3 weeks.   A. H. CORNFIELD.

Foliar leaching of cations from spinach. H. E. Hohlt and D. N. Maynard (*Proc. Am. Soc. hort. Soc.,* 1967, **90**, 296–300).—Spinach plants in sand cultures subjected to artificial rainfall lost significant amounts of Mg^{2+} and Ca^{2+}, but not of K^+, from the leaves, but usually only when nutrient Mg^{2+} supply was high. Influx and efflux rates for Mg^{2+} through isolated cuticles were related to stomatal frequency. The influx rate was greater than the efflux rate through both lower and upper cuticles.   A. H. CORNFIELD.

Manganese toxicity in celery. P. A. Gallagher (*Nature, Lond.,* 1967, **216**, 391–392).—Necrotic lesions on leaflets and petioles of *Apium graveolens* L. (var. Latham's self-blanching) occurred in ~ 20–30% of a crop grown on a deep, poorly drained, acid-peat soil (Raheen Series) to which sea-sand had been previously applied. Concn. of Mn in the affected leaflets and petioles ranged from 700 to > 10,000 ppm in mature plants, the concn. in leaflets of badly affected plants being 100-fold greater than expected. Concn. of Al in the affected parts were only slightly higher than in healthy plants. The extent of damage depended on the depth to which sea-sand had been mixed in; a depth of 6–8 in. resulted in a pH suitable for healthy growth, whereas 2–4 in. depth was insufficient. The high concn. of Mn and the resulting damage were thus caused by absorption of Mn from acid soil (pH 3·9) beneath the plants where the sand had been inadequately mixed (2–4 in. depth) with the peat.   W. J. BAKER.

Collection and partial characterisation of volatile selenium compounds from *Medicago sativa* L. C. J. Asher, C. S. Evans and C. M. Johnson (*Aust. J. biol. Sci.,* 1967, **20**, 737–748).—Tops and roots of the plants grown in solutions containing ^{75}Se-labelled

selenite or selenate lost 0·5 to 3% of their Se content when dried at 70° for 48 h. The volatile products were passed through cold-traps and absorbed on active C. Most of the volatile Se could be extracted from the C with water and the remainder with Et_2O. The water-sol. fraction was separated into two Se compounds by chromatography on Dowex 1-X8 resin: these compounds were also obtained from the intact plants, but not from the used culture solutions. (10 references.)   P. S. ARUP.

Examination of cereals fumigated with phosphine. I. The activity of glutamic acid decarboxylase after fumigation. M. Rohrlich and F. Meuser (*Getreide Mehl,* 1967, **17**, 121–125).—A simple method for the precise determination of glutamic acid decarboxylase (*GDC*) activity is described, based on titrimetric determination of trace quantities of CO_2 in the gas phase with the apparatus of Gorbach and Ehrenberger. Application of the method to samples of wheat, rye and barley fumigated with Phostoxin pellets showed no changes in *GDC* activity due to fumigation.   E. C. APLING.

Carbon dioxide limitations of plant growth in tube culture with special reference to legume nodulation studies. A. H. Gibson (*Aust. J. biol. Sci.,* 1967, **20**, 837–842).—In comparative experiments with lucerne and clovers, plants grown with unenclosed shoots thrive much better than did totally enclosed plants. The latter plants developed better under gauze caps than under cottonwool plugs or loose metal caps. The main factor, limiting growth in tubes, is attributable to restriction of the diffusion of atm. CO_2 into the tubes.   P. S. ARUP.

Influence of host on soil and rhizosphere populations of clover and lucerne root nodule bacteria in the field. A. C. Robinson (*J. Aust. Inst. agric. Sci.,* 1967, **33**, 207–209).—Rhizospheres and surrounding soils of *Trifolium subterraneum* L., *Medicago sativa* L., and *Poa australis* R.Br were studied. *Rhizobium trifolii* was detected in all the samples tested. *Rh. meliloti* was found only in the soils and rhizospheres of *M. sativa* and *P. australis*, growing in association with *M. sativa*, and was not associated with *T. subterraneum* even where *M. sativa* was found within several inches of the clover.   E. G. BRICKELL.

Examination of the numbers of nodule bacteria associated with legume seed following commercial multiple inoculation. J. Brockwell and R. J. Roughley (*J. Aust. Inst. agric. Sci.,* 1967, **33**, 204–207).—There was great variation between different samples of the same species and in only one sample out of three varieties did the level of inoculation reach that achieved in previous experimental work. (11 references.)   E. G. BRICKELL.

Translocation of sugars in *Cucurbita melopepo*. Effects of temperature change. J. A. Webb (*Pl. Physiol.,* 1967, **42**, 881–885).—The movement of ^{14}C-labelled sugars, basipetally or acropetally, ceased almost completely if portions of the stems of the plants were subjected to localised temp. of 0°; a partial inhibition occurred at 10° and a variable effect was noted at 15°. Between 15° and 35° temp. was no longer a controlling factor in translocation. Partial inhibition of translocation again occurred at 45° and movement ceased at ~ 55°. The temp. treatments had no effect on the rate of assimilation or the export of ^{14}C-compounds by the leaf blade. Temp. inhibiting movement in the initial direction caused diversions to other regions of the plant. The nature of the response to temp. was similar in all organs of the plants and was comparable with that of protoplasmic streaming in plants sensitive to low temp.   A. G. POLLARD.

Polar transport related to mobilisation of plant constituents. J. B. Zaerr and J. W. Mitchell (*Pl. Physiol.,* 1967, **42**, 863–874).—Use of ^{14}C-labelled compounds demonstrated the polar movement of indole-3-acetic acid (I), dicamba (2-methoxy-3,6-dichlorobenzoic acid), sucrose and mannitol through 5 mm segments of bean hypocotyls. Experimental data support the hypothesis of the movement of I and other endogenous and exogenous substances through stem segments to extents controlled by the mobilisation and utilisation of plant constituents at the growth centres.   A. G. POLLARD.

Transport of carbon in wheat plants. W. B. McConnell and M. Mazurek (*Can. J. Biochem.,* 1967, **45**, 1853–1861).—Using radioactive tracers, it was shown that acetate, injected into the top internodes of wheat plants, is metabolised in 24 h and that it is utilised before translocation from the stem. Movement of the tracer was largely toward the head of the plant and the kernel constituents were specially radioactive. Free glutamic acid (I) was an early metabolic product. Protein-bound (*PB*) I did not reach a max. specific activity (*SA*) until 1–3 days after injection. Max. *SA* of the kernel-protein I occurred about the same time but exceeded the *SA* of the free I in the kernels. However the relationship between the *SA* of the free and *PB* I of the other plant parts was not con-

sistent with the view that protein I was derived solely from a uniform pool in the particular plant part under consideration. ^{14}C incorporated by exposing the head of the wheat part to $^{14}CO_2$ was effectively retained in the head, but extensive transport of ^{14}C from the chaff to the kernel was observed. Exposing the leaf to $^{14}CO_2$ resulted in extensive respiration of $^{14}CO_2$; appreciable ^{14}C appeared in the head of the plant but some translocation of the tracer to the lower stem parts was observed. (15 references.)

C.V.

Uptake and metabolism of vitamins-E and -K by pea-stem sections. J. K. Gaunt and B. B. Stowe (*Pl. Physiol.*, 1967, **42**, 859–862).— Sections (10 mm long) of pea-stems were incubated with a nutrient solution, a buffer solution (pH 5·5) and α-tocophenol or vitamin K_1. The vitamins were extracted after 20–24 h (CHCl₃). Within the tissue vitamin K_1 is generally distributed and remains stable. α-Tocopherol is probably catabolised enzymically, the process requiring O_2. No association with lipoxidase was apparent.

A. G. POLLARD.

Deoxyribonucleic acid content of tobacco leaves. J. W. Lyttleton and F. A. Hole (*N.Z. Jl Sci.*, 1967, **10**, 1061–1086).—During leaf growth from 5 cm to full maturity the concn. of DNA can increase up to 30-fold. This is ascribed either to strong endomitosis leading to polysomaty, or to abnormal persistence of cell division. The concn. of DNA at maturity depends on the position of the leaf on the plant. (13 references.)

W. J. BAKER.

Characterisation of pectic substances in tobacco stems. H. Jacin, R. J. Moshy and J. V. Fiore (*J. agric. Fd Chem.*, 1967, **15**, 1057–1062).—A scheme is described for the fractional extraction of the pectic substances from the stems and their examination by t.l.c. i.r. spectrography, and a colorimetric test for galacturonic acid. No simple rules could be made for distinguishing between the pectic distribution in cigar and cigarette tobacco stems. (12 references.)

P. S. ARUP.

Protein bodies of the soyabean. M. P. Tombs (*Pl. Physiol.*, 1967, **42**, 797–813).—The protein bodies of cotyledon cells were examined microscopically, before and during germination. The bodies contained ∼ 70% of bean protein; glycinin was the only protein detected. Light and heavy fractions contained, respectively, 97·5 and 78·5% protein. Ribonucleic acid, phytic acid and lipids were also present.

A. G. POLLARD.

Rôle of ions in the etiology of maple decline. J. B. Kotheimer, A. E. Rich and W. C. Shortle, jun. (*Phytopathology*, 1967, **57**, 342).—Among 500 samples of sap and foliage of sugar maple (*Acer saccharum*) examined, a significant correlation was found between Cl^- contents and the extent of damage due to 'decline'. The Cl^- levels were relatively much higher than the corresponding Na^+ levels except in cases of terminal decline. Norway maple (*A. platanoides*) showed much greater tolerance to Cl^-. In nutritional studies of sugar maple seedlings the uptake of Cl^- was greater when the K^+ level was adequate than when it was deficient. Symptoms of 'decline' corresponded with the presence of Cl^- in the nutrient but abnormal growth was associated with high- Na^+ feeding in absence of Cl^- or with min. Ca^{2+} levels. In winter salting trials $CaCl_2$–NaCl mixtures were less harmful to the trees than was NaCl alone.

A. G. POLLARD.

Chemical control of flowering in *Bougainvillea*. W. P. Hackett and R. M. Sachs (*Proc. Am. Soc. hort. Sci.*, 1967, **90**, 361–364).— Application of Cycocel (2-chlorethyltrimethylammonium chloride, 2 g per 6 in. pot) as a soil drench or foliar sprays of B-9 (1,1-dimethylaminosuccinamic acid, 2500–10,000 ppm) promoted flowering of *Bougainvillea* under short day- but not under long day-conditions. In this way flowering occurred before excessive elongation of the plant occurred.

A. H. CORNFIELD.

Effect of pretreatment of corn [maize] seed with 6-substituted purines on growth of plants. D. Tuttle and C. G. Skinner (*J. agric. Fd Chem.*, 1967, **15**, 982–983).—Maize seeds were pre-soaked for 12 h in solutions of five different 6-substituted aminopurine deriv. (10 μg/ml) and grown as water cultures. Although the treatment stimulated early growth, it failed to give increased yields when used in field experiments. (16 references.)

P. S. ARUP.

Plant growth regulators [selected titles]. *Ann. N.Y. Acad Sci.*, 1967, **144**, 1–382).—Auxin and the mechanical properties of the cell wall. R. Cleland (15 references). Cell-wall deposition and the distribution of cytoplasmic elements after treatment of pea internodes with auxin analogue 2,3,5-tri-iodobenzoic acid. G. B. Bouck and A. W. Galston (42 references). RNA and protein biosynthesis and the regulation of cell elongation by auxin. J. L. Key, N. M. Barnett and C. Y. Lin (42 references). Steroid derivatives as native auxins in *Coleus*. J. C. Vendrig (30 references). Polarity of auxin trans-

port. A. C. Leopold and R. K. de la Fuente. **Comparison of the movement and vascular differentiation effects of the endogenous auxin and of phenoxyacetic weedkillers in stems and petioles of *Coleus* and *Phaseolus*.** W. P. Jacobs (35 references). Oxidation of indole-3-acetic acid by peroxidase enzymes. W. J. Meudt (21 references). Observations on physiology and radiation response of auxin formation by animal tissues. S. A. Gordon and E. Buess (24 references). Effect of ultra-violet radiation on auxin-controlled abcission. R. M. Klein (42 references). Relations among cell growth, DNA synthesis and gibberellin action. A. Lang and J. Nitsan (24 references). Rôle of gibberellins in the control of intercalary growth and cellular differentiation in developing *Avena* internodes. P. B. Kauffman (20 references). Gibberellin production in the fungus *Gibberella fujikuroi*. B. O. Phinney and C. Spector (31 references). The flowering hormone: physiological evidence. F. B. Salisbury (37 references). Relationship between Florigen and the flower hormones. D. J. Carr (44 references). Effects of 3-amino-1,2,4-triazole on the carbohydrate metabolism of plants. J. F. Fredrick and A. C. Gentile (25 references). 2,3,6-trichlorobenzoic and 2,6-dichlorobenzoic acid interference with phototropic and geotropic responses of seedlings. L. C. V. Beek (21 references).

C.V.

Inhibition of plant growth by halogenated benzoic acids. C. W. Huffman, E. M. Godar and D. C. Torgeson (*J. agric. Fd Chem.*, 1967, **15**, 976–981).—Of 34 halogen-substituted benzoic acids tested as sprays on tomato and bean plants (and some on soyabean plants) the 2,3,5-substituted (in various combinations of Cl, Br, and I) proved the most effective. Eight deriv. of TIBA (2,3,5-tri-iodobenzoic acid) were generally less effective than TIBA itself. As regards max. inhibition at low dosage rates and min. phytotoxicity, TIBA gave better results than any of the other compounds tested. (26 references.)

P. S. ARUP.

Disease resistance breeding [in crop plants]. R. D. Brock (*J. Aust. Inst. agric. Sci.*, 1967, **33**, 72–76).—Single-gene high-level resistance provides quick and relatively cheap protection but in most cases lacks stability. It needs to be supplemented with protection against a broad spectrum of races of pathogenic organisms and conditioned by many genes. (22 references.)

E. G. BRICKELL.

Crops and Cropping

Weather-protected nets [for crops] of high-density polyethylene monofilaments. U. Dedini (*Materieplast.*, 1968, **34**, 535–539).— The applications of polyethylene nets for protection of fruit crops (e.g. apples) against hail are described. The nets used, of high density polyethylene monofilament (0·28 mm dia.) are 2·5 m × 150 m, with a mesh size of approx. 3 × 5 mm; they cost ∼120 lire/m². Methods of application are illustrated.

C. A. FINCH.

New uses of petroleum in modifying the microenvironment of crops. G. Chambers and M. Waddell (*Industrie chim. belge, Chimie et Civilisation*, 1967, **32**, (II), 670–673).—Microenvironment modification methods in agriculture and in particular absorptive and reflective soil coatings are discussed. Field data to illustrate the performance of petroleum products in seed germination and stand establishment in cold and warm soils are presented. The protection of fruit-bearing trees under freezing conditions by solid fuel convective heaters is described. (In English.)

M. SULZBACHER.

Zinc deficiency of field and vegetable crops in the West. F. G. Viets, jun. (*Leafl. U.S. Dep. Agric.*, 1967, No. 495; 8 pp).—Sensitive crops, deficient soils, symptoms, diagnosis, corrective and preventive treatment, are described.

E. G. BRICKELL.

Effect of heredity on criteria of quality in brewing barley. G. Aufhammer, G. Fischbeck and L. Reiner (*Brauwissenschaft*, 1968, **21**, 177–182).—The relative effects of variety and environment on the total variability of corn yield and properties were studied over a period of 15 years in 15 European countries. The factors through which heredity causes high contributions to the total variation are in decreasing order of effect: Diastatic power, attenuation limit, hot water extract, Kolbach index, Brabender value and viscosity. These data can assist in selecting new strains suitable for brewing. (27 references.)

I. DICKINSON.

A new approach to fertilisation of paddy rice. H. Nicol (*Chilean Nitrate Agric. Serv. Inf.*, 1968, No. 102; 5 pp).—For growing paddy on flooded fields it has been customary to regard $(NH_4)_2SO_4$ as the most suitable fertiliser. This paper describes how rice yields can be greatly increased by fertilisation with nitrate-N, despite the fact that it is readily leached from the soil. Three 'production' methods are outlined; the paper is a summary of the work carried out earlier by Matsushima *et al.*

P.P.R.

Potato-grafting as source of virus strains dangerous for the crop. A. Rosendaal (*Proc. Third Triennial Conf. EAPR*, 1966, 232–233).—Results of laboratory experiments are recorded in which the common X-viruses from the potato Eersteling (Duke of York) acquired, presumably by mutation, widely increased host-ranges after passage to the progeny of top-necrosis-diseased plants. The new types, X-3 and X-4, caused intervenous mosaic disease of varying severity. Interactions between virus types and potato genes are considered.   P. S. ARUP.

Relation of rate of nitrogen fertilisation, seed spacing and seed size to yield of potatoes. P. F. J. Van Burg (*Neth. Nitrogen Tech. Bull.*, 1967, No. 4; 30 pp.)—Experiments with seed (Sirtema) (I) and industrial (Mentor) (II) potatoes are described. With I, tuber dry-matter content was decreased by increasing the rate of applied N and increased by increasing the planting density and the size of seed tuber. Increased N increased % of large tubers in the yield; increased planting density and size of seed tuber increased % of small tubers. With II N uptake responded strongly to applied N by mid-June when there was a clear interaction between N level and planting density. Dry-matter content and sp. gr. of the tubers increased with increasing planting density. Narrowing the spacing increased % of small tubers. It is concluded that at higher planting densities the N requirement will also be higher.
                                E. G. BRICKELL.

Enzymic browning and free tyrosine in potatoes as affected by pentachloronitrobenzene. J. P. Sweeney and P. A. Simandle (*J. agric. Fd Chem.*, 1968, **16**, 25–27).—The fungicide applied at 50 lb/ acre, tended to reduce the tyrosine content of two varieties of potato, and possibly also the degree of enzymic browning. (11 references.)   P. S. ARUP.

Effects of maleic hydrazide, nicotinic acid, and gibberellic acid on alkaloid content of potato tubers. E. V. Parups (*Am. Potato J.*, 1967, **44**, 277–280).—Spraying field potatoes with maleic hydrazide (4 weeks before harvest) had no effect on the alkaloid content of tubers stored (5°) for 6 months. Infiltrating the treated tubers with water increased their alkaloid content. Infiltrating tubers with $2–4 \times 10^{-2}$ M nicotinic acid depressed, whilst infiltration with $2 \cdot 9 \times 10^{-4}$ M gibberellic acid, increased the alkaloid content.
                                A. H. CORNFIELD.

Fertilisation and irrigation of potatoes in Virginia. E. M. Dunton, jun. (*Am. Potato J.*, 1967, **44**, 419–424).—Trials over 8 years on a fine sandy loam indicated that annual application of 100 lb per acre of N, P_2O_5, and K_2O was adequate for high yields with or without irrigation. Irrigation had little effect on yields or total solids content of tubers. In a dry year sprouting before harvest was markedly reduced by irrigation.   A. H. CORNFIELD.

Influence of Attapulgus clay on the mineral content of turnips grown in soil and sand media. C. G. del Valle, R. E. Worley and S. A. Harmon (*Proc. Am. Soc. hort. Sci.*, 1967, **90**, 342–348.)—Mixing 1–10% Attapulgus clay with a loamy sand or quartz sand increased the total uptake and tissue concn. of K, Ca, Mg and Fe in turnip roots and leaves in pot tests. Total uptake of P was increased only in the roots, whilst tissue concn. of P was reduced in roots and tops.   A. H. CORNFIELD.

Commercial growing of carrots. U. R. Boswell (*Leafl. U.S. Dep. Agric.*, 1967, No. 353; 8 pp).—Climatic requirements, varieties, soils, soil prep., manures and fertilisers, planting, thinning, irrigation, cultivation, harvesting and storage are described.
                                E. G. BRICKELL.

Grass growth in midsummer: a re-interpretation of published data. R. W. Brougham and A. C. Glenday (*J. Br. Grassld Soc.*, 1967, **22**, 100–107).—Data presented by Anslow (*ibid.*, 1965, **20**, 19) on the relationship between growth rate, leaf-area index, and light penetration in a perennial ryegrass sward are re-examined and another interpretation presented.   A. H. CORNFIELD.

Grass growth in midsummer: a reply to a re-interpretation of published data. R. C. Anslow and H. L. Black (*J. Br. Grassld Soc.*, 1967, **22**, 108–111).—Cf. preceding abstr.   A. H. CORNFIELD.

Effect of sources, rates, and time of application of fertilisers on the performance of Kentucky bluegrass turf. F. V. Juska and A. A. Hanson (*Proc. Am. Soc. hort. Sci.*, 1967, **90**, 413–419).—The incidence of leafspot in Kentucky bluegrass turf increased with level of application of N (1 lb per 1000 sq. ft). Incidence of leafspot was usually less when N was applied as sewage sludge or 'urea-form' than as $(NH_4)SO_4$ or urea alone. In general satisfactory turf density was obtained with application of 2 lb N per 1000 sq. ft in spring or autumn; 4 lb N sometimes resulted in reduced turf density during severe drought.   A. H. CORNFIELD.

Effects of soil water conditions and soil temperature on the roots of grasses. I. Effect of irrigation on the weight of root material under various swards. E. A. Garwood (*J. Br. Grassld Soc.*, 1967, **22**, 176–181).—The wt. of root material in the soil profile under grass and grass–clover swards was less under irrigation than where no irrigation was applied. Irrigation had little effect on the vertical distribution by wt. of roots within the profile. In unirrigated swards root wt. decreased with increasing level of N (0–243 lb N per acre per annum), whilst in irrigated swards the highest root wt. occurred with the intermediate level of applied N.
                                A. H. CORNFIELD.

Influence of soil and management on the botanical composition of twenty-year-old reclaimed hill pastures in mid-Wales. H. Davies (*J. Br. Grassld Soc.*, 1967, **22**, 141–147).—The botanical composition of reclaimed hill pastures was studied in relation to soil type, drainage, management and application of CaO and basic slag.
                                A. H. CORNFIELD.

Comparative effects of differential defoliation of grass plants in pure and mixed stands of two species. R. I. Jones (*S. Afr. J. agric. Sci.*, 1967, **10**, 429–444).—When cut to a height of 4 in., *Paspalum dilatatum* or the weed *Eragrostis plana* yielded more dry matter than when cut to 1 in. The important factor, however, was the ratio of the cutting height of the plant to the height of neighbouring plants, rather than the actual height of the individual plants. The frequency of cutting had little effect on the results.   P. S. ARUP.

Development of varieties of English ryegrass (*Lolium perenne* L.). Z. Hryncewicz (*Schweiz. landw. Forsch.*, 1967, **6**, 227–245).—Characteristics including leaf area, ratio of org. matter of leaves to that of stalks, and date of ripeness for pasturage are reported with comments. (16 references.)   P. S. ARUP.

Variation in concentration and composition of toxic alkaloids among strains of *Phalaris tuberosa* L. R. N. Oram and J. D. Williams (*Nature, Lond.*, 1967, **213**, 946–947).—The alkaloid concn. in the herbage of 22 *Phalaris* strains grown in a controlled environment has been assayed colorimetrically after separation by paper chromatography; six strains were also grown in a field near Canberra and assayed similarly. There was a 20-fold variation in concn. of dimethyltryptamine alkaloids and several strains were considerably lower in alkaloid concn. than the Australian commercial strain. Three compounds found in all strains were *N,N*-dimethyltryptamine, 5-methoxy-*N,N*-dimethyltryptamine and 5-hydroxy-*N,N*-dimethyltryptamine. It appears feasible to breed agronomically acceptable strains of *Phalaris* with low concn. of alkaloid.   S. A. BROOKS.

Lawn diseases and how to control them. K. W. Kreitlow and F. V. Juska (*Home Gdn Bull.*, *U.S. Dep. Agric.*, 1967, No. 61; 16 pp).—Fungus diseases are described and chemical control methods indicated. Other causes of poor turf are also listed together with recommended management practices.
                                E. G. BRICKELL.

Trefoil production for pasture and hay. Anon. (*Fmrs Bull.*, *U.S. Dep. Agric.*, 1967, No. 2191; 16 pp.)—Advantages and disadvantages, species, varieties, establishment and uses are described together with diseases and insect pests.   E. G. BRICKELL.

Effect of nitrogen, phosphorus, and potassium supply on the iso-flavone content of leaves of red clover (*Trifolium pratense*, L). G. W. Butler, M. A. Th. Steemers and E. Wong (*N.Z. Jl agric. Res.*, 1967, **10**, 312–315).—K levels do not influence the isoflavone content of red clover leaves. The effect of low N was not adequately tested. Phosphate deficiency increases isoflavone levels. A high correlation between isoflavone and total alcohol-sol. carbohydrates was observed.   E. G. BRICKELL.

Use of ionising radiations for improvement of medicinal and aromatic plants. M. L. Kapoor and S. C. Datta (*Perfum. essent. Oil Rec.*, 1967, **58**, 442–444).—Some preliminary results are given showing the effect of irradiation from a 2,000 Ci γ-source on seeds of medicinal plants planted in 1965. With the species *Cymbopogon naardus*, Rendle, increased no. of tillers produced (111 and 92 vs 40) and increases in length of leaf from 117 to 133 cm and 124 cm respectively were obtained with exposures of 5 and 17·5 kr. With the increase in leaf length, it is expected to obtain a corresponding increase in oil yield. The % germination of the seeds of *Mentha arvensis*, Linn. show that irradiation times in excess of 3 h are lethal; and the exposure of the seeds of *Eucalyptus citriodora*, Hook to any form of γ radiation produces 100% mortality. (19 references.)   G. R. WHALLEY.

Pollination range of fruit trees. K. J. Maurer (*Mitt. Klosterneuburg Rebe u. Wein Obstb. u. Früchteverwert.*, 1967, **17**, 375–385).—A review which points out the importance of pollination, and dis-

cusses the effects of metaxenia on fruit characteristics. (22 references.)   P. S. ARUP.

Storage of Sturmer [apples] in relation to date of harvest. D. I. Jackson (*N.Z. Jl agric. Res.*, 1967, **10**, 301–311).—Internal breakdown tended to increase in late picks while incidence of wilt decreased. For other disorders, however, such trends could not be established. At Hastings, fruits from the outside and upper portions of the trees stored better than fruits picked from the inside and shaded parts; this tendency was not found at Nelson.
                                    E. G. BRICKELL.

Physiological aspects of accelerated ripening and yellowing of Golden Delicious apples. C. Leblond (*Fruits d'outre mer*, 1967, **22**, 543–555).—Accelerated ripening of the immature fruit is best attained in an atm. containing $0 \cdot 1\%$ of C_2H_4 at 20°. Yellowing is best promoted in an atm. containing 50% of O_2 and $0 \cdot 1\%$ of C_2H_4. The presence of $>1\%$ of CO_2 causes deterioration of the quality. The best results as regards flavour are obtained with fruit harvested $\ll 150$ days after petal fall. (12 references.)   P. S. ARUP.

[A] Influence of pruning and results of spraying with growth retardant on leaf and shoot development of apple trees. R. Schumacher and F. Fankhauser. **[B] Influence of growth retardant Alar on fruit development, root growth, and shoot tissues of apple trees.** R. Schumacher, F. Fankhauser and E. Schläpfer (*Schweiz. landw. Forsch.*, 1967, **6**, 131–147, 148–169).—[A] The effects of spraying with succinic 2,2-dimethylhydrazide (Alar) in shortening the shoots, and reducing the length of the internodes and the no. of leaves were greater on plots on which the grass was cut than on grass-mulched plots; no differences were observed in leaf-area or -thickness as between the pruned, unsprayed and the unpruned sprayed trees. Trees that had been sprayed the previous year (but not subsequently) showed more vigorous growth than did unsprayed controls: this effect was nullified by severe pruning in the spring of the second year. (10 references.)

[B] In comparison with the fruit from pruned and unsprayed trees, that from trees sprayed with Alar ripened more slowly, and had a firmer texture and higher sp. gr. Spraying had no effect on the taste of the fruit. Other effects were differences in the distribution of parenchyma, pith, and xylem in the shoots, and a retardation of root growth, slow at first, but increasing markedly during the course of the season.   P. S. ARUP.

Colorimetric determination of Alar [growth retardant] residues in apples. L. J. Edgerton, M. L. Rockey, H. Arnold and D. J. Lisk (*J. agric. Fd Chem.*, 1967, **15**, 812–813).—Alar (*N*-dimethylaminosuccinamic acid) is simultaneously extracted and hydrolysed to dimethylhydrazine (I) with 50% aq. NaOH. The extract, with rinsings, is steam-distilled and the I is determined in the distillate by mixing with 5% aq. molybdophosphoric acid solution, heating the mixture in boiling water for 1 h, and measuring the absorbance at 625 nm. The min. detectable amount of Alar was $1 \cdot 5$ ppm. Recoveries at $2 \cdot 5$–10 ppm were $68 \cdot 114\%$.   P. S. ARUP.

Nutrient element status of leaves and quality characteristics of Valencia oranges. W. J. Fölscher and A. A. B. Bruwer (*S. Afr. J. agric. Sci.*, 1967, **10**, 267–277).—The leaves of 20 groups of trees receiving different fertiliser treatments were analysed for N, P, K, Ca, and Mg throughout the season. During fruit development the leaves of fruiting branches contained more Ca and Mg and less N, P, and K than did leaves from non-fruiting branches. Coeff. of correlation between nutrient contents are tabulated. Equations are given showing influence of nutrients on fruit and juice yields, skin-thickness, and sugar content of juice. (16 references.) P. S. ARUP.

Sampling of orange trees for foliar diagnosis. III. Influence of fructifying or non-fructifying nature of branches, of their height on the tree and of shade. P. Martin-Prével, P. Lossois, J. J. Lacœulhe and J. Del Brassine (*Fruits d'outre mer*, 1966, **21**, 577–587).—For comparative purposes it is necessary to use exclusively leaves from fructifying branches sampled at medium height and from the exterior parts of the tree.   P. S. ARUP.

Sub-soil cultivation in banana culture. J. Godefroy (*Fruits d'outre mer*, 1967, **22**, 341–350).—Experiments conducted on three African soils produced no very conclusive results except on a site with a hard pan horizon at 35 to 60 cm depth. Owing to the cost involved operations of this nature should not be undertaken without good reason.   P. S. ARUP.

***Achras sapota* Linn: Effect of preharvest sprays of: I. Growth-regulators on size, composition and storage behaviour. II. Maleic hydrazide and isopropyl phenylcarbamate** S. Lakshminarayana, H. Subramanyam and V. Surendranath (I only) (*J. Fd Sci. Technol.*, 1967, **4**, 66–69; 70–73).—I. Cultivation is difficult because fruits of different stages of development occur throughout the year, which

makes harvesting at a definite degree of maturity problematical. Growth regulators of the chlorophenoxy-acetic and chlorophenoxy-propionic acid type have been successfully employed for other fruits. Data of a study of this method applied to *Achras sapota* is presented. Application of 2,4,5-trichlorophenoxypropionic acid at 25 ppm resulted in increased fruit size and wt. and uniform ripening, the accelerated ripening however, led to over-ripeness and spoilage in some cases. (18 references.)

II. Treatment with maleic hydrazide resulted in faster ripening, higher respiration rate and greater moisture loss, while treatment with isopropyl phenylcarbamate retarded respiration, reduced spoilage and also controlled ripening. The latter compound is preferred for treating *Achras sapota*.   I. DICKINSON.

Operating conditions [vineyard management] for modern wine production. M. Dittmann (*Mitt. Klosterneuburg Rebe u. Wein Obstb. u. Früchteverwert.*, 1967, **17**, 358–367).—A rationalised scheme and time-table of operations is presented to meet the requirements of expanding markets and labour costs. Attention is given to the planning of vineyards, the training of vines, improvement of stocks, and economic aspects.   P. S. ARUP.

Genetic and histological study of blotchy ripening in segregating generations of a tomato cross. M. E. Fogleman (*Diss. Abstr.*, B, 1967, **27**, 2931).—The tomato cross Glamour × Red Top, showed the disorder to a greater extent than did either parent. Evidence obtained points to the involvement of genetic factors. The parental varieties and their F_1 and F_2 and back-cross progenies were used in an attempt to establish the heritability of resistance to blotchy ripening. Correlation coeff. for blotchy ripening with size and firmness of fruit were small and selection for size and firmness is unlikely to increase susceptibility to the disorder. Microscopical examination of individual fruits showed disorganisation and breakdown of parenchyma cells adjacent to the vascular bundle. The development of the vascular bundles was associated with the expression of blotchy ripening.   A. G. POLLARD.

Effect of phosphorus fertilisation on tomato seedling growth rate. G. E. Wilcox (*Proc. Am. Soc. hort. Sci.*, 1967, **90**, 330–334).—In pot tests tomato seedling growth was better when P was banded below or on the seed than when banded to one side of the seed or mixed with the whole soil. Addition of K^+ to the band did not affect seedling growth rate.   A. H. CORNFIELD.

Growth and nutrient absorption in greenhouse tomato and cucumber. G. M. Ward (*Proc. Am. Soc. hort. Sci.*, 1967, **90**, 335–341).—The N, P, K, Ca, and Mg contents of greenhouse tomato and cucumber plants were determined from Jan. to June. The total absorption of the five elements on the lb-per-acre basis for tomatoes was 309 for N, 80 for P, 510 for K, 220 for Ca and 37 for Mg; and for cucumbers was 521 for N, 164 for P, 770 for K, 610 for Ca, and 138 for Mg.   A. G. CORNFIELD.

Effects of phosphorus, potassium and magnesium levels on growth and chemical composition of *Chrysanthemum morifolium*. J. N. Joiner (*Proc. Am. Soc. hort. Sci.*, 1967, **90**, 389–396).—The effects of varying levels of P, K and Mg in the nutrient on stem length, flower dia., chlorosis rating and N, P, K, Ca, and Mg contents of the leaves, stems and roots at various stages of growth were studied in gravel culture. P had the greatest effect on the factors and the interaction of P and K supply dominated growth and chemical analysis results. Mg had relatively little effect. The various elements increased in tissue during floral development.
                                    A. H. CORNFIELD.

Redistribution of mineral nutrients during propagation of *Chrysanthemum morifolium*. G. L. Good and H. B. Tukey, jun. (*Proc. Am. Soc. hort. Sci.*, 1967, **90**, 384–388).—During rooting of chrysanthemum cuttings under intermittent distilled water mist, N, P, and K, but not Ca, were translocated from the mature to the new growing tissue.   A. H. CORNFIELD.

Nutrition of greenhouse chrysanthemums in soil with added peat moss and vermiculite. C. W. Dunham (*Proc. Am. Soc. hort. Sci.*, 1967, **90**, 462–466).—Addition of 40% (by vol.) of vermiculite to a soil–sand mixture increased the uptake of K and decreased that of Mg and Mn by chrysanthemums. Addition of 40% peat moss decreased the uptake of P, K and Ca and increased that of Mn. Soil with peat moss added required more frequent replenishment of nutrients, especially K, than did soil with vermiculite added.
                                    A. H. CORNFIELD.

Relationship of fertilisation frequency to chrysanthemum yield and nutrient levels in soil and foliage. J. N. Joiner and R. T. Poole (*Proc. Am. Soc. hort. Sci.*, 1967, **90**, 397–402).—The effect of applying 350 lb per acre each of N and K split in two to six biweekly applications beginning 3 days after planting out cuttings on per-

formance of chrysanthemums was studied. Extractable soil N, P, K, Ca and Mg were not correlated with leaf tissue levels of these elements. The different frequencies of fertiliser application had little effect on flower size or keeping quality. Stem length and number of flowers per stem increased slightly with frequency of fertiliser application. A. H. CORNFIELD.

Growing loblolly and shortleaf pine in the midsouth. C. X. Grano (*Fmrs' Bull., U.S. Dep. Agric.*, 1967, No. 2102; 27 pp).—Timber management, tree characteristics, fire protection, hardwood control, planting, thinning, pruning, harvesting, and marketing are described. Volume and board tables are appended.

E. G. BRICKELL.

Pest Control

Some effects of molecular structure upon anticholinesterase and insecticidal activity of substituted phenyl *N*-methylcarbamates. R. L. Metcalf and T. R. Fukuto (*J. agric. Fd Chem.*, 1967, **15**, 1022–1029).—The activities of 78 compounds (some new) have been investigated with regard to the composition of the phenolic and carbamate groups, the nature of the substituents and side chains, and other structural characteristics. (23 references.)

P. S. ARUP.

Sex attractant of sugar-beet wireworm: identification and biological activity. M. Jacobson, C. E. Lilly and C. Harding (*Science, N.Y.*, 1968, **159**, 208–210).—The sex attractant, produced by adult females [*Limonius californicus* (Mannerheim)] has been isolated and identified as valeric acid (I). In the laboratory the males are repelled by the pure attractant but in the field they are lured from a distance of 12 m in 10 sec. The pheromone occurs in unusually large amounts in the female's body. The stability, volatility and availability of I make it an ideal bait trap for population control. (12 references.) C.V.

Antifungal activity of isothiocyanates and related compounds. III. Derivatives of biphenyl, stilbene, azobenzene, and several polycondensed aromatic hydrocarbons. L. Drobnica, M. Zemanová, P. Nemec *et al.* (*Appl. Microbiol.*, 1968, **16**, 582–587).—In the investigated compounds (48), antifungal activity was observed only in the biphenyl and naphthalene compounds, the stilbene, benzene-azonaphthalenes and further polycondensed hydrocarbons being extremely insol. in water and possessing very large mol. This probably prevents them passing into the spores or mycelium of the fungi and therefore the —NCS group cannot exert its activity. (25 references.) C.V.

Constituents of the Piper sp. XVII. Kawa lactone: Chain length and fungistatic activity. R. Hänsel, D. Weiss and B. Schmidt (*Arch. Pharm., Berl.*, 1968, **301**, 369–373).—7,8-Dihydrokawain (a constituent of the rhizome and shoots of *Piper methysticum* Forster) which is an arylalkyl-substituted α-pyrone, strongly inhibits the growth of *Aspergillus niger*. Some simple mol. variations have been prepared and their activities tested; any shortening or lengthening of the C_2-bridge between the benzene and α-pyrone rings leads to a decrease in fungistatic activity. G. R. WHALLEY.

Thermodynamic properties of some carbamates and thiolcarbamates in aqueous solutions. V. H. Freed, R. Hague and J. Vernetti (*J. agric. Fd Chem.*, 1967, **15**, 1121–1123).—Solubilities of the compounds at 3° or 25° decreased linearly with increasing mol. wt. Solubilities of the carbamates in the range 275° K to 303° K increased with the temp., but those of the thiolcarbamates decreased with rising temp. From these and other calculated thermodynamic data it appears that the behaviour of the thiolcarbamates may be caused by H-bonding between water and the solute; the application of these findings to the persistence patterns of these herbicides in soils is mentioned. P. S. ARUP.

Relation between chemical structure, fungicidal properties and toxicity to warm-blooded animals, of some organic arsenic compounds. I. D. Neklesova, N. V. Egorova and M. A. Kudrina (*Izv. Akad. Nauk SSSR, Ser. Khim.*, 1967, 1998–2005).—The fungicidal properties of some thioethers of structure R-As-(SR′)₂ (I), R₂-As-SR′ (II), and As(SR)₃ (III) were investigated. Type II compounds possessed the most selective action. Radicals *S*-(2-diethylaminoethyl) attached to thioethers of different structure are more biologically active than ortho-aminophenylthio- and 3-chloro-2-acetoxy-1-propylthio groups. There was increased selective action to pathogenic fungi in the arsonium bromides of structure [(Ph)-(Pr)AsR₂]⁺Br⁻ and arsines having the formula $C_6H_5AsR_2$ where R is hydrocarbon radical, increasing with chain length up to C₇. In arsine sulphides of structure (Et)(Ph)(R)As:S, a strong selective action was noted when the hydrocarbon radical R was lengthened to C₈. Substitution of *p*-tolyl for Ph increased the biological

activity of the compounds. Cystein in excess protected pathogenic fungi and warm-blooded animals from injury caused by phenyl di-n-heptylarsine. (16 references.) A.L.B.

Effect of crop residues on soil fungi and onion growth in naturally infested soil. A. J. Latham and R. D. Watson (*Phytopathology*, 1967, **57**, 505–509).—The complex of organisms, associated with onion root rot, included *Fusarium oxysporum*, *Pyrenochaeta terrestris* and species of *Rhizoctonia*, *Pythium* and some bacteria. The effects of many crop residues on the proportions of the different organisms concerned, on the growth of onions and on the development of onion root rot are recorded. A. G. POLLARD.

Relationship between crop sequence and several diseases of Valencia peanuts. D. C. H. Hsi (*Phytopathology*, 1967, **57**, 461).—The incidence of peanut diseases is examined in relation to the sequence of preceding crops. Peanuts (I) following cotton (II) or I (first crop) showed more black hull (*Thielaviopsis basicola*) than when following sorghum (III), small grains, lucerne (IV) or fallow. These differences became more marked as the season progressed. Low moisture and high temp. in soil lowered the incidence of black hull regardless of the previous crop sequence. *Aspergillus* crown rot was more frequent when the previous crop was I, wheat or barley than when it was III, IV or fallow. Vascular wilt of I was severe on land with a history of *Verticillium* wilt of II or okra (V). Less wilt occurred in I after grain, III or IV than after II, I or V. I, following II or I showed larger proportions of rotted or damaged I than when grown after grain, III or IV. A. G. POLLARD.

Factors affecting the incidence and severity of internal browning of tomato induced by tobacco mosaic virus. J. S. Boyle and E. L. Bergman (*Phytopathology*, 1967, **57**, 354–362).—Results of numerous experiments showed that soil moisture content and the susceptibility of the cultivar were highly important factors influencing the occurrence and severity of the disorder. Effects of source of the virus and cultural treatments are also examined.

A. G. POLLARD.

Light-sensitivity of germinating uredospores of wheat brown rust. J. C. Zadoks and L. J. M. Groenewegen (*Neth. J. Pl. Path.*, 1967, **73**, 83–102; *Meded. Lab. Phytopath.*, 1967, No. 00231).—Sensitivity was measured by the rate of germ-tube elongation at 3000 lux, and the % germination at 7600 lux. Spores formed in darkness were more sensitive to light during subsequent germination than those formed in light. Exposure of the spores to moisture during storage decreased their germinating capacity in light. Spores formed in darkness at low R.H. could be classified into, (a) light-insensitive, (b) light-sensitive, and (c) non-viable; the sensitivity of population (b) could be partly reversed by keeping the spores in darkness for 2 h before germination. (12 references.)

P. S. ARUP.

Inhibitory effect of light on infection by brown leaf rust of wheat. J. C. Zadoks (*Neth. J. Pl. Path.*, 1967, **73**, 52–54).—Variable results obtained from inoculation of seedlings with *Puccinia recondita* f. sp. *triticina* are explained by the observed inhibition by light of the infection process. The inhibition occurs during the pre-penetration stage, 6–24 h after inoculation. P. S. ARUP.

Effect of lime and micronutrient soil amendments on *Fusarium* disease development in tomatoes. J. P. Jones and S. S. Woltz (*Pl. Dis. Reptr*, 1967, **51**, 645–648).—Liming a fine sand before sowing tomatoes greatly reduced the incidence and severity of wilt due to *Fusarium oxysporum* f. *lycopersici*. However, the pathogen was isolated from the stems of many of the plants grown in CaO-amended soils, even though the plants did not develop vascular browning symptoms. Addition of 10 ppm Mn²⁺ or Zn²⁺ to the soil had inconclusive effects on the disease. A. H. CORNFIELD.

Calcium nutrition in relation to the susceptibility of tobacco to black shank disease, due to *Phytophthora parasitica* var. *nicotianae* L. D. Moore and W. H. Wills (*Pl. Dis. Reptr*, 1967, **51**, 641–644).—The susceptibility of tobacco to black shank disease was very low when the nutrient solution (sand cultures) contained 20 ppm Ca²⁺, but was high when the nutrient contained 100 ppm or more of Ca²⁺. The effect was independent of the Ca nutrition of the plant before inoculation with the black shank organism. A. H. CORNFIELD.

Calcium–sterol interaction in growth of *Phytophthora parasitica* var. *nicotianae*. J. W. Hendrix and S.-M. Guttman (*Phytopathology*, 1967, **57**, 461).—Growth of the organism in a glucose-peptone medium was stimulated by addition of cholesterol when the cultures were shaken. Little or no growth occurred in glucose-asparagine or glucose–NO₃⁻ media. Addition of Ca²⁺ or cholesterol or sitosterol favoured some growth in glucose–NO₃⁻ and stimulated growth in glucose–asparagine media. In the latter, the addition of Ca²⁺ + sterol produced excellent growth. Addi-

tion of minor elements, Fe, Zn, Mn, Cu, Mo, had little effect on the growth of the fungus except in media containing Ca and sterol.

A. G. POLLARD.

Sweetpotato soil rot, due to *Streptomyces ipomoea*, in a silt loam soil as affected by annual applications of lime or sulphur over seven years. W. J. Martin, L. G. Jones and T. P. Hernandez (*Pl. Dis. Reptr*, 1967, **51**, 271–275).—Incidence of soil rot of sweetpotatoes was high on soil after annual application of CaO for 7 years (soil pH $6\cdot3$–$6\cdot8$), but was low after application of S (soil pH $4\cdot1$–$4\cdot8$), and was low to high in plots receiving neither amendment (pH $4\cdot8$–$5\cdot8$). A. H. CORNFIELD.

Effect of nutrition on resistance and susceptibility of cotton to *Verticillium alboatrum* and *Fusarium oxysporum* f. *vasinfectum*. A. Abdel-Raheem and L. S. Bird (*Phytopathology*, 1967, **57**, 457).—Cotton plants grown with various levels of N, P and K nutrition received stem inoculations of the pathogens. High-N feeding increased the incidence and severity of both diseases. The incubation period in each case was increased by high-K nutrition but was unaffected by P; it was longest with low N–high K nutrition. In general, the influence of nutrition on both wilt diseases was similar.

A. G. POLLARD.

Transformation of copper salts by wood-decaying fungi. Y. Ishii and H. Kawamura (*J. Soc. Mater. Sci., Japan*, 1967, **16**, 741–745).—Experiments described support the view that decay of wood by *Coriolellus palustris* arises from a local concn. (especially around a hole in the tissue) of Cu oxalate formed by reaction between oxalic acid (metabolised by the fungi) and the Cu salt used as preservative. Intensity of decay appears to correlate with concn. of Cu oxalate. Fungi cultured in water alone grow uniformly over filter-paper, but when $0\cdot1$–1% $CuSO_4$ is present the growth is confined to distinct concentric areas, the density of which is higher with increasing concn. of Cu. This confirms field experience that untreated wood decays more or less uniformly by shrinkage, whereas the decay of treated wood is local but serious. (From English summary.)

W. J. BAKER.

Fungistatic and fungicidal properties of an antibiotic produced by *Penicillium rubrum*. M. M. Krstic (*Pl. Dis. Reptr*, 1967, **51**, 669–671).—The purified compound (structure not yet known) produced by a strain of *Penicillium rubrum* possessed fungistatic and fungicidal properties against several plant pathogens and wood-colonising fungi. A. H. CORNFIELD.

Antibiotic production by *Streptomyces* species from the rhizosphere of desert plants. J. W. Whaley and A. M. Boyle (*Phytopathology*, 1967, **57**, 347–351).—Ten species of *Streptomyces* were isolated from the rhizosphere of desert plants. Six of the species produced candicidin (a heptaene antibiotic) and the remaining four species yielded unknown heptaenes or mixtures of cycloheximide and candicidin. Production of antibiotics by these species varied with the sources of C and N available for mycelial growth.

A. G. POLLARD.

Effect of ammonia on *Pratylenchus penetrans*. J. T. Walker and S. Mavrodineanu (*Phytopathology*, 1967, **57**, 345–346).—Previous reports showed that culture fluids of several actinomycetes and bacteria were nematocidal to adults and larvae of lesion nematodes (*P. penetrans*). The nematodes were killed by NH_3 in proportions which increased with the $[NH_3]$ used. No relationship was apparent between the amount of NH_3 in culture fluids and the mortality of the nematodes. A. G. POLLARD.

Mode of action of ammonia on *Sclerotium rolfsii*. Y. Henis and I. Chet (*Phytopathology*, 1967, **57**, 425–427).—The germinability of sclerotia of *S. rolfsii* was adversely affected in media of pH $\nleq$ $9\cdot8$ whether or not NH_3 was present. Addition of $0\cdot2\%$ NH_3 to a sandy loam completely prevented germination and increased the soil pH to toxic levels for > 24 h. A. G. POLLARD.

Altenin. V. Pathogenic activity of reductones to the pear. N. Sugiyama, C. Kashima, M. Yamamoto, T. Takano and R. Mohri (*Bull. Chem. Soc., Japan*, 1967, **40**, 2594–2596).—The three reductones, 3-hydroxy-2,4-dioxopentane, Et 2-hydroxy-1,3-dioxovalerate and Et 2-hydroxy-3-oxobutyrate were synthesised and examined for pathogenic activity to the pear by the black spot test (cf. Sugiyama *et al., ibid.*, 1966, **39**, 1573).—Pathogenic activity was related to enediol content and the centre of pathogenic activity in altenin is shown to be the enediol carbonyl grouping. E. J. H. BIRCH.

***Chenopodium* as host to viruses transmitted from apple, pear and grapevine.** D. J. Engelbrecht and L. D. Wolfswinkel (*S. Afr. J. agric. Sci.*, 1967, **10**, 419–423).—Transmissions with sap to *Chenopodium amaranticolor* and *C. quinoa* of symptoms of chlorotic leaf spot, stem-pitting, and mosaic were successfully achieved. Diseased host plants were successfully grafted to apple and pear

indicators. Back-transmission of virus from *C. amaranticolor* to a vine confirmed the association of grapevine fan-leaf with sap-transmissible viruses. The symptoms developed most readily at 16° but failed to do so at 24°. (16 references.) P. S. ARUP.

Survival of lesion nematodes in soils amended with organic residues. J. T. Walker and C. H. Specht (*Phytopathology*, 1967, **57**, 346).—Fewer lesion nematodes occurred on roots of peas grown in soil amended with plant residues than on those from unamended soil. Org. materials were added (1%) to steamed and unsteamed soil and equal amounts of a suspension of nematodes were added to each. After incubation ($25°$ for 8 weeks) the soils were sown with peas. Plants were harvested after 8 weeks and the nemas were extracted. No lesion nematodes were found in plants grown in steamed soil amended with soyabean meal or rice grain; those grown in unsteamed but amended soil showed 100 and 84% reduction in nematode population. Other org. amendments e.g., pine bark, peat moss, oat straw had similar effects but these generally, were less marked in non-steamed than in steamed soils.

A. G. POLLARD.

Symptoms and characteristics of *Pratylenchus brachyurus* infestation of stored potatoes. H. Koen and W. L. Hogewind (*S. Afr. J. agric. Sci.*, 1967, **10**, 543–549).—The lesions consist of tough, dead tissue, presumably developed as a form of resistance to invading nematodes which are eventually trapped and killed for lack of food. Infected tubers lifted early appear healthy but wither and lose wt. under storage at normal temp. When stored at 5° the tubers remain fairly intact and the *P. brachyurus* population dies completely. P. S. ARUP.

Hot water treatments control endo- and ecto-parasitic nematodes associated with turf. C. M. Heald and H. D. Wells (*Phytopathology*, 1967, **57**, 460–461).—Sprigs of Bermuda grass were infected with several species of nematodes and incubated at various temp. for 15, 30 or 45 min. Nematodes, counted after 4 months, were destroyed by the treatment at 55° for 15 or 30 min., but not at 21°. The death-pt. of the grass was 60°. A. G. POLLARD.

Invasion of wheat roots by *Pratylenchus thornei*. R. J. Baxter and C. D. Blake (*Nature, Lond.*, 1967, **215**, 1168–1169).—Seinhorst's second assumption that nematodes act independently (*Nematologica*, 1965, **11**, 137) is not entirely verified by the observed distribution of *P. thornei* in or on roots of wheat established in sand at const. temp. and moisture and inoculated with 1500 or 6000 nematodes per plant. The data fit a negative binomial distribution rather than a Poisson distribution, suggesting that some parts of the root were more suitable for invasion or that, although the initial invasion was random, other nematodes subsequently entered through the wounds (with exuded cell-contents) caused by the early invaders. Either suggestion would explain the localised regions of heavy invasion observed in wheat roots. (10 references.)

W. J. BAKER.

Viability of brown locust eggs [*Locustana pardalina* (Walker)]. D. H. Botha (*S. Afr. J. agric. Sci.*, 1967, **10**, 445–459).—Eggs kept in dry soil lost their viability after $\sim$ 10 months, but were able to survive for 31 months in soil moistened at intervals of 3 or 6 months. The contributions to outbreaks of hatchings under natural conditions, delayed for 10 months by drought, would be insignificant. (11 references.) P. S. ARUP.

Improved method for extracting leatherjackets from soil and evaluation of the Maercks method. A. M. Feeney (*Scient. Proc. R. Dubl. Soc.* B, 1967, **2**, 75–79).—A description is presented of the construction and operation of an apparatus in which sieving of the soil with water under pressure is combined with flotation in brine. The apparatus gave results that were higher and statistically more accurate than those obtained with the Salt and Hollick apparatus. Further extractions by the new method, of soils that had been treated by the Maercks brine-soaking method showed the Maercks method to be $\nleq$ 91% efficient, and suitable for routine work. (12 references.) P. S. ARUP.

Reduction of common bunt in winter wheat by 2-chloroethyltrimethylammonium chloride. W. F. Crosier and W. L. Yount (*Phytopathology*, 1967, **57**, 339).—Application of Cycocel (I) ($11\cdot8\%$ of the title substance), at 2 or 4 lb active ingredient (a.i.)/acre, to wheat at the four-leaf stage increased grain yields by reducing stem lengths and lodging. I was not effective against bunt and in some cases increased it in plants from seed pretreated with certain fungicides. Application of I ($0\cdot3$ or $0\cdot7$ lb a.i./bushel) to seed in combination with captan, PCNB, TCNA or thiram or other non-mercurials reduced bunt significantly, but, when used with mercurials, caused significant reductions of bunt only at the $0\cdot7$ lb rate.

A. G. POLLARD.

Control of nematodes in wheat soils. E. D. Kerr (*Pl. Dis. Reptr*, 1967, **51**, 637–641).—A high proportion of the many genera of nematodes found in fields of winter wheat survived summer fallowing. Fumigation, 3 weeks before sowing winter wheat, with chloropicrin (1100 lb) or 1,3-dichloropropene (20 gal per acre) gave good control of nematodes and increased yields of grain.
A. H. CORNFIELD.

Pre-emergence application of 2,4-D ester to wheat (*Triticum vulgare*). P. C. Nel (*S. Afr. J. agric. Sci.*, 1967, **10**, 283–284).—Applications of the Pr^i or Bu esters at ∼ 1 lb per acre (2 lb per morgen) at the outset of two seasons had no serious effect on the wheat and gave reasonable control of summer grasses.
P. S. ARUP.

The European corn borer. Anon. (*Fmrs' Bull.*, *U.S. Dep. Agric.*, 1967, No. 2190; 14 pp).—*Ostrinia nubilalis* (Hübner) is described, together with some of the larvae which are mistaken for it. Control measures are indicated. E. G. BRICKELL.

Control of head smut, *Sphacelotheca reiliana*, of maize with pentachloronitrobenzene (PCNB). H. S. Fenwick and W. R. Simpson (*Pl. Dis. Reptr*, 1967, **51**, 626–628).—Application of PCNB (20 lb per acre) in the seed furrow reduced the incidence of head smut in maize. The effectiveness of the treatment increased with more effective mixing of PCNB with the soil. A. H. CORNFIELD.

Triazine herbicides on maize and their residual effects on following crops. A. R. Saghir and A. H. Choudhary (*Weed Res.*, 1967, **7**, 272–280).—Pre-emergence application of 2·5 kg/ha of atrazine (I) or simazine (II) was the most effective treatment for the control of broad-leaved weeds in maize. Other triazines injured maize at all rates tested. Amongst the crops following maize treated as shown, wheat, oats, vetch, onions and soyabeans were uninjured while sugar-beet was affected. Post-emergence treatment with I or II was ineffective, there being no significant difference over the unweeded plots. Wheat, oats, vetch and sugar-beet were injured while onions and soyabeans showed no visible injuries except from I and II in concn. ≮ 5 kg/ha or from mixtures of I + prometryne or I + ametryne at 12·5 kg/ha. (13 references.) C.V.

Microscopic study of powdery mildew on barley after application of the systemic compound Wepsyn. J. F. C. Magendans and J. Dekker (*Neth. J. Pl. Path.*, 1966, **72**, 274–278).—The roots of barley seedlings infected through the leaves with conidia of *Erysiphe graminis* f. sp. *hordei* were placed in aq. suspensions of the compound 5-amino-3-phenyl-1-bis(dimethylamido)-1,2,4-phosphoryltriazole (I). At concn. of 10 or 25 ppm of I the formation of primary haustoria was decreased about thirtyfold. At concn. of 62 or 156 ppm of I the germination of the conidia was retarded, the mycelium was stunted, and the formation of primary haustoria was almost entirely suppressed. P. S. ARUP.

Chemical control of rice blast caused by *Piricularia oryzae*. M. H. Ashrafuzzaman and R. A. Frederiksen (*Phytopathology*, 1967, **57**, 457).—Comparative trials with various fungicides are recorded. Applications were made at the times of the first- and full-head stages, the two applications being shown to give better results than either alone. Blasticidin-S (I), Bufen 30 (HgPh acetate) (II) and Du Ter W50 (50% triphenyl Sn hydroxide), (III) increased yields by reducing neck infection. In greenhouse experiments plants were sprayed with an aq. suspension of the fungicide and subsequently inoculated with *P. oryzae*. Leaf blast was reduced considerably by I, II and III. Actidione II and 4FK (*s*-dichlorotetrafluoroacetone) were highly phytotoxic. None of the fungicides tested was formally approved for commercial use, although III is under consideration. A. G. POLLARD.

Wireworms on irrigated lands in the West. B. J. Landis and J. A. Onsager (*Fmrs Bull.*, *U.S. Dep. Agric.*, 1967, No. 2220; 14 pp).—Chemical and cultural methods of control of *Limonius californicus*, *L. canus*, *L. infuscatus*, *L. subauratus* and *Ctenicera pruinina* are described. E. G. BRICKELL.

Trials with insecticides for control of black beetle (*Heteronychus arator* Blanchard) larvae and adults in pasture. D. H. Todd (*N.Z. Jl agric. Res.*, 1967, **10**, 278–288).—Formulated as pellets, endrin, and isobenzan at 8 and 16-oz/acre and dieldrin at 4 and 8-oz/acre all gave a high level of control of larvae in the second season. Little effect was obtained with the treatments in the season of application. In small-plot experiments, pelleted endrin, isobenzan, and heptachlor were highly effective against the adult beetle 39 days after treatment. In similar experiments high beetle mortality was obtained with diazinon (1–2 lb/acre) and Agritox (1 lb/acre) after 26 and 13 days respectively. E. G. BRICKELL.

Influence of drying and harvesting procedures on fungus populations and aflatoxin production in peanuts in Georgia. C. R. Jackson (*Phytopathology*, 1967, **57**, 462).—Mature peanuts with moisture contents 45–50% (green) or 18–25% (semi-dry) were harvested by hand or by combine. Drying rates in the laboratory were adjusted to produce 'rapid drying' (29–35° for 2 days with continuous airflow) or 'slow drying' (27–35° for 11 days with intermittent airflow). Fungal populations on the outer surfaces of pods were estimated by a wash–dilution plate method. Aflatoxin contents of the kernels were determined. The mean no. of fungi on the total pod surface and on kernels were not influenced by the method of harvesting, but larger fungal counts were found after slow than after rapid drying. *Aspergillus flavus* was more abundant in kernels from combine-harvested than in those from hand-harvested crops and more frequent on slowly- than on rapidly-dried pods. The mean aflatoxin content of slowly-dried 'green' peanuts was 1780 ppb for combine-harvested and 140 ppb for hand-harvested pods. Corresponding slowly dried 'semi-dry' crops showed values of 1160 ppb (combine) and 140 ppb (hand-harvested). Aflatoxin B_1 was absent from or found only in traces in the rapidly dried peanuts.
A. G. POLLARD.

Evaluation of five insecticides for the control of late-season cotton pests in Uganda. W. R. Ingram (*E. Afr. agric. For. J.*, 1967, **33**, 206–211).—The whole area was treated at weekly intervals from the 5th to 14th week after emergence with DDT (1 lb in 8 gal per acre). Thereafter six treatments at weekly intervals for 6 weeks were made with carbaryl (2 lb), fenitrothion (0·5 lb), DDT (1 lb), endrin (0·25 lb), and endosulfan (1 lb per acre). Carbaryl gave good control of *Dysdercus* spp., but none of the materials controlled *Cryptophlebia leucotreta* or reduced the amount of dirty or unpickable cotton. Carbaryl, endosulfan, and fenitrothion gave good control of *Aphis gossypii* and endosulfan gave some control of *Tetranychus* sp. A. H. CORNFIELD.

Effect of environmental and biological factors on persistence of malathion applied as ultra-low volume or emulsifiable concentrate to cotton plants. T. M. Awad, S. B. Vinson and J. R. Brazzel (*J. agric. Fd Chem.*, 1967, **15**, 1009–1013).—An ultra-low vol. emulsifiable concentrate persisted longer on cotton leaves (half-life 4 days) than did the same dose of water-diluted emulsified concentrate (half-life 2 days). On glass plates the latter evaporated faster, but it was also found to penetrate the leaf surface more rapidly, resulting in increased penetration of malathion. Metabolism on the leaf surface was slight with both formulations. The dicarboxylic acid metabolite of malathion was the major decomposition product within the leaves. (21 references.) P. S. ARUP.

Apparent local systemic control of apple scab as a result of migration of dodine on the unsprayed leaf surface. B. M. Jones and M. Szkolnik (*Phytopathology*, 1967, **57**, 341).—It is shown that the control of apple scab (*Venturia inaequalis*) on ventral leaf surfaces following application of dodine to the dorsal surface is not due to a localised form of systemic action but results from the migration of dodine to the unsprayed leaf surface in amounts sufficient to provide 98–100% control of the disease. A. G. POLLARD.

Chemotherapy of *Cytospora* canker disease in peach trees. A. W. Helton and K. G. Rohrbach (*Phytopathology*, 1967, **57**, 442–446).—Various chemical treatments were compared. Prophylactic effects were more pronounced than were therapeutic effects. Most of the substances tested showed systemic activity; among these Na 2-pyridinethiol-1-oxide (I) and cycloheximide thiosemicarbazone (II) were outstanding. Prophylaxis was complete with II; healing of infected wounds was best after use of I. A. G. POLLARD.

Preharvest Botran applications in relation to post-harvest peach fruit rot control in South Carolina. H. H. Foster, C. Gambrell and W. H. Rhodes (*Phytopathology*, 1967, **57**, 459).—Applications of various fungicides were made on peach trees at different periods (18–1 days) before harvesting. The picked fruit was kept at room temp. for 5–6 days and examined for the presence of various foot rots. Data obtained suggest that captan and Botran were superior to S for controlling post-harvest fruit decay; both were free from phytotoxic action and maintained good fruit colour and finish.
A. G. POLLARD.

Effect of cycloheximide thiosemicarbazone on *Cytospora* canker disease of Italian prune trees, *Prunus domestica*. A. W. Helton and W. J. Kochan (*Pl. Dis. Reptr*, 1967, **51**, 655–658).—Old canker infections on 21-year-old trees were reduced by application of 3200 ppm cycloheximide thiosemicarbazone (CHTS) sprays. 200–800 ppm CHTS was effective for preventing or curing infections on 18-year-old trees. On 4-year-old trees preventive effects of 50–3200 ppm CHTS were excellent, and were good even in the year following application of the higher doses. Curative effects were negligible. Yields of fruit were often increased by the 200–800

ppm rates, but not by lower or higher rates. No apparent chemical injury was observed with any rate of application.

A. H. CORNFIELD.

Eradication of Exobasidium vaccinii by nickel salts. M. T. Hilborn (*Phytopathology*, 1967, **57**, 341).—Red-leaf disease (*E. vaccinii*) in blueberry fields was eliminated by spraying with solutions of Ni salts (Cl⁻, NO₃⁻ or SO₄²⁻) at 3 g per l. Healthy bushes were unaffected. Leaves of infected bushes shrivelled after spraying and were unable to disseminate the fungus to surrounding healthy bushes.

A. G. POLLARD.

Radiation–heat synergism for inactivation of market disease fungi of stone fruits. N. F. Sommer, R. J. Fortlage, P. M. Buckley and E. C. Maxie (*Phytopathology*, 1967, **57**, 428–433).—The synergistic effects of combining γ-irradiation and heat treatment in inactivating the spores of post-harvest pathogens of *Prunus* spp. are examined. Interaction between the two treatments may result in a 5- to 10-fold increase in inactivation. The amount and order of the treatments and the extent of synergism needed for effective action, varied with the pathogen concerned. With *Rhizopus stolonifera* max. effects were obtained by exposure to radiation before heat treatment. With all other species tested, the reverse order of treatments produced the greater effects. Experiments with fruit inoculated with *Monilinia fructicola* showed the advantage of heat sensitisation before irradiation in the control of brown rot.

A. G. POLLARD.

Control of onion smut with Lanstan. J. W. Lorbeer and D. P. Loparco (*Phytopathology*, 1967, **57**, 342).—On org. soils effective control of onion smut was obtained with Lanstan (1-chloro-2-nitropropane) applied in the seed furrow as granules or as a drench, at the rate of 1–2 lb a.i./acre, to rows 15 in. apart and with 15 seeds per ft.-row. In general the granular form gave better results than the drench and the granules formed on Tru-Sorb (SiO₂ + Al₂O₃) were more effective than those on vermiculite. Good control was also obtained with ethion on granules or by adding diazinon to the drench.

A. G. POLLARD.

Control of neck rot, due to Botrytis allii, of onions during storage. J. Kaufman and J. W. Lorbeer (*Pl. Dis. Reptr*, 1967, **51**, 696–699).—Of a number of materials tested by dust application CaCl₂ and 75% 2,6-dichloro-4-nitroaniline [dichloran] before storage were the most effective for controlling total decay and *Botrytis* neck rot. Captan (50%) and Difolatan (75%) were the most effective in the following year.

A. H. CORNFIELD.

Evaluation of herbicides for weed control in southern peas and snap beans. W. L. Ogle (*Proc. Am. Soc. hort. Sci.*, 1967, **90**, 290–295).—The most effective preplant soil-incorporated treatment for southern peas was trifluralin (**I**) (2,6-dinitro-*NN*-dipropyl-4-trifluoromethylaniline, 0·5–1·0 lb per acre). DCPA (dimethyl-2,3,5,6-tetrachloroterephthalate, 8 lb) and diphenamid (**II**) (*N,N*-dimethyl-2,2-diphenylacetamide, 4 lb) were also effective as soil surface sprays immediately after planting. For snap beans the most effective incorporated treatments were **I** (0·5 and 1·0 lb), EPTC (ethyl-*N,N*-dipropylthiolcarbamate, 3 lb) and PPTC (*N*-propyl-di-n-propyl-thiolcarbamate, 3 lb). **II** (4 lb), Amiben (3-amino-2,5-dichloro-benzoic acid, 8 lb), and DCPA (8 lb) were effective as surface sprays following planting.

A. H. CORNFIELD.

Chemical control of bacterial blight of dry field beans in Michigan by foliage sprays applied by ground and air equipment. A. W. Saetler and H. S. Potter (*Pl. Dis. Reptr*, 1967, **51**, 622–625).—Six Cu fungicides (inorg. and org.) were equally effective in controlling halo and common bacterial blights and increasing yields of field beans.

A. H. CORNFIELD.

Effect of sodium methyldithiocarbamate (SMDC) on wilt incidence in cumin, Cuminum cyninum. B. L. Mathur and R. L. Mathur (*Pl. Dis. Reptr*, 1967, **51**, 629–631).—Drenching the soil with SMDC 20 days before sowing cumin seed increased seedling emergence and growth and reduced the incidence of wilt, due to *Fusarium oxysporium*.

A. H. CORNFIELD.

Fungicides for Arabica coffee. V. Improved laboratory assessments and further tests against Hemileia vastatrix. D. Hocking, P. J. White and A. A. Jaffer (*E. Afr. agric. For. J.*, 1967, **33**, 136–138).—An improved laboratory method for assessing the effectiveness of fungicides for the control of coffee leaf rust, due to *Hemileia vastatrix*, is described. Of nine new fungicides tested none gave significantly better control than Colloidox (a good Cu-based fungicide).

A. H. CORNFIELD.

Control of sugar-cane frog hoppers. D. W. Fewkes (*Wld Rev. Pest Control*, 1967, **6**, 21–31).—These plant-sucking insects of the superfamily Ceropoidea (Homoptera) are important pests of several crops, lucerne, red clover, red pine, pasture grasses and

sugar-cane. Distribution, ecology and damage are briefly reviewed. A general review of chemical methods of control is given and a summary of biological control methods is presented. It would appear that no one method or insecticide is entirely satisfactory. (86 references.)

C.V.

Control of downy mildew, due to Peronospora arborescens, of opium poppy by root application of fungicides. K. L. Kothari and A. C. Verma (*Pl. Dis. Reptr*, 1967, **51**, 686–687).—Of a number of materials tested as root drenches in pot tests 200 ppm zineb, ferbam and Bis-D (Mn-Zn-dithiocarbamate) were the most effective in reducing the intensity of the disease in poppy leaves.

A. H. CORNFIELD.

Dothistroma needle blight of Austrian and ponderosa pines: Epidemiology and control. G. W. Peterson (*Phytopathology*, 1967, **57**, 437–441).—Detailed observations of the epidemiology of the disease are recorded. Current-year needles first became susceptible to *D. pini* in mid-summer. Two applications of Bordeaux mixture or other Cu fungicide, carefully timed according to climatic conditions, gave good control.

A. G. POLLARD.

Control of charcoal root disease, caused by Macrophomina phaseoli, of white fir by autumn soil fumigation. R. S. Smith, jun. and S. L. Krugman (*Pl. Dis. Reptr*, 1967, **51**, 671–674).—Autumn treatment of soil with MBR-CP-PBR (61% MeBr, 31% trichloronitromethane, 8% propargyl bromide, 200 lb) or MBR-CP (57% MeBr, 43% trichloronitromethane, 325 lb per acre) reduced the proportion of seedlings (planted in the following spring) infected with charcoal root disease and the mortality of seedlings.

A. H. CORNFIELD.

Elm bark beetles. Anon. (*Leafl. U.S. Dep. Agric.*, 1967, No. 185; 8 pp).—*Scolytus multistriatus* and *Hylurgopinus rufipes* are described and control measures indicated.

E. G. BRICKELL.

Termite control. Anon. (*Leafl. U.S. Dep. Agric.*, 1967, No. 324; 7 pp).—Soil treatment aids are described.

E. G. BRICKELL.

Preservative treatment of fence posts and farm timbers. J. O. Blew jun. and F. J. Champion (*Fmrs' Bull.*, *U.S. Dep. Agric.*, 1967, No. 2049; 33 pp).—Common preservatives and best treating methods are described.

E. G. BRICKELL.

Control of Cylindrosporium leaf spot disease of Rhus trilobata and Shepherdia argentea seedlings. G. W. Peterson (*Pl. Dis. Reptr*, 1967, **51**, 700–701).—Application of captan 50W (2 lb), maneb 80% WP (1·5 lb) or Bordeaux mixture [8 lb CuSO₄·5H₂O + 8 lb Ca(OH)₂ per 100 gal] at weekly intervals from 15 June to 31 Aug. greatly reduced the extent of defoliation in 2-year-old *Rhus trilobata* and *Shepherdia argentea*.

A. H. CORNFIELD.

Insecticides: Action and metabolism. R. D. O'Brien. 1967, 332 pp. (N.Y. and Lond.: Academic Press).—Chapters are devoted to: Physical toxicants, organophosphates: Chemistry and inhibitory activity. (60 references). Action, therapy and metabolism. (87 references). Carbamates. (58 references). DDT and related compounds. (98 references). Cyclodienes. (48 references). Nicotinoids. (26 references). Rotenoids. (13 references). Pyrethroids. (15 references). Fluorene compounds. (37 references). Lindane and other hexachlorocyclohexanes. (38 references). Miscellaneous compounds (arsenicals, silica aerogels, DNOC and dinex). (64 references). Synergism, antagonism and other interactions. (85 references). Resistance. (91 references). Selectivity: penetration. (116 references). Environmental health. (51 references).

C.V.

Tetraethylammonium chloride [TEAC] as an antidote for certain insecticides in mice. T. L. Andrews and R. P. Miskus (*Science, N.Y.*, 1968, **159**, 1367–1368).—TEAC was superior to atropine sulphate (**I**) for some carbamate (**II**) insecticides and nicotine: it does not produce traumatic effects and the sometimes fatal reactions caused by **I**. TEAC and **I** give equal protection against lethal oral doses of the **II** compounds, Zectran, NIA-10242 and Lannate [*S*-methyl-*N*(methylcarbamoyloxy)thioacetimidate]. The effects of TEAC were not evaluated against Sevin and Baygon because the acute oral toxicity of these could not be determined. The results with Matacil were inconclusive. TEAC was not an effective antidote against the organo-P compound, parathion but its use fully eliminates the effects of nicotine intoxication. (10 references.)

C.V.

Residual contact toxicity to honey bees of insecticides sprayed on to white clover [Trifolium repens. L.] in the laboratory. P. G. Clinch (*N.Z. Jl agric. Res.*, 1967, **10**, 289–300).—Direct contact toxicity tests appear of little value as guides to residual toxicity. (28 references.)

E. G. BRICKELL.

Chlorinated hydrocarbon insecticide residues in selected insects and birds found in association with cotton fields. E. I. El Sayed, J. B.

Graves and F. L. Bonner (*J. agric. Fd Chem.*, 1967, **15**, 1014–1017).—The concn. of DDT and DDE found in ten species of insects were 0·1–9·2 and trace –12·9 ppm, respectively. Five species were free from insecticide. Small concn. of other insecticides were found in some cases. In laboratory experiments with *Heliothis* spp. (5th and 6th instars) DDT disappeared within a few days, after which DDE disappeared rapidly. The residues found in eight species of birds were little different in concn. from those found in numerous species in other parts of the U.S.A. (20 references.)　　　　　　　　　　　　　　　　　P. S. ARUP.

DDT reduces photosynthesis by marine phytoplankton. C. F. Wurster, jun. (*Science, N.Y.*, 1968, **159**, 1474–1475).—Concn. as low as a few ppb (American) reduced photosynthesis in laboratory cultures of four species of coastal and oceanic phytoplankton representing four major classes of algae. It was shown that toxicity to diatoms increased as cell concn. decreased. (22 references.)　　　　　　　　　　　　　　　　　　　C.V.

Adsorption of bipyridylium herbicides in soil. B. V. Tucker, D. E. Pack and J. N. Ospenson (*J. agric. Fd Chem.*, 1967, **15**, 1005–1008).—Paraquat and diquat were separately added to five types of soil in varying concn. At low levels both herbicides were firmly bound to the soils and could only be removed by refluxing with 18N-H_2SO_4; at moderate levels they could be removed in part by leaching with saturated aq. NH_4Cl, and at high levels in part by leaching with water. The different soil types varied considerably in their capacities to bind firmly or loosely. (10 references.)　　　　　　　　　　　　　　　　　　　P. S. ARUP.

Decomposition of linuron in different soils. O. Lode (*Weed Res.*, 1967, **7**, 185–190).—In a glasshouse experiment, linuron [*N'*-(3,4-dichlorophenyl)-*N*-methoxy-*N*-methylurea] (I) was added to five soils. Chemical residue analysis and bioassay showed rate of decomposition to be different for each soil and that 1-concn. producing growth reduction in ryegrass seedlings varied with soil type. No leaching into the lower layers occurred. Initially bacterial and fungal numbers were depressed but the populations recovered later. (15 references.)　　　　　　　　　C.V.

Fate of insecticide residues. Decomposition of lindane in soil. W. N. Yule, M. Chiba and H. V. Morley (*J. agric. Fd Chem.*, 1967, **15**, 1000–1004).—Whilst DDT, dieldrin, and heptachlor proved to be stable in a mixture of five agricultural soils, lindane was found to be progressively dechlorinated (and detoxified) during 6 months; γ-pentachlorocyclohexene was formed. Dechlorination in moist, acid-to-neutral soil was ∼ 3 times faster than in dry soil. The possibility of microbial action being involved in the breakdown was examined. (24 references.)　　　　　　　　　P. S. ARUP.

Metabolism of insecticides. [A] **Microsynthesis of** ^{14}C-β-**dihydroheptachlor.** [B] **Metabolism of** ^{14}C-β-**dihydroheptachlor in soil and by micro-organisms.** N. H. Poonawalla and F. Korte (*J. agric. Fd Chem.*, 1968, **16**, 13–14, 15–16).—Directions are given for the synthesis of this compound (2-*exo*-4,5,6,7,8,8-heptachloro-4,7-methano-3a,4,7,7a-tetrahydroindan) (I) (cf. Büchel *et al.*, *Tetrahedron Letters*, 1964, **33**, 2267) by the treatment of ^{14}C-chlordene (II) with HCl under conditions modified for the micro-scale. The prep. of II from ^{14}C-hexachlorocyclopentadiene is described. The overall yield of I (purified by t.l.c.) was 84%. (10 references.)

[B] Metabolism of the compound in aq. soil suspension proceeded slowly, amounting to 3–4% in 10–15 days. Metabolism by *Aspergillus niger* and *Penicillium urticae* also proceded very slowly, and without excretion of the metabolites. These properties of the insecticide, and its low mammalian toxicity, give it an advantage over insecticides of similar structure. (11 references.)　　　　　　　　　　　　　　　　　　　P. S. ARUP.

Effect of some herbicides on the development of soil algae. L. S. Balezina (*Microbiology, [USSR]* 1967, **36**, 134–137).—CCl_3CO_2Na applied before spring cultivation to dry soil reduces the no. of algae by a factor of three. Under laboratory conditions (with a constant high water concn.) there was a stimulating effect which increased with increasing concn. Dicotex-80 and Cresonite gave similar results but simazine under laboratory conditions shows high and persistent toxicity for various groups of algae, specially for the blue-green variety. (16 references.)　　　　　　　　　C.V.

Partial hydrolysis of dieldrin by *Aerobacter aerogenes*. G. Wedmeyer (*Appl. Microbiol.*, 1968, **16**, 661–662).—The formation of 6,7-*trans*-dihydroxydihydroaldrin was studied. Conversion averaged 9% by the third day of incubation, rising to 12% by the fifth with no further change up to 10 days.　　　　　　　C.V.

Photochemical degradation of diquat. P. Slade and A. E. Smith (*Nature, Lond.*, 1967, **213**, 919–920).—When a dil. solution of ^{14}C-labelled diquat dibromide was exposed to weak sunlight there was

shown (by paper chromatography) to be one principal decomposition product. The reaction was repeated on a larger scale using a 'Hanovia 10L' photochemical reactor with a borosilicate down-tube containing a mercury vapour arc tube. The degradation product was isolated and shown by elemental analysis, i.r., u.v., nuclear magnetic resonance and mass spectra data to be 1,2,3,4-tetrahydro-1-oxo-pyrido[1,2,-α]-5-pyrazinium chloride; this was confirmed by synthesis. Although it is found on plants sprayed with diquat it is not thought to be translocated into harvested plants; it has a very low oral toxicity to mammals.　　　　　　　　S. A. BROOKS.

Animal Husbandry

Yield and chemical composition of various varieties of oat fodders. S. K. Ranjan, R. P. Singh and S. K. Talapatra (*Indian J. Dairy Sci.*, 1966, **19**, 214–215).—Data are presented on the yield (gross agricultural and dry matter) and chemical composition of 16 varieties of oats.　　　　　　　　　　　　　　　　　　M. O'LEARY.

Chemical composition of Tanzania feedstuffs. A. H. Naik (*E. Afr. agric. For. J.*, 1967, **33**, 201–205).—Dry matter, crude protein, ether extract, crude fibre, N-free extract, Ca, and P contents of 48 feedstuffs are presented.　　　　　　　　　　　A. H. CORNFIELD.

Conservation of heavily-wilted baled herbage in sealed polythene containers. W. O. Brown and N. Jackson (*J. Br. Grassld Soc.*, 1967, **22**, 135–140).—Baled herbage from a timothy-meadow-fescue sward was successfully ensiled in a completely sealed polyethylene container. Gross and metabolisable energy values of the ensiled material were high. Spraying the sward with aq. $Na_2S_2O_5$ before cutting had little effect on the ensiled material.　　　　　　　　　　　　　　　　　A. H. CORNFIELD.

Factors causing differences in digestibility of grasses measured by an *in vitro* method. R. J. K. Walters, G. ap Griffith, R. Hughes and D. I. H. Jones (*J. Br. Grassld Soc.*, 1967, **22**, 112–116).—In first (April to June) stage of growth, leafiness and the accumulation of dead material accounted only partly for the variation in digestibility of perennial ryegrass, cocksfoot and timothy. However, these factors accounted almost entirely for the variation between varieties within each species. In the regrowths, heading behaviour and leafiness were not closely associated with digestibility.　　　　　　　　　　　　　　　　　A. H. CORNFIELD.

Effects of level of nitrogen fertiliser on beef production from grazed perennial ryegrass-white clover pastures. I. Irrigation experiment. F. E. Alder, S. J. Cowlishaw, J. E. Newton and D. T. Chambers (*J. Br. Grassld Soc.*, 1967, **22**, 194–203).—The effects of level of applied N and stocking rates on soil-N levels, herbage consumption, yields, chemical and botanical composition of herbage available for grazing, and the quantities of herbage removed for conservation are reported.　　　　　　　　　　　　　　　A. H. CORNFIELD.

In vitro and faecal nitrogen techniques for predicting the voluntary intake of Rhodes grass, (*Chloris gayana*). D. J. Minson and R. Mildorf (*J. Br. Grassld Soc.*, 1967, **22**, 170–175).—Intake of dry matter (*DM*) of two varieties of Rhodes grass cut at four stages of maturity was correlated with *in vitro* digestion of *DM* after 12 h incubation. The intake of digestible *DM* was correlated (*r* = 0·99) with the sum of the digestion after 12 h and 48 h incubation. *In vitro* digestion of coarse samples was lower than that of fine samples, particularly with the more mature forages. Faecal N was correlated (*r* > 0·96) with *DM* digestibility, voluntary intake of *DM* and digestible *DM*. Different regression equations applied to the two varieties.　　　　　　　　　　　　A. H. CORNFIELD.

Effects of grazing management on yield and its components of pasture grasses. J. A. Agyare and B. R. Watkin (*J. Br. Grassld Soc.*, 1967, **22**, 182–191).—A study was made of the effects of grazing (by sheep) when swards reached heights of 3 in. and 9 in. on dry matter production, leaf area index (*LAI*), and tillering of three pasture grasses grown with white and red clovers. In the spring-summer season herbage yields were significantly higher when grazed at 9 in. than when grazed at 3 in. height. In the autumn–winter period *LAI* was linearly correlated with dry matter production. In both seasons rate of leaf production per tiller was higher in cocksfoot than in ryegrass or tall fescue. New tillers had a higher rate of leaf production than old tillers in spring–summer, but not in autumn–winter.　　　　　　　　　　　　A. H. CORNFIELD.

Nutritive value of groundnut, *Arachis hypogaea*, haulms in Uganda. R. S. Musangi and S. V. Soneji (*E. Afr. agric. For. J.*, 1967, **33**, 170–174).—Values are given for dry matter and crude protein and fibre in dry matter of the whole plant, stalks, leaves and pods of groundnut at weekly intervals during the first 2 months of growth.

Groundnut haulms should be a useful supplement to the diet of dairy cattle. A. H. CORNFIELD.

In vitro digestibility techniques used under East African conditions. M. I. E. Long (*E. Afr. agric. For. J.*, 1967, **33**, 166–169).—The *in vitro* digestibility technique (*J. Br. Grassld Soc.*, 1963, **18**, 104) was adopted with minor modifications as a suitable method for pasture assessment. Regression equations were fitted to the data for earlier *in vitro* digestibility trials and digestible energy determinations. The importance of sampling being regulated to herbage selected by the animal is discussed. A. H. CORNFIELD.

Some effects of bacterial inoculation on silage-making. K. Lesins and F. H. Schulz (*Can. J. Anim. Sci.*, 1968, **48**, 15–25).—Sedge and lucerne, inoculated with two lactic acid bacteria (rods and cocci), were incubated in laboratory silos at 30° or 43°. The lactic bacteria greatly increased the acidification of the sedge but had little effect on that of lucerne. Addition of sucrose hastened the acidification of lucerne. At 43° only the cocci survived. Possible uses of bacterial specialisation in silage-making are discussed. A. G. POLLARD.

Effect on nutritive value of crushing and lacerating hay in making of barn-dried hay. N. D. Dijkstra (*Versl. landbouwk. Onderz. Ned.*, 1967, No. 698, 33 pp).—In the new rapid process of haymaking, the newly cut grass was lacerated, tedded repeatedly until the dry matter was increased to at least 60% and finally barn-dried with unheated air, In comparison with conventionally made hay the losses in nutritive value were appreciably lower. P. S. ARUP.

Cutting management of grass–clover swards. VI. Effects of different closeness and frequency of cutting treatments on the yield and quality of herbage from a cocksfoot–white clover sward. D. Reid (*J. agric. Sci., Camb.*, 1968, **70**, 59–64).—Cutting to 1 in. from ground level with a reciprocating blade mower produced significantly higher dry matter and crude protein yields than cutting at 2·5 in. in the first year only. Cutting to 1 in. with a lawn mower gave a smaller dry matter yield at both the grazing and silage stages in the first year, but a slightly greater yield at the grazing stage in the third year. Cutting was carried out five times during the grazing stage or three times during the silage stage per season. M. LONG.

Effect of lime and calcium carbonate supplementation on the nutritive value of paddy (*Oryza sativa*) straw. K. Nath, K. Sahai and N. D. Kehar (*J. agric. Sci., Camb.*, 1968, **70**, 169–170).—Neither the dry matter, crude protein, ether extract or total carbohydrate digestibilities nor the N and P balances were affected by the inclusion of $CaCO_3$ or $Ca(OH)_2$ in paddy straw rations. The Ca balance was significantly improved and $CaCO_3$ or $Ca(OH)_2$ supplements at the rate of 28 g or 15 g respectively are considered to be economic as a means of combating negative Ca balances encountered in paddy straw feeding. M. LONG.

Effect of heat on aflatoxins in oilseed meals. G. E. Mann, L. P. Codifer, jun., and F. G. Dollear (*J. agric. Fd Chem.*, 1967, **15**, 1090–1092).—The content of aflatoxins (produced by *Aspergillus flavus*) in cottonseed meal, determined by t.l.c. (cf. Pons *et al.*, *J. Ass. off. analyt. Chem.*, 1966, **49**, 554) was reduced by ~80% by heating the meal containing 20% of moisture at 120° at atm. pressure for 120 min. A reduction of 34% was effected in contaminated soyabean meal by similar treatment. (24 references.) P. S. ARUP.

Partial purification of an antidiarrheic factor in modified cottonseed oil soapstock for milk replacer rations. F. Pinkerton and R. E. Leighton (*J. Dairy Sci.*, 1967, **50**, 1671–1674).—A fraction, 3% by vol. of the original, which was effectively antidiarrheic when added to a milk replacer diet at a level of 0·3% by wt. was extracted from a commercial feed-grade oil, Marcol B-75. M. O'LEARY.

Nutritive value of sorghum silage for growth and lactation as influenced by characteristics and treatments of the forage. G. M. Ward, F. W. Boren, E. F. Smith and J. R. Brethour (*J. Dairy Sci.*, 1967, **50**, 1634–1637).—Silages (40) were made at two stations from sorghums varying from seedless to heavily seeded, with and without wilting before ensiling, with and without grinding seed heads, and with and without added carbohydrates. Growth trials with calves revealed an average digestible energy content of 2·77 Mcal/kg dry matter. Calf wt. gains were closely correlated with silage dry matter intake. Grinding seed heads had no effect on growth of calves. Neither field wilting of the sorghum nor carbohydrate addition before ensiling effected any improvement in silage quality. M. O'LEARY.

Relationship between the digestibility of a sward and herbage consumption of grazing calves. J. Hodgson (*J. agric. Sci., Camb.*, 1968, **70**, 47–51).—*In vitro* digestibility of the herbage and the amount eaten were significantly related over the range of digestibility encountered (68–82%). Regression equations calculated within

seasons and fertiliser levels and within years did not differ significantly. No relation was found between faecal output and herbage digestibility. Young ruminants may be more sensitive than adults to changes in digestibility and chemostatic control of food intake may take full effect only at very high levels of digestibility with young, rapidly growing animals. M. LONG.

Factors affecting intra-cow variation in production of beef cattle. H. M. Jamison (*Diss. Abstr.*, B, 1967, **27**, 2931–2932).—The effects of numerous factors related to the reproductive activity of the cow on the average daily gain in wt. of the calf from birth to weaning are examined with a view to prediction of growth rates and the type of animal. A. G. POLLARD.

Factors affecting the growth and feed efficiency in beef cattle. S. Karam Shah (*Diss. Abstr.*, B, 1967, **27**, 2940–2941).—Fifty-seven Angus calves sired by four bulls in 1964–5 and 59 others sired by five bulls in 1965–6 were fed individually in an investigation of the effects of sire, sex, ration of dam, year of their performance and the interactions on initial wt., rate of gain and feed efficiency in a 'time constant' feedlot trial. The effect of daily max. and min. temp. on rate and efficiency of gain was also examined. Data showed a highly significant sire effect on initial wt., highly significant sire and sex effects on average daily gain in wt. and highly significant sex effect and sire × year effect on feed efficiency. Year effect on feed efficiency approached significance. A. G. POLLARD.

Some factors affecting feed efficiency in beef cattle. J. H. Landers, jun. (*Diss. Abstr.*, B, 1967, **27**, 2939).—Over an 11-year period 494 calves (bulls and heifers) from four in-bred lines were put on a 'constant gain' test on reaching live-wt. 500 lb until weighing 800 lb. All were fed pelletted rations (lucerne 66·6, concentrates 33·3%) individually under conditions comparable with *ad lib.* feeding. No difference between the sexes was apparent in feed consumption at any given body-wt. but the bulls gained 0·67 lb/day more than the heifers. Differences in daily gain between lines of Herefords and between Herefords and Angus were apparent. Angus calves consumed more feed and showed less gain than did the Herefords. Calves from younger cows were older on reaching the 500 lb live-wt. stage than were those from old cows. Rate and efficiency of gain of the calves were affected by the time of birth within the calving period. Feed consumption per unit body-wt. declined during the test period except for a small increase at 625 and 725 lb live-wt. for heifers and bulls respectively. Rate of gain per unit body-wt. declined continuously. A. G. POLLARD.

Dry matter consumption and digestibility. I. Adult bovines. P. Ayyaluswami, V. Jagannathan, Venkateson and V. S. Jayaramam (*Indian J. Dairy Sci.*, 1966, **19**, 146–148).—Data obtained on the dry matter intake of five dry cows of the Tharparkar, Murrah, and Kangayam breeds, fed concentrates and paddy straw, are presented. M. O'LEARY.

Urea utilisation by ruminants as affected by carbohydrate source. R. C. Joshi and B. N. Majumdar (*Indian J. exp. Biol.*, 1966, **4**, 231–234).—A comparison has been made between tapioca, maize and barley as a source of carbohydrate for improving the digestibility and retention of urea N by Hadriana calves. Tapioca was the best source although the tapioca diet supplied the least amount of energy per g of crude protein ingested. Acid and alkaline additives did not improve the retention of N, Ca or P nor did they increase the digestibility of the org. nutrients present in the urea-supplemented tapioca diet. (19 references.) S. A. BROOKS.

Cane molasses as substitute for maize in beef-finishing rations. A. W. Lishman (*S. Afr. J. agric. Sci.*, 1967, **10**, 51–59).—The introduction of molasses to represent 20–30% of the total digestible nutrients in a hay and maize diet increased the feed intake, but had no effect on the growth rate or carcass quality of 3–3·5-year old steers. (24 references.) P. S. ARUP.

Utilisation of grass fed indoors to young beef cattle, with or without supplementary barley. T. J. Forbes, A. M. Raven, J. H. D. Irwin and K. L. Robinson (*J. Br. Grassld Soc.*, 1967, **22**, 158–164).—From 5 to 9 months of age Friesian steers were fed indoors with (*i*) grass *ad lib.*, (*ii*) grass *ad lib.* with supplementary barley at 0·5 lb per 100 lb body-wt., (*iii*) grass *ad lib.* with supplementary barley at 1 lb per 100 lb body-wt. Supplementary barley increased total dry-matter intake. Mean daily live wt. gains were 1·60, 1·91, and 2·20 lb and feed dry-matter conversion ratios were 6·18, 5·67, and 5·55 for treatments *i*, *ii* and *iii* respectively. The dry matter digestibility of the diets was slightly higher when the grass was supplemented with barley than when grass alone was given. Although barley depressed the digestibility of protein, the absolute and proportionate retention of N was increased, particularly in treatment *iii*. N retention correlated with live-wt. gains. A. H. CORNFIELD.

Voluntary intake of food and water and the lactation performance of cows given diets of varying roughage content ad libitum. B. J. Owen, E. L. Miller and P. S. Bridge (*J. agric. Sci., Camb.*, 1968, **70**, 223–235).—Four dietary treatments were given to 12 Friesian heifers, from a date 2 months prior to calving until the end of the first lactation. All feeds were supplied *ad lib*. A basal concentrate was either mixed with milled hay or barley straw or else the concentrate and roughage were fed separately. One cow of each group was fitted with a ruminal cannula. The group fed roughage separately, especially the straw, consumed low amounts of roughage which led to serious deficiencies in milk butterfat content. When incorporated with the concentrate, levels were normal. The low roughage intake was associated with a smaller vol. of liquid in the rumen, a reduced flow leaving it, a lower proportion of acetic and butyric acids and a higher proportion of propionic acid in the rumen liquid and higher blood-glucose levels. When the roughage was milled and mixed with concentrate all these components were in the normal range. Food and water intakes followed a similar pattern although fluctuations in water intake were greater. M. LONG.

Effect of variety of ensiled maize and hay supplementation on milk production. V. S. Logan, L. J. Fisher and P. S. Hayden (*Can. J. Anim. Sci.*, 1968, **48**, 41–46).—Two maize varieties (Pioneer 383 and Pride 5) were grown in alternating strips in the field and, at maturity, were made into vac.-silages. The silages were fed to lactating cows with or without hay. The intake of dry matter by the cows was greater for Pride 5 than for Pioneer; it was lowered in both cases by use of hay with the silage. Milk yields were greater when Pride 5 than when Pioneer silage was fed and were significantly increased when a hay supplement was given. The relative utilisation of silage dry matter, as expressed by production of fat-corrected milk per hectare, was greater for the Pioneer silage. A. G. POLLARD.

Comparison of pearl millet and Sudan-grass as pastures for lactating dairy cows with special emphasis on the milk-fat. P. R. Peta (*Diss. Abstr.*, B, 1967, **27**, 2934–2935).—The effect of the grazing of pearl millet (*PM*) by cows on the depression of milk-fat levels is examined. Comparison of *PM* and Sudan-grass (*SG*) and of supplements of soyabean meal, lucerne hay or urea showed that (*a*) grazing *PM* resulted in lower acetic and butyric acid and higher propionic acid levels in the ruminal contents and lower milk-fat levels than when grazing *SG*; (*b*) supplementing *PM* with lucerne increased ruminal acetate and propionate and also the milk-fat level. Supplementary soyabean meal or urea tended to lower milk-fat levels, though not significantly. In a further trial *PM* and *SG* were fed as hay or silage to constitute the sole roughages. Use of *PM* hay tended to lower milk-fat levels and when fed either as hay or silage lowered ruminal acetate and increased propionate as compared with feeding *SG*. The saturated fatty acid component of the total milk-fat diminished with the % milk-fat; NaOAc or lucerne hay supplements appeared to restrict the decline. Changes in blood-glucose levels suggest a negative relation between increase in glucose level and the lowering of milk fat %; supplementary NaOAc had no apparent glucogenic effect. The non-esterified (*NE*) fatty acids in the plasma were positively related to the milk-fat %. Depression in milk-fat levels during grazing of *PM* was also associated with increased blood-glucose and decreased *NE*. A. G. POLLARD.

Milk composition as affected by intraruminal infusion of volatile fatty acids to cows on a restricted ration. G. F. Wilson, A. W. F. Davey and R. M. Dolby (*N.Z. Jl agric. Res.*, 1967, **10**, 215–225).— Infusion of dil. solutions of acetic (**I**), propionic (**II**), and butyric acid (**III**), raised milk yield. **I** increased butterfat and solids-not-fat. **II** lowered daily butterfat yield and % but increased protein yield and % and hence increased level of solids-not-fat. **III** increased yield and % butterfat and decreased % solids-not-fat. All three acids lowered the I₂-value of the butter fat. **III** increased, whereas **I** and **II** lowered, the Reichert value. (23 references.) E. G. BRICKELL.

Effect on milk yield and composition of adding the calcium salts of acetic, propionic, butyric and lactic acids to the diets of dairy cows. C. C. Balch, W. H. Broster, V. W. Johnson, C. Line, J. A. F. Rook, J. D. Sutton and V. J. Tuck (*J. Dairy Res.*, 1967, **34**, 199–206).— Calcium salts of acetic, propionic, butyric or lactic acids were added to the diets of dairy cows. Such additives are of no practical value as supplements in the diet of milking cows. (10 references.) M. O'LEARY.

Effects of pelleting concentrate mixtures of varying starch content on milk yield and composition. S. Yamdagni, R. G. Warner and J. K. Loosli (*J. Dairy Sci.*, 1967, **50**, 1606–1611).—Experiments in which Holstein cows were fed meal, pellet, and ground pellet forms of concentrate and roughage at either 1·0 or 1·5 kg hay equivalent per 100 kg body wt. revealed no differences in milk yield between the various forms of ration. Pellets and ground pellets depressed milk fat slightly but significantly. Cows on low roughage yielded milk having significantly higher solids-not-fat and protein than that of cows on high roughage. Further experiments with pelleted concentrates indicated that both the quantity and type of starch in the diet have an important effect on the fat content of milk. (23 references.) M. O'LEARY.

Effects of milking at unequal intervals for a complete lactation on milk yield and composition. E. E. Ormiston, S. L. Spahr, R. W. Touchberry and J. L. Albright (*J. Dairy Sci.*, 1967, **50**, 1597–1605).—Trials with 82 cows indicated that 3·5% more milk, 5·0% more milk fat, 4·0% more solids-not-fat, and 5·5% more total solids were produced per lactation when milking intervals were equal than when milking intervals were unequal. (19 references.) M. O'LEARY.

Vitamin A and E interrelations in high-concentrate finishing rations for beef cattle. R. H. Kohlmeier (*Diss. Abstr.*, B, 1967, **27**, 2937–2938).—Finishing rations of 90% ground ear maize and 10% supplement (but no hay) were fed to steers. A daily supplement of vitamin E (*E*) (100 or 200 I.U.) improved feedlot performance to extents which depended on the vitamin A (*A*) level of the ration and that of liver reserves. Increasing the dietary *A* level further improved the finishing performance to a limit reached with 6000 I.U./day. Dietary additions of *A* or *E* affected neither carcass grade, dressing % nor liver wt. expressed as % body-wt. Supplementary *A* lowered the proportion of abscessed livers. *E* did not affect blood- or liver-*A* during finishing trials or in a depletion–repletion test. Evidence was obtained of a sparing action of *A* on *E* reserves. In an *A*-depletion test, liver-*A* reserves declined by 50% every 28 days but blood-*A* did not fall appreciably until liver-*A* reached < 10–20 mg/g. After depletion of liver-*A* reserves, refeeding *A* at 10,000 I.U. daily greatly increased blood-*A* but increased liver-*A* only slightly; feeding at 30,000 I.U. daily markedly increased both blood- and liver-*A*. Following the withdrawal of the *A* supplement blood-*A* fell considerably in 24–48 h. Liver-*A* values of ≮ 2 mcg/g fresh liver indicate sufficient *A* nutrition although liver-*A* < 2 mcg is not in itself adequate evidence of nutritional *A* deficiency. Blood-*A* ≮ 25 mcg/100 ml plasma indicated adequate *A* nutrition, whereas ≯ 15 mcg indicated inadequacy. Intermediate levels were best evaluated by relation to the liver-*A* values. A. G. POLLARD.

Evaluation of yellow maize as supplement to mature winter-cut veld grass for woolled sheep. C. G. Coetzee and P. J. S. Pieterse (*S. Afr. J. agric. Sci.*, 1967, **10**, 203–214).—Initial body wt. could be maintained with supplements of 300 g of maize meal per day, but wool-growth decreased towards the end of a 25-week period. Results were satisfactorily improved by additional supplements of 10 g of urea per day, but not by the partial substitution of fish-meal for maize-meal. (15 references.) P. S. ARUP.

Factors affecting the voluntary intake of silage and hay by sheep and cows. J. C. Murdoch (*J. Br. Grassld Soc.*, 1967, **22**, 95–99).— Voluntary intake of hay increased with digestibility, but for silage the relationship was not so well defined. Addition of concentrates depressed the intake of hay, more so with hay of high than with that of low digestibility. Concentrates depressed the intake of hay by sheep more than by cows. A. H. CORNFIELD.

Biuretolytic activity of the ruminal flora of sheep fed practical rations containing biuret. F. M. C. Gilchrist, E. Potgieter and J. B. N. Voss (*J. agric. Sci., Camb.*, 1968, **70**, 157–163).—Sheep fed biuret (**I**) develop biuretolytic activity. On a diet of poor teff hay the activity was low but when maize meal was added it was three times as high, and was sufficient to account for the disappearance of up to 16·4 g **I** per day in a Merino sheep with ruminal ingesta amounting to 6 l. A description of a semi-micro colorimetric determination of **I**, developed for use with ingesta, is given. M. LONG.

Rates of entry and oxidation of acetate, glucose, *d*(-)-β-hydroxybutyrate, palmitate, oleate and stearate, and rates of production and oxidation of propionate and butyrate in fed and starved sheep. E. F. Annison, R. E. Brown, R. A. Leng, D. B. Lindsay and C. E. West, (*Biochem, J.*, 1967, **104**, 135–137).—Rates of entry and oxidation of some metabolites have been determined in tracheostomised sheep by a combination of isotope-dilution techniques with continuous measurement of total respiratory gas exchange, and production of ¹⁴CO₂ during intravenous or intraruminal infusion of ¹⁴C-labelled substrates. Mean entry rates in fed (*FS*) and starved sheep (*SS*) (24 h), (as mg/min./kg body wt.$^{0.75}$), were: glucose (**I**), 5·0 and 3·8; acetate (**II**) 10·8 and 5·8; *d*(-1)-β-hydroxybutyrate (**III**), 1·4 and 1·5; palmitate (**IV**), oleate

(V), and stearate (VI) (*SS*) only) 1·0, 0·9, and 0·9, respectively. Production rates of propionate **VII** and butyrate (**VIII**) in continuously feeding sheep were 6·4 and 4·3 mg/min./kg$^{0.75}$,, and in *SS* were 2·5 and 1·0 mg/min./kg$^{0.75}$, respectively. Calc. mean values, for *FS* and *SS*, of the contribution of individual substrates to overall oxidative metabolism are, respectively: **I**, 9·1 and 11·2; **II**, 31·6 and 22·1; **III**, 10·4 and 4·8; **VII**, 23·0 and 7·1; **VIII**, 16·5 and 5·3; and **IV**, **V**, and **VI** (*SS* only), 4·7, 4·0, and 4·4%. The general technique is of potential value for provision of valid quant. parameters of animal metabolism.   J. N. ASHLEY.

Haemoglobin type and blood and sodium concentrations in Sudan desert sheep. A. G. H. Kattah (*J. agric. Sci., Camb.*, 1968, **70**, 95–97).—Of 411 animals investigated 396 possessed haemoglobin B in the homozygous state, the others having AB. AA was not detected. No bimodality was found in whole blood K and Na levels.   M. LONG.

Factors associated with differences in carcass conformation in lamb. A. H. Kirton and F. S. Pickering (*N.Z. Jl agric. Res.*, 1967, **10**, 183–200).—Blocky carcasses have no superiority in terms of meat content or proportion of high-priced cuts. More emphasis should be placed on economic characteristics and less on conformation in breeding and cross-breeding programmes. (42 references.)   E. G. BRICKELL.

Influence of season and nutrition on the sulphur content of wool from Merino and Cheviot sheep. J. M. Doney and C. C. Evans. (*J. agric. Sci., Camb.*, 1968, **70**, 111–116).—Both breed and nutrient cycle have an effect on the relationships between S content and wool growth or nutrient intake. The results are explained on the basis of a constant optimum composition for each individual with S impoverishment related to lack of balance between follicular activity rate and high S substrate availability. Differences in mean max. S contents are greater between individuals within breeds than those between breeds. The seasonal depressions between individuals within a breed varies with nutrition and wool growth rate. Cheviot sheep show the greatest depressions.   M. LONG.

Relationship of nematode infection to efficiency of wool production. W. H. Southcott, D. D. Heath, and J. P. Langlands (*J. Br. Grassld Soc.*, 1967, **22**, 117–120).—Drenching sheep every two weeks with thiabendazole eliminated nematode parasites and increased wool production and the efficiency of wool production with respect to gross intake of org. matter.   A. H. CORNFIELD.

Dried brewers' grains in rations for market hogs. L. G. Young and R. H. Ingram (*Can. J. Anim. Sci.*, 1968, **48**, 83–88).—Barrows were self-fed individually with rations based on maize in which dried brewers' grains supplied 0, 25, 50, 75 or 100% of the supplemental protein. The average rate of growth of pigs receiving the experimental diets having up to 50% of dried grains was the same as in those given the control (maize–soyabean) ration. When the brewers' grains provided > 50% of the supplemental protein, growth rates and feed efficiency declined somewhat.   A. G. POLLARD.

Relationship of dietary protein levels to performance and carcass merit of market swine. L. G. Young, G. C. Ashton, R. P. Forshaw and R. H. Ingram (*Can. J. Anim. Sci.*, 1968, **48**, 71–81).—Groups of barrows and gilts were fed rations which included three different levels of protein (19·2, 17·4 and 15·5%) in the growing ration and three other levels (17·4, 15·5 and 14·2%) in the finishing ration. In the growing stage, growth rates increased linearly with the dietary protein level and feed required per kg gain in wt. diminished in a curvilinear manner. In the finishing stage, dietary protein level did not affect the gain in wt., but feed per unit gain increased linearly as the dietary protein diminished. With the diet having the lowest protein level throughout, the rate of gain diminished but the feed per unit gain increased. Efficiency of feed utilisation was similar for barrows and gilts; barrows showed the greater rate of gain and the greater daily feed consumption. The area of the *longissimus dorsi* muscle increased with the protein level in the finishing ration. An interaction is noted between sex and protein levels in the finishing period for total ham wt. and wt. of lean ham.   A. G. POLLARD.

[A] Dietary rapeseed meal for swine reproduction. [B] Composition of colostrum and milk from sows receiving dietary rapeseed meal or soyabean meal. F. W. Schuld and J. P. Bowland (*Can. J. Anim. Sci.*, 1968, **48**, 57–64, 65–69).—[A] Six litter-mate groups of four sows (live wt. 29–106 kg) were fed rations containing 0 or 8% of solvent-extracted rapeseed meal as an isonitrogenous substitute for soyabean and wheat in the original diet. The substituted diet lowered the daily gain in wt. throughout the period but the depression was significant only from 90 to 106 kg live-wt. The reproduc-

tive performance of the animals was not significantly affected, but the no. of pigs weaned was lowered. In the second cycle treatment during growth had no effect on the performance of sows or litters. Rations containing 8% of rapeseed meal should not be fed to growing gilts if required for reproduction.

[B] In the above groups of sows use of 8% rapeseed meal in the ration significantly increased the fat level in the colostrum and caused an apparent increase in milk-fat levels; total solids were also greater with the rapeseed than with the original soyabean ration. Details of the gross composition of milk and colostrum are recorded.   A. G. POLLARD.

Energy and protein levels in broiler-starting rations. L. G. Swart (*S. Afr. J. agric. Sci.*, 1967, **10**, 187–195).—Max. growth and feed conversion by 6-week chicks were obtained with a dietary energy level of 1450 kcal *ME* (metabolisable energy) per lb. A 23% protein level gave better results than did a 20% level. The optimum kcal : protein % ratio was 60–63. (18 references.)   P. S. ARUP.

Methionine and methylating agents in laying rations [for hens]. E. W. Pawson (*S. Afr. J. agric. Sci.*, 1967, **10**, 175–186).—In two trials the most favourable ratios of methionine to methylating agents (choline or betaine) with regard to size of eggs and wt. of day-old chicks were found to be 4·7 : 1 and (size of eggs only) 4·5 : 1. As it was only necessary to use methionine (without betaine) to obtain the required ratio in the second trial, it seems unlikely that either betaine, choline or methionine separately was responsible for the larger eggs. (17 references.)   P. S. ARUP.

Amino-acid supplementation of sunflower meal for feeding of chickens. J. P. H. Wessels (*S. Afr. J. agric. Sci.*, 1967, **10**, 411–417).—The only amino-acid to give a significant positive result in net protein utilisation tests was lysine, added to give a total of 0·4% in the feed. Significant depressive effects were found for isoleucine added to diets supplemented with leucine or valine.   P. S. ARUP.

Comparison of response of different peanut meals to amino-acid supplementation. J. P. H. Wessels (*S. Afr. J. agric. Sci.*, 1967, **10**, 113–121).—In six manufactured groundnut products, methionine (I) proved to be the main limiting factor as regards non-protein utilisation by chickens. I appeared to be partly inactivated or destroyed during processing. Valencia groundnut meal differed from the other unprocessed meals in being improved by added threonine. (16 references.)   P. S. ARUP.

2.—FOODS

Carbohydrate Materials

Cereals, flours, starches, baking

Nutritional value of rye and wheat, with special reference to proteins and vitamins. J. Janicki and J. Kowalczyk (*Getreide Mehl*, 1967, **7**, 73–77).—Available information is summarised in tabular form and discussed. Studies on the enzyme inhibition, natural to wheat and rye, are limited, but it is generally concluded that the overall nutritional values of the two cereals are very similar, and the choice of cereal may rationally be made on grounds of economy of production and consumer taste. (61 references.)   E. C. APLING.

Decontamination of cereals. I. P. F. Pelshenke, H.-D. Ocker, H. Bolling and H. Zwingelberg (*Getreide Mehl*, 1967, **7**, 77–80).—Removal of radioactive contamination from wheat and rye from the 1963 and 1964 harvests by pneumatic cleaning and washing is reviewed and the results obtained for five samples of Carsten VI wheat from different localities are reported in detail. The extent of removal of ^{90}Sr, ^{137}Cs and ^{54}Mn was very variable. Decontamination was not significantly improved by washing with EDTA or polyphosphate solution and only minor proportions of the radionuclides (from 3 to 38%, but generally < 20%) were removed with wash times of up to 5 min. The variability of results suggests that cleaning and washing removes contamination from the surface layers only, and that the extent of removal may depend on the stage of cereal growth in which contamination occurred. Decontamination by washing is of greater significance in the production of feeding stuffs and wholemeal products than in the milling of white flour.   E. C. APLING.

Standardisation of the enumeration of bacteria in cereals and cereal products. I. Influence of the dilution medium on the determination of total count in cereals. G. Spicher (*Getreide Mehl*, 1967, **17**, 85–89).—A comparative assessment of the suitability of seven proposed diluting media (tap water, distilled water, 0·85% NaCl,

peptone water, peptone water + 0·85% NaCl, phosphate buffer, pyrophosphate solution) is reported. Cereal micro-organisms were suspended in diluting medium and plate counts were made after setting aside for 20, 40, 80 and 120 min. Most consistent counts resulted with the use of 1% peptone, with or without the addition of 0·85% NaCl (pH 6·4 or 6·2). (11 references.)

E. C. APLING.

Investigation of the effect of wheat-bug attack on cereal protein. R. García Faure (*Getreide Mehl*, 1967, **17**, 93–96).—A comparative evaluation of methods for the detection of wheat-bug damage (*WBD*) in flour based on determinations of protein solubility or sedimentation value before and after autolysis, or on rheological measurements on dough is made. Extensograph measurements are shown to give a sensitive indication of *WBD*, which causes a marked decrease in the elastic and corresponding increase in the viscous component of the work of extension without significantly affecting the total work. The sedimentation value of damaged flour is reduced by prior incubation of the flour–water mixture for 1 h at 30°, whereas the sedimentation value of sound flour is either increased or unaltered. Estimation of protein solubility before and after autolysis did not give a satisfactory distinction between sound and damaged flour. E. C. APLING.

Interaction between wheat proteins and dextrans. R. W. Jones and S. R. Erlander (*Cereal Chem.*, 1967, **44**, 447–456).—Effects of a large number of dextrans (I) prepared from different strains of micro-organisms (mainly *Leuconostoc mesenteroides*) on dough mixing curves and on solutions of wheat proteins are reported. Some I affect the dough mixing curve, while others do not. In 0·01 M AcOH solution some I interact with gluten, gliadin and glutenin; interaction produces either a turbidity or a ppt and is concn. dependent. Sweetcorn glycogen gave a similar ppt. with glutenin; animal glycogen and levan B-523 gave only a slight turbidity while waxy maize amylopectin and levan B-512F gave no reaction. In general, I affecting the mixing curve were high mol. wt., high-viscosity polymers having relatively few branches. The I fraction producing turbidity in solution is the lowest mol. wt. component so that I purity has a marked effect on turbidity formation, but no effect on dough mixing curves. The nature of the interaction is as yet unexplained. (22 references.)

E. C. APLING.

Solubility of gliadin-like proteins. IV. Characterisation of some gamma-gliadins. P. Meredith (*Cereal Chem.*, 1967, **44**, 436–446).—Extracts of commercial wheat glutens were fractionated by chromatography on carboxymethylcellulose to yield separated β- and γ-gliadin (I) components. At least nine γ-I were observed, the γ-one type having a high tyrosine : tryptophan ratio and being resolvable into three components, and the low ratio type (γ-two and -three) which was resolvable into six components. Each γ-I showed one characteristic band in starch gel-urea electrophoresis. The triplet nature of the separated I could be a reflection of the three genomes of the genetic composition of bread wheat. Gamma-two and -three I show distinctive phase separation behaviour in aq. salt-acid media, they constitute a considerable part of gluten structure and modify the ion-sensitive swelling and shrinking properties of the gluten-like protein considered to be the skeleton of gluten and dough structure. (26 references.) E. C. APLING.

Changes in the composition of the microflora during grain storage and their effects on the technological i.e. milling and baking qualities of the grain. J. Janicki, E. Kaminski, S. Stawicki and H. Chrzanowska (*Getreide Mehl*, 1967, **17**, 113–117; 125–129; 139–141).—Wheat and rye samples were stored at 65%, 75%, and 85% R.H. and temp. of 3, 10, and 20° for wheat and 8, 20, and 30° for rye, for biochemical and microbiological examination after 12, 18, 21 and 24 months. The experimental scheme includes direct examination of the grain and of the flours produced by milling (Quadrumat Junior Model mill) before and after washing and conditioning. Changes in numbers of non-spore forming and spore forming bacteria found are reported and briefly discussed, and the enzymic activities of the isolated species of bacteria and fungi are tabulated. Changes in germination capacity, vitamin B₁ content and free −SH groups, fat acidity, diastatic and proteolytic activity, gluten content and physical dough properties, observed during storage for 2 years are reported. The biochemical characteristics of 26 bacterial and 28 fungal isolates are tabulated. Changes in the baking value and organoleptic characteristics of the bread resulting from storage of the grain are reported in detail. (94 references.) E. C. APLING.

Chemical composition of rice. IV. Distribution of sugars in the milled kernel. S. Barber, C. Benedito de Barber, J. L. Guardiola and J. Alberola (*Revta Agroquim. Tecnol. Aliment.*, 1967, **7**, 346–

353).—Determinations of total sugars (S), reducing (*RS*) and non-reducing (*NRS*) are reported for rice of different milling degrees (7·7, 9·8 and 12·0%), and for the separated outer layers of the kernel. Sucrose, glucose, fructose, xylose, galactose, maltose, arabinose and/or rhamnose were detected both in the outer layer and in the nucleus, but total *S* content was highest in the outer layer. *NRS* (mainly sucrose) were predominant in the outer layer while *RS* (mainly glucose and fructose) were predominant in the nucleus. (30 references.) E. C. APLING.

Storage of grain and flour in tropical and subtropical conditions. S. V. Pingale (*Getreide Mehl*, 1967, **17**, 104–107).—A brief review of problems and practice in India. E. C. APLING.

Methods for amino-acid analysis of wheat products. G. O. Kohler and R. Palter (*Cereal Chem.*, 1967, **44**, 512–520).—A modified hydrolysis procedure for use in the automatic amino-acid analysis of wheat products is described. Correction factors for application to the results for serine and threonine (readily liberated but unstable), and for valine and isoleucine (stable to acid but released slowly) are given; cystine and methionine (very unstable) are analysed as oxidised deriv. by the method of Moore (*J. biol. Chem.*, 1963, **238**, 235). Tryptophan, after hydrolysis with Ba(OH)₂ is determined on the basic column of the analyser at a column temp. of 65°. Complete amino-acid analyses are reported for several series of wheats and their derived flours and wheat feeds. (12 references.) E. C. APLING.

Proteolytic enzymes of wheat and flour and their effect on bread quality in the United Kingdom. J. Hanford (*Cereal Chem.*, 1967, **44**, 499–511).—Measurements of proteolytic activity of native wheat flours and proteinase (I) prep. by following the rate at which a gluten ball softened in water at 30° and by a modified Ayre-Anderson method, using indanetrione hydrate for the colorimetric determination of sol. N, are compared. The results showed no correlation. Total sol. N showed a correlation with the N supplementation requirements of sample flour doughs. It is clear that the two tests measure different enzymes; the gluten-softening enzyme is termed α-I and the sol. N-producing enzyme, β-I. The softening enzyme was affected by flour grade and was inhibited when gluten was washed with dil. NaCl solution. (16 references.)

E. C. APLING.

Distribution of phosphorus compounds in the protein fractions of various types of wheat flours. A. Bourdet and P. Feillet (*Cereal Chem.*, 1967, **44**, 457–482).—Studies of the protein and phosphorus composition of experimental flours milled from ten U.S. soft, hard and durum wheats (protein 9·2–17·6% dry basis) are reported. Significant differences in protein composition of the various wheat classes were shown, and samples showed considerable differences in contents of total-, inorg.-, lipid-, phytic-, total nucleic-, DNA- and RNA- P in both flour and gluten. Distribution of the main P compounds among the protein fractions in flour and gluten is shown. The demonstration of a particularly high content of nucleic P (70–84% of total P) in globulins invalidates the theory that flour phytates may be involved in the pptn. of this protein fraction after dialysis. (39 references.) E. C. APLING.

Studies of gluten lipids. I. Distribution of lipids in gluten fractions separated by solubility in 70% ethanol. J. G. Ponte, jun., V. A. De Stefanis and R. H. Cotton (*Cereal Chem.*, 1967, **44**, 427–435).—Lipid distributions are reported for flour, gluten, 'protein aggregates' (cf. *ibid.*, 1965, **42**, 409), gliadin (I) and glutenin (II). Flour lipids contained 46% of polar material and 37% of tri-glycerides, while I lipids contained 75% and 6% and II lipids 24% and 58% respectively of these components. I and II were separated from gluten dispersions by the classical procedure involving 70% ethanol and the possibility that differences found may have been due to lipid relocation during fractionation is considered. (24 references.) E. C. APLING.

Sulphydryl and disulphide content of wheat flour, dough and proteins. D. K. Mecham (*Brot Gebäck*, 1967, **21**, 145–149).—A review of recent studies, with particular reference to studies of the changes occurring during dough mixing. Their contribution to the understanding of dough development and ripening is discussed. (40 references.) E. C. APLING.

Dependence of baking quality on flour particle size. F. Springer (*Getreide Mehl*, 1967, **7**, 81–84).—Comparative baking quality evaluations and particle size distribution curves are reported for flours milled to different granularities from German and Canadian wheat and also for the flours after further treatment in a MIAG impact mill. Variations due to milling treatment were very much greater with the German than with the Canadian flour; impact milling increased starch damage (amylose figure), and slightly

increased water absorption, but loaf vol. (Rapid-Mix-Test) was scarcely altered. (13 references.)   E. C. APLING.

Ash as a criterion of flour quality. L. Wassermann (*Brot Gebäck*, 1967, **21**, 225–229).—The proximate and nutrient composition of the various portions of the wheat grain are tabulated and the variation in composition and properties of the flour with milling grade (as judged by ash content) is considered. (29 references.)
E. C. APLING.

Inactivation of amylolytic activity in doughs. K. Möttönen (*Getreide Mehl*, 1967, **17**, 136–139).—The Hagberg falling no. of wheat and rye flours is shown to increase with reduction of pH. Parallel studies (determinations of pH, falling no. and SKB value) in fermenting rye doughs, with or without acidification with HCl, showed that control of amylolytic activity varied with sour dough activity as well as with pH. (15 references.)   E. C. APLING.

Sorption isotherms of flour, flour products and other foodstuffs at 30°. H. M. B. Ballschmieter (*Getreide Mehl*, 1967, **17**, 118–120).—Sorption isotherms are reported graphically, and normal moisture contents and corresponding equilibrium R.H. values are tabulated for 29 powdered food materials (including wheat and maize flours, maize starch, spaghetti, dried full cream and skim milk, dried egg, pea flour, coffee, etc.). (17 references.)   E. C. APLING.

Corn dry-milling: Pre-tempering low-moisture corn [maize]. O. L. Brekke (*Cereal Chem.*, 1967, **44**, 521–531).—As determined by Beall degermination tests, the dry-milling characteristics of naturally dried yellow dent hybrid maize (moisture content $\Rightarrow 14\%$) were improved by pre-tempering, mainly as a result of reduction of the number of stress cracks formed in subsequent conventional tempering. Best results followed pre-temper to 15–17% moisture for 20 h, at room temp., but good results were obtained with 8–10 h and a moderate increase in yield of flaking grits resulted with only 3–4 h pre-temper. Pre-tempering was less effective with artificially dried maize.   E. C. APLING.

Dough and bread from starch without gluten. G. Jongh, T. Slim and H. Greve (*Brot Gebäck*, 1967, **21**, 165–173).—Effects of additions of glyceryl mono- and di-stearate (I), polyoxethylene stearate, lard, wheat gluten, egg albumen and wheat gliadin on the properties of wheat starch dough and bread are reviewed and illustrated with photographs of the cut loaves and photomicrographs of the bread crumb. All additives showed some effect in improving the structure of starch bread, and generally a characteristic optimum level of addition was found beyond which deterioration in crumb structure and/or bread vol. resulted. Microscopic observations of the crumb showed that in general increasing additive levels reduced swelling (gelatinisation) and intermingling of the starch granules, until at levels above optimum starch granules remained essentially separated from each other and showed only slight structural changes. The results are discussed in relation to the apparent rôle of starch in the development of crumb structure and the onset of staling in wheat bread. Parallel studies of effect of additions of I on properties of cassava starch dough and bread showed the importance of the gelatinisation properties of the particular starch in determining the optimum level of addition. Loaves of very promising characteristics were produced from mixtures of cassava starch (80%) and soya or groundnut meal (20%) with the addition of 1% of I. (13 references.)   E. C. APLING.

Theoretical analysis of continuous methods in bakery technology, with particular reference to the pre-ferment and dough fermentation. H. Wutzel (*Brot Gebäck*, 1967, **21**, 190–195).—Continuous bakery systems employing a pre-ferment (e.g. the American Amflow process) or continuous dough fermentation (e.g. the Russian Rabinowitsch process) are considered in the light of the theoretical analysis of continuous culture systems by Herbert (*Continuous Culture of Micro-organisms*, S.C.I. monograph No. 12, 1961).
E. C. APLING.

Dough structure—micrographical studies. I. Bassfeld and Y. Audidier (*Brot Gebäck*, 1967, **21**, 198–201).—Methods for the microscopical study of dough structure are briefly described and structural changes observed during dough development are discussed.   E. C. APLING.

Some practical aspects of dough rheology. I. Hlynka (*Brot Gebäck*, 1967, **21**, 195–198).—Newer applications of rheological techniques to the study of the effects on dough properties of flour quality, water addition, time and intensity of mixing, addition of salt, fat and improvers, rest period and reaction time are briefly discussed.   E. C. APLING.

Advances in the field of dough rheology. I. Hlynka (*Brot Gebäck*, 1967, **21**, 125–130).—Basic rheological concepts are briefly dis-

cussed and methodological advances of the last two decades are reviewed. (39 references.)   E. C. APLING.

'No-time' dough procedures in relation to different kneading conditions. Australian experiences and possible applications. E. E. Bond (*Brot Gebäck*, 1967, **21**, 173–180).—Investigations by the Bread Research Institute of Australia into the factors involved in the production of satisfactory Australian-type bread by mechanical development with a variety of mixing equipment are reviewed.
E. C. APLING.

Statistical enquiry into the use of different flour types in the production of whole and sliced bread. J. M. Brümmer (*Brot Gebäck*, 1967, **21**, 159–163).—An analysis is presented of the proportions of the various flour types used in different German States and for the production of particular bread types, based on details of materials usage disclosed by the producers of over 1000 samples submitted in the 1966 bread quality trials. Changes in the pattern of usage since 1962 are slight, but show a continuing trend towards greater use of flours of lower ash content.   E. C. APLING.

Studies of shortened baking procedures. H. M. B. Ballschmieter and H. Vlietstra (*Brot Gebäck*, 1967, **21**, 229–234).—Comparative baking test evaluations of short-time breadmaking procedures (Chorleywood Bread process, 'Camberzyme', activated development) are reported and discussed. (16 references.)
E. C. APLING.

Effects of the method of sour preparation on bread quality. H. Stephan (*Brot Gebäck*, 1967, **21**, 235–238).—Effects of propagation ratio (total dough/sour dough added, flour basis), sour dough temp., ripening time, sour dough strength, and flour quality on the properties of the final bread are systematically discussed.
E. C. APLING.

Effect of baking on retention of thiamine, riboflavin, and niacin in Arabic bread. M. Maleki and S. Daghir (*Cereal Chem.*, 1967, **44**, 483–498).—The effect of different baking conditions on vitamin retention in Arabic bread was studied using a micro-baking technique at 400°, 450° and 500°. Destruction of thiamine (I) was greater in brown than in white bread. Retention of riboflavin (II) was higher in vitamin-enriched than in unenriched bread, while loss of niacin (III) was negligible under all conditions. It was found that the addition of 1 μg of II, 5 μg of I and 10 μg of III per g of white flour (65% extraction) was sufficient to raise the vitamin content to that of brown flour (85% extraction). (15 references.)
E. C. APLING.

Influence of storage temperature on the quality of wheat bread and the stability of some of its constituents. H. Gasiorowski and S. Jankowski (*Brot Gebäck*, 1967, **21**, 137–143).—Changes in crumb compressibility (*CC*), moisture content, swelling capacity, sol. starch, dextrin content, average dextrin chain length, total and reducing sugars, and total starch content of the crumb occurring during storage of wrapped bread (dough wt. 1 kg) for 15 days at $-23°$, 18°, 45°, 55° and 65° are reported in diagrammatic form and discussed. Rate of staling, as judged by loss of *CC* was greatest at room temp., and at $-23°$ practically ceased after the first day. At the higher temp. loss of *CC* was progressively reduced, but the rate of development of undesirable taste and odour and darkening of the crumb colour increased with rise in temp. Decreases in *CC* were accompanied by decreases in swelling capacity and contents of sol. starch and long-chain dextrins, and by increases in the total starch content of the crumb, indicating the importance of the carbohydrate fractions in the staling process. (37 references.)
E. C. APLING.

Possibilities of keeping fine baked goods fresh. F. Bretschneider (*Brot Gebäck*, 1967, **21**, 238–242).—Evaluations of the effects of additions of pectin, sorbitol, glycerine, various emulsifiers, citrus fruit juices and whole raisins on the staling of fermented and unfermented biscuit-type products are briefly reported. Raisins considerably reduced moisture loss, but increased apparent staling rate (organoleptic and penetrometer tests) of goods packed in polythene foil. Possibilities of reducing staling rates by the use of moisture-resistant packaging materials, by recipe changes or additions, and by alterations in production methods are briefly reviewed. (11 references.)   E. C. APLING.

Sugars and confectionery

Quantitative determination of silyl derivatives of glucose by gas–liquid chromatography with inert internal standards. Y. Halpern, Y. Houminer and S. Patai (*Analyst, Lond.*, 1967, **92**, 714–716).—Trimethylsilyl deriv. of α- and β-D-glucose are quant. determined by g.l.c. on a 5-ft. column of 5% SE30 on Chromosorb W at 190° with He as carrier-gas and either terphenyl or triphenylethylene as

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internal standard. Results are reproducible to within $\sim\pm1\%$, and accuracy is highest when ratio of peak areas of internal standard and sample is ~1. Chrysene and pyrene can also be used as internal standards. W. J. BAKER.

Rapid quantitative anion-exchange chromatography of carbohydrates. R. B. Kesler (*Analyt Chem.*, 1967, **39**, 1416–1422).— Mixtures of mono-, di- and trisaccharides (up to 17 components) are resolved, and the sugars determined quant., in 4–6 h by use of a column (75–120 cm) of strong-base anion-exchange resin (borate form) at pH 7 and 53°. Concn. of sugars down to 1 μg are eluted by a pH-concn. gradient of borate buffers, and the eluate is analysed continuously in an Auto-Analyser by the orcinol spectrophotometric method. The strip-chart chromatogram consists of a series of nearly symmetrical peaks, the peak-area and net extinction of each being related linearly to the concn. of the specific sugar. The method, which is also applicable to mixtures of oligosaccharides, cellodextrins and sol. hemicelluloses, eliminates prep. of deriv., and re-equilibration of the resin takes only $\sim$90 min. An appropriately sized column permits simultaneous analytical and preparative work. W. J. BAKER.

Thermal capacity and specific heat of cocoa-butter, chocolate and icings at different temperatures. K. Becker and J. Bauermeister (*Fette Seifen AnstrMittel*, 1967, **69**, 593–597).—The construction of a simple copper calorimeter is described, which is suitable for determining the thermal capacity and sp. heats of chocolate, icings and cocoa-butter of different compositions, at temp. of -10 to 60°. The results obtained are shown graphically for specific mixtures and a formula is derived, by means of which the thermal capacity of chocolate doughs containing any proportions of sugar, milk powder and cocoa may be calculated between 0 and 65°. G. R. WHALLEY.

Fermentation and Alcoholic Beverages

Influence of the quality of sugar-beet and of the method of extraction of the juice on the content of volatile acids in molasses, determined by gas chromatography. S. Zagrodzki and A. Kurkowska (*Roczn. Technol. Chem. Żywn.*, 1967, **13**, 41–50).—Content of formic-, acetic- and butyric-acids in molasses was investigated from the point of view of the usefulness of molasses as raw material for production of yeast and ethanol. Highest content of volatile acids (*VA*) was found in molasses from sugar-beet harvested towards the end of the season (December), when ambient temp. were well below freezing point, down to $-17°$. The method of prep. and of juice extraction also affected the content of *VA*. Determination of individual acids was carried out by gas chromatography. T. M. BARZYKOWSKI.

Application of amylolytic fungal preparations in the brewing industry. F. Nowotny and K. Piller (*Roczn. Technol. Chem. Żywn.*, 1967, **13**, 77–90).—Based upon laboratory experiments, an attempt was made to produce beer on an industrial scale, using some amylolytic fungal prep. The mash consisted of: (1) 50% malt + 50% barley + amylolytic prep. (*AP*); (2) 50% malt + 50% huskless barley + *AP*; (3) 100% malt; (4) 80% malt + 20% huskless barley but without *AP*. Beer prepared from the mash with *AP* could hardly be distinguished from beer made from pure malt; some consumers even preferred it. Replacement of part of the malt by barley lowers production costs. (18 references.) T. M. BARZYKOWSKI.

Evaluation of the suitability of brewing yeasts. G. Krauss, C. C. Emeis and G. Sommer (*Mschr. Brau.*, 1968, **21**, 177–183).—Seven flocculent (*F*) and seven non-flocculent (*NF*) brewing yeasts have been evaluated on a pilot scale. The onset and rate of fermentation of the *NF* yeasts were, with one exception, slower than the *F* ones and more diacetyl was produced during the primary fermentation. N content of the beers produced depended mainly on yeast growth. The isohumulone loss with *NF* yeasts was always higher than the 10% max. observed with *F* strains. There were indications that shorter fermentations were accompanied by lower head retentions. The production of higher aliphatic alcohols was greater (70–80 mg/l) with *F* yeasts than *NF* types (40–60 mg/l). Probably as a result of this there were notable differences in the flavours of the two fermentation types: the *F* yeast beers were generally preferred. (27 references.) J. B. WOOF.

Gas chromatography in brewing. C. E. Dalgliesh (*Brewers' J., Lond.*, 1967, **103**, 31–37).—A review covering general principles, equipment, and applications in brewing chemistry. P. S. ARUP.

New method for determination of hot-water extract of malt. A. M. Brown (*J. Inst. Brew.*, 1967, **73**, 438–441).—A 10-g sample of malt is continuously extracted in a Soxhlet apparatus under reduced pressure with water boiling at 65·5°. After 2 h the extract is determined from the wt. and sp. gr. of the solution in the flask. The method gives lower results than does the Standard Institute of Brewing method, but it has the advantages of requiring only a 10-g instead of a 50-g sample, greater convenience in operation, and avoidance of the problem of the spent grain-vol. P. S. ARUP.

Variation of malt wort solution factors with extract concentration. A. M. Brown (*J. Inst. Brew.*, 1967, **73**, 436–438).—Determinations of sp. gr. were made of several series of dilutions of worts of initial sp. gr. 1·060–1·090. It was found that the solution factor K_0 connecting the concn. of solute with the difference between the sp. gr. of the solution and the solvent ($g-g_0$) varied with the concn. of the extract so that the extract appeared to increase as the wort was progressively diluted. The relationship between the extract concn. and sp. gr. difference was inversely rectilinear. An equation involving a second constant S allows for this variation, viz., $K = K_0 + S(g-g_0)$. For K_0 (the uncorrected solution factor) $=4\cdot0$, $S = -0\cdot488$. The corresponding factor previously reported for maltose was $-0\cdot58$. P. S. ARUP.

Effects of malt kilning on wort properties. J. Barrett, C. M. Griffiths, and B. H. Kirsop (*J. Inst. Brew.*, 1967, **73**, 445–450).— In laboratory experiments, the colour formed in malt cured at temp. in the range 82°–105° depended more on the temp. attained than on the moisture content of the sample. Colour development was associated with decrease in the free amino-acid content and in the proteolytic and amylolytic activity during mashing. Curing decreased the maltose content, increased the dextrin content, and thus caused a decrease in fermentability. The decreases in aminoacids were not accompanied by corresponding losses in total N. P. S. ARUP.

Effect of suspended solids on fermentation of distillers' malt wort. N. R. Merritt (*J. Inst. Brew.*, 1967, **73**, 484–488).—The addition of as little as 0·005% (dry basis) of solids from spent grains to clear wort increased yeast growth by > 10% and also increased the production of glycerol and higher alcohols, especially Bu^{i}OH and 2-Me butanol. The stimulation was independent of the state of aeration of the wort, particle size or chemical nature of the suspended matter; it occurred in worts in which amino -N concn., not carbohydrate concn., was the limiting factor for growth. The effects are attributed to the concn. of amino-acids at the solid-liquid interfaces of the cells by electrostatic and absorption forces. (10 references.) P. S. ARUP.

Hop substance and yeast behaviour. I. J. Dixon (*J. Inst. Brew.*, 1967, **73**, 488–493).—Many top yeasts failed to form yeast heads in unhopped wort but were enabled to do so after the addition of isohumulone (40 ppm) or humulone to the wort. Hop bitters amounting to 6 and 14% of the total bitters could be extracted with 6 M-urea from the cells in heads from two yeasts. Evidence was obtained that the bitters were transported to the surface by CO_2 bubbles: even in bottom fermentation the bitters were transported to the top and deposited on the sides of the vessels. In comparison with hopped wort, fermentation in unhopped wort was characterised by an increased amount of yeast in suspension without, however any increase in the rate of attenuation. (12 references.) P. S. ARUP.

Toxicological investigation of the effects on beer prepared with hop extracts containing dichloromethane. G. Bornmann, E. Herold, A. Loeser and K. Opitz (*Dt. LebensmittRdsch.*, 1968, **64**, 167–171).—Beers brewed with (*i*) hop extract prepared with CH_2Cl_2 and (*ii*) natural hops were fed to rats over a period of 3 months. In both male and female rats growth rates, blood analyses, liver and kidney wt. and blood plasma enzyme levels showed that beers brewed with (*i*) had no toxic effects. J. B. WOOF.

Storage and brewing trials with natural hops, Hopfix and hop extracts. H. Schilfarth and G. Sommer (*Mschr. Brau.*, 1968, **21**, 204–205).—A 20 cm deep layer of fresh Hallertau hops and samples of Hopfix and hop extract kept in their original containers were stored for 11 months at 0° and then compared in pilot brewery trials with similar samples stored at 20°. Hopfix and the extract stored equally well and both were better than the natural hop in retaining bittering ability. J. B. WOOF.

Effects of some hop oil components on flavour and aroma of beers. K. Silbereisen, E. Krüger, B. Wagner and M. Forch (*Mschr. Brau.*, 1968, **21**, 206–211).—Hops (10 kg) yielded 55·94g of hop oil after steam distillation; this was separated into oxygenated and hydrocarbon fractions by column chromatography on silica gel. The following components were then isolated by preparative scale g.l.c. on polypropylene glycol-coated columns; myrcene, caryophyllene, farnesene, humulene, methylnonylketone, Me dec-4-enoate, and

Me dec-4,8-dienoate. These components, together with commercial Bu^i isobutyrate, and linalool, were added to beer individually and as mixtures at different levels. Myrcene and linalool were found to give aromas resembling natural hops but no combination tested was found to give a typical hop aroma. (22 references.)

J. B. Woof.

Relationship between composition and viscosity of beers. P. Kolbach (*Mschr. Brau.*, 1968, **21**, 171–176).—The viscosity (η) of beer is determined by a number of sol. constituents and is affected considerably by malting and mashing conditions. The η of 66 beers of varying gravity were studied and related to the concn. of main viscous components. In each case the analyses are related to a standard 12% Plato wort. Of the η contributed by the total dissolved matter, on average alcohol contributes 24·3%, dextrin 26·6%, fermentable sugar ('maltose') 1·1% and protein 4·2%. Unknown components therefore are responsible for 43·8% of the total η and the differences observed in the commercial beers lie mainly in this fraction. The contributions of each of the components is readily calculated from its sp. η and concn. (14 references.)

J. B. Woof.

Phenolic constituents of beer and brewing materials. II. Rôle of polyphenols in formation of non-biological haze. J. W. Gramshaw (*J. Inst. Brew.*, 1967, **73**, 455–471).—The addition to beer of polyphenols (I) from three sources caused immediate and intense turbidities, whilst the addition of a monoflavonoid anthocyanogen (II) increased the rate of haze formation. Attention is drawn to the complexity and functional variety of the II and I constituents of beer as accounting for the failure to correlate the tendency to haze formation with any simple II or I variant. The nature and origin of the I in beer and possible processes in haze formation are discussed. (74 references.)

P. S. Arup.

Comparative studies on protein distribution in malt, wort and beer by different protein fractionation methods, particularly gel filtration. L. Narziss, E. Reicheneder and R. Nonhoff (*Brauwissenschaft*, 1968, **21**, 184–190).—Methods for the fractionation of protein are reviewed and the use of gel filtration for mol. sieving in column chromatography and in batch procedures is described. Three different barleys were malted with different levels of moisture during germination; with increasing moisture content the proportion of nitrogenous substances of high mol. wt. decreased and that of the lower mol. wt. compounds increased. Changes in the proportion of compounds of intermediate mol. wt. depended on barley variety. The coagulable N depended on the N content of the malt and on the variety; the amount of nitrogenous material pptd by tannin gave some indication of the extent of protein degradation, while that pptd by MgSO$_4$ gave highly reproducible values. (19 references.)

I. Dickinson.

Detection of formaldehyde in beer. E. Krüger (*Mschr. Brau.*, 1968, **21**, 155–156).—Degassed beer (1–3 ml) is distilled in a flask fitted with a Vigreux column and a condenser; the condensed aq. CH$_2$O passes into an 81% H$_2$SO$_4$ solution. After addition of 1 ml of 1·5% chromtropic acid to the acid distillate, the resultant colour is measured spectrophotometrically at 570 mμ. A blank is prepared by distilling over the same vol. of a CH$_2$O- free beer. The relationship: mg/l CH$_2$O = (24·2 E−0·477)/sample vol. is then used to determine concn.; from pure aq. solutions the relationship is slightly different. Down to 0·25 mg/l can be detected and the accuracy is ±4·5%.

J. B. Woof.

Formaldehyde, a method for producing protein stable beers? H. Schilfarth and G. Sommer (*Mschr. Brau.*, 1968, **21**, 149–154).—Pilot brews have been analysed to determine the effect of CH$_2$O at levels of 0, 125, 250, 375, and 500 mg/kg malt, added at the beginning of mashing. At the two highest levels 0·3 to 0·4 mg/l of CH$_2$O were detected in the finished beer. At 250 mg/kg the colour was reduced by 0·8 EBC units, the head retention (as measured by the Kolbach and Schilfarth method) was 13 sec. better and the anthocyanogen content was reduced by 85%. A loss of 13% in isohumulones was recorded. The treated beers could be stored for 28 days before showing a chill haze of 2·0 EBC-formazin-haze units, compared with 3 days for the control. The taste of the treated beers was normal and the observed effects could be enhanced by the use of the usual proteolytic enzymes and adsorbants.

J. B. Woof.

Treatment of 'last runnings' with active charcoal. L. Narziss and F. M. Klein (*Mschr. Brau.*, 1968, **21**, 185–195).—The effects of treatment with Purocarbon under different conditions on the composition of 'last runnings' has been investigated. In all cases there was a decrease in the N, anthocyanogen and tannin content with a resultant improvement in the flavour of the finished beer. At 70°

with a contact time of 15 min. there was a max. effect. The amount of anthocyanogen, tannin and N pptd. by MgSO$_4$ removed, increased as the dosage was raised but at 100 g/hl most of the desired improvements had been achieved. There was little effect on formol-N, coagulable-N or isohumulone content but the colour was significantly reduced. In large scale tests, addition of more than 100 g/hl of Purocarbon to 'last runnings' to be used as mash liquor resulted in reduced tannin and protein without affecting the saccharification times and whilst clarification and stability were not significantly different from the controls (without using 'last runnings') the head retention was lowered. It is recommended that the charcoal be used at mashing.

J. B. Woof.

Aroma constituents of grapes and wines of *Vitis vinifera* var. White Riesling. C. J. Van Wyk (*Diss. Abstr.*, B, 1967, **27**, 3136).—The grape and wine aroma constituents were recovered by reduced pressure steam distillation, solvent extraction, concentration and then separation by gas chromatography. Individual compounds were identified by combined functional group and gas chromatographic analyses, appropriate deriv., and i.r. spectroscopy. The major grape aroma constituents are EtOH, Bu^{i}OH, amyl- and isoamyl-alcohol, 2-hexenal, n-hexanol, *trans*-2-hexen-1-ol and 2-phenethanol. The chief classes of compounds in the wine extract were alcohols, acids, and esters with alcohols predominating. Components of different functionality than those listed were γ-butyrolactone, *N*-ethylacetamide, diethyl acetal and acetaldehyde. A no. of these compounds have not been reported in grapes and wines before.

F. C. Sutton.

Characterisation of wines by their volatile constituents. F. Prillinger, H. Horwatitsch and A. Madner (*Mitt. Klosterneuburg Rebe u. Wein Obstb. u. Früchteverwert.*, 1967, **17**, 271–279).—G.l.c. analyses were carried out on the volatile components extracted with pentane–ethyl ether (2 : 1) from seven wines. Use was made of a support-coated open tubular column with Carbowax 1540 as stationary phase, temp.-programming, flame ionisation detection, and with BzOH as inner reference standard. Wines of the finest bouquet were characterised by the prevalence of certain esters and alcohols, and their low content of certain other alcohols. (12 references.)

P. S. Arup.

New fluorimetric method for determination of malvin in wine. H. Hadorn, K. Zurcher, and V. Ragnarson (*Mitt. Geb. Lebensmittelunters. u. Hyg.*, 1967, **58**, 1–30).—Determinations of malvin (I) by paper chromatography are unreliable. Satisfactory results are obtained by applying the Dorier and Verelle reaction (cf. *Analyt. Abstr.*, 1967, **14**, 5048) to the sample, previously freed from interfering substances by pptn. with Pb acetate. The green fluorescence produced in the clear supernatant liquid after addition of N-H$_2$SO$_4$, NaNO$_2$, and methanolic NH$_3$ is proportional to the I content. Background fluorescence values were determined for a large no. of commercial wines (mostly European), most of which contained traces of I. Authentic hybrid wines (21) contained 0–950 mg/l of I. The max. permissible content of I in non-hybrid wines should be fixed at 5 mg/l. (12 references.)

P. S. Arup.

The 'grade number' method as a simple possibility for organoleptic comparison of two or more samples [of wines]. F. Paul (*Mitt. Klosterneuburg Rebe u. Wein Obstb. u. Früchteverwert.*, 1967, **17**, 280–288).—A system of grading and score evaluation is described in which the errors associated with present systems are minimised. The tasters, working in separate compartments, are presented with not more than five samples at a time. Longer series of samples are divided into groups of 2–5 approx. equal in quality.

P. S. Arup.

Apple cider. J. F. Robinson, C. F. Woodward, C. H. Hills, K. M. Hayes and T. Nold (*Fmrs' Bull.*, U.S. *Dep. Agric.*, 1967, No. 2125; 16 pp).—Prep., preservation, containers, plant and equipment design, sanitation and fly control are described.

E. G. Brickell.

New investigations on aniseed aperitifs. H. Bouscharain (*C. r. hebd. Séanc. Acad. Agric. Fr.*, 1968, **54**, 363–367).—A chromatographic method on paper impregnated with NH$_4$Cl is used for the detection of colouring matters in the aperitifs, by comparison of the R_F values obtained with those of samples of the dyes.

P. S. Arup.

Volatile fatty acids in some brands of whisky, cognac and rum. L. Nykänen, E. Puputti and H. Suomalainen (*J. Fd Sci.*, 1968, **33**, 88–92).—Gas chromatographic analysis showed that the max. content of volatile acids was in rum, with 600 mg/l, and the min. in whisky, with 90 mg/l. Acetic acid represented 40 to 95% of the total amount of volatile acids in whisky, in cognac and brandy 50–75% and in rum 75–90%. Rum contained the largest amount

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of lower fatty acids, particularly propionic and butyric acid; the main acidic component of Jamaican rum was propionic acid. Long-chain fatty acids were mainly myristic, palmitic and palmitoleic acids. Scotch whisky contained equal amounts of palmitic and palmitoleic acid. (25 references.) I. DICKINSON.

Analysis of spirits by the Komarowsky reaction: I. Determination of higher alcohols. II. Determination of these in potable spirits. B. R. Glutz (*Mitt. Geb. Lebensmittelunters. u.-Hyg.*, 1967, **58**, 114–128; 129–145).—I. *p*-Dimethylaminobenzaldehyde (I) or salicylaldehyde (II) are to be preferred as reagents to *o*-nitrobenzaldehyde. An EtOH solution of I or II and the sample is mixed with H_2SO_4 and heated to 80° for 30 min. Determinations are made by visual or spectrophotometric comparison with standard solutions of Bu^iOH in pure EtOH.

II. Potable spirits often contain aldehydes (III) in sufficient concn. to interfere with the test; III can be removed by *m*-phenylenediamine, by Ag_2O or by the author's distillation method which is described. All are equally satisfactory. When III are removed, the recovery of the higher alcohols follows as described in part I.

P. S. ARUP.

Fruits, Vegetables, etc.

Microchromatographic investigation of citrus fruits. A micromethod for vitamin C determination in citrus fruits and its application to assessment of keeping quality. G. Gorbach and I. Hekal (*Dt. LebensmittRdsch.*, 1968, **64**, 203–207).—Fruit juice (5 μl) containing 1% oxalic acid is loaded onto a small paper strip resting on a heated plate. Ten strips are run in parallel in stoppered tubes using n-butanol/water (2 : 3) containing 2% oxalic acid as solvent and the ascorbic acid (I) is detected on one of them by spraying with ethanolic 2,6-dichlorophenolindophenol (II) and heating at 105° for 3 min. The I on the other strips is eluted from the excised spots and determined quant. by titration with 10^{-3}-N II solution. With SO_2 as preservative I was not degraded in the juice. Aeration with CO_2 was better than N_2 for stabilising juice. J. B. WOOF.

Quantitative methods for anthocyanins. I. Extraction and determination of total anthocyanin in cranberries. II. Determination of total anthocyanin and degradation index for cranberry juice. T. Fuleki and F. J. Francis (*J. Fd Sci.*, 1968, **33**, 72–77, 78–83).—I. The mol. extinction coeff. for anthocyanins and anthocyanidins reported in literature are tabulated and discussed. The method described consists of extracting the anthocyanins (pigments responsible for the colour in cranberries and many other fruits) with ethanol–1·5 N HCl (85 : 15) and measuring the absorbance of the diluted sample (1-cm cell) at 535 nm. The total anthocyanin content was calculated in absolute quantities with the aid of the extinction coefficients established for the four major cranberry anthocyanins dissolved in the alcoholic solvent system. (23 references.)

II. The development of an improved method for total anthocyanin determination, which minimises interference due to brownish-coloured degradation products is described. The absorbance at 510 nm is measured on samples diluted with pH 1·0 buffer (0·2 N KCl–0·2 N HCl (25 : 67)) and 4·5 buffer (N sodium acetate–N HCl–water (100 : 60 : 90)). This pH differential method has the advantage that an index can be calculated which is indicative of the proportion of degraded anthocyanin in the sample when the original anthocyanin content is not known. Method: 10 ml aliquots of cranberry juice were diluted to 250 and 50 ml with the pH 1·0 and pH 4·5 buffers respectively. The diluted samples were equilibrated in the dark at room temp. for 2 h. Absorbances of the samples were measured at 510 nm, and the absorbance difference was obtained by subtracting the total absorbance at pH 4·5 from the total absorbance at pH 1·0. (18 references.)

I. DICKINSON.

Isolation and identification of flavonoid pigments in cranberries 'Vaccinium macrocarpon, Ait.'. G. Puski (*Diss. Abstr.*, B, 1966, **27**, 1505).—Pigments were extracted from the cranberries with 70% aq. MeOH. After removal of MeOH the aq. solution was extracted with light petroleum, Et_2O and EtOAc in successive steps. The light petroleum extract was discarded. The remaining three extracts and aq. fractions were concentrated, and purified by polyamide chromatography. Final purification of the pigments was done by preparative paper chromatography. Methods of identification of the pigments are given together with their names. The major pigment was quercetin-3-galactoside. Leucocyanidin and leucodelphinidin deriv. were also shown to be present.

F. C. SUTTON.

Sorbitol in stone fruits. U. Stoll (*Mitt. Geb. Lebensmittelunters. u. -Hyg.*, 1967, **58**, 145–150).—Sorbitol (I) was determined in conc. 80% EtOH extracts of apples and pears (three varieties of each). Increases in the content of I became more marked with the size of the fruit. Max. I was found near the epidermis, and min. near the core. Marked decrease in I occurred during the first three months in ordinary storage; the decrease was followed by a slow increase; these changes were slightly less marked in fruits stored in an atm. modified by CO_2. (10 references.) P. S. ARUP.

Mechanism of enhancement of lactic acid fermentation of green olives by alkali and heat treatments. B. Juven, Z. Samish, Y. Henis and B. Jacoby (*J. appl. Bact.*, 1968, **31**, 200–207).—A treatment of Merhavia and Manzanillo green olives with hot alkali resulted in a marked increase in lactic fermentation rate; this arose chiefly from the destruction of the antilactic factor (*ALF*). The press juice from olives contains an ether-extractable, hot alkali-labile factor which inhibits acid production by *Lactobacillus plantarum* in APT broth. A rapid method for detecting the *ALF* is described; *ALF* is present in intact green olives but this may not be identical with that found in the press juice. (14 references.)

C.V.

Changes in lipid components of broccoli as the result of various heat treatments. L. C. Dahlke (*Diss. Abstr.*, B, 1966, **27**, 1530).—The total amount of lipid in broccoli was determined (0·5%), and changes were noted with cooking and storing. The proportions of the tentatively identified fatty acids (found by g.l.c. of the Me esters) were determined in all three types (described) of lypholised broccoli after 40, 97, and 150 days of storage at −25°. Effects of cooking were most pronounced in the neutral lipid fraction; the free fatty acid fraction and the phospholipid fraction were not greatly affected. F. C. SUTTON.

Volatile flavour compounds involved in the processing of snap beans (*Phaseolus vulgaris*, L.). J. G. Fairbrother (*Diss. Abstr.*, B, 1967, **27**, 3135).—An ether extract of the steam distillate of fresh snap beans, when analysed by gas chromatography, showed 28 distinct chemical compounds present, the following probably contributing significantly to the flavour of fresh snap beans: PrOH, 2-pentanol, 1-hexanal (I), *trans*-2-hexenal-1 (II), *cis*-3-hexenol-1 (III), 1-heptanol, and an unknown compound (IV). The compounds responsible for the 'green' odour of fresh and frozen beans, namely I, II, and III were not present in the volatiles of canned snap beans. The characteristic odour of the canned beans was due to IV, together with other minor components. A second unknown compound, in combination with IV imparted a 'viney' odour to dehydrated snap beans. The retention time of the unidentified IV indicated a saturated alcohol (8C) or a saturated aldehyde (9C); it might, however, be an unsaturated (7-9C) compound, due to its intense odour.

F. C. SUTTON.

Non-alcoholic beverages

Influence of sugar concentrations on the vapour pressure of food odour volatiles in aqueous solutions. A. G. Wientjes (*J. Fd Sci.*, 1968, **33**, 1–2).—Observations by W. W. Nawar (*Fd Technol. Champaign*, 1966, **20**, 115) on increased pressures after addition of NaCl in concn. up to saturation, and of different sugars in concn. up to the 40% wt./wt. level, are confirmed. Experiments on the use of sugars at concn. in excess of those used by Nawar, showed that with some types of volatile compounds (from strawberries) no increase but a marked decrease of peak heights was observed in gas chromatograms of vapour over aq. solutions. This may be of interest for the study of flavour retention in juice concentrates.

I. DICKINSON.

Objective method for evaluation of content of pulp in suspension in fruit beverages. P. Dupaigne (*Fruits d'outre mer*, 1967, **22**, 305–308).—The available methods are critically examined. An improvement in the method of centrifugation is introduced by a preliminary dilution of the sample with $COMe_2$ (1 : 1). P. S. ARUP.

Spectrophotometric determination of limonin in orange juice. K. W. Wilson and C. A. Crutchfield (*J. agric. Fd Chem.*, 1968, **16**, 118–124).—The method involves extraction of the limonin with CH_2Cl_2, treatment of the extract with Al_2O_3, filtration and washing with $CHCl_3$, submission of the solids (obtained by evaporation of filtrate and washings) to partition between MeCN and light petroleum, and finally measurement at 510 nm of the colour developed on adding alkaline hydroxylamine and $Fe(ClO_4)_3$ to the MeCN solution. Results obtained by this method (5–40 ppm of limonin) agreed well with the degree of bitterness of navel orange juice. The method was not applicable to orange peel or other plant parts, or to grapefruit juice, because of interference from coumarins. (18 references.) P. S. ARUP.

Detection of adulterations in citrus juices. IX. Mineral composition of the whey of single strength orange juices manufactured in Spain and the United States. E. Primo Yúfera and J. Royo Iranzo (*Revta Agroquim. Tecnol. Aliment.*, 1967, 7, 364–375).—Determinations of degrees Brix, acidity, maturity index, pulp, ash, K, Na, Ca, Mg and P are reported for the whey (filtrate) of industrial orange juices from Spain (25 samples), California (22 samples) and Florida (28 samples). The results are statistically analysed and limit values proposed for use in the detection of adulteration.

E. C. APLING.

Detection of adulterations in orange juices and beverages. J. Royo Iranzo and A. Aranda (*Revta Agroquim. Tecnol. Aliment.*, 1967, 7, 376–380).—Proportions of pure orange juice in ten canned 'orange juices' and fifteen commercial brands of orange soft drinks were determined by the methods previously reported (*ibid.*, 1965, 5, 216 and 471; 1967, 7, 364). Only three of the canned products were 100% orange juice; another three contained only 40–50%. Four of the orange soft drinks contained no orange juice, while juice content of the remainder varied from 3 to 15%.

E. C. APLING.

Volatile from grapes. Identification of volatiles from Concord essence. D. J. Stern, A. Lee, W. H. McFadden and K. L. Stevens (*J. agric. Fd Chem.*, 1967, 15, 1100–1103).—Sixty compounds identified by capillary g.l.c. and mass spectrometry were mainly esters, with EtOAc and Me anthranilate predominating. A series of alkyl (mainly ethyl) crotonates and a possible ethyl alkylthioate were also detected. (14 references.)

P. S. ARUP.

Detection of addition of *dl*-malic acid to apple juice. W. Pilnik and M. Faddegon (*Mitt. Geb. Lebensmittelunters. u.-Hyg.*, 1967, 58, 151–154).—The detection depends on the selective fermentation of *l*-malic acid (naturally present or added with the synthetic *dl*-acid) with *Leuconostoc mesenteroides*. The unchanged *d*-acid is then detected by a well-known paper chromatographic method.

P. S. ARUP.

Dehydrated 'horchata'. II. Influence of some variables on the freeze-drying process. E. Primo, B. Lafuente and F. Piñaga. (*Revta Agroquim. Tecnol. Aliment,.* 1967, 7, 354–363).—Freeze-drying curves are reported for extract of chufa (*Cyperus esculentus*), the basis of the Spanish soft-drink 'horchata'. Variables considered were extract concn., layer thickness, and plate temp. Best results were obtained by drying a 1 cm layer of deep frozen 20° Brix extract with a plate temp. of 60°; under these conditions the drying cycle was 11·5 h and the yield was 0·262 kg of dry product per hour per m². E. C. APLING.

Tea, coffee, cocoa

Composition of black tea aroma. II. J. Bricout, R. Viani, F. Müggler-Chavan, J. P. Marion, D. Reymond and R. H. Egli (*Helv. chim. Acta*, 1967, 50, 1517–1522).—After presentation of an additional list of constituents of black tea aroma, the isolation of seven new components from fractionation of the aroma extract by g.l.c. and their identification by i.r. and mass spectrometry are described. The compounds are *trans*-2-penten-1-ol, 1-ethyl-2-formyl-pyrrole, 2-*trans*,4-*cis*-heptadienal, phenylacetonitrile, methyl benzoate, 2-phenyl-but-2-enal and the lactone of 2,6,6-trimethyl-2-hydroxy-cyclohexylideneacetic acid. (14 references.)

M. SULZBACHER.

Volatile constituents of coffee. Pyrazines and other compounds. H. A. Bondarovich, P. Friedel, V. Krampl, J. A. Renner, F. W. Shephard and M. A. Gianturco (*J. agric. Fd Chem.*, 1967, 15, 1093–1099).—Mass and i.r. spectrographic and g.l.c. data are tabulated for numerous pyrazine deriv. of possible interest in food flavour research. (15 references.) P. S. ARUP.

Composition of cocoa aroma. J. P. Marion, F. Müggler-Chavan, R. Viani, J. Bricout, D. Reymond and R. H. Egli (*Helv. chim. Acta*, 1967, 50, 1509–1516).—An aroma extract of roasted cocoa from Ghana, made by steam distillation, extraction with dichloromethane and fractional vac. distillation, was submitted to gas chromatography, the individual substances being identified by i.r. and mass spectrometry. A full list of the compounds, aliphatic, alicyclic, aromatic, heterocyclic, pyrrol and pyrazine deriv. is given. Besides 22 of the 91 previously described constituents, 35 new substances viz. eight aliphatic, one alicyclic, seven aromatic, nine *O*-heterocyclic, four pyrrolic and six pyrazinic, have been isolated. The synthesis of several reference compounds is outlined. (25 references.) M. SULZBACHER.

Milk, Dairy Products, Eggs

The secretion of water and of water-soluble constituents in milk. J. A. F. Rook and J. V. Wheelock (*J. Dairy Res.*, 1967, 34, 273–

287).—The influence of such factors as breed, stage of lactation, age, feeding, intervals between milking, and udder infections on secretion of water and water-sol. constituents in milk is reviewed. (87 references.) M. O'LEARY.

Influence of the dissolved oxygen in milk on the stability of some vitamins towards heating and during subsequent exposure to sunlight. J. E. Ford (*J. Dairy Res.*, 1967, 34, 239–247).—Destruction of folic acid and vitamin B_{12} during heat sterilisation of milk and subsequent exposure to sunlight was shown to be a consequence of the oxidative destruction of the ascorbic acid in the milk. Loss of these vitamins was prevented by thorough exclusion of O_2 from milk during heating and storage. Oxygen tension was shown to have little, if any effect, on stability of thiamin, riboflavin, nicotinic acid, vitamin B_6 and biotin during heating and exposure to sunlight. (16 references.) M. O'LEARY.

Effects of repeated heat treatment on the levels of some vitamins of the B-complex in milk. H. Burton, J. E. Ford, J. G. Franklin and J. W. G. Porter (*J. Dairy Res.*, 1967, 34, 193–197).—Losses of thiamin and vitamin B_6 were shown to be cumulative and to increase with frequency and severity of heating of milk. The first heat treatment magnified the effect on folic acid of subsequent treatments, whereas with vitamin B_{12} preliminary *HTST* pasteurisation markedly reduced losses suffered in subsequent in-bottle sterilisation. These anomalous effects may be due to a specific interaction of ascorbic acid with each of these two vitamins. (10 references.) M. O'LEARY.

Factors affecting the design of milk total solids testing schemes. M. G. O'Keeffe (*J. Dairy Res.*, 1967, 34, 207–210).—This analysis was applied to the 'within herd' variance (*WHV*) of milk total solids to determine the contribution due to sampling, testing and biological variances on a balanced 8 × 8 two factor (herds and days) experiment with replicate sampling and testing. Testing samples from every consignment from 10 herds over a 12-month period showed the mean monthly *WHV* to be 0·039 and the yearly *WHV* to be 0·085. A formula is presented which shows how the variance of the mean for compositional quality testing can be obtained and on which the design of testing schemes can be based. In the selection of test methods, less importance should be attached to accuracy and more emphasis should be placed on ease of application and possible automation. (15 references.) M. O'LEARY.

A suggested correction to the British Standards hydrometric method for the estimation of total solids in milk. M. G. O'Keeffe (*J. Dairy Res.*, 1967, 34, 211–214).—A comparison of the gravimetric method (*British Standards Institution*, 1963, B.S.1741) for the estimation of milk total solids with the hydrometric method (*ibid.*, 1959, B.S. 734, Part II), showed that the latter method over-estimated the total solids on average by 0·096 with 1082 drip samples of fresh creamery milk over a 3-year period. It is suggested that the formula which was applied before October 1957 is correct and should replace the formula currently being used. (15 references.) M. O'LEARY.

Electrophoretic mobility of milk fat globules. III. Centre cell observations and effect of viscosity, field strength, diluting media, and pH. R. Tjepkema and T. Richardson (*J. Dairy Sci.*, 1967, 50, 1566–1571).—Average electrophoretic mobility (*AEM*) multiplied by viscosity was shown to be a constant (1·00) when mobility of milk fat globules was determined in the viscosity range 1·060 to 1·493 cP in Jenness and Koops solution with added lactose. Variation of the field strength to provide a velocity range of 1·6 μ/sec to 10 μ/sec showed that field strength had no significant influence on *AEM* until the fat globule velocity exceeded 7 μ/sec. *AEM* increased 0·35 μ/sec/V/cm when the pH of the solution was increased from 6·08 to 7·44. Comparison of mobilities at 21% and 79% of cell depth with mobilities at 50% of cell depth showed that mobilities were equal when the mobilities determined at the centre of the cell were multiplied by 0·67. (10 references.) M. O'LEARY.

Effect of oxidation on the iodine values of phospholipid in milk, butter and washed cream serum. E. G. Pont and G. L. Holloway (*J. Dairy Res.*, 1967, 34, 231–238).—Iodine values of phospholipid (I) from milk, butter, and washed cream serum were determined by a micro-Wijs technique. Values from fresh samples ranged from 70 to 86 for the cephalin fraction, from 44 to 55 for the lecithin, and from 36 to 44 for the sphingomyelin. Oxidation with Cu and ascorbic acid resulted, in the case of washed cream serum in significant falls in I_2-values of the three I fractions. In milk, slight, not consistently significant reductions in I_2-values of I were observed following Cu-catalysed oxidation. I_2-values of butter-I were not affected even by gross oxidative quality deterioration. (12 references.) M. O'LEARY.

Effect of oxygen removal technique on flavour stability of low-heat foam spray dried whole milk. A. Tamsma, F. E. Kurtz and M. J. Pallansch (*J. Dairy Sci.*, 1967, **50**, 1562–1565).—Holding of low-heat foam spray dried whole milk powder for 18 h at 1 mm pressure prior to packing in N_2 ($O_2 < 0.002\%$) reduced but did not eliminate oxidised flavour development during six months storage at 4°. Similar treatment stabilised the flavour of foam vac. dried whole milk. Development of oxidised flavour in foam spray dried whole milk was prevented by packing the powder in cans containing a noble metal catalyst pellet plus N_2 containing 5% H_2. Holding the powder at 1 mm pressure for 15 min. before packing in the $N_2 + H_2$ mixture stabilised the flavour of both types of foam dried powder. (12 references.)   M. O'LEARY.

Factors affecting growth of *Streptococcus agalactiae* in milk. R. W. Brown (*J. Dairy Sci.*, 1967, **50**, 1572–1584).—Acid production after 24 h at 37° was used to measure the growth of *Streptococcus agalactiae* in raw or pasteurised milk serially diluted in steamed milk (100°, 30 min.). The inhibitory titre (reciprocal of the highest dilution of milk showing max. inhibition of acid production) was lowered when the diluent milk was heated for 30 min. at 80 or 90°, 10 min. at 100°, or 15 min. at 121°, and also when the incubation period of the test was increased to 48 h. The inhibitory titre was progressively increased when 5 to 30 μg NH_4CNS per ml was added to pasteurised milk and the diluent. The presence in milk of a stimulatory substance (I) that counteracted the inhibitory substance (II) and which could be removed from whey by dialysis and ultrafiltration was demonstrated. Colostrum samples tested (20) were shown to have more I but less lactoperoxidase than milk. The II in milk was shown to be inactivated by addition of cysteine, glutathione, catalase and horseradish peroxidase. (30 references.)   M. O'LEARY.

Estimation of catalase activity in milk, with particular reference to its application as a screening test in bovine mastitis and milk quality control. D. S. Kalra (*Diss. Abstr.*, B, 1966, **27**, 1529).—A rapid procedure for the estimation of catalase (*C*) in milk was developed. The time is determined for a paper disc, soaked with 0·1 ml of milk, to rise to the surface in a 1·0% Na-perborate solution (pH 7·0). The *C* values can be expressed in terms of beef-liver *C* activity by interpolating on a calibration curve prepared on the basis of determinations made previously. The log values of disc flotation time and log values of corresponding enzyme concn. show a linear relationship. Limitations in the evaluation of different tests for *C* in milk are discussed. The rapid test described is considered suitable for screening in mastitis and for milk quality control.
                                         F. C. SUTTON.

Relationships between California mastitis test reaction and leucocyte count, catalase activity, and A-esterase activity of milk from opposite quarters. L. O. Luedecke, T. L. Forster and U. S. Ashworth (*J. Dairy Sci.*, 1967, **50**, 1592–1596).—Results of 1,122 comparisons of milk from opposite quarters of the same udder indicated that as the severity of the California mastitis test reaction increased the leucocyte count, catalase activity, and A-esterase activity also increased. (18 references.)   M. O'LEARY.

Incidence of *Salmonella* and *Shigella* in samples of raw milk. R. S. Singh and I. P. Singh (*Indian J. Dairy Sci.*, 1966, **19**, 149–150).—205 pooled samples of raw buffalo milk from villages were examined for the presence of *Salmonella* (*SA*) and *Shigella* (*SH*) Two *SA* strains were isolated and were identified as being *S. paratyphi* A (1, 2, 12: a:-) and *S. newport* (6, 8: e, h: 1, 2). Two *SH* strains were also isolated but only one (*Sh. flexeneri*) was identified. (12 references.)   M. O'LEARY.

Reliability of predictions of contamination of milk with fission products in the United Kingdom. B. O. Bartlett (*Nature, Lond.* 1967, **216**, 385–386).—The approx. equation previously established, containing three proportionality factors (rate, lag-rate, soil) (cf. *ibid.*, 1966, **209**, 1062) is used to determine the annual average level (*C*) of contamination with ^{90}Sr and ^{137}Cs from the deposition of fall-out (mean annual deposit, deposit in second half of previous year, and cumulative deposit). Calculated and observed levels of *C* are in good agreement for each of the years 1958–1966, except for ^{137}Cs in 1965 and 1966, for which the equation yields values $\sim20\%$ too low. The results confirm that the contamination of milk by both nuclides is strongly influenced by the rate of fall-out in the preceding year.   W. J. BAKER.

Dependence of strontium-90 in milk on its concentrations in air and surface deposition. B. Y. Lalit (*J. scient. ind. Res.*, 1967, **26**, 372–375).—Regression analysis was carried out to determine the dependence of ^{90}Sr content of milk on its concn. in air, its monthly rate of deposition, and cumulative deposition in soil in Bombay.

One or two months delay is noticed between deposition of activity and its appearance in the milk. Dry season and monsoon season deposition are treated individually, and only about 3% of the annual ^{90}Sr deposition is noted per month in the dry season. Estimated values agree to within 30% of the experimentally determined values. (14 references.)   J. LAMBORN.

Quantitative recovery of carboxymethylcellulose [stabiliser] from milk. P. M. T. Hansen and J. C. Chang (*J. agric. Fd Chem.*, 1968, **16**, 77–79).—The sample is subjected to tryptic digestion, and the proteins are pptd. by the addition of trichloroacetic acid to give a concn. of 12·5% of the acid. After removal of the ppt. the carboxymethylcellulose (CMC) is pptd. from the solution by the addition of EtOAc and EtOH. The mean recovery of CMC (0·05–0·4% added to milk or ice cream) was $92.7\% \pm 3.4\%$. The presence of chocolate causes interference, but if the amount of pectin and starch introduced with the chocolate is known, appropriate correction can be made.   P. S. ARUP.

Effect of some methods of heating cream on its reducing systems and the oxidative stability of butter. K. Domalewska, J. Chuchlowa and E. Pijanowski (*Roczn. Technol. Chem. Żywn.*, 1967, **13**, 91–104).—Laboratory investigation on the effect of four methods of cream pasteurisation on butter stability is described. Cream was heated quickly to 85° and 95° followed by a rapid cooling to 90° and maintained for 5 min. at that temp. before cooling, and also to 115° in an autoclave and maintained at this temp. for 10 min. Butter from this treated cream was kept for nine weeks at 5 to 7°. The cream, buttermilk and butter were subjected to analysis, described in detail (for sulphydryl groups, ferricyanate reducing tests, peroxides etc.). Results indicate that butter manufactured from cream pasteurised at higher temp. is more resistant to auto-oxidation and therefore shows better storage qualities. (13 references.)   T. M. BARZYKOWSKI.

Effect of milk from cows with mastitis on the activity of butter culture. S. Szakály (*Acta microbiol. hung.*, 1967, **14**, 261–269).—Milk from such a source showed a considerably lower acidity and total bacterial count. Culture made from pathological samples possessed a bitter, nauseating taste and showed a soft, flaky clot expelling whey. Probably because of the lower buffer capacity, growth of *Streptococcus lactis, S. cremoris* and *Leuconostoc* was inhibited. As shown by acid content determination, the inhibitory effect was not demonstrated by normal milk unless $>5\%$ pathological milk was present. (21 references.)   C.V.

Effect of the microbial flora on the flavour and free fatty acid composition of Cheddar cheese. B. Reiter, T. F. Fryer, A. Pickering, H. R. Chapman, R. C. Lawrence and M. E. Sharpe (*J. Dairy Res.*, 1967, **34**, 257–272).—The results are presented of trials in which comparisons were made of the flavour, free fatty acids and bacterial flora of commercial cheeses and experimental cheese made under aseptic conditions: (*i*) with δ-gluconic acid lactone instead of starter (*S*), (*ii*) with *S* only, (*iii*) with *S* and added flora from commercial cheese curd. The results are considered to show unequivocally that basic Cheddar flavour is produced by *S*. (29 references.)   M. O'LEARY.

Dehydrogenase activity of *Lactobacillus* species. T. W. Keenan and R. C. Lindsay (*J. Dairy Sci.*, 1967, **50**, 1585–1588).—Tests with single-strain cultures of *Lactobacillus brevis, L. casei, L. lactis*, and *L. plantarum* showed that both growth and production of acetaldehyde (I) and ethanol were very slow at 8°. At optimum growth temp. production of these compounds varied significantly both between species and between strains within a species. All the organisms were capable of reducing added I and propionaldehyde to the corresponding alcohols. *L. brevis* strains reduced added butanone to 2-butanol. A strain of the same species produced n-propanol as a normal metabolite when grown in milk. Significance of these results to development of flavour in Cheddar cheese is discussed. (19 references.)   M. O'LEARY.

Curd-forming techniques for making Pizza cheese by direct acidification procedures. W. A. Larson, N. F. Olson, C. A. Ernstrom and W. M. Breene (*J. Dairy Sci.*, 1967, **50**, 1711–1712).—A description is given of a direct acidification procedure for making Pizza cheese, employing continuous agitation of milk and rennet coagulation. (12 references.)   M. O'LEARY.

***In situ* identification of crystals on process cheese by *X*-ray diffraction.** L. G. Scharpf, jun. and M. J. Michnick (*J. Dairy Sci.*, 1967, 1989–1991).—In a comparison of the rapid *in situ* method described, with the Nujol *X*-ray diffraction method, analyses of crystals from this cheese, containing 2% NaCl and made with 3% anhyd. Na_3PO_4 as emulsifier, gave nearly identical diffraction patterns which corresponded to the α-form of $Na_2HPO_4\cdot12H_2O$. M. O'LEARY.

Edible Oils and Fats

Dielectric properties of commercial cooking oils. W. E. Pace, W. B. Westphal and S. A. Goldblith (*J. Fd Sci.*, 1968, **33**, 30–36).— The dielectric constants (ϵ'_r) and loss tangents (tan δ) of 11 fats and oils were determined at three different temp. and at three different microwave frequencies. The differences in dielectric properties appear to be due to the phase of the material and generally correspond to the degree of unsaturation as determined by iodine values. The differences in loss factors among these fats and oils at any given temp. and frequency are too small to be of any practical importance in selecting any of them for use in heating processes where microwaves are used, or for choosing an optimal frequency. (21 references.)　　　　　　　　　　　　　　I. DICKINSON.

Flavour stability of soyabean oil. J. C. Cowan (*Infciones Grasas aceit*, 1966, **4**, 259–273).—Factors affecting flavour stability, e.g., presence of linolenate, metallic impurities, air etc., are considered and the production of oils of improved stability, e.g., by hydrogenation-winterisation, selective hydrogenation (of the linolenate), and protection with N_2 are discussed. (34 references.)　　　　　　　　　　　　　　L. A. O'NEILL.

Studies on the influence of steroid fraction on the reversion of soyabean oil flavour. H. Niewiadomski and M. Budny (*Roczn. Technol. Żywn.*, 1967, **13**, 23–40).—Influence of steroid fraction on the reversion of soyabean oil flavour is described. It has been ascertained that β-sitosterol (I) influences the oxidation rate of the fatty acid methyl esters of soyabean oil. The relation between the flavour reversion and the degree of oxidative I transformation has been established by organoleptic tests. Results show that these changes are negligible and cannot be detected by conventional analytical methods. (26 references.)　　　　　　T. M. BARZYKOWSKI.

Determination of butter fat in margarine fat by transesterification and gas chromatography. D. F. Withington (*Analyst, Lond.*, 1967, **92**, 705–710).—The sample (0·3–3 g) is submitted to transesterification with ethanolic NaOH and the resulting Et esters are examined by temp.-programmed (55°–200°) gas chromatography, with Me hexanoate (I) as internal standard. The concn. of butter-fat is calculated from peak-height ratio of Et butyrate to I, which ratio is linearly related to % Et butyrate. Results agree with those obtained by the much longer Reichert-Polenske-Kirschner method.　　　　　　　　　　　　　　W. J. BAKER.

Action of micro-organisms on the peroxides and carbonyls of rancid fat. J. L. Smith and J. A. Alford (*J. Fd Sci.*, 1968, **33**, 93–97).— The activities of 26 species of bacteria, moulds and yeast on the monocarbonyl content of rancid fat were determined. They were quite varied and could be divided into (1) micro-organisms which produced large increases in at least two monocarbonyl classes; (2) micro-organisms which removed 2,4-dienals; (3) micro-organisms which removed 2,4-dienals and 2-enals and (4) micro-organisms which caused decreases in at least two classes of monocarbonyls without destroying completely any class. Two micro-organisms produced methyl ketones, a monocarbonyl class which does not appear in rancid lard. A relationship between the ability to decompose peroxides and the ability to produce a great increase in monocarbonyl content was established, but no relationship between the ability to decompose peroxides and lipolytic activity was discovered. (21 references.)　　　　　　I. DICKINSON.

Determination of vitamin A, and α- and β-carotene in margarine, including the results of a collaborative test. C. D. Usher, D. J. Favell and H. Lavery (*Analyst, Lond.*, 1968, **93**, 107–110).—The statutory method for determination of total vitamin A (I) in margarine has been modified to permit separate determination of I, and α- and β-carotene. The sample is heated under reflux with ethanolic KOH to extract the unsaponifiable matter, which after treatment is chromatographed on a column of neutral Al_2O_3. The initial (yellow) eluate is again chromatographed, in a 4–12% solution of ether in light petroleum on a column of MgO, to give two sharp bands of α- and β-carotene, whilst the subsequent (colourless) eluate is passed through alkaline Al_2O_3 to give I in the percolate. Concn. in each eluate are then determined spectrophotometrically. Results from six laboratories show that the accuracy is higher than that of the statutory method, particularly for samples coloured with β-carotene.　　　　　　W. J. BAKER.

Meat and Poultry

Slaughtering, cutting and processing pork. Anon. (*Fmrs' Bull., U.S. Dep. Agric.*, 1967, No. 2138; 48 pp).—Methods for farm use are described.　　　　　　　　　　　　　　E. G. BRICKELL.

Meat tenderness. IV. Changes in the extractability of myofibrillar proteins during meat aging. C. L. Davey and K. V. Gilbert. **V. The effects on tenderness of carcass cooling and freezing before the completion of rigor mortis.** B. B. Marsh, P. R. Woodhams and N. G. Leet (*J. Fd Sci.*, 1968, **33**, 2–7; 12–18).—IV. Myofibrillar proteins (*MP*) from beef and rabbit were extracted with a buffer which dissociates the actomyosin complex of the muscle cell; ~52% of the *MP* of unaged meat was extracted in 40 min. at 2°, and 78% from aged meat. The rate and extent of these changes are determined largely by the ultimate pH of the meat. Similar increases in protein extraction which display the same pH dependence, occur during the aging of well-washed myofibrillar prep. The increase in the % of *MP* extracted during aging results from either a progressive weakening of the fibrous protein linkages with the insol. stroma of the meat cell, or from a disintegration of the insol. stroma itself. (29 references.) V. The time-temp. pattern on the carcass during the onset of rigor mortis greatly affects the tenderness of lamb loin. Toughness develops in the *longissimus dorsi* muscles exposed to low temp. within about 16 h of slaughter. This processing toughness is unrelated to the lack of aging, it appears to be due to muscle fibre shortening. Cold and thaw shortening are capable of producing toughness, the latter becomes prominent when meat previously frozen before rigor completion, is cooked without preliminary thawing. (15 references.)　　　　　　　　I. DICKINSON.

Catheptic enzymes and meat tenderisation. I. Purification of cathepsin D and its action on actomyosin. C. B. Martins and J. R. Whitaker (*J. Fd Sci.*, 1968, **33**, 59–64).—A cathepsin was purified from chicken leg muscle by $(NH_4)_2SO_4$ fractionation and by chromatography on carboxymethyl- and diethylaminoethyl-cellulose. The preparation was probably cathepsin D and did not contain cathepsins A, B and C. It had no activity on actomyosin at pH 4·95 and 5·90 as measured by viscosity and gel-filtration methods. Trypsin, with 0·014 the potential activity of the cathepsin used hydrolysed actomyosin readily at pH 7·9. The cathepsin prepared did not hydrolyse actomyosin, but the results do not exclude the possibility of hydrolysis of actomyosin by other cathepsins. (34 references.)　　　　　　I. DICKINSON.

Effects of freezing, frozen storage conditions and degree of doneness on lamb palatability characteristics. G. C. Smith, C. W. Spaeth, Z. L. Carpenter, G. T. King and K. E. Hoke (*J. Fd Sci.*, 1968, **33**, 19–24).—Frozen and fresh chops and roasts from 190 lamb carcasses were compared. Freezing resulted in significant increase in Warner-Bratzler shear force values for loin chops and significant decreases in flavour and tenderness for leg roasts. In contrast, freezing rib chops resulted in a decrease in shear force values which indicated an increase in tenderness as the result of freezing. Explanations for these findings are suggested and discussed. Variance ratios between chops treated differently were not significantly altered. (16 references.)　　　　　　I. DICKINSON.

Changes in oxalate-stimulated calcium accumulation in particulate fractions from post-mortem muscle. M. L. Greaser, R. G. Cassens, and W. G. Hoekstra (*J. agric. Fd Chem.*, 1967, **15**, 1112–1117).— Sedimented porcine muscle fractions were suspended in a medium containing 0·1 M-KCl, 5 mM-histidine, 5 mM-$MgCl_2$, 5 mM-$K_2C_2O_4$, 5 mM ATP, and 0·1 mM-$CaCl_2$ (containing ^{45}Ca); measurements of the ^{45}Ca remaining in the Millipore-filtered solution showed 40% or ≮ 90% decreases in the Ca-absorbing capacity at 3 h or 24 h post-mortem, respectively. Electron-microscopic examination of the particulate fractions at 0 and 24 h post-mortem showed no differences in their vesicular composition or morphology. (40 references.)　　　　　　P. S. ARUP.

Conversion ratio of meat pigments during the curing process. A. Borys (*Roczn. Technol. Chem. Żywn.*, 1967, **13**, 51–70).—The problem of nitrite curing with special reference to pigmentation and time of cure is discussed. The effect of concn. of NO_2^- or NO_2^-/NO_3^- on curing time appeared to be inconclusive. (25 references.)　　　　　　　　　　　　T. M. BARZYKOWSKI.

Improved device for insertion of meat samples into butyrometers used in the Gerber test for fat in meat. C. W. B. Armstrong (*S. Afr. J. agric. Sci.*, 1967, **10**, 563–565).—The device for use in the De Langen method (cf. *N.Z. Jl agric. Sci.*, 1963, **6**, 452) is made by sawing off the tip of a 10-ml nylon syringe and enlarging the hole in the remainder of the metal part of the tube or the nylon shoulder to a dia. of 3 mm. The syringe is filled by removing the plunger and pressing the open end into the minced meat contained in a plastic bag. Two 5-g samples are delivered, by operating the plunger into separate Gerber tubes for duplicate tests. For 930 samples the mean delivery was 5·05 g with a standard deviation of 0·1 g.　　　　　　　　　　　　P. S. ARUP.

2.—FOODS

Direct spectrophotometric determination of fat and moisture in meat products. I. Ben-Gera and K. H. Norris (*J. Fd Sci.*, 1968, **33**, 64–67).—The near-i.r. spectra of 2 mm thick samples of meat emulsions were measured by direct spectrophotometric techniques for the $1\cdot5$–$1\cdot85$ μ region. The spectra obtained are interpreted in terms of absorptions from O–H and C–H stretching vibrations combined with scatter losses. Optical density differences are correlated with fat and moisture contents. The difference in optical density between $1\cdot80$ and $1\cdot725$ μ gave a high correlation with moisture content and the difference between $1\cdot725$ and $1\cdot65$ μ gave a high correlation with fat content. Direct spectrophotometric analysis predicted fat content within $\pm2\cdot1\%$ and moisture content within $\pm1\cdot4\%$. The problems of developing a rapid, accurate method are discussed. I. DICKINSON.

A simple method for quantitative determination of glucono-δ-lactone in meat products and additives. R. Hamm and F. Schneider (*Dt. LebensmittRdsch.*, 1968, **64**, 207–210).—The lactone is extracted with water and the deproteinised solution is mixed with AcOH, H_2O_2 and NH_4-molybdate. An optically active complex is formed which is estimated polarimetrically. Citric-, malonic-, glutamic-, and sorbic-acids, sorbitol, 5′-ribonucleotides and polyphosphates do not interfere and the effects of ascorbic acid and ascorbates at levels normally encountered is small. The rotation due to sugars can be corrected for and tartrates can be pptd. as K salts. Under normal conditions, added gluconolactone was found not to be degraded after several weeks storage. (10 references.) J. B. WOOF.

Heat-induced changes in turkey muscle as related to moisture retention. P. J. B. Rogers (*Diss. Abstr.*, B, 1966, **27**, 1531).—An incomplete block design was used to measure moisture by selected methods, pH, and Warner-Bratzler shear values for unheated (10°) and heated (dry heat, 176° to internal temp. of 25 to 65°) breasts and thigh legs. Greatest moisture changes occurred between 10 and 25°. Shear and pH values and wt. losses were not related to the majority of moisture measurements. F. C. SUTTON.

Tenderness of freeze-dried chicken with emphasis on enzyme treatments. G. H. Well, jun. (*Diss. Abstr.*, B, 1966, **27**, 1506).—The effect of age on the tenderness of freeze-dried chicken breast muscle was determined. The old birds were selected for proteolytic enzyme treatments and evaluation of muscle tissues. Tenderness was determined by relating shear force to area of breast muscle sheared. % Water uptake during rehydration was directly related to tenderness. Enzyme-induced tenderness seemed to be more related to muscle fibre destruction than to dissolution of the connective tissue. F. C. SUTTON.

Identification of some chemical components in chicken flavour. L. J. Minor (*Diss. Abstr.*, B, 1967, **27**, 2739–2740).—Some of the chemical components from cooked light and dark chicken tissue (derived from roasters, light and heavy hens) have been identified using gas, column, thin-layer and paper chromatography, u.v. and i.r. absorbtion spectra, a trap reaction technique utilising functional group analysis, and a solubility classification method. Precursors and non-volatiles were studied by chemical methods. The protein, fat, moisture and ash contents were determined for the raw muscle and cooked-freeze-dried slurry from light and dark meat for three classes of birds. Concn. of inosinic acid, creatine/creatinine, diacetyl/acetoin, sulphydryl compounds, inorg. sulphides, cystine and methionine, and pH values were also determined for these samples. Gas chromatography of the volatile fraction released upon cooking fryer breasts resulted in identification of ethane, propane and CO_2. H_2S and CO_2 were identified in the fraction distilling off at $-140°$F. NH_3 was identified chemically in the cooked volatile fraction. Odour tests indicated the presence of carbonyl sulphide. A steam distillate from heavy hen breast contained two phosphatidyl lipid components, tentatively identified by t.l.c. as cardiolipin and either phosphatidic acid or phosphatidyl inositol. Volatiles derived by cooking-distillation of young birds in an O_2-containing atm. were trapped in 2,4-dinitrophenyl-hydrazine and Pb acetate solution. Nine carbonyls were identified. Comments and deductions are given. F. C. SUTTON.

Degradation of inosinic acid in chicken muscle during aseptic storage and its possible use as an index of quality. A. W. Khan, J. Davídek and C. P. Lentz (*J. Fd Sci.*, 1968, **33**, 25–27).—Ribonucleotides exert a major influence on the flavour of flesh foods. The most abundant ribonucleotide in chicken muscle is inosinic acid which accumulates as a result of degradation and deamination of adenosine triphosphate. Inosinic acid is slowly degraded to inosine and hypoxanthine by the action of intrinsic muscle enzymes. Storage of chicken breast and legs at 0, 5 and 10° showed that the

breakdown of inosinic acid and formation of hypoxanthine depends on storage temp. and time. Over 75% loss of inosinic acid occurred in 3–5 weeks at 0°, in 2–3 weeks at 5° and in about 1 week at 10°. These periods correspond to the lengths of time after which deterioration can be detected by sensory evaluation. Since the level of inosinic acid in fresh muscle is fairly constant and related to flavour, the results indicate that an objective method of quality assessment based on inosinic acid content is feasible. (11 references.) I. DICKINSON.

Fish

Extraction and some properties of adenosine-5′-monophosphate aminohydrolase from pre-rigor and post-rigor muscle of cod. J. R. Dingle and J. A. Hines (*J. Fish. Res. Bd Can.*, 1967, **24**, 1717–1730).—The enzyme could be extracted from pre-rigor muscle with water or $0\cdot02$ M succinate buffer in about 90% yield; $0\cdot02$ M-KCl gave a low yield. With post-rigor muscle it tended to be associated with the fibrillar protein fractions. The enzyme was sensitive to glass and other substances, leading to loss of activity during dialysis. Activation energy was 10 kcal/mol. and the Michaelis-Menten const. $1\cdot4 \times 10^{-3}$ mol. per l. E. G. BRICKELL.

Preparation of light-salted fish by brining. R. Legendre (*J. Fish. Res. Bd Can.*, 1967, **24**, 1693–1699).—In a batch operation using an equal wt. of cod and brine (25% by wt.), good results were obtained after 5 h. With continuous circulation of brine (18%) optimum conditions were achieved at 8 h. In both cases the salted product had to be 'water horsed' for 24 h before drying and after efficient drying resembled the regular Gaspé-cure in appearance and in chemical characteristics. E. G. BRICKELL.

Post-mortem degradation of adenine nucleotides in muscle of the lobster, *Homarus americanus*. J. R. Dingle, J. A. Hines and D. I. Fraser (*J. Fd Sci.*, 1968, **33**, 100–103).—T.l.c. showed that the degradation of adenine nucleotides followed the route: adenosine 5′-triphosphate (ATP)→adenosine 5′-diphosphate (ADP)→adenosine 5′-monophosphate (AMP)→inosine 5′-monophosphate (IMP) →inosine (Ino)→hypoxanthine (Hx). KCl extracts ($0\cdot6$ M) also degraded ATP by this route. Such extracts contained a weak AMP-aminohydrolase activity that was activated by ATP, but no adenosine aminohydrolase could be detected. Neither of these aminohydrolases were found in extracts made with water or $0\cdot002$ M K-succinate. Details of preparation of the samples from muscle and of the development of the chromatograms were described by D. I. Fraser et al., (*J. Fish. Res. Bd Can.*, 1967, **24**, 1837–1841). I. DICKINSON.

Spices, Flavours, etc.

Dihydrochalcones. Synthesis of potential sweetening agents. L. Krbechek, G. Inglett, M. Holik, B. Dowling, R. Wagner and R. Riter (*J. agric. Fd Chem.*, 1968, **16**, 108–112).—Synthetic methods are described by which the commercially available flavonoid naringin was converted into other phenolic glycosides. One such compound, 2′,4′,6′,3-tetrahydroxy-4-n-propoxydihydrochalcone 4′-β-neohesperidoside had a sweetening effect ~2000 times that of sucrose. (18 references.) P. S. ARUP.

Minor sesquiterpene hydrocarbons of black pepper. C. J. Muller, R. K. Creveling and W. G. Jennings (*J. agric. Fd Chem.*, 1968, **16**, 113–117).—Further to a previous investigation by Muller and Jennings (cf. *ibid.*, 1967, **15**, 762) the following compounds have been identified as minor constituents: α-cubebene, isocaryophyllene, and γ-muurolene; β-farnesene has been isolated as an additional major constituent. (23 references.) P. S. ARUP.

Identification of essential oils of citrus fruits by gas chromatography. R. Huet (*Fruits d'outre mer*, 1967, **22**, 177–181).—Chromatograms were recorded for 35 varieties of the fruits. The technique used was that of Rasquinho (*J. Gas Chromat.*, 1965, 340–344), with temp.-programming and flame-ionisation detection. P. S. ARUP.

Isolation and characterisation of terpenes from *Citrus reticulata* Blanco and their comparative distribution among other citrus species. S. H. M. Ashoor and R. A. Bernhard (*J. agric. Fd Chem.*, 1967, **15**, 1044–1047).—The monoterpenes of tangerine and mandarin oils were investigated by g.l.c. with katharometer and with flame-ionisation detection and with the use of five different stationary liquid phases. In addition to known components, $\triangle^3$-carene, α-phellandrene, and β-phellandrene were identified by their retention times and by i.r. spectroscopy. (17 references.) P. S. ARUP.

Concentration of volatile compounds [flavours] from dilute aqueous solutions. D. A. Forss, V. M. Jacobsen and E. H. Ramshaw (*J.*

agric. Fd Chem., 1967, **15**, 1104–1107).—Two forms of apparatus are described by means of which the compounds can be concentrated without the use of org. solvents or exposure to high temp. In the first apparatus the compounds are distilled from the aq. solutions (5–5000 ml) through a vertical condenser maintained at 0° and into a liquid N_2 trap; the temp. of the aq. solution is gradually increased to 20° whilst the pressure is increased from 6 to 15 torr. In the second apparatus further concentration is effected by freezing the distillates from the first apparatus at 55° and subliming them at $< 10^{-4}$ torr into a liquid N_2-trap. Concn. ratios of 400-fold were achieved. Yields from aq. solutions of n-alkan-2-ones, n-alkan-1-ols and Et n-alkanoates in concn. of $0·1 \mu l$ per ml were $> 80\%$ for compounds of b.p. $< 175°$, and $> 40\%$ for those of higher b.p. (14 references.)　　　　　　　　　　　　　　　P. S. ARUP.

Preservatives

Effects of processing, storage and incorporation of antioxidants on canned mackerel and ravas [*Polynemus tetradactylus*]. S. S. Pawar and N. G. Magar (*J. Fd Sci. Technol.*, 1967, **4**, 8–11).—The canned fish was divided into three groups, (1) $2·5\%$ brine, (2) $2·5\%$ brine+ $0·01\%$ citric acid (I) and $0·01\%$ ascorbic acid per 100 g of fish muscle, (3) $2·5\%$ brine $+ 0·01$ I and $0·01\%$ nordihydroguariaratic acid (II) per 100 g fish muscle. The cans were stored at room temp. and at 37°. II in combination with I was found to be an effective preservative. (21 references.)　　　　　　　I. DICKINSON.

Rôle of tocopherol as an antioxidant in safflower oil. M. K. Govind Rao and K. T. Achaya (*Fette Seifen AnstrMittel*, 1967, **69**, 711–714).—When equal parts of oleic and linoleic acids are mixed, the autoxidation of the latter dominates, and at 63° the γ-tocopherol has the best antioxidant action followed by α- and δ-tocopherols respectively. Safflower oil is insufficiently protected by its own tocopherol content, and doubling the natural content only brings about a slight improvement. The practical way to stabilise this oil effectively is by addition of $0·02\%$ of synthetic phenolic antioxidants (BHA or BHT) together with $0·05\%$ of a phosphoric- or citric-acid synergist. (21 references.) (In English.)
　　　　　　　　　　　　　　　　　　　G. R. WHALLEY.

Use of sulphite and antioxidants in the preparation of potato purée flakes. S. van Gorkom (*Dt. LebensmittRdsch.*, 1968, **64**, 178–180).—Potato flakes prepared with $0·66\%$ monoglyceride, 200 ppm BHA and 40–400 ppm SO_2 were packed in air and N_2 and stored for up to 12 months and the changes in colour, flavour and aroma noted. Addition of BHA and SO_2 had a marked beneficial effect both in air and N_2 but increasing the SO_2 level provided no additional advantage. Very good results could also be obtained using BHA, BHT and SO_2. Packaging under N_2 gave improved keeping qualities. (13 references.)　　　　　　　　J. B. WOOF.

Brine-fermentation of cucumbers treated with sodium *o*-phenyl-phenate. T. A. Bell, J. L. Etchells and R. M. Swindell (*J. agric. Fd Chem.*, 1967, **15**, 1108–1111).—The use of Na *o*-phenylphenate (I) in the cooling water and in the pickling brine had no undesirable effect on the brine fermentation or the quality of the fermented cucumbers. The I was mostly adsorbed from the brine on to the cucumber skins. Some difficulty could be foreseen in controlling the tolerance level at 10 ppm. (13 references.)　　　　P. S. ARUP.

Effects of the food additive butylated hydroxytoluene on monolayer cultures of primate cells. S. M. Milner (*Nature, Lond.*, 1967, **216**, 557–560).—Experiments described show that inhibition of monkey kidney-cell nos. by butylated hydroxytoluene (I) is caused by rapid depression of cellular metabolism. Inhibition is proportional to concn. of I ($0·034–0·136$ mM) and the rate of RNA synthesis is most affected; male cells show ~50% inhibition and female 95% inhibition. In male cells protein synthesis is least (15%) inhibited. Complete and rapid reversal of RNA inhibition occurs on removal of I. The differential sex effect is probably due to more rapid metabolism of I in male cells. The two I metabolites (3,5-ditert-butyl-4-hydroxybenzoic acid and 3,5-ditertbutyl-4-hydroxybenzyl alcohol) and also α-tocopherol do not inhibit RNA synthesis at concn. $\sim 0·14$ mM. These results are discussed in respect of the findings for *in vivo* metabolism of I in rats and rabbits and the permitted concn. of I in foods. (34 references.)　W. J. BAKER.

Pesticides in Foods

Toxicity of pesticides in foodstuffs. G. Baluja Marcos (*Revta Ciencia apl.*, 1967, **21**, 305–314).—The nature of pesticide residues in foodstuffs, their toxicity and permissible level are considered. (13 references.)　　　　　　　　　　　　　L. A. O'NEILL.

Determination of quassin residues in plums. E. Kroller (*Dt. LebensmittRdsch.*, 1968, **64**, 183–185).—The bitter substance quassin, a mol. compound of neo- and iso-quassin is extracted from quassia woods and is used as an insecticide against the plum saw fly. The chemical properties are described. No suitable colour reactions are known and the ppt. given with alkaloid reagents are not suitable for quant. determinations. Pure neo- and iso-compounds have been prepared and have m.p. of 212° and 224° respectively, with an absorption max. at 255 mμ. Residues of these compounds are extracted from plums with nitromethane and the washed extracts subjected to t.l.c. on Kieselgel F 254 plates with $CHCl_3$/acetone (9 : 1) as solvent. The quassin bands are detected under a u.v. lamp and after extraction with 50% ethanol the extinction is measured at 255 mμ. The detection limit is 100 μg and errors are within $\pm15\%$.　　　　　　　J. B. WOOF.

Effect of chlorophenoxyacetic acid growth-regulator sprays on residues in canned apricots and grapes. Y. N. Lee and B. S. Luh (*J. Fd Sci.*, 1968, **33**, 104–108).—A gas–liquid chromatographic method coupled with an electron capture detector is described. The technique involves the conversion of chlorinated phenoxyacetic acid residue to its methyl ester using diazomethane, chromatography on a 5% silicone grease SE-30 column at 210°, and subsequent detection of the compound by an electron-capture detector. $0·02$ ppm of the residue could be detected. *p*-Chlorophenoxyacetic acid (I), 2,4-dichlorophenoxyacetic acid (II) and 2,4,5,-trichlorophenoxyacetic acid (III) can be separated and quant. determined simultaneously. Levels of II and III residues in canned apricots and of I in canned grapes are reported. (14 references.)
　　　　　　　　　　　　　　　　　　I. DICKINSON.

Improved microwave-emission gas chromatography detector for pesticide-residue analysis. H. A. Moye (*Analyt. Chem.*, 1967, **39**, 1441–1445).—Improvement is based on use of Ar–He mixtures for both carrier and microwave-discharge gases and on establishing optimum values and conditions for the operating parameters. Max. sensitivity and selectivity for org.-P pesticides, p,p'-DDT (I), lindane (II), and 2-iodobutane were obtained with an 85% He mixture, 25-torr discharge-pressure, slit-width 6 nm and a relatively high flow-rate. The lines P 253·57, I 206·2 nm were used, but for I and II an emission spectrum run from 200 to 700 nm on $CHCl_3$ (band head at 221·0 nm) was necessary. Sensitivity for parathion in orange juice is 0·5 ppb.; in comparison with the electron-capture detector no extraneous peaks were present in the chromatogram for celery extract containing 10 ppb of parathion.
　　　　　　　　　　　　　　　　　　W. J. BAKER.

Food Processing, Refrigeration

Changes in the nitrate and nitrite contents of fresh and processed spinach during storage. W. E. J. Phillips (*J. agric. Fd Chem.*, 1968, **16**, 88–91).—Under retail sale conditions, NO_2-N accumulated in some samples of fresh spinach (S), but not in tinned infants' food or frozen S. Accumulations of NO_2-N occurred if frozen S was thawed and then kept at room temp. for unduly long periods. Cooking decreased the NO_2^- and NO_3^- content of S by extraction into the water. The possible production of methemoglobinemia in infants due to ingestion of NO_3^- is discussed in relation to these findings. (21 references.)　　　　　　　P. S. ARUP.

Absorption spectra of copper and zinc complexes of pheophytins and pheophorbides. I. D. Jones, R. C. White, E. Gibbs and C. D. Denard (*J. agric. Fd Chem.*, 1968, **16**, 80–83).—The complexes of the pheophytins *a* and *b* and the pheophorbins *a* and *b* were prepared by addition of $CuCl_2$ or $ZnCl_2$; the reactions were completed in aq. $COMe_2$ solution within 30–90 min. The purity of the complexes was checked by means of t.l.c. Before determining the spectral characteristics and mol. absorbance coeff. it was necessary to purify the complexes by chromatography on a sugar column with appropriate mixtures of Et_2O and light petroleum as eluents. The complexes may be formed during food processing of certain vegetables. (17 references.)　　　　　　　P. S. ARUP.

Preservation of foods by irradiation. A. de Jong (*Landbouwvoorlichting*, 1967, **24**, 315–318).—A pilot installation is described in which packages of fruit, vegetables, or grain are transported by a conveyor belt through a field of γ-radiation. The use of this technique on a commercial scale is considered feasible.
　　　　　　　　　　　　　　　　　　P. S. ARUP.

Packaging

Engineering analysis of the controlled atmosphere (*CA*) storage. D. Gurevitz (*Diss. Abstr.*, B, 1967, **27**, 2739).—The *CA* storage of

apples is described, but most of the observations are applicable to other types of produce. The problems of moisture migration through the insulation, the effects of weather, reversible vapour flow and room operational conditions are discussed. Other possible solutions to water vapour transfer, CA gas tightness and pressure and temp. cycles in the CA storage is presented. It is suggested that all the spaces between the pallet boxes be closed, and the stacking arranged so that the air is forced to flow through the void spaces between the apples in the boxes. Suction fans distributed uniformly on a false ceiling will be used to move air through the system and give even distribution in the stack. The time to reduce the O_2 level in the room is shorter than in a regular CA room. The study was based on a theoretical analysis only, and the proposed new air distribution method is only speculative at this stage.
F. C. SUTTON.

Protection of packaged foods from insects. J. P. Brooke and P. H. Lomax (*Pyrethrum Post*, 1967, **9**, No. 1, 36–39).—Good protection from insect infestation for at least 6 months is given by a high gloss varnish prepared from a styrene copolymer, containing synergised pyrethrins.
J. D. WALPOLE.

Plastics in the dairy industry—a critical study. III. Behaviour of the packed milk products in relation to the impervious properties of plastic films. G. Wildbrett (*Fette Seifen AnstrMittel*, 1967, **69**, 781–794).—The permeability of water vapour, gases and various liquids encountered in the foodstuffs industry through a variety of packaging films is discussed. It is demonstrated by reference to published data, how the uptake and liberation of water vapour, CO_2, O_2 and aromas affect the quality of the milk products; the relationship between optical clarity and oxidative changes is also pointed out. For the effective protection of milk products the use of combination foils is recommended. (185 references.)
G. R. WHALLEY.

Plastic films in the storage and distribution of agricultural and horticultural products. G. C. Pratella (*Materie plast.*, 1967, **33**, 1039–1044).—The use and applications of plastic films, especially polyethylene, in packaging Italian fruit and vegetables is described. Satisfactory temp. for storage, and the evolution of O_2 and CO_2 in enclosed conditions by different vegetables are indicated. Use of mould-prevention agents is discussed.
C. A. FINCH.

Gas chromatographic procedure for measuring isostatic permeation of volatile aroma components of food through packaging films. A. G. Wientjes, H. Maarse and S. Van Straten (*Mitt. Geb. Lebensmittelunters. u.-Hyg.*, 1967, **58**, 61–70).—Volatile compounds permeating the foil from the upper to the lower chamber of a permeating vessel at 30° are conveyed by a stream of N_2 to an absorption column cooled with solid CO_2, and allowed to accumulate for a specified time. A special flow arrangement securing isostatic permeation is described. The compounds are then liberated from the column by heating it in a water-bath, and conveyed by N_2 to a g.l.c. column; the two columns consist of Lac I-R-296 (25%) supported on Chromosorb W. An experiment is described in which the alteration of the proportions of the volatile compounds of roasted coffee, stored in a light-density foil was demonstrated. Reproducibility of the results was satisfactory. (20 references.) (In English.)
P. S. ARUP.

Solid electrode chronopotentiometry for studying the reaction of erythrosin on tinplate. P. W. Board, R. V. Holland and D. Britz (*Br. Corros. J.*, 1968, **3**, 31–33).—Chronopotentiometry using a solid Sn cathode was used to study the reaction of erythrosin (2′,4′,5′,7′-tetraiodofluorescein) on tinplate, after rapid detinning was observed in cans containing cherries which had been coloured with this dye. Erythrosin is reduced by Sn in acidic solution to fluorescin, the reduced form of fluorescein, with the exchange of 10 electron/mol. In the concn. normally used in foods, erythrosin makes only a slight contribution to the corrosion of tinplate.
J.A.C. ABSTRACT.

Miscellaneous

Nutrition, proteins, amino-acids, vitamins

Protein–lipid interaction affecting the quality of protein foods. T. N. R. Varma (*J. Fd Sci. Technol.*, 1967, **4**, 12–13).—A review. (14 references.)
I. DICKINSON.

Biological ceilings and economic efficiencies for production of animal protein, A.D.2000. P. N. Wilson (*Chemy Ind.*, 1968, 899–902).—Future trends and limitations are discussed with reference to reproductive efficiency, longevity, generation interval, growth rate and food-conversion efficiency, and environmental adaptation for the rabbit, chicken, goat/sheep, cow/buffalo and pig. Present and probable future protein sources are compared.
W. J. BAKER.

Enzymic extraction of protein from coconut flour 'Cocos nucifera'. A. Chandrasekaran (*Diss. Abstr.*, B, 1967, **27**, 2984).—*Postalotiopsis westerdijkii*, a cellulolytic mould, produced a freely diffusing, extracellular enzyme capable of solubilising hydrocellulose and amorphous cellulose when grown on coconut flour–solka-floc (2 : 1) medium at 31° for 11 days. Activation of enzyme by cysteine hydrochloride was observed. Five-fold increases in specific activity with the elimination of most of the non-enzyme N was achieved by $(NH_4)_2SO_4$ pptn and gel filtration of the crude enzyme. The most limiting amino-acid in the coconut milk prep. is lysine, followed by threonine, sulphur amino-acids and isoleucine. Animal feeding experiments did not show any significant difference between the protein qualities of the control and enzyme-treated milk prep.; however, the animals on the residue diets all lost wt.
F. C. SUTTON.

Preparation and composition of a tofu-like product from groundnut. M. A. Krishnaswamy and T. Shantha (*J. Fd Sci. Technol.*, 1967, **4**, 6–7).—Tofu (I) is a product of pptn. of soya milk with $CaSO_4$; it consists mainly of protein and oil of soyabean and is therefore a valuable source of protein. Analytical figures for I were: Moisture: 3·2%, protein (N × 6·25): 43·75%, ether extractives: 37·2%, total ash: 8·7%, water-insol. ash: 5·4%, P: 601 mg%, Ca: 1362 mg %, Fe: 0·78 mg %, lysine: 2·49, available lysine: 1·75, methionine: 0·91 and cystine 0·81 g/16g N. Freeze-dried I was spongy, with a bland flavour, and an agreeable colour; deep-fat fried it was crisp and tasty.
I. DICKINSON.

Nutritive values of some oilseed proteins. R. J. Evans and S. L. Bandemer (*Cereal Chem.*, 1967, **44**, 417–426).—Determinations of essential amino-acids and of nutritive value by rat assay and by assay with *Tetrahymena pyriformis* W. are reported for hexane-extracted peanuts (*P*), safflower seed (*SA*), sesame seed (*SE*), soyabean (*SB*) and sunflower seed (*SU*). *P* were deficient in methionine (I), isoleucine (II) and lysine (III), *SA* in I, II and III, *SE* in III and I, *SU* in I and III and heated *SB* was deficient only in S-containing amino-acids. Very good growth in rats resulted with equal parts of *SE* and *SB*, heated *SB* + I, or *SE* + III. Supplementation of *P* did not improve their growth-promoting properties; supplementation of *SA* gave some improvement, but did not raise it to the protein nutritive value of casein. *Tetrahymena* assay results agreed poorly with rat growth; the microbiological assay gave particularly low values for *P* and tended to overestimate the effect of supplementation. (26 references.)
E. C. APLING.

Determination of tryptophan in proteins. J. R. Spies (*Analyt. Chem.*, 1967, **39**, 1412–1416).—Errors inherent in the intact-protein method (*ibid.*, 1949, **21**, 1249) are eliminated by either of the two new methods described. In one, the protein is hydrolysed at ~150° with O_2-free NaOH saturated with basic Pb acetate containing histidine, whilst in the other the hydrolysis is effected with Pronase. The free tryptophan (I) in each hydrolysate is determined spectrophotometrically at 590 nm after formation of its complex with p-dimethylaminobenzaldehyde in H_2SO_4 solution (*ibid.*, 1948, **20**, 30). The histidine prevents destruction of I by serine during hydrolysis, whilst basic Pb acetate partly protects I from cystine and decreases interference by S-compounds in the spectrophotometry (addition of $AgSO_4$ to the hydrolysate eliminates this interference completely). Results obtained by both methods for I in milk and other proteins and in some enzymes are in satisfactory agreement; the coeff. of variation was ±1·2% for each method (167 determinations on 61 proteins).
W. J. BAKER.

Detection and location of N-methylamino-acid residues in N-acyl-oligopeptide methyl esters by mass spectrometry. B. C. Das, S. D. Géro and E. Lederer (*Nature, Lond.*, 1968, **217**, 547–548).—Because N-methylamino-acid residues in a peptide cannot be satisfactorily detected and located by mass spectrometry, it is necessary to make use of the mass spectrometric shift procedure. By comparison of the mass spectra of the sample and its permethylated deriv. the following information is obtained: (1) the no. of Me groups introduced by complete N-methylation and hence the no. of CONH groups in the original ester, (2) each amino-acid residue which has gained a Me group (indicated by an increase of 14 in mass no. as determined from the peaks due to peptide-bond linkage), (3) any amino-acid residue having its peptide-N initially methylated or fully substituted (indicated by no change in mass no. after methylation). This approach was applied to fortuitine, a naturally occurring peptidolipid containing nine amino-acids, and was confirmed by analysing the mass spectrum of perdeuteriomethylfortuitine. The method is inapplicable when the oligopeptide deriv. do not give mass spectra before methylation; for these the mass spectra of the permethylated and perdeuteriomethylated deriv. must be compared.
W. J. BAKER.

Changes of the content of vitamin B₁ and B₂ during fermentation cycle of brewers yeast. K. Maslowski (*Roczn. Technol. Chem. Żywn.*, 1967, **13**, 17–21).—Thiamin content decreases considerably in brewers yeast which has been used in 12–13 cycles of wort fermentation; loss may be of the order of 50%. A lesser decrease amounting to only 22% is found in total flavine content, but the loss of riboflavine can be as high as 70%. Analytical methods applied in this research are described in detail.

T. M. BARZYKOWSKI.

Nutritionally unavailable niacin in corn [maize]. Isolation and biological activity. D. D. Christianson, J. S. Wall, R. J. Dimler and A. N. Booth (*J. agric. Fd Chem.*, 1968, **16**, 100–104).—A substance containing bound niacin (I), unavailable to rats, was extracted from commercial maize gluten with 50% EtOH at 4°. The substance, after purification by lyophilisation and passage through a column of cation-exchange resin still contained carbohydrate and nitrogenous compounds in addition to I; I was rendered available to rats after treatment with 0·1 N-NaOH at room temp. or with N-H₂SO₄ at 120°. (24 references.)   P. S. ARUP.

Influence of ascorbic acid on some quality factors for macaroni goods. Lj. Milatović and M. Martinek (*Getreide Mehl*, 1967, **17**, 97–103).—Studies are reported of the effects of additions of *l*-ascorbic acid on the rheological properties of durum and vulgare pastes made with or without the addition of egg, and on the quality of the resulting macaroni goods. Optimum improvement in rheological properties resulted with addition of 15–20 mg % to durum semolina or 15 mg % to vulgare flour. For pastes containing egg addition is unimportant from the rheological point of view, but additions also significantly reduced loss of pigment, development of non-enzymic browning, and (in egg goods) loss of tocopherols.

E. C. APLING.

Unclassified

Food for fitness. Anon. (*Leafl. U.S. Dep. Agric.*, 1967, No. 424, 7 pp).—A daily guide is listed.   E. G. BRICKELL.

Biological efficiency of food-producing systems in A.D.2000. A. N. Duckham (*Chemy Ind.*, 1968, 903–906).—The improvement of future food yields by increasing total photosynthesis and decreasing phytic and animal wastages, are discussed, together with social and economic factors influencing the reliability and persistence of each system, taking the changing agrarian structure into account. A simple energy model for a food-production system is given. (19 references.)   W. J. BAKER.

Feeding the world. (*Science J.*, 1968, **4**, No. 5; 3–106).—**The earth's potential.** H. L. Penman (43–47). **Nutritional requirements.** J. Yudkin (48–52). **Animal harvest.** K. L. Blaxter (53–59). **Improving traditional agriculture.** A. H. Bunting and A. Harrison (60–65). **Novel routes to plant protein.** H. A. B. Parpia (66–71). Much protein malnutrition can be overcome if unconventional sources such as oilseed meals (*OSM*) are used; these are presently mainly used as cattle feed or fertiliser but they can be upgraded to high class human food. The *OSM* are the dry protein-rich residues left after the vegetable oil has been extracted and although deficient in certain amino-acids, they can be enriched with legumes or synthetic amino-acids. Examples are given. **Farming the desert.** H. Boyko (72–78). **Ocean reservoir.** J. A. Gulland (81–86). **Single cell protein.** S. R. Tannenbaum and R. I. Mateles (87–92). The production of foods from cultivated bacteria, algae, yeasts, protozoa or fungi is discussed. The various industrial programmes are indicated and a simplified flow-sheet is given. **Synthetic foods.** M. Pyke (93–98).   C.V.

Identification of gelatinising and thickening agents 'permitted in Switzerland' by means of electrophoresis and staining on cellulose acetate foils. M. Padmoyo and A. Miserez (*Mitt. Geb. Lebensmittelunters u-Hyg.*, 1967, **58**, 31–49).—Directions are given for the electrophoretic separation of these substances, isolated from defatted foods by pptn. with EtOH by the official AOAC method (*10th Edn.*, 1965, 255). Four staining methods are specified for the distinction between substances of the same electrophoretic mobility and for colorimetric determinations in comparison with reference standards; for the latter purpose, the electropherograms are rendered transparent by treatment with MeOH and with MeOH–AcOH, and heating at 110°. The procedure permits the detection of 2–10 μg of the gelatinisers or thickeners. (14 references.)

P. S. ARUP.

Moving boundary electrophoresis of food stabilisers. J. Hidalgo and P. M. T. Hansen (*J. Fd Sci.*, 1968, **33**, 7–11).—The electrophoretic characteristics of locust bean gum, guar gum, arabic gum, carboxymethylcellulose (CMC), κ- and λ-carrageenan, alginate and the non-dialysable fraction of corn syrup solids were studied. A modified technique was used to eliminate boundary disturbances which otherwise might be interpreted as heterogeneity of composition. CMC, arabic gum, alginate and λ-carrageenan showed a high degree of homogeneity, while κ-carrageenan was a mixture of migrating components (of different ester sulphate content). The colloidal fractions of guar gum, locust bean gum and corn syrup solids did not migrate in the electrical field. Mixtures of CMC with guar gum or gum arabic could be separated at pH 7, and mixtures of CMC with carrageenan at pH 2. Electrophoretic separation at 23° of different stabiliser mixtures was unsatisfactory and the patterns showed evidence of interactions. (11 references.)

I. DICKINSON.

Determination of microgram amounts of lead in food with a radioactive tracer. D. C. Bogen and M. T. Kleinman (*Analyst, Lond.*, 1967, **92**, 611–613).—The sample (1 kg) is freeze-dried, wet-ashed, and ²¹²Pb [prepared from Th(NO₃)₄] is added as tracer. The Pb is concentrated by solvent extraction of its tetraiodo complex from 5 M-HCl into 3-methyl-2-butanone. The residue from the dried org. phase is wet-ashed and, finally, Pb is extracted as dithizonate into CHCl₃ for spectrometric determination at 510 nm. The chemical yield (~94%) is obtained by γ-scintillation counting of ²¹²Pb. The accuracy is within ~10% for 5–700 μg of Pb per kg of milk or vegetables.   W. J. BAKER.

Determination of iron, cobalt and nickel when present together in beverages with 1-(2-pyridylazo)-2-naphthol to form 'panates'. Th. Vondenhof and H. Beindorf (*Mschr. Brau.*, 1968, **21**, 156–157).—The method of Püschel *et al.* has been applied to fruit juices and to beer. The sample is evaporated to dryness and ashed; the residue is dissolved in HCl (1 : 1) and 0·25% ethanolic PAN solution is added. After neutralisation with NH₄OH, acetate buffer (pH 5) is added and the coloured complex extracted with CHCl₃. The extract is washed free of interfering metals with buffered EDTA and made up to standard vol. before measuring the extinction at 764 mμ (Fe), 628 mμ (Co) and 565 mμ (Ni). Regression equations for calculating concn. are given.   J. B. WOOF.

Diffusion of aflatoxins in foodstuffs. H. K. Frank (*J. Fd Sci.*, 1968, **33**, 98–100).—Fruit juices, sliced prepacked bread and cheese, which turn mouldy under household conditions, were examined. Aflatoxins are excreted by the mycelium, and studies were made of their production in apple juice, their diffusion in rye and wheat breads and cheese, and the kinetics of their diffusion in a model substrate composed of 'Cream of Wheat', sucrose and tap-water. The matrix was cut into 3-cm cubes and autoclaved. The fermented substrates were dialysed in methanol–water and the solution extracted with CHCl₃, dried over Na₂SO₄, and the highest dilutions of aflatoxin B₁ and G₁ were determined under u.v. light on thin-layer silica gel plates. Results show a possible danger for consumers who eat foods with closely cut-off mouldy spots. The content of aflatoxin below the fungus mat depends on the strain growing on the surface, the moisture content and the storage time and temp.   I. DICKINSON.

Food poisoning and food hygiene. B. C. Hobbs. 1968, 2nd edition, 252 pp. (London: Edward Arnold, Publishers).—Seven chapters dealing with food poisoning and food-borne infection, and eight chapters on food hygiene.   P.P.R.

Typhoid in Aberdeen, 1964. J. W. Howie (*J. appl. Bact.*, 1968, **31**, 171–178).—A review of the outbreak. Two special findings are stressed: (*a*) typhoid is not a highly communicable disease and (*b*) typhoid bacilli in a can of corned beef can outgrow many competing contaminants introduced at the same time without causing any visible damage to the can or the meat.   C.V.

3.—SANITATION, WATER etc.

Roach control key to salmonella control. P. G. Bartlett (*Soap chem. Spec.*, 1967, **43**, No. 9; 62–64, 66).—The literature sources relating to the incidence of salmonella-type diseases and cockroach infection are listed and reviewed, together with the general measures required to eliminate this insect vector. The properties of typical food plant sanitisers are presented in a tabular form; they include the Ca- and Na-hypochlorites, chlorinated-trisodium phosphate, the K or Na chloroisocyanurates, iodophors and the quaternary ammonium compound formulations. (18 references.)

G. R. WHALLEY.

Pyrethrum as an insect repellent. II. A laboratory technique for its evaluation as a mosquito repellent and the influence of formulation on persistence. A. J. S. Weaving and N. K. Sylvester (*Pyrethrum Post*, 1967, **9**, No. 1; 31–35).—Carefully controlled experiments involving exposure of treated and untreated forearms to *Aedes*

aegypti adults and recording biting rates at hourly intervals over a period of 5 h, showed that 2% pyrethrins + 2% piperonyl butoxide in a white oil gave protection for 5 h and was markedly superior to isopropyl alcohol- and water-based formulations. Descriptions of apparatus and experimental procedures are provided. (10 references.)
J. L. WALPOLE.

Properties and biological activity of some asymmetrically substituted 2,2′-dihydroxydiphenylmethane derivatives. J. Arct, E. Czerwińska, Z. Eckstein, R. Kowalik and H. Krzywicka (*Bull. Acad. pol. Sci. Sér. Sci. chim.*, 1967, **15**, 233–238).—2,2′-Dihydroxydiphenylmethane deriv. were synthesised, using various substituents (Cl, Br, F, CH₃), and 22 compounds were obtained in yields of 35 to 98%. All (except one), were examined for their bacteriostatic activity against *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis* and for fungicidal activity against *Fusarium culmorum*, *Rhizoctonia solani* and *Alternaria tenuis*. All tested compounds showed promise as disinfectants; some were equal, or better than, dichlorophene and hexachlorophene which were used as standards.
T. M. BARZYKOWSKI.

Pesticides: Transatlantic movements in the Northeast Trades. R. J. Huggett, J. J. Griffen and E. D. Goldberg (*Science, N.Y.*, 1968, **159**, 1233–1236).—The amounts contributed are comparable to those carried to the sea by the major river systems. (14 references.)
C.V.

Comparison of various methods of effluent examination. K. Vogl, G. Schumann and H. Kolb (*Mschr. Brau.*, 1968, **21**, 212–215).—In order to estimate the level of contamination in domestic and industrial effluent, river and lake water the following methods have been compared: (*i*) permanganate oxidation method of Kubel and the Schulze modification in acid and alkaline solution respectively; (*ii*) chromate oxidation procedure of Beuthe; (*iii*) determination of BOD by the dilution method in which the dissolved O₂ reacts in alkaline medium with Mn²⁺ to give higher hydroxides, which in turn yield Mn³⁺ in acid medium. This liberates an equiv. amount of iodine from KI solution which is titrated with thiosulphate. Methods (*i*) were unsatisfactory because of the relatively low oxidation effect but the Kubel method may be of use for approx. determinations. So as to take into account the BOD of micro-organisms and substances not easily broken down, method (*iii*) should be used in conjunction with (*ii*). (15 references.)
J. B. WOOF.

Biological treatment of phenol-bearing wastes. R. R. Prasad and B. K. Dutta (*Technology, Q. Bull. Fertil. Corp. India*, 1966, **3**, 188–191).—A biological process for destruction of phenols by a bacterial culture prepared from garden soil is proposed. Studies of the effect of temp., pH, and air rate on the oxidation of phenol are reported. At 37° and an air rate of 0·2 cu. ft/min./l a phenol concn. of 1170 mg/l was reduced to 5 mg/l in 10–11 h. (10 references.)
E. C. APLING.

4.—APPARATUS AND UNCLASSIFIED

Imunofluorescent studies on yeasts. P. Geek and E. K. Novák (*Acta microbiol., hung.*, 1967, **14**, 13–22).—This technique carried out on some 107 strains enabled *Procandida albicans* and *P. stellatoidea* to be differentiated from other yeasts. The significance of this approach is discussed. (35 references.)
C.V.

Improved method of determining cellulytic activity in anaerobic bacteria. S. O. Mann (*J. appl. Bact.*, 1968, **31**, 241–244).—The disintegration of a filter strip indicates activity. The paper containing media is inoculated from dilutions of material such as rumen contents and estimates of the number of cellulytic bacteria in the original sample can be made.
C.V.

Fungi isolated from damaged flue-cured tobacco. R. E. Welty and G. B. Lucas (*Appl. Microbiol.*, 1968, **16**, 851–854).—Species of *Aspergillus* were the most prevalent fungi isolated from 51 samples and comprised 57% of the total isolates. *Penicillium* (I) (16%), *Alternaria* (8%) (II), *Cladosporium* (III) (4%) and *Chaetomium* (4%) were also found. When moisture content was 18–28% *A. glaucus* was chiefly present; and *A. niger* occurred at 18–30%

moisture, while **I**, **II** and **III** were consistently found at 24–32% moisture.
C.V.

Spark-source mass spectrographic analysis of tobacco ash. R. M. Jones, W. F. Kuhn and C. Varsel (*Analyt. Chem.*, 1968, **40**, 10–13).—Detection of 30–35 elements, and the semi-quant. determination of eight of them, were effected by pelletising the ashed sample and sparking pellet strips against high purity Au wire as counter-electrode. Optimum conditions for sample handling, spectrography, and interpretation of densitometer traces, are discussed. Concn. of Mg, Al, Si, P, Cl, Mn, Fe and Br ranged from 0·07 (Mn) to 8·35% (Cl), and the coeff. of variation ranged from 8·5 to 27%.
W. J. BAKER.

Assay of ³²P in biological material using a Čerenkov counter. F. J. Haasbroek and E. v. d. S. Lotz (*S. Afr. J. agric. Sci.*, 1967, **10**, 285–287).—The advantages of this counter as regards convenience in operation and accuracy of results are pointed out.
P. S. ARUP.

Automated neutron-activation analysis of biological material with high radiation levels. K. Samsahl (*Analyst, Lond.*, 1968, **93**, 101–106).—The apparatus and procedure described permits, in ~40 min, the radiochemical separation and subsequent γ-spectrometry of alkali and alkaline earth metals, Lu, Ce, Sm, Cr, Hf, La, Mn, P, Sc, and Ag in the irradiated sample. In sequence, Hf and Sc are adsorbed from conc. HCl on kieselguhr impregnated with di(2-ethylhexyl)orthophosphate, the lanthanides on a similar column but from dil. HCl, Ag is removed as the bromide complex on Dowex-2 resin at pH 3·5, Cr and Mn on Chelex-100 resin at pH ~4, the alkaline-earth metals on the same resin from aq. 8N-NaOH, and finally K, Rb and Cs on a column of Zr orthophosphate at pH 4–4·5. The final eluate contains Na and P. The max. error for each element is 3%. Applications and limitations of the method are discussed.
W. J. BAKER.

Automatic group separation system for simultaneous determination of a large number of elements in biological material. K. Samsahl, P. O. Wester and O. Landström (*Analyt. Chem.*, 1968, **40**, 181–187).—The complete scheme for determination of ~40 elements is based on ion-exchange separations, partition and extraction column chromatography and, finally, γ-spectrometry of 16 groups of 1–3 elements each so obtained. With the exception of Se, In, Th and U the recoveries were ≮ 90% for added activities of 32 common elements in biological samples; max. error was ±3%, but was more for Th and Ba. Reproducibility was within 10% for all elements except Sb and Th and was generally within 5%. An analysis takes ~ 2 h and the piston-driven Perspex apparatus is claimed to have advantages over apparatus operated by peristaltic pumps. (20 references.)
W. J. BAKER.

Microdetermination of calcium in biological material by automatic fluorometric titration. A. B. Borle and F. N. Briggs (*Analyt. Chem.*, 1968, **40**, 339–344).—A Ca–calcein complex is titrated with 0·25 m M-EDTA in a Turner fluorometer, the titrant being delivered in 1 μl pulses by a lambda pump, with magnetic stirring. Titration of 1 μg of Ca takes 2 min. and of 0·2 μg ~40 sec.; sensitivity is 0·04 μg, and the coeff. of variation is ~1·8%. Up to mol. ratios of 200, 20,000 and 40,000, Mg, PO₄³⁻ and ATP do not interfere, neither do albumin, troponin, tropomyosin, actin nor cell sonicates when present in max. mol. ratios of ~40,000. The only sample prep. required is sonication for ~30 sec.
W. J. BAKER.

Bacterial inhibitors in milk and other biological fluids. B. Reiter and J. D. Oram (*Nature, Lond.*, 1967, **216**, 328–330).—Based mainly on the literature, the following inhibitory systems (lactenins) are discussed: (1) lactoperoxidase-SCN⁻–H₂O₂ (active against certain streptococci; completely inhibits hexokinase but not phosphohexokinase), (2) xanthine oxidase, (3) lactotransferrin (inhibits growth of *B. subtilis* and *B. stearothermophilus*, except in presence of Fe²⁺), (4) complements and antibodies (both are bacteriostatically active against Gram-negative organisms in bovine blood-sera, milk and dry-udder secretions), (5) leucocytes (active against mastitis pathogens). High no. of polymorphonuclear leucocytes prevent udder infection by staphylo- or strepto-cocci irrespective of the presence of (4). Systems (1) and (3) probably act as minor defence mechanisms in other animals and in man. (34 references.)
W. J. BAKER.

The general arrangement of the abstracts is as follows: 1.—AGRICULTURE AND HORTICULTURE. 2.—FOOD; also appropriate Microbiological Processes; Essential Oils. 3.—SANITATION, including Water; Sewage; Atmospheric Pollution, etc. 4.—APPARATUS AND UNCLASSIFIED.

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