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PLANT RESISTANCE TO APHIDS INDUCED BY CHEMICALS*

By H. F. VAN EMDEN

Evidence is presented that the effect on aphids of various chemicals applied to plants, e.g. water, N and K fertilisers, chlormequat chloride, and the amino acid antagonist 1-amino-2-nitrocyclopentane-1-carboxylic acid, is dependent on a common nutritional mechanism. The value of content of soluble nitrogen compounds in a plant as an indicator of suitability for attack by aphids is demonstrated. Plant resistance as an added restraint contributing to integrated pest control is discussed.

Introduction

Research in the early 1960s on the influence of plant growth regulators on various pests and diseases[1-7] has stimulated considerable interest, and has been followed by extensive work on pest species in several countries.[8-14]

Although the symposium title refers to chemicals in general, there is a strong emphasis on plant growth regulators.

There is perhaps a danger that the use of plant growth regulators and other chemicals for increasing the resistance of plants to pests and diseases may be regarded as a new concept in plant protection. However, the way in which the various chemicals have their effect is diverse, and the mechanisms can individually often be better related, not to each other, but to certain familiar and well established plant protection principles. Moreover, the idea is not new; as early as 1923, pest resistance was induced in plants by the external application of non-toxic chemicals.[15] Fig. 1 shows a normal classification of methods for pest control marked with the diverse points of attachment of the use of external chemicals (excluding recognised insecticides, etc.) applied to the plant. The present paper deals with the point of attachment relating to nutritional plant resistance. Even for one chemical, the potential contribution to pest control may not be contained in a unique category.

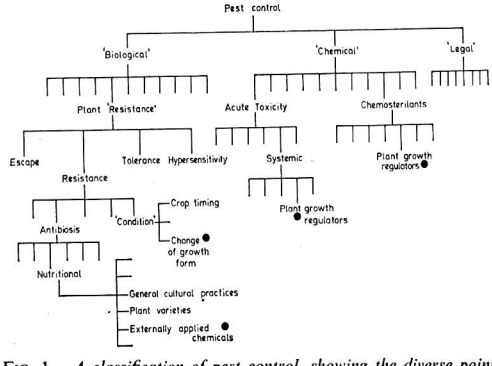

FIG. 1. *A classification of pest control, showing the diverse points of attachment* (●) *for 'chemically induced' plant resistance*

* Presented at a symposium on 'Chemically induced resistance of plants to pests and diseases', organised by the Pesticides Group of the Society of Chemical Industry on 27 September, 1968

The present paper is also limited to examples taken from work on aphids. There is evidence from a very wide range of sources[16] that susceptibility of plants to aphid multiplication is often correlated with the plant's content of soluble nitrogen and particularly amino acids. Two examples of this correlation may perhaps be mentioned; they concern the two points of attachment, other than externally applied chemicals, of nutritional plant resistance shown in Fig. 1.

The first example is perhaps particularly relevant to a consideration of plant resistance—the variability in resistance to pea aphid (*Acyrthosiphon pisum*) shown by pea varieties. The plant characteristics influencing this resistance have been studied extensively in Canada.[16,17] A five-fold difference in the resistance of three varieties was negatively correlated with total levels of soluble nitrogen in the varieties.

The second example concerns plant age as a variable influencing aphid performance (Fig. 2). As the age of Brussels sprout plants increased between 10 and 22 weeks after sowing, soluble nitrogen levels in the plant decreased and the plants became more resistant to the peach potato aphid (*Myzus persicae*). In these experiments aphid performance was expressed as the mean relative growth rate of the aphid (present author, unpublished).

These two examples concern correlations, but the very many correlations established between soluble nitrogen in the

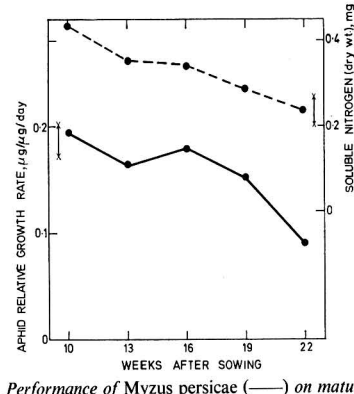

FIG. 2. *Performance of* Myzus persicae (——) *on mature leaves of different aged Brussels sprout plants*

— — — Soluble nitrogen in mature leaves

×——× least significant difference (P = 0·05)

plant and aphid performance are indicative either of a direct importance to the aphids of soluble nitrogen or of soluble nitrogen as a useful indicator of some other correlated nutritional variable.

The second example of this correlation is closely connected with a consideration of nutritional plant resistance induced by externally applied chemicals, for the latter again does not involve different plant seed packets but involves, as for plant age, altering the conditions under which plants from the same seed packet are grown.

Water

Possibly the chemical most frequently applied to plants by man is water. Wearing[18] has studied the effect of water on *M. persicae* and the cabbage aphid (*Brevicoryne brassicae*) feeding on Brussels sprout plants grown in pots at three water regimes (achieved by restoring the soil to field capacity whenever a predetermined deficit of 10, 50 or 90% of available water between field capacity and the permanent wilting point had been reached).

Young and medium aged leaves became more susceptible to the aphids as water stress increased, and this may have been related to enrichment of the phloem sap with soluble nitrogen resulting from proteolysis in the senescing leaves.

On old leaves the picture was more complicated. *M. persicae*, which normally occupies the old leaf stratum of brassicas, appeared to derive some benefit from the sap enrichment accompanying water stress. However, aphids on the leaves in the dry regime showed a considerable drop in fecundity which may have been associated with reduced phloem turgor limiting food uptake by the aphid. On the old leaves *B. brassicae*, which normally feeds on the young leaves, seemed increasingly unsuccessful as phloem turgor fell.

At high doses, water applied externally to the plant therefore appears to reduce the fecundity of both aphids on the parts of the brassica plant they normally occupy.

Fertilisers

Fertilisers represent another category of commonly applied chemicals which affect the susceptibility of plants to aphids,[16] including *M. persicae* and *B. brassicae* feeding on Brussels sprout plants.[19]

As was to be expected, the reduced nitrogen uptake and nitrate accumulation in plants given reduced nitrogen fertilisation (as ammonium sulphate) resulted in reduced levels of soluble nitrogen in the plant, and aphid fecundity fell progressively as nitrogen fertilisation was reduced.

Reduction in the level of potassium, on the other hand, increases soluble nitrogen levels partly by increasing proteolysis and partly by an inhibition of protein synthesis. Correlated with this increase in soluble nitrogen, the fecundity of the aphids (particularly of *M. persicae*) was increased as potassium fertilisation (as potassium chloride) was reduced.

The reduction of aphid fecundity with low nitrogen fertilisation is obviously of fundamental rather than of economic interest: however, experiments with combinations of the nutrients have shown that, even with high nitrogen doses, high potassium fertilisation gives a useful increase in resistance of the plants to both aphid species.

Plant growth regulators

Chlormequat chloride ((2-chloroethyl)-trimethylammonium chloride) clearly affects aphids in more than one way[2,20] and its effects on soluble nitrogen in the plant are also far from clear. However, van Emden[2] noticed reductions of several amino acids in chromatograms of extracts of treated Brussel sprout plants on which the response of aphids had been measured; reductions in the soluble nitrogen content of treated wheat seedlings have also been reported.[21] Both *M. persicae* and *B. brassicae* showed reduced populations on treated Brussels sprout plants (Fig. 3), although there was no apparent reduction in the fecundity of *B. brassicae* which had been placed on treated plants as adults.[1] This perhaps indicates that one effect of chlormequat chloride on the aphids was via the food quality of the host plant.

1-Amino-2-nitrocyclopentane-1-carboxylic acid (ANCPA) has been reported as an amino acid antagonist[22] and was therefore likely to prove of particular interest. In experiments with aphids (present author, unpublished), Brussels sprout plants treated with ANCPA certainly showed marked growth effects; they were stunted, and severe curling of the leaves was apparent. At the concentrations used, however, ANCPA had no significant effect on soluble nitrogen levels in the plant and similarly no significant effects on aphid performance (mean relative growth rate) could be shown (Fig. 3). These inconclusive results from clearly affected plants do, however, demonstrate the value of soluble nitrogen as an indicator of host plant suitability for aphids, and provide further evidence for a common nutritional mechanism in the effect, on aphids, of the various externally applied chemicals studied.

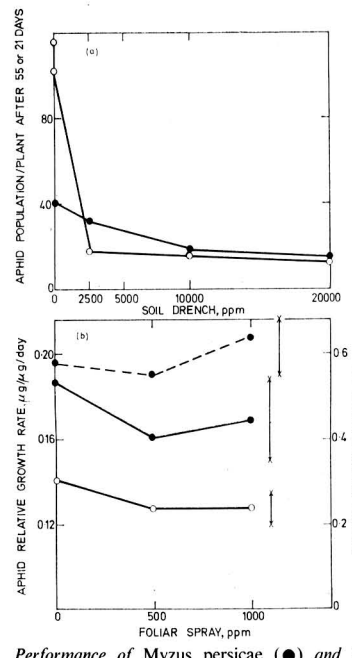

FIG. 3. *Performance of* Myzus persicae (●) *and* Brevicoryne brassicae (○) *on mature leaves of Brussels sprout plants treated with* (a) *chlormequat chloride and* (b) *ANCPA*

● — — — ● Soluble nitrogen in mature leaves
× —— × least significant difference (P = 0·05)
Aphid population after 21 days for *M. persicae* and 55 days for *B. brassicae*

Discussion

Finally, some comment should be made on the status of induced plant resistance to pests as a control measure and on its possible potential. Such resistance may be induced not only by chemicals, but by a whole variety of factors.[23]

Plant resistance and susceptibility are not antonyms, but describe relative positions on a continuous scale of plant suitability for pest attack. Even so-called 'susceptible' crop varieties may really be quite 'resistant' in the sense that they can be made considerably more susceptible by environmental treatments[19] and may not be accepted by a high proportion of immigrating alate aphids.[24,25] A small change in the growth and physiology of the host plant may therefore sometimes produce a valuable increase in the plant's resistance to a pest.

With chemicals, particularly plant growth regulators, it is often undesirable that any pest control properties a chemical may have should be accompanied by gross effects on plant growth. If such a chemical is applied at low concentrations to minimise effects on plant growth, it will often produce only a small increase in plant resistance. In the field this increase may or may not be adequate in itself; if inadequate, however, satisfactory pest control may still be achieved when a small increase in plant resistance is combined with the action of the natural enemies of a pest.[23]

With the current interest of applied entomologists in the integration of biological control with the use of selective insecticides, any approach to pest control which can play a part in an integrated control programme is of considerable interest. Induced plant resistance based on the mechanisms of plant growth and nutrient status has such a part to play. The concept of integrated control[36] is that it may be possible to control a pest (Fig. 4) by the application of selective insecticides (restraint 3) which cause minimum damage to natural enemy populations (restraint 1). For many crop pests the average population level is well above the economic threshold, and it is therefore necessary to add a further restraint (2) to reduce the average level of the population to a base line on which the integrated control principle can operate. This further restraint may be an imported natural enemy, but plant resistance is a clear alternative as it is less likely to be affected by the use of insecticides than is a predator or parasite.

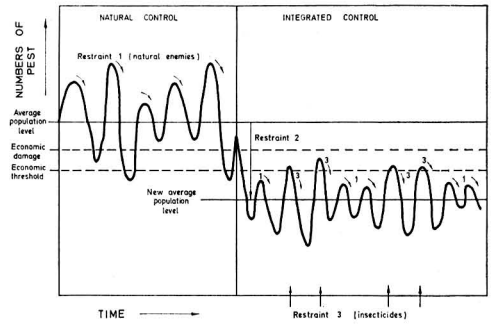

FIG. 4. *Diagrammatic representation of the rôle of three restraints in an integrated control programme (see text)*

Induced plant resistance is of value here to substitute for or augment varietal resistance because: (a) it is another source of resistance; (b) it can usually be developed fairly quickly in research with the retention of the desirable cropping characters of a variety; (c) it is extremely flexible in mechanism, and can be regulated in time so as to be particularly effective at the critical point in the pest infestation; and (d) high plant resistance may not be necessary or even desirable in combination with the other restraints of an integrated control programme.

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References

1. van Emden, H. F., *Nature, Lond.*, 1964, **201,** 946
2. van Emden, H. F., *CCC Research Symposium, Geneva*, 1964 (Wayne, U.S.A.: Cyanamid International)
3. Maxwell, R. C., & Harwood, R. F., *Ann. Entomol. Soc. Am.*, 1960, **53,** 205
4. Robinson, A. G., *Can. Ent.*, 1959, **91,** 527
5. Robinson, A. G., *Can. Ent.*, 1960, **92,** 494
6. Robinson, A. G., *Can. J. Pl. Sci.*, 1961, **41,** 413
7. Sinha, A. K., & Wood, R. K. S., *Nature, Lond.*, 1964, **202,** 824
8. Adolphi, H., *Z. PflKrankh. PflPath. PflSchutz*, 1967, **74,** 684
9. Bhalla, O. P., & Robinson, A. G., *J. econ. Ent.*, 1968, **61,** 552
10. Smith, B. D., *Proc. 4th Br. Insecticide & Fungicide Conf., Brighton*, 1967, p. 364
11. Smith, B. D., & Corke, A. T. K., *Nature, Lond.*, 1966, **212,** 643
12. Tahori, A. S., Halevy, A. H., & Zeidler, G., *J. Sci. Fd Agric.*, 1965, **16,** 568
13. Tahori, A. S., Zeidler, G., & Halevy, A. H., *J. Sci. Fd Agric.*, 1965, **16,** 570
14. Webster, J. M., *Nematologica*, 1967, **13,** 256
15. Andrews, E. A., 'Factors affecting the control of the tea mosquito bug (*Helopeltis theivora* Waterh.)', 1923 (London: Indian Tea Association)
16. van Emden, H. F., Eastop, V. F., Hughes, R. D., & Way, M. J., *A. Rev. Ent.*, 1969, **14,** 197
17. Maltais, J. B., *Can. Ent.*, 1951, **83,** 29
18. Wearing, C. H., *Nature, Lond.*, 1967, **213,** 1051
19. van Emden, H. F., *Entomologia exp. appl.*, 1966, **9,** 444
20. Honeyborne, C. H. B., *J. Sci. Fd Agric.*, 1969, **20,** 388
21. Linser, H., Neumann, K. H., & el Damaty, H., *Nature, Lond.*, 1965, **206,** 893
22. Brian, P. W., Elson, G. W., Hemming, H. G., & Radley, M., *Nature, Lond.*, 1965, **207,** 998
23. van Emden, H. F., *Wld Rev. Pest Control*, 1966, **5,** 115
24. Müller, H. J., *Züchter*, 1953, **23,** 176
25. Müller, H. J., *Entomologia exp. appl.*, 1958, **1,** 67
26. Stern, V. M., Smith, R. F., van den Bosch, R., & Hagen, K. S., *Hilgardia*, 1959, **29,** 81

PERFORMANCE OF *APHIS FABAE* AND *BREVICORYNE BRASSICAE* ON PLANTS TREATED WITH GROWTH REGULATORS*

By C. H. B. HONEYBORNE**

It was shown that the fecundity of aphids may be altered on plants treated with plant growth stimulators and retardants. There was evidence that the reduced fecundity and smaller size of *Aphis fabae* on broad beans treated with ethylene bisnitrourethane was related to the reduced nutritional value of the plants to the aphids, levels of soluble nitrogen being reduced. The reduced fecundity of *A. fabae* on broad beans treated with gibberellic acid may be related to the apparent toxicity of the compound when fed directly to the aphid. CCC (chlormequat chloride) seemed toxic to both *A. fabae* and *Brevicoryne brassicae*, and the number of embryos per adult *A. fabae* reared on CCC-treated beans was reduced, although this did not reduce fecundity. Apart from any direct action of CCC on the aphids, there is evidence that the substance may reduce the availability of nutrients from the phloem of treated plants to the insects by reducing translocation, and there is further evidence that amino acid content of the sap may be reduced.

Introduction

Changes in aphid performance resulting from applications of growth regulators to host plants were first reported for compounds used as herbicides. Maxwell & Harwood[1] showed that the reproduction rate of *Acyrthosiphon pisum* was increased on broad beans treated with sub-lethal doses of 2,4-D, and Robinson[2] found that, of 30 herbicides screened for an effect on this aphid, only maleic hydrazide, amitrole and zytron were active, and these increased mortality of adults and nymphs within the five-day observation period following application. Growth retardants were first shown to affect aphids by van Emden,[3,4] who found that the population increase of both *Brevicoryne brassicae* and *Myzus persicae* was reduced on Brussels sprouts treated with CCC (chlormequat chloride). Tahori *et al.*[5] showed that there was a similar fall in the rate of population increase of *Aphis nerii* on oleander leaves standing in solutions of Phosphon, CCC and B995, and B. D. Smith (unpublished) found the same effect on *Aphis pomi* on apple shoots growing in solutions of CCC and B995. Although there is no published record of growth stimulators affecting aphids, Carlisle *et al.*[6] have shown that injections of gibberellin affect the rate of maturation of locusts, and Ellis *et al.*[7] showed that gibberellin-deficient diets delayed sexual maturation of *Schistocerca gregaria*. Eichmeier & Guyer[8] and Rodriguez & Campbell[9] found both reductions and increases in mite populations on plants given different concentrations of gibberellin. Edel'man & Efros[10] found that various substituted benzimidazoles, which stimulate plant growth, increased the rate of development and the final size of various caterpillars.

An investigation of the performance of aphids on treated plants centres around a triangle of inter-relationships between the growth substance, the plant and the aphid. Any response shown by the aphid may result from a direct effect of the substance on the insect or from an indirect effect, the plant being altered by the substance and so having a changed value as a host for the insect. In this investigation several triangular inter-relations were involved resulting from the application of four growth regulators to two plant–aphid combinations. The aphids were *Aphis fabae* on broad beans and *Brevicoryne brassicae* on Brussels sprouts. Two growth stimulators, namely gibberellic acid (GA) and ethylene-bis-nitrourethane (EBNU), and two growth retardants, namely (2-chloroethyl) trimethylammonium chloride (CCC) and *N*-dimethylaminosuccinamic acid (B995), were applied to host plants.

Experimental and Results

Aphid response was first assessed by caging individual aphids from clonal stocks on treated plants. The introduced aphid was reared to maturity and when it produced young it was removed. One of its nymphs was retained and reared; the young of this individual were counted. This procedure was adopted so that fecundity was assessed on aphids reared throughout their development on the treated plant. This was thought necessary as the work of van Emden[3] had shown that when *B. brassicae* was reared on CCC-treated sprouts, treatment effects were not apparent until the second generation.

To normalise the distribution of the data and at the same time to convert them, where aphids did not survive to reproduce, the data were transformed before analysis by adding half to each individual fecundity value and taking the square root. The treatment means based on these transformed data were called 'mean fecundities' for convenience. The result (Fig. 1) showed that although the regulators had no effect in many cases each of them reduced the fecundity of one or other aphid. Progressive reductions with increasing dosage were found with *A. fabae* on plants treated with GA and with *B. brassicae* on CCC-treated plants. A curvilinear effect was seen in the cases of *A. fabae* on EBNU-treated beans and *B. brassicae* on sprouts treated with B995. It was noted that individuals of *A. fabae* reared on the EBNU-treated beans were much paler in colour than the aphids on untreated plants.

These effects on the fecundity of aphids having been shown, the responses of one aphid (*A. fabae*) were studied further,

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* Presented at a symposium on 'Chemically induced resistance of plants to pests and diseases', organised by the Pesticides Group of the Society of Chemical Industry on 27 September, 1968.

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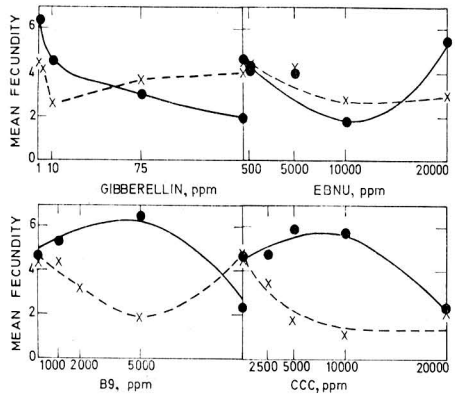

FIG. 1. *Mean fecundities of* Aphis fabae *and* Brevicoryne brassicae *reared on plants treated with growth regulators*

———— *Aphis fabae*
- - - - - - *Brevicoryne brassicae*

partly to obtain more information on the response and partly to see if a more convenient method of assessment could be developed. The size of nulliparous adult aphids reared on plants treated with growth regulators at those dosages which had given the greatest effect on fecundity was measured. Only EBNU affected size, reducing both length and weight. The numbers of embryos in mature *A. fabae* from plants treated at a range of concentrations of the regulators was investigated by dissecting the aphids in liquid paraffin on a microscope slide. Only CCC showed a significant effect giving a linear reduction with increasing dosage. This contrasted with the fecundity result for *A. fabae* on CCC-treated beans, for which only at the highest dose did fecundity tend to be reduced though this was not significant. This difference may be related to the ability of *A. fabae* to develop more embryos after maturity: in this way aphids, which at maturity might contain less embryos, could have an apparently normal fecundity. There was some evidence from the fecundity results that aphids on CCC-treated plants reproduced more slowly than did those on untreated plants.

In an attempt to differentiate between the direct and indirect action of growth regulators in producing the responses observed, aphids were fed on artificial diets containing the substances. Feeding units were made in which it was possible to feed adult aphids through Parafilm membranes on 20% sucrose solutions in tap water. A number of aphids were caged in each unit, and survival times to 50% mortality of the aphids on diets with and without the regulators[11] were compared. Of the four substances only GA and CCC reduced survival. This appeared to be the result of toxicity of the compounds, for no evidence could be obtained in diet selection experiments that aphids were merely repelled from the diets containing these substances and were dying of starvation. In these experiments, GA affected only *A. fabae* the fecundity of which it had affected in the previous experiment; CCC also affected *A. fabae* the fecundity of which had been unaffected on CCC-treated plants.

In addition to this investigation of direct effects on aphids, possible indirect actions of growth regulators were investigated. Broad beans treated with EBNU and Brussels sprouts treated with CCC were analysed for some of the substances generally held to be important to feeding aphids. Analyses were based on freeze-dried leaf powders. Investigation of beans treated with EBNU at the dosage which had reduced fecundity showed that the soluble nitrogen level was reduced and the ratio of reducing sugar to soluble nitrogen was increased. Such changes would reduce the nutritional value of the treated plants to the aphids, and provide the most likely explanation of the reduced size of the aphids on treated plants. EBNU showed no direct toxicity when fed to aphids through Parafilm. Analyses of leaves from Brussels sprouts treated with CCC at a range of doses showed increases in the levels of both soluble sugars and soluble nitrogen. The ratio of sugar/nitrogen was not significantly altered. Humphries[12] found that treating plants of *Phaseolus vulgaris* with CCC delayed the time when total nitrogen and protein nitrogen diminished in the developing primary leaves. Khan & Faust[13] found that the coleoptiles of barley seedlings treated with CCC had an increased content of soluble protein. These observations would suggest that CCC is unlikely to decrease the nutritional value of host plants to aphids; however, there is evidence that translocation is reduced in CCC-treated plants, and as aphids are phloem feeders this may mean that there is reduced availability of nutrients to aphids on treated plants. Shindy & Weaver[14] found reduced translocation of photosynthetic products from leaves of plants treated with CCC, and Humphries[12] suggests that nitrogen compounds move more slowly from primary leaves of CCC-treated beans because there is a reduced demand for nitrogen related to the slower growth of the shoot. Linser *et al.*[15] and van Emden[4] found changes in the amino acid content of the soluble nitrogen fraction of young wheat plants treated with CCC, and it is possible that the availability of amino acids important to the aphids may be altered. For example the level of arginine was reduced in treated wheat seedlings, and this is one of the amino acids found by Maltais & Auclair[16] to occur at a reduced level in resistant (compared with susceptible) pea varieties.

The reduced pigmentation of *Aphis fabae* on plants treated with EBNU was investigated and it was found, by transferring aphids from treated to untreated plants, that the effect persisted through the lifetime of an individual but was not transmitted to offspring. The coloration of *A. fabae* depends on the presence of pigments in the haemolymph, and the two most important pigments have been extracted and identified.[17] These pigments were extracted from aphids, and comparison of the absorbence of the final extracts showed that the levels of the pigments were reduced in aphids from EBNU-treated plants on the basis of both weight and numbers of aphids. Reichmuth & Klink[18] have reported that naturally occurring pale clones of *A. fabae* are more susceptible to insecticides than are blackfly of more normal coloration.

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References

1. Maxwell, R. C., & Harwood, R. F., *Ann. ent. Soc. Am.*, 1960, **53,** 199
2. Robinson, A. G., *Can. J. Pl Sci.*, 1961, **41**, 413
3. van Emden, H. F., *Nature, Lond.*, 1964, **201,** 946
4. van Emden, H. F., CCC Research Symposium, Geneva 1964 (Cyanamid International: Wayne, U.S.A.)
5. Tahori, A. S., Halevy, A. H., & Zeidler, G., *J. Sci. Fd Agric.*, 1965, **16,** 568
6. Carlisle, D. B., Osborne, D. J., Ellis, P. E., & Moorhouse, J. E., *Nature, Lond.*, 1963, **200,** 1230
7. Ellis, P. E., Carlisle, D. B., & Osborne, D. J., *Science, N.Y.*, 1965, **149,** 546
8. Eichmeier, J., & Guyer, G., *J. econ. Ent.*, 1960, **53,** 661
9. Rodriguez, J. G., & Campbell, J. M., *J. econ. Ent.*, 1961, **54,** 984
10. Edel'man, N. M., & Efros, A. M., *Dokl. Akad. Nauk. SSSR*, 1962, **142,** 1172
11. Wearing, C. H., & van Emden, H. F., *Nature, Lond.*, 1967, **213,** 1051
12. Humphries, E. C., *Ann. Bot.*, 1968, **32,** 497
13. Khan, A. A., & Faust, Maria A., *Nature, Lond.*, 1966, **211,** 1215
14. Shindy, W., & Weaver, R. J., *Nature, Lond.*, 1967, **214,** 1024
15. Linser, H., Neumann, K. H., & el Damaty, H., *Nature, Lond.*, 1965, **206,** 893
16. Maltais, J. B., & Auclair, J. L., *Can. Ent.*, 1957, **89,** 365
17. Bowie, J. H., Cameron, D. W., Findlay, J. A., & Quartey, J. A. K., *Nature, Lond.*, 1966, **210,** 395
18. Reichmuth, W., & Klink, G., *Biol. Zbl.*, 1961, **80,** 281

EFFECTS OF PLANT GROWTH REGULATORS ON LOCUSTS AND COTTON STAINER BUGS*

By D. B. CARLISLE, PEGGY E. ELLIS and DAPHNE J. OSBORNE

Gibberellic acid is a necessary dietary constituent for normal fast maturation in the desert locust. Diets short of gibberellins, such as senescent vegetation, produce a state of adult diapause. The synthetic plant growth retardant (2-chloroethyl)-trimethylammonium chloride (CCC), either injected into locusts or fed to them as a separate dietary constituent, inhibits meiosis and so retards or prevents sexual maturation. Once this stage of gametogenesis has passed (late in nymphal life) CCC appears to have no further effect, but a single injection just before the onset of meiosis will sterilise locusts completely. Cotton stainers provided with a solution of CCC in their drinking water during the same period of larval life show impaired reproductive function and produce deformed offspring. CCC acts directly upon the insects, quite apart from any secondary effect produced by the altered physiology of the food plant.

Locusts are short-horned grasshoppers that have the habit of swarming. The two species investigated, the migratory locust, *Locusta migratoria migratorioides* R & F, and the desert locust, *Schistocerca gregaria* (Forskål), pass through five nymphal instars, each one terminated by a moult. At the final moult, winged adults are produced which, under favourable conditions, breed about 3 weeks later. A female will probe below the surface of moist sand and bury a pod containing 50–100 eggs. The female will produce several pods at intervals of 10–15 days before dying of old age at about 3 months.

Under unfavourable conditions, however, locusts do not mature as quickly as this and it has been suggested that, in the desert locust at least, the crucial factor regulating the onset of sexual maturation is the nutritive value of the vegetation.[1,2] In the laboratory it has been found that locusts fed from the middle of the fifth instar on senescent leaves mature very slowly. In the semi-desert areas frequented by the desert locust the eggs are normally laid at the onset of the brief sporadic rains. The flush of green vegetation, which has already begun to flourish by the time the eggs hatch about two weeks later, normally lasts only a few weeks and will have already started to become senescent by the time the locusts have reached the last nymphal instar. Only if the rains have been exceptionally heavy and prolonged does the vegetation remain green, and the locusts able to produce a second generation in that place.

In laboratory experiments[2] locusts fed green leaves began laying about three weeks after the final moult (Table I). By four and a half weeks they had produced an average of three pods per female. The earliest eggs laid by locusts fed on senescent vegetation did not appear until six weeks of adult life had elapsed. Even at three months (by which time they were beginning to die of old age) only 60% of females had laid even a single egg pod. Supplementing this diet of senescent vegetation with a dose of 1 mg of gibberellic acid (GA_3) per day enabled animals to mature as quickly as those fed green leaves (Table I). The one difference was that the eggs were smaller in size and fewer in number. This diet based on senescent vegetation may be quite adequate for fast maturation but is deficient in many factors present in a green diet, such as protein and ascorbic acid as well as the plant growth hormones such as GA. In these experiments GA acted directly on locusts and not through the altered physiology of the plant, for the insects were eating the two separately. The technique used was adopted as standard in all the experiments discussed here for feeding substances to locusts. Locusts readily eat filter paper that has been soaked in a concentrated solution of sucrose and then dried. Controls were given rectangles of this paper together with a diet of leaves. Pieces of such sugared paper were sprayed with measured amounts of GA solution and then dried again. Known areas (and hence known doses) of these papers were fed daily to the locusts.

TABLE I

Reproductive performance of desert locusts fed on green vegetation, senescent vegetation plus sucrose, or senescent vegetation plus sucrose and GA (1 mg/locust per day)

Figures indicate the number of days after the final moult

	Green leaves	Senescent leaves	Senescent leaves + GA
First eggs laid	22	42	22
60% of females have laid once	23	80	23
One egg pod per female	26	—	26
Three egg pods per female	32	—	34

This technique was used to investigate the effects of natural and synthetic plant regulators on locusts. So far no significant effects were obtained from abscisic acid, a naturally occurring antagonist of GA.[3,4] The synthetic growth retardant (2-chloroethyl)-trimethylammonium chloride (CCC) however, has a direct effect, either by feeding or by injection, upon gametogenesis of locusts and hence upon their reproductive performance.

In locusts, as in many insects, the spermatogonia undergo mitotic divisions in the first three nymphal instars.[5] Meiosis begins early in the fourth instar and is completed by the middle of the fifth. By the time the animals undergo the final moult to become adult their testes are filled with a mixture of spermatids and spermatozoa. The proximal part of each testis tubule matures first and a wave of maturation passes distally along each one. Once fully mature the locusts copulate at intervals, the more mature proximal parts of the

* Presented at a symposium on 'Chemically induced resistance of plants to pests and diseases', organised by the Pesticides Group of the Society of Chemical Industry on 27 September, 1968

testes providing the first packet of sperm. Little information is available at present about structural changes in the testis. This seems to be true of all insects since investigators have confined their attention to the larval instars and the process of spermatogenesis.

When CCC was injected into locusts early in the fourth instar, i.e. before meiosis had begun, spermatogenesis was delayed and prolonged well into adult life. At a high dose rate (2000 ppm) the process was blocked completely, the animals dying (usually of old age) before meiosis was completed. Most of these animals did not produce any sperm at all.[6] In general it was found that meiosis ceased at second metaphase, no part of the testis progressing beyond this stage and the distal parts of the tubules not even reaching it. With some individuals the same effect was obtained with a tenth of this dose (200 ppm); in others it appeared that spermatogenesis was only delayed at this dose level and some of the locusts finally copulated and produced viable offspring. Feeding CCC to locusts, at a dose of 200 μg per day, from the beginning of the fourth instar to the middle of the fifth (a period of about 10 days) likewise blocked spermatogenesis at second metaphase and such locusts failed to breed, even though dosing ceased several days before they became adult.

When male locusts were injected at the beginning of the fifth instar (a time when the proximal part of the testis tubules has already completed the reduction divisions) spermatogenesis was delayed but not blocked. These animals appeared finally to behave like normal adults, except that the duration of the act of copulation was shortened and the amount of sperm produced was reduced. Even these slight effects were absent in animals dosed as adults, whether by feeding or by injection, and such locusts showed perfectly normal breeding behaviour.

In females it was found that the higher dose level of CCC administered during the fourth instar produced sterility. None of the experimental locusts laid eggs and a number killed on the twentieth day of adult life (when normal animals have oocytes 4–8 mm long) showed no macroscopic signs of oogenesis. At the lower dose rate (200 ppm) some 70% of females died without laying and without showing any signs of egg development, though adult life was not noticeably curtailed. Those that did lay showed no delay and did not produce significantly fewer eggs per pod. CCC injected into fifth instar adult females or fed to them at either of these stages had no significant effect on breeding performance.

The cotton stainer, *Dysdercus cardinalis* Gerth., a heteropteran bug which is a serious pest of cotton in parts of Africa and America, piercing the cotton boll to reach the seeds on which it feeds, passes, like locusts, through five nymphal instars before the final moult at which it becomes adult. In the laboratory it was fed on dry cotton seeds and was given drinking water in tubes plugged with cottonwool. These experimental animals received 2000 ppm of CCC in their drinking water. There is no information on the amounts of water the stainers drink, so it was not known how much CCC they might have received. Cotton stainers which had been drinking CCC from the start of the fourth instar until the final moult were housed in pairs from the beginning of adult life and then given plain water to drink. They were normal in appearance but their reproductive performance was impaired (Table II). This treatment reduced the number of eggs laid ($P < 0{\cdot}05$) by reducing the number of clutches ($P < 0{\cdot}05$) and by reducing the number of eggs especially in the first and largest clutch ($P < 0{\cdot}05$). The fertility, moreover, of the eggs laid by the treated animals (Fig. 1) was much less than in the normal stainers ($P < 0{\cdot}001$). In control pairs the first clutch of eggs was laid at a mean of $10{\cdot}1 \pm 0{\cdot}85$ days after the final moult; in bugs treated with CCC it was not laid until $13{\cdot}0 \pm 0{\cdot}71$ days, i.e. 3 days later ($P < 0{\cdot}05$). A proportion of the offspring produced by treated parents developed abnormalities, although they themselves received no exposure to CCC. The abnormalities were of the type which has come to be associated with an excess of juvenile hormone or of its mimics.[7]

TABLE II

Number of eggs in each clutch of *Dysdercus cardinalis* supplied either with plain drinking water or with 2000 ppm of CCC in their water

Pair number	Drinking supply	Clutch number					Total number of eggs
		1	2	3	4	5	
1	Water	176	172	61	30	6	435
2	Water	156	96	46	26	—	324
3	Water	186	78	47	12	—	323
4	Water	121	84	52	26	4	287
5	Water	128	78	48	—	—	254
6	Water	106	65	40	—	—	211
Means		145·5	95·5	49·0	15·7	1·7	305·7
7	CCC	191	127	6	—	—	324
8	CCC	86	46	94	62	12	300
9	CCC	124	80	64	—	—	268
10	CCC	108	68	58	12	—	246
11	CCC	85	58	22	—	—	165
12	CCC	46	40	39	—	—	125
13	CCC	25	—	—	—	—	25
14	CCC	15	—	—	—	—	15
15	CCC	12	—	—	—	—	12
16	CCC	4	—	—	—	—	4
Means		69·6	41·9	28·3	7·4	1·2	148·4

In these two unrelated insects, the cotton staining bug (Heteroptera) and the locust (Orthoptera), CCC supplied directly to the animal, whether by injection or fed, delayed maturation, reduced fertility, or produced complete sterility. By contrast, GA hastens maturation in locusts. Since CCC inhibits GA synthesis in plants[8–10] it will have secondary effects (on locusts at least) if it is applied to crops which they eat. When insects are fed upon plants treated with CCC any changes in reproductive performance[11] may be due either to a direct effect of this substance upon the insect or to the altered physiological state of the plant.

The direct effect of CCC is upon meiosis, and exposure to the substance after this process is completed seems to be without any effect on reproduction, or indeed any toxic effect. There appears to be no reports of toxicity towards mammals, but in this class the first meiotic division in the female is completed before birth.

It is possible that the daughters of mothers which have, during pregnancy, eaten crops sprayed with CCC, may be at risk.

Since this paper was presented, Mr. P. C. Williams and Mrs. Barbara E. Burford, of the Imperial Cancer Research Fund, London, have tested CCC on mice, providing a dose of 50 mg/kg/day, without finding any signs of toxicity or of interference with reproductive capacity over two generations. The manufacturers of CCC, Cyanamid Corporation, have also made available unpublished data showing that twice this dose administered to rats over a period of two years (three generations) had no effect on mortality, fertility or growth. It did, however, result in the appearance of giant cells, of unknown significance, in the testis.

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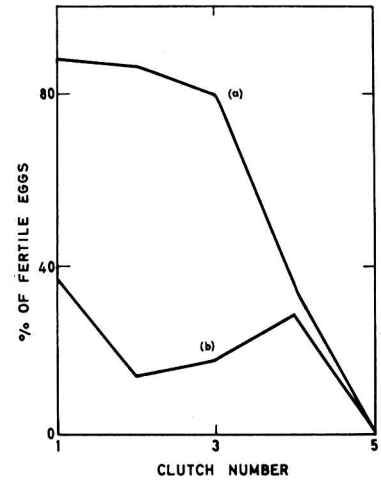

FIG. 1. *Mean percentage fertility in successive egg clutches laid by* Dysdercus cardinalis

(a) Controls (plain water); (b) 2000 ppm of CCC in the water during the last two nymphal instars

References

1. Carlisle, D. B., Ellis, P. E., & Betts, E., *J. Insect Physiol.*, 1965, **11**, 1541
2. Ellis, P. E., Carlisle, D. B., & Osborne, D. J., *Science, N.Y.*, 1965, **149**, 546
3. Thomas, T. H., Wareing, P. F., & Robinson, P. M., *Nature, Lond.*, 1965, **205**, 1270
4. Chrispeels, M. J., & Varner, J. E., *Nature, Lond.*, 1966, **212**, 1066
5. Nelsen, O. E., *J. Morph.*, 1941, **51**, 467
6. Carlisle, D. B., & Ellis, P. E., *Nature, Lond.*, 1968, **220**, 189
7. Carlisle, D. B., & Ellis, P. E., *Bull. ent. Res.*, 1967, **57**, 405
8. Kende, H., Ninnemann, H., & Lang, A., *Naturwissenschaften*, 1963, **50**, 599
9. Ninnemann, H., Zeevaart, J. A. D., Kende, H., & Lang, A., *Planta*, 1964, **61**, 229
10. Felippe, G. M., & Dale, J. E., *Planta*, 1968, **80**, 344
11. van Emden, H. F., *Nature, Lond.*, 1964, **201**, 946

USE OF GROWTH RETARDANTS ON CHRYSANTHEMUMS: EFFECT ON PEST POPULATIONS*

By C. R. WORTHING

Numbers of the aphid, *Myzus persicae*, and the spider mite, *Tetranychus urticae*, were compared on two chrysanthemum cultivars treated with commercial rates of B995, chlormequat chloride and chlorphonium chloride. The growth retardants did not give effective pest control on this host, but in some trials the populations were significantly lower where chlormequat chloride and chlorphonium chloride was used. They appear to act indirectly and reduced the survival rate of nymphal *M. persicae*.

Introduction

Growth retardants have been reported to affect the severity of attacks on plants by several pests and diseases, including the aphids, *Brevicoryne brassicae* (L.)[1] and *Aphis nerii* (Boyer),[2,3] and the mite *Cecidophysopsis ribis* (Nalepa).[4,5] They have also been claimed to inhibit the feeding of the cutworm, *Spodoptera littoralis* Boisduval, on chrysanthemum.[3,6]

The growth retardants B 995 (*N*-dimethylaminosuccinamic acid) and chlorphonium chloride are used as a matter of routine in the commercial culture of pot chrysanthemums to obtain plants of a suitable height, the dosage and time of treatment depending on the cultivar and season. These compounds could also have possible effects on pests on this host. The two most important pests on this crop are the aphid, *Myzus persicae* (Sulzer), and the red spider mite, *Tetranychus urticae* (Koch). These two pests were, therefore, examined on chrysanthemum plants dwarfed by commercial rates of B 995 and chlorphonium chloride. Chlormequat chloride is not recommended for use on chrysanthemums but was also included in the trial because of its reported activity against several pests on other hosts.

Experimental

The growth retardants used were B 995 (5% active ingredient (a.i.) solution), chlormequat chloride (40% a.i. solution) and chlorphonium chloride (1·5% a.i. dust). A liquid formulation (10% a.i.) of chlorphonium chloride was included for the study of direct effect on *M. persicae*.

Seven rooted cuttings (cultivars No. 4 Yellow Indianapolis and Golden Princess Anne) were planted in John Innes No. 2 compost in each 5·5 in half-pot. The chlorphonium chloride was mixed with the compost before the cuttings were planted. A soil drench (110 ml) of chlormequat chloride or a foliar spray of B 995 was applied 2–3 weeks after planting. The plants were grown as standard chrysanthemums with short days from the time of planting as in normal commercial practice. A randomised block layout was used for the experimental treatments.

The effect on *M. persicae* was studied by caging[7] this aphid on leaves in the region where the length of the internode was reduced. The strain of *M. persicae* used had been found on chrysanthemums at a local nursery and was known to show resistance to several organophosphorus aphicides. Five adult apterae were used per cage and the number of live aphids was recorded after seven days.

In another trial, plants were infested with adult female *T. urticae*, 30 per pot. Population counts were made on two leaves per pot at the twelfth node below the flower bud. Egg viability studies were included.

Aqueous sucrose (15%) containing various proportions of the growth retardants was tested on *M. persicae* using the Parafilm membrane technique,[8] to observe any direct effect on this pest. Three adult apterae were used per cage and six cages per treatment, the number of aphids being noted after four days.

Square root and angular transformations were used for the analysis of the aphid populations and the nymph survivals, respectively. Significance of treatment differences was judged by Hartley's method[9] following analysis of variance. The de-transformed means are given in the Tables.

Results

Observations on *T. urticae*

The cuttings were planted on 9 March, 1967, and infested with *T. urticae* after eleven days. The populations were assessed five weeks later, most of the mites being present as eggs or immature stages. The numbers were lower for both cultivars when chlormequat chloride or chlorphonium chloride was used (Table I). For Golden Princess Anne the reductions with these two compounds were just significant ($P = 0{\cdot}05$) compared with untreated plants or those treated with B 995. On No. 4 Yellow Indianapolis the reductions in mite numbers were not significant at this level.

Observations on *M. persicae*

The cuttings were planted on 17 July and *M. persicae* were caged on the plants 4–5 weeks later. Aphid numbers after seven days' exposure were lower (Table II) when chlormequat chloride or chlorphonium chloride was used but the only difference significant at $P = 0{\cdot}05$ was that between untreated No. 4 Yellow Indianapolis plants and those treated with chlormequat chloride.

On combining the results for the two cultivars, however, the populations were significantly lower ($P = 0{\cdot}05$) when chlormequat chloride or chlorphonium chloride was used.

* Presented at a symposium on 'Chemically induced resistance of plants to pests and diseases', organised by the Pesticides Group of the Society of Chemical Industry on 27 September, 1968.

TABLE I

Populations of *T. urticae* on plants treated with growth retardants

Cultivar and treatment	Plant height* at flowering, mm	Mean no. mites/leaf, eggs and active stages	Time of sampling, days after treatment
		(2 leaves/pot)	
No. 4 Yellow Indianapolis		(5 pots/treatment)	
Untreated	307	80·2 [b]	—
B 995**	239 [a]	75·9 [b]	32
Chlormequat chloride†	226 [a]	58·4 [b]	32
Chlorphonium chloride††	223 [a]	72·8 [b]	43
Standard error of means	10·5	10·2	
		(2 leaves/pot)	
Golden Princess Anne		(3 pots/ treatment)	
Untreated	385	103 [d]	—
B 995**	327 [c]	110 [d]	35
Chlormequat chloride†	331 [c]	58·2 [e]	35
Chlorphonium chloride††	275	53·8 [e]	48
Standard error of means	10·5	13·3	
Combined results from both cultivars		(8 pots/treatment)	
Untreated		88·7 [f]	
B 995**		88·7 [f]	
Chlormequat chloride†		58·3 [g]	
Chlorphonium chloride††		65·7 [f g]	
Standard error of means		8·1	

Figures with a letter suffix in common do not differ at P = 0·05
* Mean of 7 plants/pot, 5 pots/treatment; within a cultivar figures lacking a common suffix differ at P = 0·01
** Spray (0·25% a.i.) applied 11 days after planting
† Drench (750 mg a.i./pot) applied 11 days after planting
†† Dust (80 mg a.i./pot) mixed with compost at planting

The difference between untreated plants and those receiving chlorphonium chloride was significant at P = 0·01.

The total of live and dead aphids was reasonably constant for each treatment so the survival of nymphs was examined (Table II). On No. 4 Yellow Indianapolis their percentage survivals were significantly lowered (P = 0·05) by chlormequat chloride or chlorphonium chloride, and on Golden Princess Anne all three growth retardants produced an effect significant at this level. The combined results for the two cultivars showed a reduction in the survival rate, compared with untreated plants, for chlormequat chloride and chlorphonium chloride that was significant at P = 0·01.

Direct effect on *M. persicae*

Extracts prepared by macerating untreated foliage or pressing the juice from the leaves were toxic to *M. persicae* when tested in 15% sucrose by the Parafilm membrane technique. Therefore, the direct effect on caged aphids was observed for various proportions of growth retardants in 15% sucrose (Table III). Under these conditions the aphids seemed little affected by solutions containing up to 80 ppm B 995 or chlormequat chloride, but chlorphonium chloride (160 ppm) produced a marked reduction in the number of live aphids after four days' exposure and showed some activity at lower dosages. At the higher concentrations all three compounds showed a trend towards lower survival rates of the nymphs, but too few nymphs were born under these conditions to be certain of this aspect.

TABLE II

Numbers of *M. persicae* on plants treated with growth retardants

Cultivar and treatment	Plant height* at flowering, mm	7 days' exposure begun, days after treatment	Live aphids, square root & no./cage transform	Nymph survival, angular & % transform
No. 4 Yellow Indianapolis			(10 cages/treatment)	(10 cages/treatment)
Untreated	343	—	23·7 *4·87* [b]	52 *62·1* [d]
B 995**	260 [a]	9	19·7 *4·44* [b c]	53 *62·5* [d]
Chlormequat chloride†	245 [a]	9	6·55 *2·56* [c]	31 *26·1* [e]
Chlorphonium chloride‡	255 [a]	24	11·4 *3·38* [b c]	33 *30·3* [e]
Golden Princess Anne				
Untreated	403	—	39·4 *6·28* [g]	69 *87·5*
B 995**	331 [f]	20	26·5 *5·15* [g]	51 *61·3* [h]
Chlormequat chloride†	346 [f]	20	18·2 *4·27* [g]	45 *50·2* [h]
Chlorphonium chloride‡	222	35	18·0 *4·24* [g]	49 *56·3* [h]
Standard error of means	9·8		*0·559*	*5·61*
Combined results from both cultivars			(20 cages/treatment)	(20 cages/treatment)
Untreated			*5·57* [i]	*60·6* [l]
B 995**			*4·97* [i j]	*52·1* [l m]
Chlormequat chloride†			*3·81* [j k]	*37·9**** [n]
Chlorphonium chloride‡			*3·41**** [k]	*41·0**** [m n]
Standard error of means			*0·394*	*3·97*

Figures with a letter suffix in common do not differ at P = 0·05. Transforms are shown in italics
* Mean of 7 plants/pot, 5 pots/treatment; within a cultivar figures lacking a common suffix differ at P = 0·01
† Applied as drench (750 mg a.i./pot) 15 days after planting
‡ Dust (80 mg a.i./pot) mixed with compost at planting
** Spray (0·25% a.i.) applied 15 days after planting
*** Significantly lower than on untreated plants at P = 0·01

TABLE III

Direct effect of growth retardants on *M. persicae*

Treatment	Rate, ppm	No. of aphids in 6 cages after exposure†			
		Adults (Live)	Nymphs (Live)	Nymphs (Dead)	Total (Live)
B 995	40	15	12	4	27
	80	12	12	2	24
	160	12	6	4	18
	320	10	5	2	15
Chlormequat chloride	10	12	14	0	26
	40	15	16	1	31
	80	15	12	1	27
	320	9	4	1	13
Chlorphonium chloride	10	9	3	7	12
	40	7	5	8	12
	160	1	0	6	1
	320	1	2	1	3
Untreated*	—	11	11	0	22
Standard error					5·16

* 15% aqueous sucrose
† 3 adult apterae/cage exposed for 4 days

Discussion

The results given above support the data obtained by other workers using different pests or hosts. In these trials the growth retardants used did not give effective control of *M. persicae* and *T. urticae* although, in general, the pest numbers were lower when chlormequat chloride or chlorphonium chloride was used than they were on untreated plants. The differences were usually significant at about the 5% level. The magnitude of the effect may vary with the time after treatment, and in view of the known differences between cultivars in responses to growth retardants[10] it seems unlikely that effects on pests will be exaggerated by combining the results for a treatment observed on two cultivars. For these pooled results the effects were sometimes significant at $P = 0{\cdot}01$. Foliar sprays of chlormequat chloride are too phytotoxic to chrysanthemums[11] to warrant investigation, so the conditions used so effectively for blackcurrants[4,5] could not be simulated.

Extracts of untreated chrysanthemums were toxic to *M. persicae* when tested by the Parafilm membrane technique, so it was not feasible to compare extracts from plants that received different treatments. These might have indicated whether the effect on aphids was due to a physical or morphological change in the foliage produced by the growth retardants. For example, leaves are thicker in plants treated with chlormequat chloride.[11]

The compounds were, therefore, examined in 15% aqueous sucrose to observe any direct action on this aphid, though the artificial diet used was not the optimum recommended.[12] There was little effect on aphid numbers after four days' exposure to 80 ppm B 995 or chlormequat chloride, and it seems unlikely that the content of growth retardants or their metabolites in the plants would reach the high levels needed to show a direct toxicity to this strain of *M. persicae*. Chlorphonium chloride, however, showed some effect at lower dosages. Presumably the compounds affected the pests indirectly.

Many plant constituents are of nutritional value to pests[13] and one of several possible ways in which the retardants could act is by modifying their levels within the host. The dependence of the fecundity and lifespan of *M. persicae* on the sugar, amino acid and metal ion content of artificial diets has been thoroughly studied by Mittler & Dadd.[12] Numerous studies of pests have been made on plants receiving various levels of nutrients, including those with *M. persicae* on Brussels sprouts[14] and radish[15]. The influence of the nitrogen nutrition of chrysanthemums on attack by the leaf miner, *Liriomyza pusilla* (Meigen), has also been studied.[16] Cabler[17] and Poole[18] have followed changes in the levels of amino acids and metal ions in the foliage and stems of the chrysanthemum Bluechip treated with chlormequat chloride or chlorphonium chloride. These changes varied with the time elapsing after treatment and with the part of the plant examined. Such investigations have dealt with the mean content for several leaves on a given part of the plant, but it is the level of these nutrients in the phloem or other localised regions where aphids probe and feed that is really important.

The effect on *M. persicae* seemed to be due, at least in part, to a reduced survival of nymphs born and reared on treated plants. van Emden[1] observed a similar effect with *B. brassicae* on Brussels sprouts plants that had been treated with chlormequat chloride. Possibly the nymphal stages are more sensitive to changes in the host plant. Honeyborne[19] has also reported effects on nymphs.

Considerable differences between cultivars in susceptibility to pests and diseases have been reported for many plant species, for example, *M. persicae*[20] and *T. urticae*[21] on chrysanthemums. These differences, too, may be related to the levels of nutrients within the plants. Even if this is so, it is possible that treatment with growth retardants might modify some cultivars so as to make them more susceptible to attack by pests and diseases. Examples of such increases are, in fact, known.[5,22] The results obtained do not, however, exclude physical mechanisms such as the development of a thicker plant cuticle rendering it more difficult for the aphids, and especially the nymphs, to probe for food.

It was noticed in both trials that all plants became heavily infested with *M. persicae*. These growth retardants alone will not give adequate control of the pests examined so far. Reduction in pest numbers with the aid of growth retardants might, however, increase the efficiency of biological control measures provided that there is no adverse interaction on parasites or predators, an aspect not yet examined. Though chlorphonium chloride gave rise to lower populations of aphids and mites than B 995, too few cultivars and pests have been examined to make a recommendation to growers.

Conclusions

The routine use of growth retardants in the culture of pot chrysanthemums does not give effective control of *M. persicae* and *T. urticae*, but populations of these pests may increase more slowly following the use of chlormequat chloride or chlorphonium chloride. B 995 was less effective against these pests on the two cultivars tested. The growth retardants are believed to exert their effect by an indirect mechanism; they reduce the survival rate of *M. persicae* nymphs on treated plants.

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References

1. van Emden, H. M., *Nature, Lond.*, 1964, **201,** 946; *J. Sci. Fd Agric.*, 1969, **20,** 385
2. Tahori, A. S., Halevy, A. H., & Zeidler, G., *J. Sci. Fd Agric.*, 1965, **16,** 568
3. Tahori, A. S., Zeidler, G., & Halevy, A. H., *Naturwissenschaften*, 1965, **52,** 191
4. Smith, B. D., & Corke, A. T. K., *Nature, Lond.*, 1966, **212,** 643
5. Smith, B. D., *Proc. 4th Br. Insect. Fungic. Conf.*, 1967, **1,** 364
6. Tahori, A. S., Zeidler, G., & Halevy, A. H., *J. Sci. Fd Agric.*, 1965, **16,** 570
7. Worthing, C. R., *J. hort. Sci.*, 1968, **43,** 197
8. Mittler, T. E., & Pennell, J. T., *J. econ. Ent.*, 1964, **57,** 302
9. Hartley, H. O., *Communs. appl. Math.*, 1955, 8/47
10. Cathey, H. M., *A. Rev. Pl. Physiol.*, 1964, **15,** 271
11. Lindstrom, R. S., & Tolbert, N. E., *Q. Bull. Mich. St. Univ. agric. Exp. Stn*, 1960, **42,** 917
12. Mittler, T. E., & Dadd, R. H., *Ann. ent. Soc. Am.*, 1965, **58,** 396; 1966, **59,** 1162; *Entomologia exp. appl.*, 1964, **7,** 315; 1965, **8,** 107; Mittler, T. E., *ibid.*, 1967, **10,** 39; 1967, **10,** 87; Dadd, R. H., & Mittler, T. E., *J. Insect Physiol.*, 1965, **11,** 717; *Experimentia*, 1966, **22,** 832; Dadd, R. H., Kriegler, D. L., & Mittler, T. E., *J. Insect Physiol.*, 1967, **13,** 249
13. Kennedy, J. S., & Stroyan, H. L. G., *A. Rev. Ent.*, 1959, **4,** 139; Auclair, J. L., *ibid.*, 1963, **8,** 439; van Emden, H. F., *Wld Rev. Pest Control*, 1966, **5,** 115
14. van Emden, H. F., *Entomologia exp. appl.*, 1966, **9,** 444
15. Quoilin, J., *Bull. Rech. agron. Gembloux*, 1966, **1,** 140
16. Woltz, S. S., & Kelsheimer, E. G., *Proc. Fla St. hort. Soc.*, 1959, **71,** 404
17. Cabler, J. F., *Diss. Abstr.*, 1965, **25,** 6910; *Proc. Fla St. hort. Soc.*, 1964, **77,** 543
18. Poole, R. T., *Diss. Abstr.*, 1965, **25,** 6915
19. Honeyborne, C. H. B., *J. Sci. Fd Agric.*, 1969, **20,** 388
20. Wyatt, I. J., *Ann. appl. Biol.*, 1965, **56,** 439; 1969, **63,** 331; *Commil Grow.*, 1965, No. 5643, 29 Oct., p. 779
21. Hussey, N. W., & Parr, W. J., *Rep. Glasshouse Crops Res. Inst., 1965*, 1966, p. 87
22. Tahori, A. S., Zeidler, G., & Halevy, A. H., *Pl. Dis. Reptr*, 1965, **49,** 775

SPECTRA OF ACTIVITY OF PLANT GROWTH RETARDANTS AGAINST VARIOUS PARASITES OF ONE HOST SPECIES*

By B. D. SMITH

Effects are described of foliar applications of the growth retardants, chlormequat (Cycocel), and *N*-dimethylaminosuccinamic acid (B995), on the major pests and diseases of blackcurrants. These compounds have a wide spectrum of activity; they both reduce the severity of attack by the mite *Cecidophyopsis ribis* and the aphid *Aphis varians* but they differ in their effects against the pathogenic fungi, *Pseudopeziza ribis* and *Sphaerotheca mors-uvae* and in their effects on the host plants. Some possible ways in which they may increase plant resistance are discussed.

Introduction

There have been a number of reports in the literature of the effects on pests and diseases of applications of growth retarding compounds to plants,[1-3] but none has been concerned with the wide range of activity against a number of different parasites which can attack the same plant species. By examining the effects of growth retardants on those parasites which simultaneously live and feed on different tissues at different sites on the same plant it may be possible to learn more about the persistence of such compounds in these tissues, more about their movement through the plant from the site of application, and ultimately, more about their mode of action. The effects which have been observed on the major parasites of blackcurrants following applications of either chlormequat chloride (2-chloroethyltrimethyl ammonium chloride) or B995 (*N*-dimethylaminosuccinamic acid) are described. The effects of varying dosage rate, timing of application, and varietal difference, on blackcurrant gall mite, leaf spot, mildew and grey mould, have been in part described elsewhere[1-5] and will only briefly be mentioned here.

Experimental

To test the ability of the growth retardants to protect healthy bushes from infestation with blackcurrant gall mite (*Cecidophyopsis ribis*), heavily infested plants were planted at intervals along rows of healthy one-year old plants and left unsprayed so that each plot consisted of 5 bushes on either side of a source of infestation. The foliage on the healthy plants was sprayed to run-off with chlormequat or B995 at concentrations varying from 1000–5000 ppm, on three occasions, at fortnightly intervals, beginning at the first open-flower stage. Plant growth rate was determined from weekly measurements of shoot length; the number of mite-infested buds on each plant was counted during the winter, and plants infected with the virus Reversion were recorded in the following spring.

To find if there was any direct toxicity through contact with chlormequat, mites were allowed to walk over glass surfaces treated with the compound, whilst others walked over untreated surfaces.

On fruiting bushes, which had received the spray treatments previously described, assessments were made at intervals throughout the growing period of the infection levels of blackcurrant leaf spot (*Pseudopeziza ribis*) and American gooseberry mildew (*Sphaerotheca mors-uvae*); grey mould (*Botrytis cinerea*) was assessed on the fruit at picking time. None of these fungi were deliberately introduced into the experimental area but populations of the red spider mite, *Tetranychus urticae* were released. Fifty leaves, infected with mites, were attached to each of 40 fruiting bushes which had received sprays of either chlormequat, at 5000 ppm, or water only. All leaves were removed from some of these plants after either one or two months and the remaining mites were counted.

The effects on the aphid, *Aphis varians*, were investigated under more artificial conditions, primarily to avoid attack by natural enemies. Groups of 12 potted plants were sprayed with either chlormequat or B995 at 2500 ppm, chlorphonium chloride (Phosfon) at 400 ppm, or water only. On the following day five first-instar nymphs of the aphid were placed on each plant, and the pots were stood in a tray of water to prevent aphids escaping. The plants were kept in an outdoor insectary and the total population on each plant was counted after one and two months. This experiment was designed to determine the persistence of treatment effects on a population and it was not suitable for assessment of the performance of individual aphids.

Results and Discussion

The degree to which plants have been protected from invasion by blackcurrant gall mite has varied with season, variety, the compound used and its concentration. The relative growth rate of different varieties varies considerably from month to month.[6] In 1965, chlormequat at 5000 ppm applied to the variety Wellington XXX, reduced the number of buds invaded by 73%; in 1966, on Malvern Cross, a more vigorous variety, chlormequat had little effect whereas B995 at 1000 ppm reduced the number of buds invaded by approximately 50%. In 1967 neither chlormequat nor B995 gave any significant degree of protection to Wellington XXX or Malvern Cross when used at 2500 ppm; this is believed to be mainly due to the unusually early migration in that year. Apparently, once mites have entered the new buds they are little affected by subsequent applications of these compounds. The mite feeds on the surface of the very young

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tissues surrounding the growing point in the bud. Increased movement of the population in the bud usually indicates that the environment is unfavourable, but such movements were not seen in buds on plants treated with growth retardants and this, together with the fact that virus transmission is not affected, is perhaps indicative that feeding behaviour is not much affected. There was no evidence of a direct toxic effect by contact with chlormequat. It seems probable that the reduction in shoot growth, and consequently of axillary buds available for invasion, is the main way by which the plant 'escapes' or resists mite attack.

The mortality rate did not increase in populations of the red spider mite on chlormequat-treated leaves; this species is entirely free-living and feeds on tissues near the surface in expanded leaves. It was found (Lloyd-Jones, C.P., personal communication) that most chlormequat remained in the leaf tissues following foliar application but it is not yet known whether it is taken up by the mites when they feed.

By contrast, the incidence of two fungal parasites, blackcurrant leaf spot and American gooseberry mildew, which infect the surface tissues of the leaves, can be reduced by chlormequat treatment. However, as the growth check disappears, the growth rate of the treated plants usually exceeds that of the untreated ones; this fast-growing tissue favours the development of the mildew, and by the end of the growing season the extent of the fungal growth may be greater than that on the untreated plants, whereas with leaf spot, which is not favoured by such growth, the earlier control of infection is sustained. On B995-treated plants there was a significantly higher level of leaf spot than on the untreated plants. A reduction in the incidence of a third fungal parasite, grey mould, infections of which may penetrate more deeply, was found on one occasion following chlormequat treatment.[4]

Aphis varians feeds on phloem tissue, mainly near the shoot tips, and, unlike the aphids in the experiments of van Emden,[1] Tahori *et al.*[2] and Bhalla & Robinson,[7] which were restricted in some way to certain feeding sites or diets, the blackcurrant aphids could move freely over the plants and leave them if conditions became intolerable. Very few aphids attempted to leave the plants, and then only in the second month when they were obviously very crowded. The rate of population increase (Table I) on the chlormequat-treated plants was much lower than on those with other treatments, and two months after treatment was only 40% of that on the untreated controls. The aphids on the B995-treated plants were less affected than on the chlormequat-treated ones and during the second month this effect rapidly diminished.

Two main ways have been suggested by which growth retardants affect the development, longevity and reproductive rate of aphids through their food supply;[1,7] but the time spent feeding is also an important factor affecting reproduction and if aphids do not find a suitable feeding site they continue to move and make exploratory probes. *Aphis varians* forms very dense colonies under natural conditions and is not a suitable species on which to observe the time during which individuals feed.

The quantity of food taken in by an aphid on a treated plant may be less in a given time, and may be further affected by the smaller cell size, and thicker cell walls which have been reported on plants treated with growth retardants. Changes have also been found in the cuticle of retardant-treated plants. Chlormequat, for example, was found to reduce greatly the production of surface wax and increase the thickness of the cuticular membrane.[8] Treated leaves are usually darker green in colour and this, together with the changes in the cuticle, may affect host plant selection by insects such as aphids. It is not possible to state what contribution was made, by direct toxicity, quality or quantity of food or other factors, to the lower rate of population increase of *Aphis varians* found on retardant-treated plants, but the relative importance of such factors under natural conditions will need to be determined if the basis of resistance of plants is to be fully understood.

Growth retardants are being used commercially, (e.g. chlormequat on cereals, B995 on apples and both compounds on ornamental plants), at concentrations similar to those at which anti-parasite activity has been found experimentally. Not all activity against parasites is likely to be beneficial to the plant, as has been shown with chlormequat and American gooseberry mildew, and with B995 and blackcurrant leaf spot. Also, some varieties of blackcurrant react differently to the same growth retardant. Chlormequat and B995 differ in their effects on the plant, the former was found to increase the ratios of flowers/vegetative buds and ultimately the yield, whilst the latter was found to do the reverse. At present, it is not possible to generalise and to predict the effects of growth retardants on host–parasite interactions without at least some preliminary experimentation on the interaction concerned.

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TABLE I

Average number of aphids produced by 5 adults on each of 12 plants

	Treatment							
	Chlormequat		Phosfon		B995		Control	
	Apterous	Alate	Apterous	Alate	Apterous	Alate	Apterous	Alate
After 1 month	9·5	—	31·2	—	21·3	—	31·6	—
After 2 months	214·6	1·0	429·0	13·4	472·3	7·1	546·4	10·4

Significant differences: After 1 month CCC < all others (0·1%)
B995 < Control (5%)
After 2 months CCC < all others (0·1%)
Alatae CCC < all others (0·1%)
B995 < Phosfon (1%)

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References

1. van Emden, H. F., *Nature Lond.*, 1964, **201,** 946
2. Tahori, A. S., Zeidler, G., & Halevy, A. H., *J. Sci. Fd Agric.*, 1965, **16,** 570
3. Sinha, A. K., & Wood, R. K. S., *Nature, Lond.*, 1964, **202,** 824
4. Smith, B. D., & Corke, A. T. K., *Nature, Lond.*, 1966, **212,** 643
5. Smith, B. D., *Proc. IV Br. Insectic. Fungic. Conf. 1967*, 1968, **1,** 364
6. Wilson, D., & Adam, J., *Rep. Long Ashton Res. Stn 1966*, 1967, p. 104
7. Bhalla, O. P., & Robinson, A. G., *J. econ. Ent.*, 1968, **61,** 552
8. Baker, E. A., Dawkins, D., & Smith, B. D., *Rep. Long Ashton Res. Stn 1967*, 1968, p. 116

OBSERVATIONS ON DISEASES OF BLACKCURRANTS*

By A. T. K. CORKE

Changes in the relative importance of the fungi attacking blackcurrants have possibly been due, in part, to the replacement of lime sulphur for insect control. The dwarfing due to sulphur can also be induced by the use of (2-chloroethyl)-trimethylammonium chloride or *N*-dimethylaminosuccinamic acid, and these chemicals may also affect the incidence and severity of certain fungal diseases. While some growth regulators may be generally beneficial in reducing infection, the results are unpredictable, and the effects are examined in the light of other ways in which host resistance may be altered.

Until about five years ago the leaf spot fungus (*Pseudopeziza ribis*) was the only economically important fungus attacking blackcurrant bushes. American gooseberry mildew (*Sphaerotheca mors-uvae*) and grey mould (*Botrytis cinerea*) were familiar diseases of gooseberry, but uncommon on blackcurrant bushes. Then, within about two years blackcurrant plantations became heavily infected by mildew and *Botrytis*, and crop losses due to these fungi increased rapidly.

A possible explanation for the sudden rise in the severity of infection by these two fungi on blackcurrant bushes may be found in the modifications to horticultural practice which have recently been introduced.[1,2] Cultivation of the soil has been replaced by the use of herbicides for the control of weeds; tar oil winter wash and lime sulphur have been replaced by more specific insecticides for pest control in the spring and summer; and a programme of several pre-harvest fungicidal sprays against leaf spot has been widely adopted in place of post-harvest copper sprays.

The change which appeared most likely to influence the infection of bushes by mildew was the abandonment of lime sulphur for the control of the blackcurrant gall-mite (*Cecidophyopsis ribis*). For many years lime sulphur had been the standard fungicide for the control of mildew on gooseberries, and in addition to its insecticidal and fungicidal properties, it was known to restrict shoot growth and to alter the texture of the leaves of blackcurrants.

The susceptibility to infection of the blackcurrant bush can be influenced to a considerable extent by the amount of pruning carried out in the winter. Heavy pruning, which induces rapid growth in the following spring, favours mildew but reduces the amount of leaf spot infection; it also provides an open bush in which conditions are less favourable to infection, particularly by *Botrytis*, than in a thick compact bush.

Other factors such as nutrition, and sprays used for the control of weeds, insects and even fungi, may also affect the physiology of the host plant, and exert some influence on the degree of susceptibility to a particular disease. Apart from the direct effects of fertilisers, experience suggests that clean cultivation obtained by the use of herbicides can affect the nutritional status of the host. There appears to be a relationship between the amount of simazine used and the incidence of mildew, which is distinct from that which is due to the removal of weeds competing for the same nutrients. The increasing use of pre-harvest sprays to reduce infection by leaf spot may also have brought about physiological changes which favour infection by mildew and *Botrytis*. It has been found, for example, that the fruit of tomato plants treated with zineb to prevent fungal infection of the leaves is more heavily infected by *Botrytis* than the fruit on untreated plants.[3]

Treatment with non-fungitoxic oil has been used successfully in banana plantations to reduce infection by the leaf spot fungus (*Mycosphaerella musicola*). Oil has been found to alter the rates of metabolic processes taking place so that conditions approximate to those in shaded banana leaves, which are much less heavily infected than exposed ones. These changes are sufficient to influence the development of the fungus within the tissues.[4] Treatment of Blacksmith blackcurrant leaves with oil was almost as effective in reducing the level of mildew infection as an equal number of sprays of fungicides found to be particularly effective against mildew.[5,6]

There are clearly many factors which can induce changes in the host plant sufficient to upset the delicate balance of a host-parasite relationship. Even seasonal differences in disease intensity may be due as much to the effects of the environment on the host as on the fungus.

Alternatives to fungicides for reducing the annual crop losses caused by fungus diseases have long been investigated. While the use of sulphur has always been complicated by the sensitivity of some plants to it, such treatment of blackcurrant bushes induced an apparent immunity to mildew infection to an unexpected extent. Speculation on the part played in reducing infection by physiological changes in the host plant induced by the restriction on shoot growth, led to an examination of the ability of growth regulators to achieve a comparable effect.

Experiments with growth regulators carried out at Long Ashton since 1965 have included several varieties of blackcurrant and a number of concentrations of several chemicals, as well as trials of the number and timing of applications. Varieties generally differed little in their response to a particular regulator, but the effects of different regulators on the incidence of diseases were very marked. Young non-fruiting bushes treated with 0·25% (2-chloroethyl)-trimethylammonium chloride (chlormequat or CCC) were less heavily infected with mildew than untreated bushes, while those treated with 0·25% *N*-dimethylaminosuccinamic acid (B995) were more heavily infected. The differences were more marked where more applications were made, or higher concentrations of B995 were used. Bushes treated with B995 were also more heavily infected by leaf spot than those treated with CCC, which were not significantly different from untreated bushes. The level of infection decreased with increasing concentrations of CCC, but increased with higher concentrations of B995.

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Treatment of fruiting bushes with CCC in the spring also reduced, but not significantly, the level of mildew infection at harvest time; a subsequent increase in the rate of shoot growth was followed by a marked increase in mildew infection. Bushes treated with CCC, however, had significantly shorter shoots ($P = 0{\cdot}001$) carrying more buds ($P = 0{\cdot}01$) and more crop ($P = 0{\cdot}01$) than untreated bushes. There was also 43% less leaf spot infection and 66% less *Botrytis* infection of the fruit on treated bushes.[7]

It may be concluded that although the treatment of blackcurrant bushes with CCC is generally beneficial from the point of view of reducing disease, a depressing effect on mildew is achieved by spraying only during the period of active shoot growth, when susceptibility to infection is normally at a maximum. A direct fungicidal effect of CCC on the spores of mildew has not been demonstrated but Jordan[8] has shown that the development of mildew on blackcurrant leaves is affected by treatment with 0·5% CCC both before and after infection. Baldwin cuttings with twelve leaves were sprayed to run-off and dusted with conidia 24 hours later. After seven days there were some mildew infections on the CCC-treated leaves, but infection was not statistically different from that on leaves treated with established mildew fungicides, and all were significantly different from untreated leaves ($P = 0{\cdot}001$). Seven days after the treatment of foliage already heavily infected with mildew, there was more mildew on CCC-treated leaves than on those treated with mildew fungicides ($P = 0{\cdot}001$), but there was also an equally significant difference between those treated with CCC and those left untreated.

The influence exerted by a number of cultural measures on the susceptibility of blackcurrant bushes to disease has already been discussed. It was expected that treatment with growth regulators would alter susceptibility to disease, but it is clear from the experiments that the magnitude of this effect is unpredictable. With so many chemicals now being used on crop plants for a variety of reasons, it is worth considering whether some may affect the physiology of the host plant, and thus enhance or detract from the performance of others. Some fungicides may exert part of their effect by influencing the host's physiology; they may also stimulate detoxication mechanisms in the host.

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References

1. Corke, A. T. K., *Proc. Third Br. Insect. Fungic. Conf.*, 1965, p. 150
2. Corke, A. T. K., *Proc. Third Br. Insect. Fungic. Conf.*, 1965, p. 336
3. Cox, R. S., & Hayslip, N. C., *Pl. Dis. Reptr.*, 1957, **41**, 878
4. Calpouzos, L., & Corke, A. T. K., *A. Rep. Long Ashton Res. Stn for 1962*, 1963, p. 106
5. Corke, A. T. K., & Jordan, V. W. L., *A. Rep. Long Ashton Res. Stn for 1965*, 1966, p. 184
6. Corke, A. T. K., & Jordan, V. W. L., *A. Rep. Long Ashton Res. Stn for 1967*, 1968, p. 128
7. Smith, B. D., & Corke, A. T. K., *Nature, Lond.*, 1966, **212**, 643
8. Jordan, V. W. L., Thesis, Univ. London, 1967

MODE OF ACTION OF PHENYLTHIOUREA, A THERAPEUTIC AGENT FOR CUCUMBER SCAB*

By A. KAARS SIJPESTEIJN

In laboratory tests, phenylthiourea (PTU) induced resistance in cucumber seedlings to infection by spores of cucumber scab (*Cladosporium cucumerinum*). At the same time it inhibited polyphenol oxidase activity in the plant, increased peroxidase activity, and caused lignification of the cell walls in the parenchyma around the penetrating hyphae of *Cladosporium*. Possible association and interaction between these effects are discussed.

Introduction

For many years workers at the Institute for Organic Chemistry T.N.O., Utrecht and at the Laboratory for Phytopathology, Wageningen have co-operated in a common research programme to develop and study systemic fungicides. The most frequently used test in this joint study was a test for the protection of cucumber seedlings against cucumber scab, a disease caused by *Cladosporium cucumerinum*. One of the few compounds found to give complete protection in this test was a well known and fairly simple compound, phenylthiourea (PTU).[1]

Experimental

The test was carried out in the following manner. Seedlings were placed with their roots either in water or in a solution of 50 ppm PTU for two days. They were then transferred to water and a spore suspension of the fungus was sprayed on to them. Seven days after inoculation the water-treated control plants were highly diseased; the PTU-treated plants, in contrast, were completely protected.

Results

The following account of the mode of action of PTU is based on work carried out in collaboration with Sisler and Dieleman.

The antifungal activity of PTU[1,2] under various conditions was investigated. The concentration required for complete growth inhibition of *Cladosporium cucumerinum* appeared to be as much as 500 ppm. This concentration far exceeds the concentration present in fully protected plants, for after treatment of the roots with 50 ppm of PTU, the concentration in the sap of the hypocotyl was only 10–20 ppm. It appeared also, that PTU did not give rise to the formation of a fungicide in the plant nor did it inhibit the pectolytic enzymes of *Cladosporium* which are essential to the parasitism of this organism.

It thus appeared that PTU is one of those compounds which do not act by inhibition of growth of the fungus, but by changing host metabolism in a way which renders the plant resistant. Such compounds, because of their mode of action, may provide information about the host–parasite relationship and perhaps about the mechanism of natural resistance.

PTU has at least three effects on the plant and these are probably interconnected and co-operate in bringing about protection.

In the first place PTU is known to be a very strong inhibitor of polyphenol oxidase.[1,3,4] A colorimetric determination using a partly purified potato polyphenol oxidase, with DOPA as the substrate showed that in a homogeneous medium about 0·2 ppm PTU was required for 50% inhibition of the enzyme.[2] In sap from plants protected by treatment with 50 ppm PTU, sufficient PTU (10–20 ppm) is present to suppress all polyphenol oxidase activity. The activity of polyphenol oxidase in sap of untreated cucumber seedings is, in any case, quite low.[1]

PTU also has an effect on the enzyme peroxidase in cucumber.[2] Like polyphenol oxidase, peroxidase oxidises phenols, but requires for oxidation the presence of hydrogen peroxide. PTU does not inhibit peroxidase, nor does it stimulate peroxidase *in vitro*. However, in PTU-treated plants the activity of peroxidase was consistently higher than in untreated plants. This effect was looked for especially in the top halves (± 1·5 cm) of the hypocotyls because this is the part of the seedlings in which the disease develops.

Homogenates were made of hypocotyl tips, and peroxidase activity was measured spectrophotometrically using *p*-phenylene diamine as a substrate. If the average peroxidase activity in the homogenates of the control plants is taken as 100%, the activity in the homogenates of plants treated for 2 days with 50 ppm PTU was 168% in 6 different experiments. After PTU treatment for 5 days an increase of peroxidase activity to 300% of that of control plants could be obtained.

In 1963, Hijwegen[5] looked for histological differences between cucumber varieties which are susceptible to *Cladosporium* and those which are resistant. He found no difference between susceptible plants and resistant plants which had not been inoculated. However, when inoculated, resistant plants showed considerable lignification; in the susceptible plants lignin formation was absent in the etiolated plants. Hijwegen postulated that this lignification might constitute a barrier to the invading fungus.

Following these observations on cucumbers with natural resistance, the possibility that the resistance induced by PTU might have a similar cause was examined. Cross-sections were made of the upper part of hypotocotyls of plants treated in various ways. Microscopic examination

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after staining with phloroglucin–HCl showed that in plants not sprayed with *Cladosporium* whether or not they had been treated with 50 ppm PTU for 2 days lignin was present only in the walls of the xylem vessels. In sections of plants which had been sprayed with the parasite but not treated with PTU the fungus could frequently be seen in tissues without any lignin nearby. Sometimes lignification was seen in the walls of the parenchyma; only in plants with well-advanced lesions was lignification marked. This contrasts with the picture shown by PTU-treated plants after infection by the fungus.

In plants completely protected by treatment with PTU at 50 ppm smaller or larger lignified spots were frequently found in, and near, the epidermis. They presumably are the sites where the fungus was prevented from entering by the quick lignification in the treated plant. In plants which received somewhat less PTU (20 ppm) a lesion was occasionally seen. A cross-section made as soon as the lesion became visible showed strong lignification of the cells around the fungus through several cell layers, and it appeared that a barrier had been formed in time to prevent further penetration of hyphae; presumably this lignified tissue was also rather resistant to attack by pectolytic enzymes of the fungus.

Discussion

Three effects of PTU on the cucumber plant have been recognised: it inhibits polyphenol oxidase, it causes an increase of peroxidase activity and it causes strong lignification of the cell walls in the parenchyma situated around the penetrating hyphae of *Cladosporium*.

It remained to be resolved whether these three effects could explain the resistance induced by PTU and how far the three effects were interrelated. The following is a tentative explanation.

In these tests seedlings were treated for 2 days with water or with PTU. This did not change the histology. However, peroxidase activity was increased in the latter case and polyphenol oxidase activity was inhibited. Histological changes occurred only in those seedlings which were subsequently infected with *Cladosporium*. *Cladosporium* readily attacked plants that had been treated with water only and there was little lignification in these plants.

This lignification is not a specific effect of *Cladosporium* invasion, for according to Behr[6] even mechanical damage brings about some lignification in cucumber.

In PTU-treated plants *Cladosporium* has to attack tissues containing higher peroxidase activity. Moreover, polyphenol oxidase activity is completely inhibited in the plants as well as in the invading fungus. Consequently more phenols may remain available as substrates for lignification by peroxidase. In PTU-treated plants this higher peroxidase activity and the inhibition of polyphenol oxidase may together be responsible for the fact that the tissues around the invading fungal hyphae are able to lignify more rapidly and more intensely than the untreated tissues. This rapid lignification of the host tissues forms a barrier to the invading fungus and stops further spread. At high PTU concentration there is complete protection; only microscopically can the sites of infection be located as sites of lignification. With lower concentrations of PTU a small lesion is sometimes formed, but the lignin barrier prevents further attack.

It is convenient to suppose that this is the way in which PTU protects plants. However, many questions remain to be answered. It is known[7,8] that peroxidase and indole acetic acid oxidase are closely related enzymes and that the activity of both is frequently affected by chemicals (e.g. gibberellic acid, growth retardants) in one particular way. There is, however, no convincing experimental proof that PTU stimulates indole acetic acid oxidase activity in the plant. For this reason it is still uncertain whether there is a similarity between the mode of action of PTU and that of phenylserine (cf. van Andel[9,10]).

It is not clear in what way mechanical damage or the invading fungus incite lignin formation. According to Behr[6] mechanical damage led to lignification only in the case of cucumber although lignin formation following penetration by a fungus has been described by Behr[6] for cucumber and by Asada & Matsumoto[11] for Japanese radish.

It is not clear either in what way PTU treatment brings about an increase in peroxidase activity in the host tissues. Increase in activity of an enzyme may be due to synthesis of more enzyme; it can also be due to removal of an inhibitor or to an increase in the level of some co-factor.

All the plant tissues so far investigated have not one peroxidase but rather a number of isoperoxidases which can be separated by gel electrophoresis;[7,12] this is also the case in cucumber. The question then arises as to the way in which PTU treatment changes this isoenzyme pattern. Certainly the PTU treatment did not give rise to any new peroxidase bands in the gel. It appeared, however, that all isoenzymes showed higher activity in PTU-treated plants than in the controls. Some, possibly, were more stimulated than others; and this depended also on the substrate used. This may mean that synthesis of each of the isoenzymes has taken place in the plant following PTU treatment or alternatively that for each of the enzymes more of a common co-factor is present in PTU-treated plants or that more of a common inhibitor has been removed. There is also the possibility that the inhibition of polyphenol oxidase in the plant by PTU gives rise to accumulation of phenols, which might perhaps act as inducers ('derepressors') of the synthesis of the peroxidases.

The hypocotyl peroxidase is located particularly in the vessels and in the outer cell layers; it is not certain if the isoenzymes are equally distributed in these different sites, for the different isoenzymes may have different functions.

Other chemicals are also known to increase peroxidase activity. Halevy[8] found that growth retardants are able to do so in cucumber hypocotyls, but the isoenzyme pattern was not studied. Nickel salts and mercuric salts have been reported by Farkas & Stahmann[12] to increase peroxidase activity in bean plants. New isoperoxidases were observed in this case, but this work has been criticised by Novacky & Hampton.[13] They pointed out that erroneous results may be obtained if the hydrogen peroxide concentration used in the experiments is too high.

It is relevant to note that Stahmann *et al.*[14,15] and co-workers found an increase in peroxidase activity in sweet-potato following treatment with ethylene. This treatment at the same time rendered the tissues more resistant to attack by *Ceratocystis fimbriata*. It would be useful to discover whether lignification had taken place in this case as well.

Natural resistance to *Cladosporium* in resistant cucumber varieties is conditioned by the presence of one dominant gene. It has already been mentioned that Hijwegen found a great difference in lignification when penetration into a susceptible variety was compared with penetration into a resistant variety. He therefore suggested that the lignification in the resistant plant might be the immediate cause of the resistance.

For this reason it was considered important to discover if the level of peroxidase activity in the hypocotyls of two resistant varieties (Vios, Trias) is higher than that of the susceptible variety (Lange Gele Tros). There was, however, no essential difference and the isoenzyme pattern in resistant and susceptible varieties did not differ.

It may be possible that for some other reason lignification takes place more rapidly in the resistant plant than in the susceptible plant when the fungus penetrates. For example, more phenols may be available as substrates in the resistant tissue or this tissue may be able to increase more rapidly its peroxidase activity in response to penetration of the fungus. It still remains to be discovered how the mechanism of natural resistance is related to the mechanism by which PTU induces resistance to *Cladosporium cucumerinum*.

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References

1. Kaars Sijpesteijn, A., & Pluygers, C. W., *Meded. Landb. Hoogesch. OpzoekStns Gent.*, 1962, **27,** 1199
2. Kaars Sijpesteijn, A., & Sisler, H. D., *Neth. J. Pl. Path.*, 1968 (Suppl. 1), **74,** 121
3. Bernheim, F., & Bernheim, M. L. C., *J. biol. Chem.*, 1942, **145,** 213
4. Dubois, K. P., & Erway, W. F., *J. biol. Chem.*, 1946, **165,** 711
5. Hijwegen, T., *Neth. J. Pl. Path.*, 1963, **69,** 314
6. Behr, L., *Phytopath. Z.*, 1949, **15,** 92
7. McCune, D. C., *Ann. N.Y. Acad. Sci.*, 1961, **94,** 723
8. Halevy, A. H., *Pl. Physiol., Lancaster*, 1963, **38,** 731
9. van Andel, O. M., *Nature, Lond.*, 1966, **211,** 326
10. van Andel, O. M., *Neth. J. Pl. Path.*, 1968 (Suppl. 1), **74,** 113
11. Asada, Y., & Matsumoto, I., *Phytopathology*, 1967, **57,** 1339
12. Farkas, G. L., & Stahmann, M. A., *Phytopathology*, 1966, **56,** 669
13. Novacky, A., & Hampton, R. E., *Phytopathology*, 1968, **58,** 301
14. Stahmann, M. A., Clare, B. G., & Woodbury, W., *Pl. Physiol., Lancaster*, 1966, **44,** 1505
15. Stahmann, M. A., in 'The dynamic rôle of molecular constituents in plant-parasite interaction', (Eds Mirocha, C. J., & Uritani, I.) 1967, p. 357 (St. Paul, Minn: Am. Phytopath. Soc. Inc.)

INTERACTIONS BETWEEN STEROIDS IN THE GROWTH OF *PHYTOPHTHORA**

By C. G. ELLIOTT and B. A. KNIGHTS

It is shown that several steroids in mixture with cholesterol inhibit the cholesterol-dependent oospore production by *Phytophthora cactorum* in culture. It is suggested that in plants, precursors of the phytosterols might compete with sterols to reduce the sporulation of pathogens, such as *Phytophthora* and *Pythium* spp., which are dependent on their hosts for the sterols required for their reproduction. A high proportion of precursor compared with sterol would thus enhance the disease resistance of the host.

Species of the fungus genera *Phytophthora* and *Pythium* grow vegetatively on a medium comprising simple sources of carbon and nitrogen (e.g. sucrose and asparagine), mineral salts and thiamine. For the formation of sexual organs (oogonia and antheridia), and of the oospores which develop from them, sterols must be added to the medium.[1-5] In addition to promoting sexual reproduction, sterols increase growth rate,[6-8] and are required for the production of viable zoospores[9] (the asexual reproductive bodies). It would appear that these fungi are unable to synthesise sterols,[5,10,11] and that they are totally dependent on their host or substrate for them.

The ability of a sterol to promote sexual reproduction in *Phytophthora cactorum* was found to depend on its configuration in both the ring system and side chain.[6] For activity, a hydroxyl group at position 3, a B ring with one double bond and a hydrocarbon side-chain of at least five carbon atoms are required. The arrangement of double bonds in the side-chain is also critical. Cholesterol (cholest-5-en-3β-ol), for example, promotes the formation of numerous oospores, while with cholestanol (5α-cholestan-3β-ol) oogonia and antheridia are formed, but very few oospores develop. The effect of supplying the fungus with mixtures of cholesterol and cholestanol was examined.[7] It was found that with excess cholestanol the production of oospores was greatly reduced. It was argued that cholestanol competes with cholesterol for sites in the cellular structure at which sterols control oospore formation, and when present at these sites the cholestanol is unable to promote effectively the later stages in oospore development.

Table I gives further data on the inhibition of oospore production in *Phytophthora cactorum* with various steroids mixed with cholesterol. These data were obtained by the same methods as were used previously.[6,7] The fungus was grown in small (5 cm) Petri dishes on a sucrose asparagine agar medium. The numbers of oospores were counted in radial transects of the dishes in one focal plane with the lower-power microscope objective.

In previous work,[6] substances which promote oospore development were classified as active, substances which promote oogonium development but which give few oospores were classified as partially active, and substances which give no sex organs when added to the basal sucrose asparagine medium as inactive. Table I shows that the partially active substance Δ^5-cholesten-3-one, added to the medium with cholesterol, inhibits oospore production, just as the partially active cholestanol does. Δ^7-Cholesten-3β-ol is an active compound, but with it fewer oospores are formed than with cholesterol and they take longer to develop.[6] Table I shows that when a large amount of Δ^7-cholestenol is added to the cholesterol, the number of oospores is lower than in the cholesterol controls, agreeing with the number with Δ^7-cholestenol alone. The Δ^7-cholestenol must dilute the cholesterol at the sites controlling oospore development. The second experiment with Δ^7-cholestenol shows a synergistic effect on oospore numbers at a low ratio of cholestenol:cholesterol (cf. Elliott[7]).

The two inactive compounds, Δ^8-lanosten-3β-ol and lanosterol ($\Delta^{8,24}$-lanostadienol) were also tested. Table I shows that both compounds inhibit oospore production in mixtures with cholesterol. Δ^8-Lanostenol has the same side-chain configuration as cholesterol, and is considerably more effective in reducing spore formation than lanosterol, which has a double bond at C_{24}. It was previously[6] found that desmosterol, which also has a double bond at C_{24}, and differs from cholesterol in this respect only, is inactive in promoting oospore formation. Evidently molecules with a double bond at C_{24} are unable to occupy oospore-determining sites effectively.

Table II shows that the 'partially active' substance Δ^5-cholesten-3-one promotes vegetative growth (measured as dry weight of mycelium), and that the 'inactive' Δ^8-lanostenol does not. But it may be noted that with the 'active' compound Δ^7-cholestenol there is no significant effect on vegetative growth.

The above results suggest that it might be possible to affect the growth and sporulation of sterol-deficient pathogenic fungi by manipulation of the sterol content of their hosts. The biosynthetic pathway from squalene to β-sitosterol (or other active sterols) passes through a number of compounds which might compete with β-sitosterol for the sites controlling sporulation. In a host plant with a high ratio of inactive precursor to active sterol, sporulation might be reduced compared with that in one in which this ratio was lower. A high ratio of precursors to active sterols might be brought about by treatment of the plant with a compound inhibiting the later stages of sterol synthesis, or by breeding, i.e. by selecting plants where this increase is the result of the genetical constitution.

* Presented at a symposium on 'Chemically induced resistance of plants to pests and diseases', organised by the Pesticides Group of the Society of Chemical Industry on 27 September, 1968.

TABLE I

Oospore formation in *Phytophthora cactorum* in media with various steroids

Each value is the mean of five counts in each of eight Petri dishes (cholestenone and cholestenol) or six (lanostenol and lanosterol)

Test substance	Concentration of cholesterol, mg/l	Concentration of test substance, mg/l					
		0	3·13	12·5	25	50	100
Δ^5-Cholesten-3-one	0	—	—	0·1	0·03	0·2	0·3
	3	29·9	—	14·9	8·9	8·5	2·5
Δ^7-Cholesten-3β-ol	0	—	—	26·8	22·3	19·8	10·6
	3	23·9	—	21·4	18·5	16·8	9·2
Δ^7-Cholesten-3β-ol	0	—	3·0	14·2	—	19·3	16·6
	3	24·0	46·5	24·0	—	14·9	14·6
Δ^8-Lanostenol	3	26·5	—	5·7	0·8	1·7	0·8
	5	34·7	—	17·2	5·1	2·6	1·1
Lanosterol	3	26·5	—	19·1	18·0	19·1	13·2
	5	34·7	—	22·9	28·0	23·9	23·5

Analysis of variance

Component of variation	Degrees of freedom	Mean squares	
		A	B
Concentrations of test substance	4	736·6*	680·5**
Petri dishes at same concentration	25	251·6*	133·0
Counts in same Petri dish	120	151·6	92·4

A, lanosterol: cholesterol 5 mg/l
B, lanosterol: cholesterol 3 mg/l
The analyses of other experiments are comparable
* Significant at 5% level, ** at 1% level

TABLE II

Growth of *Phytophthora cactorum* in medium with various steroids

Values are mean dry weights (mg) of mycelium after 14 days' growth in 25 ml liquid medium in 100 ml conical flasks; stationary culture

Steroid added	Concentration of added steroid, mg/l			
	0	2·5	10	40
Cholesterol	40 ± 4·8	82 ± 5·7	93 ± 6·3	100 ± 9·4
Δ^5-Cholesten-3-one	34 ± 5·6	52 ± 5·2	61 ± 7·1	81 ± 6·2
Δ^7-Cholesten-3β-ol	34 ± 5·6	38 ± 2·4	46 ± 4·3	45 ± 5·2
Δ^8-Lanosten-3β-ol	62 ± 4·8	61 ± 5·1	60 ± 5·5	54 ± 5·2

To test this idea, six varieties of potato sent by Dr. W. Black of the Scottish Plant Breeding Station as differing in their field resistance to blight (*Phytophthora infestans*) were analysed. The dried haulms of the potatoes were extracted with petroleum ether. After removal of the solvent, sterols were isolated by saponification followed by digitonin precipitation from the non-saponifiable fraction, and examined by gas-liquid chromatography. The results (Fig. 1 and Table III) show that there are considerable differences between varieties in the ratio of *cyclo*artenol to β-sitosterol. The triterpene *cyclo*arternol is believed to be a precursor of the phytosterols.[12–14] The agreement with the suggested correlation between the degree of blight resistance and the precursor/sterol ratio is good. There is some discrepancy between the two sets of results for Variety 1, but it has been found (Clarke, D. D., personal communication) that this variety is considerably more susceptible to blight in the tubers than in the leaves, ranking fourth in the tests on tubers; thus its place in the resistance series is uncertain. Also there is a considerably higher proportion of β-sitosterol in the samples B than in A; the sterol composition doubtless varies with the external environment and with the stage of growth of the plant.[15]

This encouraging correlation must be treated with caution. The results with *P. cactorum* in culture suggest that *cyclo*artenol might not be a very effective competitor with the active sterols, as it resembles lanosterol, and like lanosterol it has a double bond at C_{24} (Fig. 2). It is possible, however, that the sterol glycosides or the alkaloids such as solanidine might compete with the sterols which they more closely resemble.

Another difficulty is that the work with *P. cactorum* has been concerned with oospores. Oospore production is not a factor in pathogenicity with *P. infestans* (except perhaps in Mexico alone, where the two mating types of the heterothallic fungus occur[16]), but reduced production of the asexual

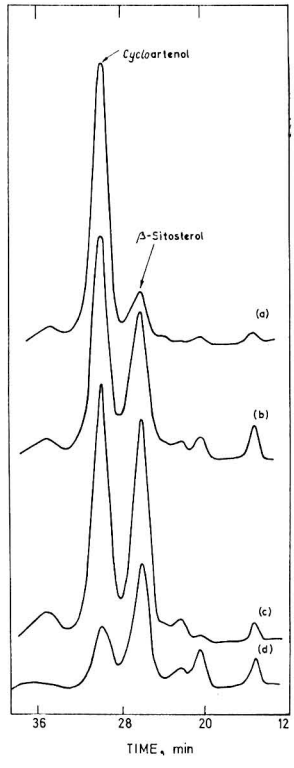

FIG. 1. *Gas-liquid chromatograms of sterol fractions of four potato varieties*
(a) 3278(3); (b) 3267ac(2); (c) 3268ac(2); (d) Craig's Defiance (varieties 1,3,4 and 5 of Table III)

TABLE III

Analysis of potato haulms for *cyclo*artenol and β-sitosterol

Variety	Blight resistance*	Ratio *cyclo*artenol : β-sitosterol	
		A†	B†
1. 3278(3)	3−	6·30 : 1	0·14 : 1
2. M109–3	3	5·30 : 1	0·29 : 1
3. 3267ac(2)	3+	1·40 : 1	0·40 : 1
4. 3268ac(2)	4−	1·10 : 1	0·34 : 1
5. Craigs Defiance	4	0·53 : 1	0·15 : 1
6. 2558a(2)	4+	0·75 : 1	0·10 : 1

* Blight resistance determined on detached leaves. Values on scale 1 (highly resistant) to 5 (very susceptible) according to Black.[18]

† A and B, samples assayed on two different occasions. A, by g.l.c. of sterol fraction on 1% SE-30, 225°. B, by g.l.c. of sterol fraction as trimethylsilyl ethers on 3% OV-17, 254°

zoosporangia by the fungus is one of the chief characteristics of high field resistance to blight in the potato.[17,18] Whether the interaction between sterols described also applies to the production of sporangia and zoospores has not been examined.

It has, however, been shown that in *Phytophthora cactorum*, interaction between a sterol, active in promoting oospore production, and various inactive or partially active substances can reduce the number of oospores. It is suggested that this system may operate in the host plant, and that its exploitation may be of value in protecting plants against attack by *Phytophthora* and *Pythium*.

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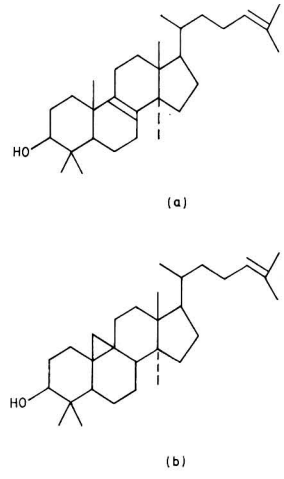


FIG. 2. *Formulae of* (a) *lanosterol and* (b) cyclo*artenol*

References

1. Haskins, R. H., Tulloch, A. P., & Micetich, R. G., *Can. J. Microbiol.*, 1964, **10,** 187
2. Hendrix, J. W., *Science, N.Y.*, 1964, **144,** 1028
3. Leal, J. A., Friend, J., & Holliday, P., *Nature, Lond.*, 1964, **203,** 545
4. Harnish, W. N., Berg, L. A., & Lilly, V. G., *Phytopathology*, 1964, **54,** 895
5. Elliott, C. G., Hendrie, M. R., Knights, B. A., & Parker, W., *Nature, Lond.*, 1964, **203,** 427
6. Elliott, C. G., Hendrie, M. R., & Knights, B. A., *J. gen. Microbiol.*, 1966, **42,** 425
7. Elliott, C. G., *J. gen. Microbiol.*, 1968, **51,** 137
8. Schlosser, E., & Gottlieb, D., *Arch. Mikrobiol.*, 1968, **61,** 246
9. Hendrix, J. W., *Phytopathology*, 1965, **55,** 790
10. Hendrix, J. W., *Mycologia*, 1966, **58,** 397
11. Schlosser, E., & Gottlieb, D., *J. Bact.*, 1966, **91,** 1080
12. Rees, H. H., Goad, L. J., & Goodwin, T. W., *Biochem. J.*, 1968, **107,** 417
13. Ponsinet, G., & Ourisson, G., *Phytochemistry*, 1968, **7,** 757
14. Baisted, D. J., Gardner, R. L., & McReynolds, L. A., *Phytochemistry*, 1968, **7,** 945
15. Ingram, D. S., Knights, B. A., McEvoy, I. J., & McKay, P., *Phytochemistry*, 1968, **7,** 1241
16. Niederhauser, J., *Trans. N.Y. Acad. Sci.*, 1956, **19,** 55
17. Lapwood, D. H., *Ann. appl. Biol.*, 1961, **49,** 316
18. Black, W., *Rep. Scott. Pl. Breed. Stn.*, 1960, 29

FACTORS AFFECTING THE SYSTEMIC ANTIFUNGAL ACTIVITY OF SOME CHEMICALLY RELATED COMPOUNDS*

By J. L. GARRAWAY and S. E. COOK

Some phenols, phenolic glycosides and derivatives of sulphanilic acid were tested for direct (*in vitro*) and systemic activity against some rust and mildew fungi. Results against *Uromyces fabae* are quoted. Some of the highly substituted phenols were toxic to *U. fabae*, but there was little systemic activity in broad beans and wheat shoots with phenols or phenolic glycosides. Although 4-aminobenzenesulphonamides and 4-aminobenzenesulphonylureas were relatively inactive *in vitro*, they showed appreciable systemic activity. Modification of the structure of compounds to confer solubility in water did not necessarily improve systemic activity.

Introduction

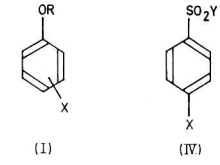
In the control of crop diseases growers rely mainly on preventative measures using chemicals which are effective at the surface of the plant. Such chemicals are usually present as water-insoluble deposits and show little tendency to enter and move within the plant itself. The 'chemotherapeutic' control of plant disease, by the introduction of chemicals into the plant, has long been recognised as a desirable development in the search for more effective and selective fungitoxicants. Parallels already exist in medicine where suitable therapeutic agents are administered to control the invasion of the animal body by pathogenic organisms, and in other areas of crop protection where systemic insecticides are employed on plants to prevent insect attack. Many compounds have been shown to be effective as therapeutic agents in plants[1–4] but it is only more recently that compounds with some promise on field crops[2–7] have been discovered. In general it can be said that success has been somewhat limited and research in this area of crop protection has always lagged behind achievements in related medical fields. Nevertheless it would seem that some of the advances in pharmacology can serve as useful guide-lines in the search for plant therapeutic agents as the numerous investigations using sulphonamides and antibiotics have shown. More recently, thiabendazole, originally reported as an anthelmintic,[7] has been shown to have systemic[8] antifungal activity.

Therapeutic agents for plants can be divided into two broad groups;[4] those which are directly or indirectly toxic to the fungus, i.e. systemic fungicides, and those compounds which enable the host plant, through modification of its metabolism or structure, to resist the process of pathogenesis. A potential systemic fungicide must be mobile within the plant, be non-toxic to the plant, only slowly metabolised to inactive fragments and, of course, be fungitoxic when required. Bearing these conditions in mind an investigation was carried out with simple compounds in an attempt to establish some of the specific chemical structure requirements for systemic antifungal activity. Compounds known to be fungitoxic, e.g. phenol, sulphanilic acid, were selected and modified by combination with groups generally recognised for their ability either to reduce phytotoxicity and/or promote mobility, e.g. glucose, urea, amino acids.

Experimental

Details of the tests used for direct and systemic antifungal activities will be published in due course but, in general, they followed techniques already established at Wye. For direct tests on glass slides, *Uromyces fabae*, *Puccinia recondita*, *Alternaria brassicicola* and *Botrytis fabae* were used, whilst *U. fabae*, *P. recondita*, *Erysiphe graminis* and *B. fabae* were used in tests for systemic antifungal activity. Results for *U. fabae* are quoted throughout since this organism reflects the response of the other fungi to compounds used in the direct tests and was the most sensitive organism in the systemic tests. The tests for systemic antifungal activity were conducted with cut shoots or segments of broad bean or wheat plants after initial treatment of the shoot by standing it in a solution of the compound for four hours. During the treatment period the plants were kept in growth cabinets, illumination 600 ft candles, relative humidity $< 80\%$ and temperature 20°. A qualitative assessment of uptake by and stability of the compound in plant tissue was obtained by extracting separate plants after the initial four hour treatment and chromatographic examination of the extracts.

Results and Discussion



(I) (IV)

II R = H
III R = glucosyl
V Y = NHR
VI Y = NH·CO·NHR

Phenols and phenol-β-D-glucosides

Phenolic glucosides appeared attractive compounds for study since it is possible for hydrolysis by fungal or plant glucosidases[9–17] to take place in diseased tissue releasing fungitoxic or phytotoxic aglycones. As with some naturally

* Presented at a symposium on 'Chemically induced resistance of plants to pests and diseases', organised by the Pesticides Group of the Society of Chemical Industry on 27 September, 1968.

occurring phenolic compounds, the aglycone could then be directly toxic to the fungus,[1-11] cause death of the invaded tissue localising the infection,[12-16] or take part in further reactions, e.g. oxidation, providing alternative barriers to the infection.[17] On the other hand, irrespective of reactions in connexion with the infection syndrome the less phytotoxic glucoside may be a convenient way of increasing the plant's resistance to disease by increasing its content of fungitoxic materials.

From Table I it can be seen that some of the highly substituted phenols are quite toxic *in vitro* to *U. fabae* whilst the glucosides exhibit very little fungitoxicity: this indicates inability of the fungus to bring about hydrolysis. In the tests for systemic antifungal activity no clear pattern exists for the phenols whilst glucosides of the more fungitoxic phenols are weakly active. Phenols of lower molecular weight and their glucosides appear to exist in equilibrium in both broad bean and wheat tissues, however, 2,4,5-trichlorophenol and pentachlorophenol showed little tendency to undergo rapid glycosylation and neither did their glucosides appear to be appreciably hydrolysed.

At first sight the tendency for the infection to be reduced in broad bean by glucosides of phenols with greater direct fungitoxicity would seem to indicate that the glucoside concentration in the tissue was too low to be effective therapeutically. On the other hand, treatment of broad bean plants with higher concentrations of glucoside often led to phytotoxic effects. It would seem therefore with *U. fabae* infection of broad bean tissue that this type of synthetic glucoside is unable to fulfil the rôle of many naturally occurring glucosides against pathogens of other plants.

Derivatives of sulphanilic acid

In medicine the antibacterial properties of derivatives of benzenesulphonamide have been extensively investigated.[18,19] Some derivatives have also shown promise under laboratory conditions as therapeutic agents for plants, in particular against rusts and mildews.[20] However, this group of compounds suffers from several disadvantages in so far as they are fungistatic rather than fungicidal, are steadily detoxified by plant tissues, and sometimes phytotoxic. They also tend to be selective towards obligate parasitic fungi rather than facultative organisms. Nevertheless this type of structure provided a pattern for further investigation and encouraged an investigation of simpler compounds, since many of the sulphonamides hitherto examined have been selected from compounds used in medicine.[21,22]

TABLE I

Direct and systemic antifungal activities of some substituted phenols (II) and their corresponding β-D-glucosides (III) against *Uromyces fabae*

Substituent X in II or III	Direct antifungal activity ED_{50}, $\mu g/ml^{-1}$		Systemic antifungal activity* % reduction in infection	
	Phenol	Glucoside	Phenol	Glucoside
4-OH	217	>500	8	2
4-Cl	386	>500	43	14
2,4-Cl_2	109	>500	12(P)	23
2,4,5-Cl_3	48	193	26(P)	32(P)
Cl_5	16	>500	23	48
2,3,5,6-Cl_4-4-OH	3·4	>500	0	13
Chloranil	4·1	—	0	—

* Broad bean shoots treated with solution of the compound at 100 $\mu g/ml^{-1}$ for 4 h. In cases where water solubility < 100 $\mu g/ml^{-1}$ saturated solutions were used.

(P) = Phytotoxic

TABLE II

Direct and systemic antifungal activities of some 4-aminobenzene- sulphonamides (V) and -sulphonylureas (VI), and their N^1-acetyl derivatives against *Uromyces fabae*

Substituent R in V or VI	Direct antifungal activity ED_{50}, $\mu g/ml^{-1}$		Systemic antifungal activity* % reduction in infection	
	4-amino compounds (X = NH_2)	4-acetamido compounds (X = $NH{\cdot}CO{\cdot}CH_3$)	4-amino compounds (X = NH_2)	4-acetamido compounds (X = $NH{\cdot}CO{\cdot}CH_3$)
V, H	>500	>500	100	—**
V, Me	>500	>500	64	51
VI, H	281	152	100	15
VI, Me	>500	193	100	45
Sulphanilic acid	49	—	73	—

* Broad bean shoots treated with solution of compound at 100 $\mu g/ml^{-1}$ for 4 h

** 98% at 250 $\mu g/ml^{-1}$

From Table II it can be seen that *p*-aminobenzenesulphonamide and *p*-aminobenzenesulphonylurea, as well as their *N*-methyl and N^4-acetyl derivatives, showed little antifungal activity in *in vitro* tests. However, both 4-aminobenzenesulphonamides and -sulphonylureas showed appreciable systemic antifungal activity whilst their N^4-acetyl derivatives were less effective. Table III shows the range of antifungal activity of sulphanilic acid, 4-aminobenzene- sulphonamide and -sulphonylurea in the direct and systemic antifungal tests. 4-Aminobenzenesulphonylurea was more toxic than the other two compounds against three of the fungi used in systemic fungicide tests, but none had any inhibitory effect on *B. fabae*.

Over the 4 h treatment period there was no indication that the 4-aminobenzene- sulphonamides or -sulphonylureas underwent acetylation in the shoot or that their N^4-acetyl underwent de-acetylation as has been demonstrated following longer periods of treatment.[26] The possible conversion of the sulphonylurea to sulphonamide has not been investigated but the therapeutic activity of N^1-methyl 4-aminobenzenesulphonylurea (Table II), which would be expected to be less easily hydrolysed in plant tissue, seems to indicate that activity is independent of such a conversion.

The direct and systemic antifungal activities of some other nuclear-substituted benzene- sulphonamides and -sulphonylureas are presented in Table IV. Excluding 4-nitro substituted compounds the benzene sulphonamides showed little *in vitro* fungitoxicity, whilst the fungitoxicity of the corresponding benzenesulphonylureas tended to increase with increasing electronegativity of the nuclear substituent. Compared with 4-aminobenzene- sulphonamide and -sulphonylurea these compounds showed no systemic antifungal activity. 4-Nitrobenzene- sulphonamide and -sulphonylurea, however, were fairly active in the systemic fungicide test and were similar to the 4-amino compounds in the direct test. Investigation of extracts of plants treated with both 4-nitrobenzene- sulphonamide and -sulphonylurea revealed the presence of the corresponding 4-amino compounds. Since the ability of plant and animal tissues to reduce the nitro group to amino is well known[27] it would seem that the systemic antifungal activity of 4-nitrobenzene- sulphonamide and -sulphonylurea is realised by conversion to the corresponding 4-amino analogues. The inability of other 4-substituted compounds to control *U. fabae* infection of broad bean highlights the importance of the 4-amino group.

In contrast Table V contains data on some amino acid derivatives of sulphanilic acid and their corresponding N^4-acetyl analogues. Both the 4-amino and 4-acetamido compounds have moderate and comparable antifungal activity *in vitro*. Since it is generally recognised that acetylated derivatives of sulphanilamides are less fungitoxic it would seem that the acidic nature of these compounds partly accounts for their activity. Even at high concentrations this group of compounds exhibited no systemic antifungal activity in contrast to their moderate antifungal activity *in vitro*.

TABLE III

Direct and systemic antifungal activities of sulphanilic acid (IV, X = NH_2, Y = OH), 4-aminobenzenesulphonamide (IV, X = NH_2, Y = NH_2) and 4-aminobenzenesulphonylurea (VI, X = NH_2, R = H) against various fungi

Fungus	Sulphanilic acid	4-Aminobenzenesulphonamide	4-Aminobenzenesulphonylurea
Direct antifungal activity, ED_{50}, $\mu g/ml^{-1}$			
Uromyces fabae	49	> 500	281
Puccinia recondita	26	> 500	> 500
Alternaria brassicicola	27	> 500	> 500
Botrytis fabae	188	> 500	> 500
Systemic antifungal activity,* % reduction in infection			
Uromyces fabae	73	100	100
Puccinia recondita	67	23***	100**
Erysiphe graminis	0	16***	90**
Botrytis fabae	0	0	0

* Plant shoots treated with solutions of the compound at 100 $\mu g/ml^{-1}$ (**250 $\mu g/ml^{-1}$, ***500 $\mu g/ml^{-1}$) for 4 h

TABLE IV

Direct and systemic antifungal activities of some nuclear-substituted benzene- sulphonamides (V) and -sulphonylureas (VI) against *Uromyces fabae*

Substituent X in V or VI	Direct antifungal activity ED_{50}, $\mu g/ml^{-1}$		Systemic antifungal activity,* % reduction in infection	
	Sulphonamide (V)	Sulphonylurea (VI)	Sulphonamide (V)	Sulphonylurea (VI)
OMe	> 500	124	0	2
Me	> 500	41	6 (P)	11 (P)
H	> 500	59	0	1
Cl	373	16	11	54
NO_2	> 500	178	80	76

* Broad bean shoots treated with solution of the compound at 100 $\mu g/ml^{-1}$ for 4 h
(P) Phytotoxic

TABLE V

Direct and systemic antifungal activities of some *N*-(4-aminobenzenesulphonyl)- substituted amino acids (IV) and their N^4-acetyl derivatives against *Uromyces fabae*

Grouping Y in IV	Direct antifungal activity, ED_{50}, $\mu g/ml^{-1}$		Systemic antifungal activity,* % reduction in infection	
	4-amino compounds (X = NH_2)	4-acetamido compounds (X = $NH \cdot CO \cdot CH_3$)	4-amino compounds (X = NH_2)	4-acetamido compounds (X = $NH \cdot CO \cdot CH_3$)
Glycine	37	58	0	0**
Alanine	50	88	7	7
Aspartic acid	17	32	0	26 (P)**
Glutamic acid	19	22	24	5

* Broad bean shoots treated with solution of the compound at 500 $\mu g/ml^{-1}$ (or **1000 ug/ml^{-1})
(P) = Phytotoxic

This group of compounds showed no tendency to be rapidly metabolised by plant tissue in comparison with *N*-acyl derivatives of amino acids, e.g. *N*-(2,4-dichlorophenoxyacetyl)-glycine which can be hydrolysed by plants to yield the parent acid. It would appear, therefore, that any advantage to be gained from greater solubility in water is offset by other characteristics of the substituent which do not permit therapeutic activity with or without intervention of the host or fungus.

Conclusion

Modification of *in vitro* fungitoxic molecules by combination with polar groupings which lead to increased solubility in water and/or decreased phytotoxicity does not necessarily lead to substances with therapeutic properties.

Systemic antifungal activity is unrelated to direct antifungal activity, possibly indicating the rôle of the plant in the action of the former.

Attention should be focused on host–parasite–chemical interactions rather than on separate host–chemical and parasite–chemical interactions.

The investigation, of course, is far removed from the practical problem of disease control in crop plants because the need for information has necessitated the use of the simplest standard conditions. Many facets remain unexplored. Shoots were used rather than intact plants—consequently the plant's metabolism was upset; the problem of uptake through roots and soil application was avoided; persistence and the status of the chemical at the stage when the fungus actually enters the plant were not considered. Such aspects of the problem are of great importance and they cannot be ignored if a complete assessment of the factors underlying therapeutic activity is to be made.

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References

1. Pluijgers, C. W., 'Direct and systemic antifungal action of dithiocarbamic acid derivatives'. Thesis; (Schotanus & Jens: Utrecht) 1959
2. Kaars Sijpesteijn, A. *Tijdschr PlZiekt*, 1961, **67,** 11
3. Wain, R. L., & Carter, G. A., in 'Fungicides: an advanced treatise'. (Ed. Torgeson, D. C.), 1967, Vol. 1, p. 561 (Academic Press: New York)
4. Dimond, A. E., *Adv. Pest. Control Res.*, 1967, **6,** 127
5. Erwin, D. C., Mee, H., & Sims, J. J., *Phytopathology*, 1968, **58,** 528
6. von Schmeling, B., & Kulka, M., *Science, N.Y.*, 1966, **152,** 659
7. Brown, H. D., Matzuk, A. R., Ilves, I. R., Peterson, L. H., Harris, S. A., Sarett, L. H., Edgerton, J. R., Yakstis, J. J., Campbell, W. C., & Cuckler, A. C., *J. Am. chem. Soc.*, 1961, **83,** 1764
8. Erwin, D. C., Sims, J. J., & Partridge, J., *Phytopathology*, 1968, **58,** 860
9. Molot, P., *C.r. hebd. Séanc Acad. Sci., Paris*, D. 1967, **264,** 1724
10. Oku, H., *Ann. Rep. Takamine Lab.*, 1959, **11,** 190
11. Siebs, E., *Phytopath. Z.*, 1955, **23,** 37
12. Elnaghy, M. A., & Linko, P., *Physiologia Pl.*, 1962, **15,** 764
13. Hildebrand, D. C., & Schroth, M. N., *Phytopathology*, 1964, **54,** 640
14. Holowczak, J., Kuć, J., & Williams, E. B., *Phytopathology*, 1960, **50,** 640
15. Noveroske, R. L., Kuć, J., & Williams, E. B., *Phytopathology*, 1964, **54,** 92, 98
16. Schroth, M. N., & Hildebrand, D. C., *Phytopathology*, 1964, **54,** 906
17. Davis, D., Waggoner, P. E., & Dimond, A. E., *Nature, Lond.*, 1953, **172,** 959
18. Northey, E. H., 'Sulphonamides and allied compounds', 1948, (Reinhold: New York)
19. Zbinden, G., 'Drug Design'. *Adv. Chem. Ser.*, 1964, **45,** 25
20. Rudd Jones, D., *Outl. Agric.*, 1956, **1,** 111
21. Crowdy, S. H., Elias, R. S., & Rudd Jones, D., *Ann. appl. Biol.*, 1958, **46,** 149
22. Hassebrauk, K., *Phytopath. Z.*, 1951, **17,** 384
23. Hotson, H. H., *Phytopathology*, 1953, **43,** 659
24. Livingston, J. E., *Phytopathology*, 1953, **43,** 496
25. Mitchell, J. E., Hotson, H. H., & Bell, F. H., *Phytopathology*, 1950, **40,** 873
26. Crowdy, S. H., & Rudd Jones, D., *J. exp. Bot.*, 1958, **9,** 220
27. McKee, H. S., 'Nitrogen metabolism in plants', 1962, (Clarenden Press: Oxford)

ASSESSING THE RESPONSE OF FOREST TREES TO FERTILISERS*

By W. H. JACK

Some of the problems of statistical design of fertiliser experiments with forest plots are described, and the significance of apparently anomalous results from the customary methods of measuring growth of forest trees is discussed.

Introduction

The main difference between a forest and other crops is that trees are long-lived. It is this feature which governs the design of forest fertiliser trials. This paper will deal with those aspects of forestry which are practised in the field, and will not consider pot experiments on individual trees.

The principal object of any fertiliser experiment must be to determine if the treatment is economically worth while. Because of the long-term nature of forestry this poses the problem of what interest rate should be used. Thus, at 6% compound interest, fertilising costing £10 per acre 50 years before a crop is felled must produce an added value of £184 to be worth while, whereas the same treatment over the same time period at only 3% would require an added value of only £44. An improved financial return from a tree crop may be based on the production of more wood, on greater diameter growth because of the effect of price gradient which tends to increase as size increases, and the reaching of a particular size in a shorter period of time.

Spacing and size of plots

While American, Australian and South African practice often involves planting tree seedlings some 12 ft apart, in Europe spacings of between 3 and 7 ft are more common. Not allowing for drains, roads, etc., the closer spacing is equivalent to just under 5,000 plants per acre, and the wider spacing, to just under 1,000 plants per acre. Therefore, for a given amount of fertiliser per acre, individual trees will have access to very different quantities depending on their initial spacing. On the same theme, an important point is the amount of root spread. In Northern Ireland it has been found that the root radius of Sitka spruce on deep peat is frequently twice the height of the tree up to a maximum radius of 20 ft, suggesting that interaction between experimental plots is possible unless the plot surrounds are greater than 20 ft. An apparent anomaly has occurred in some of the experiments laid down in 1961 without such plot boundaries. The trees which had a good initial start from the fertiliser have stayed the biggest and now appear to be feeding on the poorer ground nearby at the expense of the trees which did not have a good start.

Within a plot it is preferable to measure about 25 individual trees to obtain a reasonable estimate of the mean. At 6 ft spacing and with a 20 ft surround each plot would need about one-ninth of an acre and therefore a total of 9 acres would be required for a $3 \times 3 \times 3$ factorial experiment with 3 replicates. A compromise is usually adopted and it has been found that a satisfactory size of plot is one of 7 trees $\times$ 7 trees (about one-thirtieth of an acre) with spiral numbering of the trees and separate recording of each, so that boundary effects can be determined. Even with these small plots the effect of soil variation can still be extremely high, and trials using various provenances of a number of species in Latin square layouts have shown highly significant between-row and between column differences even where sites were carefully selected.

Within a 50 acre block it has been possible to assess exposure using the technique of measuring the loss by tattering of flags. This has indicated, on the basis of other research using the same methods, that the height of 5 year-old trees with the standard fertiliser regime would be likely to vary between 30 in and 60 in. It is therefore necessary to minimise as far as possible the exposure effects within blocks in an experiment. As no definite relationship between fertiliser response and exposure has been investigated, replicates in both exposed and sheltered sites are being set out in an attempt to determine what interaction, if any, is to be found. To minimise variability it is common practice to use plants from one seed source, the same age of plant, and the same method planting, in an experiment. There are, however, suggestions that different provenances of the same species may react differently in certain circumstances, and this feature is also being investigated. As weather affects the establishment and growth of the young trees soon after planting, replication over time is also to be recommended as well as replication within any planting season.

Significance of customary measurements

With young trees the effect of fertiliser is usually assessed by measuring the growth of the leading shoot, largely because length is the easiest variable to measure. Growth in fertilised plots is often expressed as a percentage of the growth in control (unfertilised) plots but if the trees have gone into 'check' (growth retardation) an improvement of 300% is possible but may, however, be quite meaningless in economic terms.

* Presented at a meeting of the Agriculture Group, 21 November, 1967

Growth of these young plantations is generally given in terms of the mean height. Within a stand individuals tend to become dominant either because they have been more fortunate in their nursery treatment or in their very local site variation or because they are from better parents. When certain experiments have been analysed to determine the response to fertiliser treatment of both overall averages and averages of the dominant trees, it has generally been found that at 10 years of age the dominant trees in the best treatment are only some 12 in better than the dominant trees in the poorest treatment, although the general means of these two treatments can differ by as much as 3 ft.

Continuous thinning as the trees get older is a fairly common forestry practice. During this operation different trees within the general hierarchy of trees in the stand can be removed, but in general the better dominants are untouched and so it is desirable to use the height of a few dominants (currently 40 per acre in the U.K.) as the index of height. It is perhaps useful at this stage to give further consideration to the value of height as a predictor of subsequent growth. From studies in the U.K. a general relationship between the height of a few dominants per acre and the total volume production per unit area has been obtained for each species. This is a general relationship, and local variations can be quite large. To allow for this, the British Forestry Commission have introduced the concept of production classes allowing for quite considerable variation from the general line. Regional differences can be considerable: for example, in Northern Ireland, Japanese larch is usually one production class above the U.K. mean. The effect of site in its widest sense is usually expressed by a group of curves of dominant height *versus* age. These again are derived from average figures based on a number of repeatedly measured plots. In assessing the response to fertilisers there is obviously a considerable difference in total production depending on whether the height growth continues along the new curve, whether it merely goes up for a few years and then remains parallel to the old curve, or whether it goes up and then rejoins the original curve with all other parameters such as total crop yield remaining similar to those of the general curve. In general all these tables of expected growth have been derived from stands of trees which have been thinned.

Once the crop has got beyond the initial establishment stage it is necessary to consider the individual tree as there are frequently only some 150 stems per acre left at time of final felling. A tree is generally an extremely awkward shape to measure; although it has been described as part parabolic, part rectilinear, and part neiloid (semi-cubic parabola) in outline rotated about an axis to give its volume, this still gives only a very rough approximation to the volume of the individual tree itself, and the coefficients of the mathematical functions differ from tree to tree. For various reasons many trees grow with a curved stem or with an elliptical, rather than a circular, cross-section; or they may even, like older Sitka spruce, develop peculiar buttressing at the bottom of the tree. All this has led the practising forester to make use of general volume tables which are based on the easily measured parameters of the total height of the tree and the diameter at 4 ft 3 in ('breast height') above ground level. While these can be quite satisfactory for general use they cannot be considered very good tools for determining responses to fertiliser. In some circumstances, therefore, detailed measurements on individual trees have been related by means of regression analysis to the diameters, at 4 ft 3 in, of all the trees in a plot. As these regression lines are usually based on a very small number of trees (e.g. 10) they cannot be considered to be very reliable either. It is found that the greatest diameter growth normally occurs near the base of the live crown. As a result of this the size and shape of the crown has a very strong influence on where the greatest ring width and, following this, the greatest volume, will be gained by any individual. A single measuring point can therefore be misleading. Examples have been obtained of fertilised trees appearing from diameter measurements to be growing less than unfertilised trees because the fertilised trees grew fastest at one stage in their development and then, after the canopy closed, put on their main growth at some distance above ground level; meanwhile the trees in the unfertilised plot were putting on their maximum growth near the measurement point.

Thinning also influences a stand of trees. In general it has been found that within fairly wide limits thinnings have no effect on the stand volume production. However, a more detailed investigation of 132 individual trees which were sampled in an area that had been thinned once during the last 5 years showed that only half of these trees had been 'released' by the thinning while the other half had none of their immediate neighbours removed during thinning operations. The trees which had had some of their neighbours removed during the last five years had also appeared to grow differently in the intervening period from those which had not received any release at all.

Trees can be uniquely identified and can be easily sampled with probability proportional to size factor. By looking at the closeness of the neighbours to any sample tree and also the size of the neighbours it is possible to determine important parameters of competition affecting the growth of that individual. An investigation is being undertaken to determine in greater detail the effect of removing competing trees. The better these relationships are understood the easier will it be to assess results from fertiliser trials on older plantations.

Conclusion

Even apparently short-term experiments have long-term implications and must be designed with these in mind. The large areas of ground which must be covered in any one experiment and the many factors whose effects still remain unknown or uncertain require the best statistical designs. While it is possible to obtain statistical significance which is of no practical importance in the field, it is also true that differences should be ignored unless found to be statistically significant. So many factors have an influence on the growth of any individual tree over the whole of its lifetime that techniques such as those of covariance, multivariate regression analysis, and time series can be extremely valuable in reducing the variance of experimental treatments imposed at one time or different times in the life of this tree. The more that can be learned about the basic competition between trees the easier it will be to determine true fertiliser effects. By collecting information at the basic level of individual trees rather than averages to which general tables are applied, it should be possible to make better use of simulation models to help in economic analysis of the results of fertiliser and other trials.

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FERTILISER RESEARCH IN SCOTTISH NURSERIES AND FORESTS*

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The use of fertilisers, particularly slow-release nitrogen fertilisers, in forest nurseries is discussed. In forests, trials have shown unforeseen interactions between applied fertilisers and unforeseen effects of different constituents of particular fertilisers. Future requirements of phosphate rock, phosphate-potash, and urea, the main fertilisers used, are considered.

Fertiliser research in forest nurseries

Forest nurseries are generally situated on freely draining sandy acid soils where nutrient retention is poor. Annual application of fertiliser is essential and, if soluble forms are used, several dressings must be given throughout the growing season. Research carried out in Scotland during the 1950s to find the correct fertiliser regimes is summarised by Faulkner[1] who worked mainly with soluble forms of N, P and K. He found that P and K could be applied before sowing or transplanting without deficiencies occurring at the end of that growing season, but that with nitrogen, top-dressings during summer and autumn were necessary.

Since 1959 research has been concentrated on finding a source of nitrogen which would last in the soil for a complete growing season, and the following substances have been tried: urea–formaldehyde, in many forms including 'Nitroform' and plastic waste; thiourea; sulphur-coated ammonium sulphate; sulphur-coated urea; latex-coated granules of soluble NPK fertiliser; linseed oil-treated granules of soluble NPK fertiliser; isobutylidene diurea; and magnesium ammonium phosphate. None of the above substances showed much promise except the last; this proved to be not only a long-lasting source of nitrogen, but also a valuable source of magnesium—an element which was becoming more and more deficient in acid soils owing to the continuous cropping and removal of whole plants, i.e. including roots. Most of the above substances were provided by Scottish Agricultural Industries Ltd., who now produce a magnesium ammonium phosphate-sulphate of potash mixture known as 'Enmag' which has been found to be a good basal fertiliser supplying all the essential major nutrients required by young trees. Unfortunately, this fertiliser supplies too much phosphorus and magnesium when it is applied at the rate required to give the correct quantity of nitrogen, and this may lead to a build-up of P and Mg in the soil. One way in which the NPK ratio of applied nutrients could be improved when 'Enmag' is used would be to add another slow-release nitrogen fertiliser to 'Enmag'. Sulphur-coated urea, isobutylidene diurea, and urea-formaldehyde were tried in this way, but without success. The search for another slow-release nitrogen fertiliser is still continuing. Details of this work on slow-release fertilisers are available in recent reports of the research carried out by the Forestry Commission.[2]

Forest fertiliser research

In Scotland forest fertiliser research began in Forestry Commission forests in 1925 with trials on peat at Fort Augustus and heathland in N.E. Scotland; these trials proved that phosphate was essential for adequate tree growth on such sites, and that no other nutrient was limiting in the initial stages of plantation growth on the sites afforested at that time. Most of the subsequent fertiliser research was, therefore, concerned with testing different types of phosphate on a wide variety of sites at different rates, methods and times of application throughout the rotation. This research is continuing. For example, in recent experiments four forms of rock phosphate, namely ground phosphate rock from Gafsa in N. Africa, from the Kola Peninsula in N.W. Russia and from Nauru Island in the Pacific, and also unground Gafsa phosphate rock, have been compared. Ground Gafsa rock has been the type normally used and was applied by hand, but it was found unsuitable for machine application. Unground Gafsa can be spread by machine, but it was thought it might be less effective because of its larger particle size. Kola and Nauru rocks have higher phosphate contents than Gafsa, but contain less citric acid-soluble phosphate so that they too may be less effective than ground Gafsa; this applies particularly to Kola rock. Preliminary results indicate that all these rocks are equally effective on acid peat.

Another aspect of research is the possible importance of calcium for tree growth on acid peats. In the past, liming experiments were designed to see if altering the pH value of acid peat had any influence on growth of coniferous trees, and rates of up to 4 tons of limestone per acre were added. There was no apparent effect on trees grown on the peat, and it was realised that control plots which received no limestone did, however, receive calcium in the basal phosphate dressing, as calcium phosphate. It was not until potassium metaphosphate was tested as a source of phosphate that calcium was shown to be important on such sites. All of the elements contained in a fertiliser have to be considered. For example, comparisons made between potassium metaphosphate and potassic superphosphate showed that both materials increased the P and K concentrations in the needles of Lodgepole pine and Sitka spruce while neither altered the calcium concentration. When the potassic superphosphate was applied the nitrogen concentration of the needles of both species increased markedly, in some cases doubling, but this did not happen when metaphosphate was applied. This could have been caused by the sulphur in the superphosphate, but as rock phosphate produces a similar increase in nitrogen in tree foliage and does not contain sulphur, this element

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TABLE I

Fertiliser consumption in Forestry Commission forests in Scotland

Tons fertiliser per year

	Actual			Estimate		Maximum after normality
	1960	1963	1966	1969	1975	
Phosphate rock	650	1000	1500	3500	7500	4500
Phosphate + potash (20 : 20)	0	120	450	600	1000	7000
Nitrogen (urea)	0	0	45	1000	2000	9000

cannot be essential in this case. Presumably the effect is directly or indirectly attributable to calcium in the superphosphate, and experiments are being designed to establish the minimum quantity of calcium necessary to produce this effect on a variety of peat types. In other experiments magnesium is being substituted for calcium to determine whether the calcium is acting as a nutrient or is locally increasing the pH.

The availability of nitrogen on such sites or the ability to utilise it seems to differ so much between tree species, that on many peats Lodgepole pine can be grown satisfactorily without added nitrogen whereas Sitka spruce cannot. As the spruce is a potentially higher yielding species than the pine, it would be profitable if an economic way of providing sufficient nitrogen for the spruce could be found. It is known that on most peat soils, spruce can be made to grow very well if sufficient nitrogen is added at intervals of 3–4 years, but the applications have not been continued over a sufficiently long period of time for an economic crop to be certain. Theoretical calculations, however, indicate that if assumptions regarding the yield classes obtainable using correct NPK dressings are correct, it is better economically to grow spruce than pine. It may be possible to obtain high yields of spruce, but on the other hand, if pine is planted, the maximum yield obtainable is low and the net discounted revenue is small or even negative.

Nitrogen is being used on a large scale in older tree stands in Scandinavia, and economic returns from such dressings have been found. Hagner described this work to the Fertiliser Society.[3] Some trials have been started in Scotland in pole-stage and older crops on mineral soils using an NPK compound fertiliser. Responses which have been obtained on Scots pine appear to be due mainly to nitrogen, but phosphate may also be contributing on some sites. Potash does not seem to be necessary on such sites. It is too soon to say whether the response obtained will be economic except on the sands and gravels of the N.E. coastal plain, of which the Culbin Sands is the prime example.

Of the other nutrients, potassium is known to be limiting sooner or later on most acid peats 2 ft or more in depth, and routine dressings of 100 lb K_2O/acre are given when symptoms of the deficiency become apparent. Magnesium deficiency has only been noticed on one or two very poor peat sites so far and only a colour difference was found between trees given, and trees not given, magnesium. No trace element deficiencies have so far been found. Nevertheless, it is possible that, if increasing use is made of N, P and K on the deeper peats, magnesium and/or trace element deficiencies may be induced either directly by the fertilisers applied or by the faster growth obtained.

Consumption of fertilisers by the Forestry Commission in Scotland

In Scotland the Forestry Commission has about 660 acres of nursery and the total consumption of fertiliser on this relatively small acreage is not of great importance, but the types of fertiliser in use may be of interest.

The main basal fertilisers in use are potassic superphosphate and 'Enmag', and those used for top-dressing during the summer are ammonium sulphate, ammonium nitrate–calcium carbonate mixtures, and nitrogen–potash mixtures. The types of nitrogen–potash mixtures available are limited and the choice could with advantage to the nurseryman be widened. Because techniques in nursery management here already improved, it is unlikely that fertiliser consumption in forest nurseries will increase even though planting programmes are increasing.

In Forestry Commission forests in Scotland, the main fertilisers used at present are rock phosphate at the time of planting and phosphate–potash mixtures for later top-dressing of trees on deep peat. It is possible that, if the planting of pure spruce in place of pine or pine–spruce mixtures on poor peat gains momentum, nitrogen will be used in relatively large quantities in the not-too-distant future. Table I gives some approximate figures for past, present and possible future consumption of fertilisers in Forestry Commission forests in Scotland, and is intended to show trends only.

In the Table, 'normality' is meant to indicate consumption after large planting programmes are completed and the 'back-log' of phosphating has been made good.

The increase in use of phosphate rock will be due partly to an increase in the rate applied per acre from 2 to 3 cwt because of a probable increase in broadcasting by machine. Also future programmes envisage increased planting, and also top-dressing of areas which should have received phosphate at planting but did not because phosphate was not considered essential on such sites until recently.

The large increase in nitrogen consumption envisaged may not take place as it largely depends on the change from pine to spruce on poor peat; some nitrogen will undoubtedly be used for top-dressing slow-growing stands on mineral soil.

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References

1. Faulkner, R., *Rep. For. Res. For. Comm., Lond.*, 1959, 1960, p. 126 and 141
2. *Rep. For. Res. For. Comm., Lond.*, 1960–1967
3. Hagner, S., *Proc. Fertil. Soc., Lond.*, 1966, **94**, 38

NITROGEN NUTRITION OF PINES ON THE SANDS OF CULBIN FOREST, MORAYSHIRE*

By H. G. MILLER

Although conifers usually seem able to obtain sufficient nitrogen for establishment, it was shown that 36 year-old Corsican pine on infertile sands responded to application of ammonium sulphate at rates greater than 84 kg N/ha by greatly increased height growth and diameter growth. After canopy closure, increasing amounts of nitrogen became immobilised in the litter, so that nitrogen deficiency arose less from increased demand for nitrogen by the tree than from a decrease in the available supplies.

Introduction

Fertilisers have long been used in British forests to stimulate the growth of young trees. On most poor hill sites and on peatlands phosphate is applied as a routine at the time of planting; indeed without such an application successful establishment of a tree crop may prove impossible. It is now also realised that many forests on peat are liable to suffer from a deficiency of potassium, and the application of this element in the first decade after planting is becoming increasingly common. The use of nitrogen fertilisers, however, has been less usual. Applications of nitrogen to young crops on typical forest land have occasionally given limited growth increases, but only when applied together with phosphate,[1] and in general it seems that there are few sites in Britain, except where heather checks spruce, that cannot supply sufficient available nitrogen to enable at least the establishment of a tree crop. However it is only now, as the early Forestry Commission plantings on poor land approach middle age, that interest is being shown in the nutrition of older crops. Evidence from mature managed forests in many other parts of the world suggests that this is the stage at which nitrogen deficiency may occur.[2–7]

In recent years a study of the nitrogen nutrition of coniferous trees has been carried out at Culbin Forest, which is atypical in that nitrogen deficiency is the rule rather than the exception, since the trees have been planted on wind-blown sand dunes very low in reserves of organic matter and nitrogen. Two broad degrees of severity of the deficiency can be recognised in this forest. On the worst areas the planted Scots and Corsican pines exhibited nitrogen deficiency from the outset, while, over much of the forest, growth during the establishment phase was fairly rapid, but the deficiency has developed and become progressively severe following canopy closure.

Response to nitrogen fertilisers

On the poorest areas, which represent only a small portion of the forest, Corsican pine became chlorotic soon after being planted and though survival has been good the trees are making very little growth, only about 5 to 10 cm in height per year. These trees were found to have a foliar nitrogen concentration of 0·7% and in pot trials, with sand taken from the forest, Corsican pine seedlings showed acute nitrogen deficiency. Accordingly a series of experiments were laid out in 1961 to study the effect of nitrogen, applied as 'Nitrochalk', on young trees at various rates up to 195 kg N/ha, a number of the treatments being repeated annually. The initial response was marked and height growth and diameter growth more than doubled in those plots given repeated heavy rates. Growth in any one year was found to be significantly correlated with the concentration of nitrogen in the foliage at the previous autumn, which was raised to as high as 1·2%. This level, however, is still below that considered to be optimum.

The success of these experiments stimulated interest in older crops in the forest that were showing signs of nitrogen deficiency. In these the canopy had closed and decaying litter and humus were accumulating on the forest floor. One such crop was a stand of 36 year-old Corsican pine the needles on which were short, yellow-green in colour, and contained only 0·9% nitrogen. Growth of these trees had become very slow despite rapid early establishment. In 1964 an experiment was laid out to investigate the effect of applications of ammonium sulphate at rates of 84, 168, 336 and 504 kg N/ha per year, the higher rates to be given as split dressings at intervals during the growing season. After three years the heaviest treatment was found to have raised the concentration of nitrogen in the top-whorl needles to 2·6%, and over the same period the basal area increment of these trees was 5·8 m^2/ha as against 2·8 m^2/ha in the untreated control plots. Growth in the control plots is below that given for the lowest Yield Class (80) of this species in the Forestry Commission Management Tables[8] whereas growth rates in all the treated plots have exceeded those given for the highest Yield Class (220). The stimulation of growth by nitrogen applications, therefore, has been considerable.

Development of nitrogen deficiency after establishment

The question remains as to how trees that were increasing in height by 45 cm per year prior to and during canopy closure could have reached a state of virtual stagnation some 15 to 20 years later. The fact that this condition can be rapidly and effectively alleviated by the application of nitrogen suggests that somehow the demand for available nitrogen must have increased beyond the rate of supply or that the supply must have decreased. The rate of nitrogen uptake by the tree would increase progressively during the establishment stage, but subsequent to canopy closure it can be assumed that the weight per unit area of crown and

* Presented at a meeting of the Agriculture Group, 21 November, 1967

root tissues would remain fairly constant, the formation of new tissue being largely balanced by the death of old (though there would be a slow increase in the weight of major roots). At this stage the main increase in nitrogen uptake would be that required to produce new stem wood and bark, which is periodically harvested and removed from the forest. Table I shows the distribution of nutrients in the tree crop at the time immediately prior to the first applications of fertilisers. With the exception of calcium the nutrients in the stem represent a fairly low proportion of the total incorporated in the trees. For nitrogen the stem accounts for 33% which is equivalent to a mean annual incorporation of 1·6 kg/ha, though when thinnings already removed are taken into account this figure becomes 2·1 kg/ha.

Thus, subsequent to canopy closure the net drain of nitrogen from the mineral soil is fairly low in relation to the quantity it contains, which was found to be 701 kg/ha to a depth of one metre or 65·5% of the total amount of nitrogen in the ecosystem. However, this assumes that the nitrogen contained in the dead and discarded portions of the crowns and roots is rapidly made available for the formation of their replacements and that the only net immobilisation is that within the actual trees. That this is not the case can be seen from Table II in which the weights of nutrients contained within the organic layers on the forest floor are given. Nitrogen has accumulated on the forest floor to a greater extent than in the living crop, the forest floor representing 18·0% of the nitrogen in the ecosystem as against 16·5% in the trees, whereas the reverse is the case with the mineral nutrients.

The extent of this immobilisation can be further illustrated by comparing the concentration of nutrients in the waste organic matter on the forest floor with that in the freshly fallen litter collected at monthly intervals over a thirty month period in the control plots (Table III). Whereas the concentration of the mineral elements is lower in the decomposing litter than in the falling leaves, that of nitrogen is higher. In part this may reflect the decrease of nitrogen concentration in freshly fallen litter that followed initiation of deficiency conditions within the trees, but this is insufficient to explain the extent of the observed difference which must be due largely to a marked resistance of nitrogen-rich portions of the litter to decay.

Thus, because of a very low rate of mineralisation much of the nitrogen incorporated into the crown components of the tree must be regarded as a net removal from the nitrogen cycle within the forest. It follows that the onset of nitrogen deficiency in this stand is due less to an increasing demand by the tree crop than to a decrease in the rate of supply resulting from the trees rendering much of the nitrogen that passes through them incapable of re-entering the cycle. This nitrogen accumulates in the waste organic matter on the forest floor, although the mineral elements in this layer, with the possible exception of phosphorus, appear to be released sufficiently rapidly to cause little concern.

TABLE I

Weight of nutrients contained in a 36 year-old plantation of Corsican pine, kg/ha

	N	P	K	Ca	Mg
Crowns	86	10	54	69	16
Stems	58	6	38	62	15
Roots	32	11	28	9	12
Total	176	27	120	140	43

TABLE II

Weight of nutrients contained in the organic layers beneath a 36 year-old plantation of Corsican pine, kg/ha

	N	P	K	Ca	Mg
Litter	37	4	5	26	5
Humus	155	12	11	29	12
Total	192	16	16	55	17

TABLE III

Mean concentration of nutrients in existing organic material on the forest floor, and in freshly fallen litter collected between May, 1964 and November, 1966, % dry weight

	N	P	K	Ca	Mg
Freshly fallen litter	0·42	0·08	0·13	0·53	0·12
Organic layers on forest floor	0·66	0·05	0·05	0·15	0·05

Conclusions

The problem of immobilisation of nitrogen in mor humus layers derived from coniferous trees is one which has been recognised for some time, particularly in Scandinavia and North America,[3,9–11] though its causes are not clear and are undoubtedly complex. However, the occurrence of the deficiency at Culbin shows that this form of biologically induced nitrogen deficiency leading to progressive site degradation is not unique to natural spruce woods of extreme northern latitudes; the processes involved are equally operative in artificial plantations in Scotland, even in relatively mild climatic conditions such as those prevailing on the Moray Firth. The nitrogen capital at Culbin was found to be low, only about 1,070 kg/ha, and hence nitrogen deficiency appeared at a very early stage in the history of the trees on this site. However there is no reason to suppose that other coniferous plantations on poor sites in Scotland will not ultimately suffer from such an induced nitrogen deficiency provided that the mor humus continues to accumulate undisturbed until a significant proportion of the nitrogen capital of the site becomes fixed within this layer. Indeed such deficiencies have already been diagnosed in a number of the few existing mature stands of coniferous trees on poor land, as for example at Alltcailleach forest in Aberdeenshire, where mature 80 year-old Scots pine has shown an economic response to nitrogen fertilisation.[1,12] It can be expected, therefore, that nitrogen fertilisation, though practised little at present, will become increasingly common as new forests pass middle age or are succeeded by subsequent rotations of coniferous species on the same sites.

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References

1. Craig, J. B., & Miller, H. G., *Rep. For. Res., Lond.*, 1967, 1966; 94
2. Gessel, S. P., & Shareeff, A., *Proc. Soil Sci. Soc. Am.*, 1957, **21 (2),** 236
3. Weetman, G. F., *Woodld. Res. Index No. 129*, 1962, pp. 112
4. Stone, E. L., & Will, G. M., In 'Forest soil relationships in North America', 1965, pp. 117 (ed. C. T. Youngberg) (Oregon State University Press)
5. Viro, P. J., *Meddr norske SkogsforsVes.*, 1967, **23 (85),** 113
6. Kreutzer, K., *Fortwiss. ZentBl.*, 1967, **86 (1),** 28
7. Kreutzer, K., *Fortwiss. ZentBl.*, 1967, **86 (3),** 176
8. Bradley, R. T., Christie, J. M., & Johnston, D. R., Forest Management Tables, *Bookl. For. Commn.*, 1966, No. 16
9. Tamm, C. O., Carbonnier, C., & Hagberg, N., *Proc. Fifth Wld. For. Congr.*, 1960, **1,** 558
10. Weetman, G. F., & Nykvist, N. B., *Woodld. Res. Index No. 139*, 1963, pp. 15
11. Heilman, P. E., *Ecology*, 1966, **47 (5),** 825
12. Binns, W. O., & Grayson, A. J., *Scott. For.*, 1967, **21 (2),** 81

UPTAKE OF NUTRIENTS FOLLOWING FERTILISATION OF SITKA SPRUCE ON DEEP PEAT IN NORTHERN IRELAND*

By D. A. DICKSON

The effects are described of broadcast application of mineral phosphate at rates from 0–400 units† P_2O_5 per acre and top-dressing treatments with nitrogen and potassium at rates up to 30 units N and 180 units K_2O per acre on the growth and nutrient uptake of newly planted Sitka spruce on deep oligotrophic peat in western Northern Ireland. Rate of tree growth and P uptake increased progressively over four years with increasing phosphate application. Applied K appreciably increased rate of growth, length of growing season and K uptake, but tree response decreased in the fifth season after application. Nitrogen application has had little effect except in the year of planting.

Levels of N and K in Sitka foliage at the end of the first growing season were very much higher than in subsequent years.

Introduction

Because of the high proportion of peat land, as opposed to mineral soils, which has been planted, and is still potentially available for afforestation, in Northern Ireland[1] much of the research effort of the Forestry and other Research Divisions of the N.I. Ministry of Agriculture is devoted to the problems of growing trees on deep peat. Unlike the position in other parts of the British Isles the main species being planted is Sitka spruce (*Picea sitchensis* Carr.) and the mineral nutrition of this species on ombrogenous peat forms one of the major forestry research projects in the Province. The present paper summarises the results of an experiment involving one aspect of this work.

Experimental

The experiment (Ballintempo C.R.D. 1/63) is located at Ballintempo forest, Co. Fermanagh in the extreme S.W. of the Province, only some 14 miles from the Atlantic seaboard. The site of the experiment is at an altitude of approx. 900 ft O.D. and is moderately exposed. The peat averages about 7 ft in depth and is typical unflushed hill blanket peat supporting a mixed vegetation of *Calluna*, *Scirpus*, *Eriophorum* and *Sphagnum* before planting.

Sitka spruce was planted in 1963 at 5 × 5 ft spacing. A split-plot design was used with levels of phosphate (applied broadcast before ploughing) as main-plot treatments and factorial combinations of three levels of applied nitrogen × three levels of applied potassium as sub-plot treatments. Phosphate (in various forms) was applied at 8 levels from 0–400 units† P_2O_5 per acre. Sub-plot treatments were applied as top-dressings round individual trees, N being applied at 0, $\frac{3}{4}$ and $1\frac{1}{2}$ oz sulphate of ammonia per tree and K at rates of 0, $1\frac{1}{2}$ and 3 oz muriate of potash per tree (equivalent broadcast rates are approximately 0, 15 and 30 units N, and 0, 90 and 180 units K_2O per acre).

Average growth of the leading shoot has been assessed annually since the beginning of the experiment, and foliar samples from the topmost whorl of branches have been collected at the end of each growing season. The total concentrations of the major nutrient ions in the foliage samples have been estimated using standard laboratory analytical techniques described previously.[2]

Results and Discussion

Effect of level of applied phosphate on growth and foliar P concentration

The effect of level of applied phosphate on foliar P concentration over the four years since planting is shown in Table I. Although the foliar P concentration in all treated plots is above the generally accepted minimum value of 0·14% dry matter (*DM*) for the satisfactory growth of spruce,[3] the difference in P uptake between the trees receiving the highest and lowest rates of phosphate is already appreciable and, on such a recognised P-deficient site, is likely to increase with time. Four years after planting, the P level in the trees receiving 10 cwt/acre ground rock phosphate for instance, is almost 50% greater than in those receiving 2·5 cwt/ac.

A feature of the results is the course of P uptake shown by trees which received no phosphate at time of planting. Even at the end of the first growing season the foliar P concentration was considerably below that in any of the treated plots. By the end of the second season the average P concentration had dropped to 0·10% *DM*. At this stage it was obvious from the appearance of the trees that they would not continue to grow without treatment, and 1 cwt/ac ground rock phosphate was applied broadcast at the beginning of the third growing season. This treatment evoked an immediate response and by the end of the season of treatment the average P concentration had doubled to 0·20% *DM* although growth, apart from colour, was not noticeably affected. Growth improved slightly during the following year but at the end of that season the level of foliar P had again dropped to the very low value of 0·11% *DM*.

As shown in Table II, the effect of level of applied phosphate on tree growth was not very noticeable until the end of the third season after planting but, since then, differences between treatments have become more apparent. Annual growth has always been slightly better at the higher rates of phosphate application but, as is borne out by the results of the foliar analyses, none of the trees in the treated plots appears to

* Presented at a meeting of the Agriculture Group, 21 November, 1967

† 1 unit = 1·12 lb

TABLE I

Effect of level of phosphate application on foliar P concentration

Fertiliser	Rate of application (cwt/ac)	Units P_2O_5 supplied	Total foliar phosphorus concn. (% *DM*) 1963	1964	1965	1966
None*	—	—	0·15	0·10	0·20	0·11
Ground rock phosphate	2·5	83	0·24	0·19	0·14	0·16
" " "	5	165	0·27	0·21	0·22	0·21
" " "	10	330	0·25	0·21	0·25	0·23
Basic slag	5	100	0·25	0·23	0·20	0·21
" "	20	400	0·24	0·19	0·22	0·24
Superphosphate	5	100	0·23	0·17	0·14	0·19
Triple superphosphate	2	94	0·22	0·17	0·17	0·19

* 1 cwt/ac ground rock phosphate applied at beginning 1965 growing season

TABLE II

Effect of applied phosphate on annual growth of the leader of Sitka spruce

Fertiliser	Rate of application (cwt/ac)	Units P_2O_5 supplied in fertiliser	Average leader length, cm 1963	1964	1965	1966
Nil	—	—	7·2	7·7	—	—
Ground rock phosphate	1	33	—	—	7·0	11·9
" " "	2·5	83	7·2	14·4	16·1	23·3
" " "	5	165	7·0	16·8	16·0	23·6
" " "	10	330	7·0	18·2	19·7	30·0
Basic slag	5	100	7·6	15·9	13·1	16·6
" "	20	400	7·0	17·1	18·8	25·9
Superphosphate	5	100	7·7	16·2	19·7	27·2
Triple superphosphate	2	94	7·4	15·2	16·4	23·0

TABLE III

Effect of N and K top-dressing on average foliar K concentration

Year	N_0K_0	N_0K_1	N_0K_2	N_1K_0	N_1K_1	N_1K_2	N_2K_0	N_2K_1	N_2K_2
	Foliar-K concentration (K as % *DM*)								
1963	0·78	0·86	0·87	0·79	0·87	0·88	0·84	0·92	0·93
1964	0·41	0·59	0·64	0·35	0·59	0·62	0·35	0·57	0·59
1965	0·33	0·56	0·58	0·31	0·55	0·59	0·32	0·52	0·58
1966	0·41	0·57	0·55	0·36	0·55	0·55	0·41	0·56	0·56

have reached the stage of actual P deficiency. Despite this, the current growth rate at the highest rate of phosphate application is some 28% greater than at the lowest rate.

Effect of N and K top dressing on growth and foliar-K concentration

The effect of N and K top-dressing treatment on the foliar K concentration (averaged over all main-plot phosphate treatments) since time of planting is shown in Table III. The most obvious feature of these results is that the foliar K concentration at the end of the first growing season is very much higher, irrespective of K treatment, than in any subsequent year for which data are available. In the absence of applied K, for instance, the K concentration is almost twice as high at the end of the first season as it is at the end of the second and subsequent seasons. A similar situation also exists in the K-treated plots. This feature is also apparent in the results of the N analysis and it throws grave doubts on the value of establishing 'critical' nutrient concentration levels for planted-out trees using data derived from pot cultures of seedlings. A commonly quoted 'optimum' K concentration for such seedlings is about 1·0% *DM*.[4] It is not valid, however, to suggest that this figure represents the 'optimum' K concentration in the foliage of spruce in the forest.[5] Indeed, the only values from adult Sitka spruce found to approach this figure in Northern Ireland have been from trees growing on shallow peat overlying granitic soils where there is an inverse relationship between tree growth and foliar K concentration over the range 0·7–1·1% *DM*.[6]

The effect of K applied in 1963 on foliar K levels has progressively decreased with time. This is not unexpected in view of the high solubility of muriate of potash and the consequent likelihood of its being leached quite rapidly from deep acid peat. It is somewhat surprising, however, that the foliar K levels show only a slight increase from the K_1 to K_2 treatments even at the end of the fourth year after application.

The effect of applied N on the uptake of K is also illustrated in Table III. In the first season both levels of applied N increased the foliar K concentration slightly but in subsequent years the effect of applied N on K uptake has been negligible.

A very close correlation exists between level of applied K, foliar K concentration and annual growth of the leader. This is shown in Table IV. At the end of the first season there was little response to applied K but since then the K-treated trees have grown at a significantly greater rate than those receiving no potassic fertiliser. Rates of growth at the two levels of K application have been very similar. Taken in conjunction with the results of the foliar analyses, which showed no increase in K uptake at the higher level of application, this lack of growth response between the two levels of applied K would indicate that the exchange capacity of this type of peat for K is relatively low and that above the level of application at which the exchange complex is saturated with respect to K the rate of leaching of K is directly related to the quantity of K applied as fertiliser. The level of application at which this occurs on peat of the type studied corresponds to approximately 90 units K_2O/ac as muriate of potash.

As well as its direct effect on annual growth the effect of K treatment on the cessation of growth in autumn has been studied. The number of trees showing lammas, or extension, growth on approximately the same date each season has been recorded since 1963. The data are illustrated in Table V.

As previously, the data for the first growing season differ from those for subsequent years. In the first year, although K had an appreciable effect on the incidence of lammas growth, the effect due to applied N was even greater. For example, 11% of the trees in the N_0K_0 treatment showed lammas growth but 62% in the N_2K_0 plot had not formed dormant buds at the time of assessment. Potassium treatment alone gave only about half this response. In later years, however, the effect of K was predominant, the response to N then being slight. At the end of the 1965 season the incidence of lammas growth was 5 times greater in the N_0K_2 plot than in the N_0K_0 plot.

Another significant feature shown in Table V is that the magnitude of response to K decreased rather abruptly. By autumn 1967 the differences in incidence of lammas growth between the K and non-K plots were comparatively slight. The suggestion from this is that by the end of the fifth season after application the residual effects of K were slight.

TABLE IV

Effect of applied potassium on average leader length of Sitka spruce

Potassium treatment	Average leader length, cm			
	1963	1964	1965	1966
K_0	6·5	13·8	11·4	17·7
K_1	6·9	16·5	20·9	26·0
K_2	7·4	17·2	19·5	26·0

Effect of top-dressing treatment on growth and foliar nitrogen concentration

The effect of applied N and K on foliar N concentration is shown in Table VI. As with K, a decrease in N concentration with time is apparent. At the end of the first season the highest foliar-N concentration found was 2·86% *DM* but at the end of the fourth season 1·72% *DM* was the highest recorded. This again indicates that the relatively high nutrient levels occurring in seedling material are not maintained in trees after planting. In the first year there was a general increase in foliar N levels with increasing rates of applied N but in the second and subsequent years no such relationship was apparent. Addition of K increased N uptake slightly in the third and fourth growing seasons.

The effects of applied N on the incidence of lammas growth have been discussed; no effect was apparent after the first season.

Growth response of Sitka to applied N is shown in Table VII.

Apart from a small consistent increase in leader length with increasing N application at the end of the first season the effects in subsequent years are inconsistent and in some years adverse.

Indeed, significant positive growth responses to applied inorganic N have not been recorded in Sitka spruce growing

TABLE V

Effect of N and K top-dressing treatment on incidence of lammas growth

Year	Percentage of trees showing lammas growth								
	N_0K_0	N_0K_1	N_0K_2	N_1K_0	N_1K_1	N_1K_2	N_2K_0	N_2K_1	N_2K_2
1963	11	35	32	42	56	50	62	75	68
1964	41	62	62	25	60	47	28	52	50
1965	12	58	60	7	60	56	10	60	60
1966	27	40	46	21	52	50	29	46	46
1967	23	25	36	25	37	40	31	36	33

TABLE VI

Effect of N and K top-dressing treatment on average foliar N concentration

Year	Foliar-N concentration (N as % *DM*)								
	N_0K_0	N_0K_1	N_0K_2	N_1K_0	N_1K_1	N_1K_2	N_2K_0	N_2K_1	N_2K_2
1963	2·54	2·66	2·64	2·65	2·65	2·72	2·86	2·74	2·76
1964	2·39	2·24	2·23	2·28	2·22	2·26	2·38	2·28	2·25
1965	1·64	1·76	1·81	1·73	1·82	1·84	1·74	1·78	1·85
1966	1·54	1·64	1·69	1·58	1·68	1·72	1·63	1·72	1·60

TABLE VII

Effect of applied N on average leader length of Sitka spruce

Nitrogen treatment	Average leader length, cm			
	1963	1964	1965	1966
N_0	6·5	13·8	11·4	14·7
N_1	6·9	13·0	9·3	14·5
N_2	7·4	13·8	10·7	15·9

on deep peat in Northern Ireland. Previous experience suggests that at least in the early stages of the rotation and, provided that an adequate supply of P is maintained, sufficient N becomes available to meet the demands of a spruce crop. Even in checked Sitka, where the N uptake is low, the addition of N, either alone or in combination with P and K, has not given an economically worthwhile response. Whether this situation can be maintained throughout the rotation has not yet been determined but there is no evidence yet to support the contention that repeated applications of mineral N will be necessary to maintain the growth of Sitka plantations on deep peat in N. Ireland.

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References

1. Parkin, K. F., *Irish Forestry*, 1957, **14,** 111
2. Dickson, D. A., *Rec. Agric. Res. Min. Agric., N. Ire.*, 1965, **XIV (2),** 61
3. Leyton, L., *Encyclopedia Pl. Physiol.*, 1958, **IV,** 1026
4. Ingestadt, T., *Physiol. Pl.*, 1959, **XII,** 568
5. Parker, R. E., *Irish Forestry*, 1962, **XIX (1),** 60
6. Dickson, D. A., *Ann. Rep. Res. Min. Agric., N. Ire.*, 1967

FERTILISERS IN FORESTRY – THE FUTURE*

By W. O. BINNS

Present results with fertilisers in Forestry Commission forests are used, with several qualifications, as a basis for estimating the approximate fertiliser requirements by the Commission up to the year 2010.

Introduction

It is important to outline some major differences between agricultural and forest crops which affect the way fertilisers are used. Tree crops are usually on the ground for 50 to 100 years; in this country most are felled between 50 and 70 years after planting. The age at which a crop of any tree species is finally cut will depend on its rate of growth; the faster it grows, the earlier it will be harvested, and the difference between the best and worst crops growing in a given region will range from 5 to 15 years, depending on species.

There is a very long period before there are any returns on the money invested in a crop. When thinning is practised, the first cuts are made at the 12-year stage but cuts are commonly made at 20 years, and occur at up to 30 years with the slowest growing crops. It follows that the interest on the money invested becomes important for many forest operations, e.g. cultivation, draining, fertilising, weeding. This means that the time money is invested is critical, and no operation is done before it has to be. This affects fertilisation practice in the following way: if a treatment produces a constant effect, in terms of increased yield, regardless of the age at which it is applied, then if the extra yield (or a proportion of it) has to be left until the crop is finally cleared the later the treatment is applied the more profitable it will be. There will always be financial pressure to harvest increased yields as soon as possible: the shorter the time that money is tied up the less is the accumulated cost that counts against the increased output. There is one further marked difference which follows from the longevity of trees. An agriculturist might reasonably estimate the sort of fertiliser regimes which would be used for potatoes, sugar-beet, and wheat in 1980s but would be uncommitted as to the likely acreages of these crops. In forestry on the other hand, the acreages in 1980 of spruce, Scots pine, and Douglas fir planted between 1950 and 1960 can be estimated quite accurately, although because of current uncertainty about the fertiliser responses in a wide variety of species and site combinations only approximate forecasts can be made about the sort of fertiliser regimes likely to be used on these crops at that date.

Nurseries

The total area of forest nurseries in the immediate future is likely to be 1,300 to 1,600 acres (800–1,000 acres in the Forestry Commission, and 500–600 in private hands), the total annual use of fertilisers in them is unlikely to exceed 250 tons of a 21% N fertiliser, and about the same weight of a 0–20–20 PK compound. Even if curious and unorthodox forms of fertilisers are found to be effective and profitable, the amounts required are unlikely to result in manufacturers making special provision for the needs of forest nurseries.

Forests

In the forest, however, the areas are much greater. The 1943 Forest Policy Report,[1] recommended that the country should have 5 million acres of productive forest. Though the long-term strategic objectives considered at that time have become irrelevant, and economic considerations have largely taken their place, the areas already planted plus the plans announced suggest that by the end of the century there may well be about 5 million acres of forest.

Establishment of plantations

Up to the present time, the only nutrient used in any quantity has been phosphorus, usually in the form of Gafsa or other ground rock phosphates. This is now normally applied once only shortly after planting, and seldom again on all acid peat soils, and most podsolic soils (including almost all sites characterised by ericaceous plants), and on peaty gleys and gleys. There are, however, still large areas of forest on these soil types which were not treated at planting, and which urgently need phosphate. This problem should have been dealt with in the next ten to fifteen years.

The use of potash fertilisers at planting, or within a few years afterwards, is restricted to raised and blanket peats, though there are indications that crops on shallow soils over chalky tills may also respond to potash.

Nitrogenous fertilisers are not used at all at planting. This may seem surprising when one considers the enormous responses of agricultural crops, and also the very marked responses in the nurseries of trees, both as seedlings and transplants. There seem to be several reasons for this. Until the advent of effective contact herbicides and selective herbicides the existing plants, especially on grassy sites, overwhelmed the trees and greatly increased weeding costs, so that there is little incentive to boost nutrient supplies in this stage of a crop's life. Also, many conifers suffer from planting check, and seem unable to use added nitrogen, at least in the first two years following planting. If nitrogen is to be used to speed up establishment then the production of plants by different means, with root systems able to exploit the soil more rapidly, may first be necessary.

* Presented at a meeting of the Agriculture Group, 21 November, 1967

The rate of phosphorus used at planting is about 20 to 30 lb P/acre, and only on the most phosphate-responsive (or phosphate-fixing) soils has thus been appreciably increased. This rate is likely to be used for much future planting, and the total amount in Forestry Commission forests will probably be 3,000 to 5,000 tons of rock phosphate or its equivalent a year.

It is difficult to forecast with any certainty the use of potash on young crops but it is bound to increase with more and more planting on peatland.

Older crops

It may seem strange that work on the use of fertilisers to improve the yield of established crops which are in the thinning stage has only been going on for a few years. This is due partly to the original idea of establishing forests as a primary need, and partly because the problems of establishment seemed so large that all energy was expended on them.

As in agriculture, a wide range of yields are found for a given crop with a mean which falls far below the highest recorded yields. Table I, for example, shows the distribution of Yield Classes in Commission plantations of the highest-yielding of the commonly planted conifers, namely Sitka spruce, which accounts for over 40% of current Forestry Commission planting. The Yield Class is defined[2] as the maximum mean annual volume increment; i.e. the maximum value of the quotient $\frac{\text{total volume production}}{\text{age}}$. The classes represent intervals of 20 hoppus feet* in volume to 3 inches top diameter of log.

*1 hoppus foot = 1·273 cubic feet.

TABLE I

Sitka spruce in Forestry Commission plantations, as at September, 1967

Yield class	Area, thousand acres	% of total area
< 60 (in check)	24	4
60–100	59	11
120–160	400	74
180–220	58	10·5
240–280	2·5	0·5
Total area	543·5	

The small proportion of the three highest Yield Classes, 0·5%, is striking: however the greatest contrast with present day agricultural practice is brought out when it is realised that all the highest yields have been achieved without any additional fertilisers. It is known that many crops in the lowest Yield Classes, 60 to 100, are associated with soils of low fertility: but it is not known how many of the crops in the range 120 to 160—which comprise three-quarters of the Forestry Commission's area of Sitka spruce—would respond to fertilisers (few have had any so far) or whether the crops in the top Yield Classes are limited by factors which can be remedied. (Even if the situation of the crops in the top Yield Classes could be improved, it may not of course be economic to boost the Yield Class to 300 or over.)

Scottish experiments suggest that Scots and Corsican pines in the pole-stage (i.e. 30 to 50 feet tall) are likely to respond to nitrogen applications, but it is not yet known if these responses will be large enough to justify such treatment.

In Welsh experiments on Sitka spruce, phosphorus is the only major nutrient found to improve the growth of pole-stage stands of moderate Yield Classes (130 to 180): there have been no responses to N, K, Ca, or Mg in seven factorial trails. Analysis of the foliage has shown that the trees are reasonably supplied with N and K ($N \gg 1{\cdot}7\%$ for 5 trials, 1·5% for two ill-drained sites; K from 0·85 to 1·01%). As in Scotland, Corsican pine seems responsive to N, and Scots pine in England shows responses both to N and P. More detailed results for these trials are given by Binns & Grayson.[3]

There have been few experiments on older crops but there are some suggestions that nitrogen may become increasingly important with age.

Potential use of fertilisers

These results are too few for generalisations to be possible, but it seems that there may be large areas of forest where N and P fertilisation will be effective.

If it is assumed that crops will not be fertilised (other than at establishment) before the first thinning, which is likely to be at 20–25 years, and that the mean rotation is 60 years, the areas of crops coming up for first thinning and entering the last ten years of the rotation, each year, for ten year periods can be tabulated (Table II).

Phosphorus is a slow-acting fertiliser, and the effects of a single application last many years. It is assumed therefore that, following establishment, only one application will be made, probably at the time of the first thinning. In contrast,

TABLE II

Acreages (in thousands) per year of Forestry Commission crops coming into the thinning stage, and into the final crop phase

	1971–80		1981–90		1991–2000		2001–10	
	Thinning stage	Final crop	Thinning stage	Final crop	Thinning stage	Final crop	Thinning stage	Final crop
Spruces	24	3	27	10	33*	13	35*	24
Pines	18	5	20	5	15*	5	15*	18
Larches and Douglas fir	10	2	5	3	5*	3	5*	10
Total	52	10	52	18	53	21	55	52

Assuming: Rotation 60 years; first thinning 20 years; final crop phase from ten years before final felling
* estimated from present planting trends

TABLE III

Potential annual use of N and P fertilisers in Forestry Commission forests, in terms of urea and rock phosphate, tons

	N as urea				P as rock phosphate			
	1971–80	1981–90	1991–2000	2001–10	1971–80	1981–90	1991–2000	2001–10
At planting	—	—	—	—	5000	5000	5000	5000
Thinning stage, spruces	—	—	—	—	1800	2000	2400	2500
Thinning stage, pines	1200	2500	3400	3200	1300	1400	1200	1200
Final crops	400	750	900	2100	—	—	—	—
Total	1600	3250	4300	5300	8100	8400	8600	8700

nitrogen effects last only a few years, and applications at intervals of about seven years, i.e. about five applications in all, are likely for responsive crops.

It is assumed for the purpose of these predictions that half of all pines and spruces will respond to P and that half the pines will respond to N from the thinning stage onwards. It is also assumed that half of the spruce crops will respond to N in the final ten years, i.e. they will be treated once only at the same time as the fifth application on the pines. Practically nothing is known about the responses of larch and Douglas fir, so they have been left out of the calculations.

The rates assumed are 44 lb P and 100 lb N per acre, corresponding to 3 cwt rock phosphate and 2 cwt urea per acre. Using the acreages in Table II and the assumptions set out above, the maximum potential use of N and P fertilisers in Forestry Commission forests is set out in Table III, up to the year 2010.

There are of course a number of uncertainties about the figures. The planting pattern of the 1980s can only be guessed at; it is likely that N will not be used much on lodgepole pine, but will be used on pole-stage Norway spruce; changes in timber prices, fertilisation costs and rates of return required on capital may radically alter the profitability of fertilising by one method or the other. Many plantations may be felled early, for a variety of reasons. Nothing has been said about potassium, which will be needed on nearly all peat soils at some stage or other, nor have private forests been mentioned, which include nearly 650,000 acres of conifers.

One of the great attractions of fertilisation (at the pole-stage or later) lies in the fact that (once responsive crops are identified) its profitability is less in doubt than that of planting. If more jobs are to be provided in the countryside and in wood-using industries and if at the same time Britain's dependence on imported wood products is to be reduced, then it is likely to be more profitable to boost timber production by fertilisation than by planting the poorest sites.

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References

1. 'Post-war forest policy', *Rep. H.M. For. Commissioners*, 1943, Cmd. 6447 (London: H.M.S.O.) 114 pp.
2. Bradley, R. T., Christie, J. M., & Johnston, D. R., Forest Management Tables, *Bookl. For. Commn., Lond.*, 1966, (London: H.M.S.O.) 219 pp.
3. Binns, W. O., & Grayson, A. J., *Scott. For.*, 1967, **21**, 81

PUROTHIONIN ANALOGUES FROM BARLEY FLOUR

By D. G. REDMAN and N. FISHER

Acid treatment of a light-petroleum extract of barley flour yielded two proteins (termed hordothionins) closely similar in electrophoretic behaviour to the purothionin doublet obtained from wheat by the same procedure. The major (faster) component, hordothionin α has been obtained apparently pure. Comparison of its amino acid composition, peptide maps, immunological reactions and C-terminal end group with those of purothionin α has confirmed the relationship between the proteins from the two genera although some differences in amino acid composition and sequence have been found. Extraction of barley flour with buffered saline followed by acid treatment yielded proteins with similar properties to the hordothionins; this supports previous observations on the relationship between the purothionins and their corresponding globulins.

Introduction

The purothionins, proteins obtained after acid treatment of light-petroleum extracts of wheat flour[1–3] and subsequently separated into two closely similar components,[4] have been shown to bear a very close relationship to the 'fast-moving globulins' of wheat flour.[2–4] Since it is known that barley flour contains proteins of similar mobility to these 'globulins'* on starch-gel electrophoresis,[5] it was considered of interest to determine if barley and other members of the Gramineae likewise contained compounds corresponding to the purothionins. Preliminary experiments on light-petroleum extracts of barley flour showed the presence of such components, but these were not detectable in the flours of rye, maize and oats, and were also absent from wheat germ. In the present paper, work on the fractionation and characterisation of the products obtained from barley is reported. Barley is shown to contain α- and β-analogues of the wheat purothionins, and the separation of the α-component of barley in virtually pure form has been achieved. A comparison of its composition and properties with those of purothionin α will be described. (While the name 'purothionin' is derived from the Greek,[6] it is felt that the common usage of 'hordein' to refer to barley protein makes the term 'hordothionin' more acceptable, though derived from a Latin root, than a Greek-based alternative, e.g. 'krithothionin'.)

Experimental

Materials

Barley flour was milled to 51·2% extraction from Proctor variety. The protein content was 8·4% and moisture content 9·9%. An untreated commercial wheat flour used for comparative isolation of purothionins had a protein content of 11·5% and a moisture content of 14·7%. Ionagar No. 2 was obtained from Oxoid Ltd. Trypsin ('crystalline') and α-chymotrypsin ('4× crystallised') were B.D.H. products.

Light-petroleum extraction

Barley or wheat flours were extracted by repeated shaking with light petroleum (60–80°) until very little yellow colour was observed in the extracts. Solvent was removed from the filtered extracts by rotary evaporation under reduced pressure. The residual oil from 10 kg flour was diluted with peroxide-free diethyl ether (200 ml), extracted for 7 h by magnetic stirring with 8% (by vol.) lactic acid (200 ml) and allowed to settle. Two major phases were obtained, separated by an interfacial emulsion. The aqueous phase was removed and the remaining phases were re-extracted for 16 h with a further 200 ml 8% lactic acid and allowed to separate. The aqueous phases and second interfacial emulsion were combined and extracted with diethyl ether which eliminated most of the emulsion. After removal of the dissolved ether by rotary evaporation the aqueous phase was dialysed against distilled water to remove lactic acid and freeze-dried. Fractionation of this material on carboxymethyl cellulose columns was carried out as reported earlier.[4]

Barley globulins

The barley globulins were extracted and treated with acid and then fractionated on carboxymethyl cellulose columns in the same manner as used for wheat globulins.[2,4]

Starch-gel electrophoresis

The method of Elton & Ewart[5] was used with aluminium lactate buffer pH 3·3, 0·0083 M at 7V/cm for 4–4½ h.

Amino acid analyses

Samples were hydrolysed and analysed as described earlier.[4] Correction factors for destruction or incomplete liberation of amino acids were obtained by a study of hydrolysates after 25, 46 and 75 h of purothionin α and hordothionin α respectively. These factors were then used for the calculation of the composition of the barley 'globulin' (25 h) hydrolysates.

Tryptophan determinations were carried out by the methods of Goodwin & Morton[7] and Spies & Chambers[8] (procedure N). Nitrogen content was determined by the semi-micro Kjeldahl technique.

C-terminal end group

The hydrazinolysis method of Akabori *et al.*[9] as applied by Press *et al.*[10] was employed, with a reaction time of 24 h. Correction factors for destruction of lysine were obtained by applying the same conditions to the free amino acid.

Peptide maps

Performic acid-oxidised proteins (4–5 mg) were dissolved in 0·5 ml of a solution of 0·5% ammonium bicarbonate (pH 7·7), in which trypsin had been dissolved at a concentration of 0·02%. After addition of 1 drop of toluene, samples were

*Inverted commas are used to refer to acid-heated proteins

incubated at 37° for 72 h. The clear solutions were freeze-dried before being dissolved for the preparation of (two) peptide maps from each digest.

Samples (4–5 mg) which had not been oxidised with performic acid were dissolved in water (0·5 ml) and the pH was adjusted to ~ 8·0 with triethylamine using indicator papers. 0·1 ml of a trypsin solution (1 mg/ml in 0·001 N-HCl) and 1 drop of toluene were added before incubation at 37° for 72 h. The clear solutions were dried over phosphorus pentoxide *in vacuo* before being dissolved in the electrophoresis buffer and application to the papers.

For chymotryptic hydrolysis, performic acid-oxidised samples (4–5 mg) were dissolved in 0·5 ml 0·5% solution of ammonium bicarbonate (pH 7·7) in which α-chymotrypsin had been dissolved at a concentration of 0·02%. Incubation after addition of 1 drop of toluene was for 2 h at 37°. The pH was then adjusted to about 3·5 with acetic acid, the solution was freeze-dried, and the product was taken up for the preparation of two peptide maps.

Peptide maps were prepared from tryptic or chymotryptic digests as described previously,[4] except that solutions were applied to the papers as spots instead of streaks.

Immuno-diffusion

An almost pure wheat purothionin doublet preparation was dissolved in saline (0·85% wt./vol.) at a concentration of 8 mg/ml. Two rabbits received daily 1 ml doses subcutaneously for 5 days, followed by a second course of daily doses two weeks later, so that each rabbit received a total of 80 mg purothionin. Rabbits were bled 10 days after the final injections. Antisera were concentrated five times by per-evaporation and then cleared and further concentrated in the following manner. After removal of a slight precipitate by centrifugation at 2000 *g*, an equal volume of saturated ammonium sulphate was added, and the mixture was centrifuged. The solid was dissolved in water, dialysed against water until free from sulphate, freeze-dried and finally taken up in 0·85% saline (1 volume for every 10 volumes antiserum before ammonium sulphate precipitation). Agar (2 g) was placed in 200 ml water and thiomersal (20 mg) was added. The mixture was left at room temperature for 15 min and then autoclaved at 15 lb in^{-2} for 15 min and allowed to cool at room temperature for 30 min. To 180 ml of the resulting solution *either* 20 ml of an acetate buffer (0·35 M sodium acetate, 0·15 M acetic acid pH 5·0) *or* 20 ml of 8·5% sodium chloride solution were added just before pouring. Plates were filled to a depth of 2·5 mm. The centre well contained antiserum and the peripheral wells contained the samples to be tested (8 mg/ml in 0·85% saline). The radial distance between the edge of the wells was 9 mm. Plates were observed for periods up to six weeks. (Materials of unspecified preparation mentioned in further sections of this work, were those described previously.[4])

Results

Carboxymethyl cellulose fractionation

Retention properties of wheat purothionins α and β were similar to those reported earlier[4] for the corresponding proteins from 'Snowdown' wheat flour. Yields from 10 kg flour were α 250 mg, β 150 mg, approximately.

The elution pattern for the hordothionins is shown in Fig. 1. Yields from 10 kg flour were α 550 mg, β 35 mg. Re-chromatography of the α-peak gave no further resolution. Fractionation of barley 'globulins' gave a more complicated pattern shown in Fig. 2. Yields of the incompletely separated materials from 750 g flour were A 120 mg, B 55 mg, C 30 mg, D 30 mg.

Starch-gel electrophoresis

Starch-gel patterns of hordothionin α, purothionin α and the purothionin doublet are shown in Fig. 3. When the materials are run the whole length of the gel (20 cm) hordothionin α has a very slightly (< 1%) lower mobility than its wheat counterpart. The peak in Fig. 1 designated β was shown to consist of a slower-moving band of corresponding mobility to purothionin β but contaminated with material which was probably the α-compound.

Patterns from peaks A, B, C, and D of Fig. 2 showed that A and B possessed mobilities equal to each other and to that of hordothionin α, while C and D were in the β-region for mobility (D very slightly slower and contaminated with slower-moving impurities).

Amino acid compositions

Amino acid analyses are presented in Tables I and II. The values for 'Snowdown' purothionin α are calculated from those in a previous paper.[4]

Tryptophan was undetected in hordothionin α, purothionin α and the barley globulin sample, when measured by the Goodwin & Morton method based on the ultra-violet absorption of proteins in 0·1 N sodium hydroxide. In all cases equilibrium was not reached for several hours (possibly

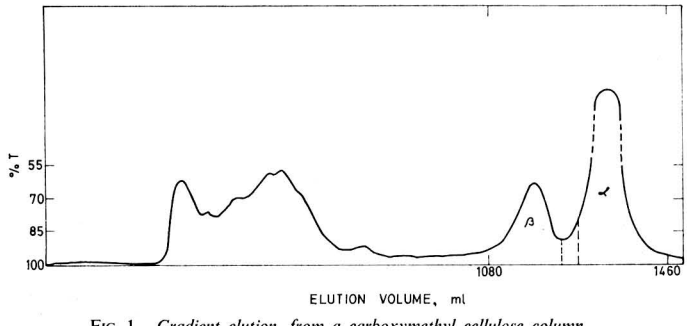


FIG. 1. *Gradient elution, from a carboxymethyl cellulose column, of materials obtained by acid treatment of a light petroleum extract of barley flour*

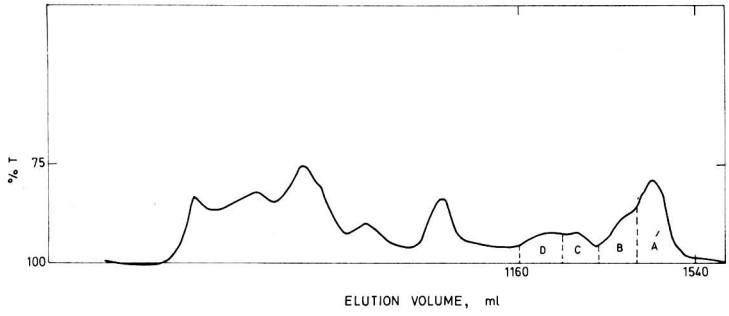
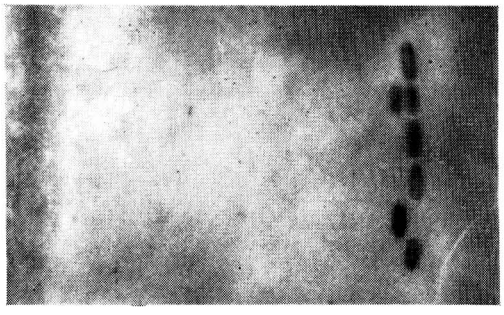


FIG. 2. *Gradient elution of barley 'globulins' from a carboxymethyl cellulose column*



↑ Slot

FIG. 3. *Starch-gel electrophoretic patterns*

From top to bottom; purothionin α, purothionin doublet, hordothionin α purothionin α, purothionin β, hordothionin α

owing to alkaline scission of the numerous disulphide bonds); the tryptophan content of the hordothionin was therefore checked by the colorimetric method of Spies & Chambers which proved the absence of this amino acid. (Nimmo *et al.*[3] have shown previously by fluorescence measurements that a purified wheat purothionin preparation lacked tryptophan.)

C-terminal end group

Hordothionin α was found to contain C-terminal lysine, the amount obtained corresponding approximately to a molecular weight of 7,500 for every C-terminal residue.

Peptide maps

'Fingerprints' of tryptic hydrolysates of hordothionin α and its wheat counterpart are shown in Figs 4(a) and 4(b). The corresponding peptide maps obtained after tryptic hydrolysis of performic acid-oxidised samples are shown in

TABLE I

Amino acid compositions of hordothionin α, purothionin α and the 'fast-moving barley globulin' fraction

	moles/10^5g recovered anhydro amino acid			
	Hordothionin α N=16·61%	Purothionin α N=16·23%	Purothionin α ('Snowdown') Calc. from ref. 4	Barley globulin A N=13·77%
Asp	47·81	42·37	42·66	41·75
Thr	54·80	52·14	50·74	68·70
Ser	92·00	100·98	102·09	83·59
Glu	23·90	23·93	27·71	22·54
Pro	44·31	42·00	49·42	38·86
Gly	84·19	90·72	93·60	98·99
Ala	48·97	43·71	45·16	40·69
Val	31·48	17·82	17·96	34·95
½ CyS	156·48	156·90	144·56	171·41
Met	—	—	Trace	—
Ile	—	10·13	10·25	—
Leu	92·70	93·77	94·52	82·72
Tyr	15·86	16·85	15·50	16·85
Phe	21·45	22·95	23·91	23·73
Lys	106·11	104·40	103·67	104·33
His	—	—	Trace	Trace
Arg	104·82	106·10	104·99	101·25
Trp	—	—	not determined	—
% N recovery	97·2	95·3		94·3

Figs 5(a) and 5(b) and those for α-chymotrypic hydrolysis of performic acid-oxidised samples in Figs 6(a) and 6(b).

Immunological reactions

In double diffusion experiments with antiserum to wheat purothionin doublet, purothionin α gave a single precipitin line which was confluent with precipitin lines arising from hordothionin α, purothionin β and the wheat globulins α and β, irrespective of the medium used.

TABLE II

Estimated number of amino acid residues for minimum molecular weight of hordothionin α and purothionin α

	Hordothionin α		Purothionin α	
		Nearest integer		Nearest integer
Asp	6·25	6	5·32	5
Thr	7·16	7	6·54	7
Ser	12·02	12	12·67	13
Glu	3·12	3	3·00	3
Pro	5·79	6	5·27	5
Gly	11·00	11	11·39	11
Ala	6·40	6	5·49	5
Val	4·11	4	2·24	2
½ CyS	20·45	20	19·69	20
Met	—	—	—	—
Ile	—	—	1·27	1
Leu	12·12	12	11·77	12
Tyr	2·07	2	2·11	2
Phe	2·80	3	2·88	3
Lys	13·87	14	13·10	13
His	—	—	—	—
Arg	13·70	14	13·32	13
Trp	—	—	—	—
Total		120		115
Calculated molecular weight		13,000		12,500

Discussion

Since barley flour forms only a very weak gluten, it is unlikely that the purothionins or the hordothionins now isolated in similar concentrations from barley, can be essential to the strength of dough structures. However, in the biochemical comparison of cereals, it is of interest that such proteins should be found in wheat and barley but not in rye, oats or maize. The similarity between the purothionins and the corresponding wheat globulins has previously been discussed[2–4] and a similar situation appears to exist for the hordothionins and their globulin analogues, although there is a possibility that more than two 'fast-moving globulins' may be present in barley.

Hordothionin has now been isolated for the first time, and the α-component has been obtained apparently free from other components. The ratio of the yields of hordothionin α to hordothionin β was considerably greater than the corresponding ratio for the purothionins and it may be noted that the proportion of α to β for the wheat flour used in the present work is similar to the value obtained with an earlier flour ('Snowdown') of very different protein content. The purothionin α of 'Snowdown' flour was higher in glutamic acid and proline contents than the corresponding α-component of the present work, and contained trace amounts of methionine and histidine, but the two preparations were otherwise very similar. The use of lactic acid solution in the isolation of the protein from light-petroleum extracts was found to be more convenient than the method given earlier, which involved the use of anhydrous alcoholic hydrogen chloride.

The very slight difference in the starch-gel electrophoretic mobilities of the α-components of hordothionin and purothionin indicates that there can be little difference in charge or molecular size between the two compounds, and this is borne out by the amino acid data given in Tables I and II. It should be emphasised that the rounded values of Table II are only very approximate, i.e. the same data for wheat could give a total number of residues of 120 (equal to that of barley) and 14

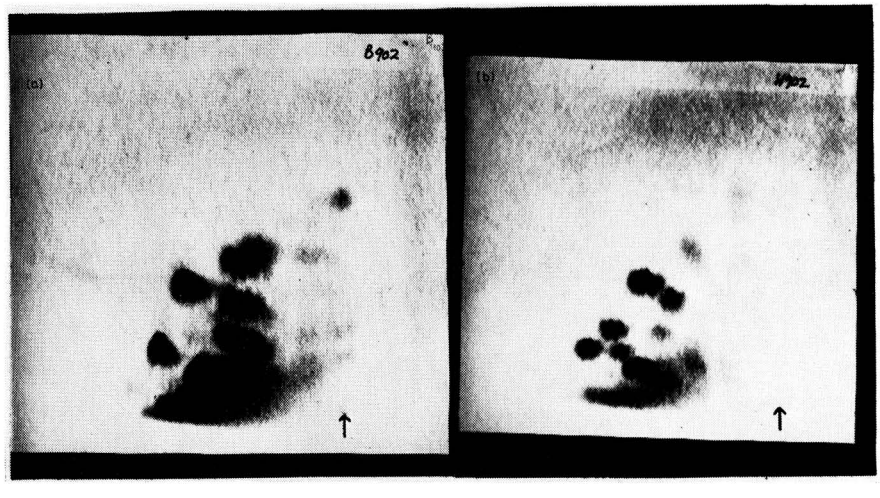


FIG. 4. *Peptide maps of tryptic hydrolysates of* (a) *hordothionin α and* (b) *purothionin α respectively*

The papers were loaded as indicated by the arrow. The left of the photographs represents the negative electrode, subsequent chromatography being from bottom to top

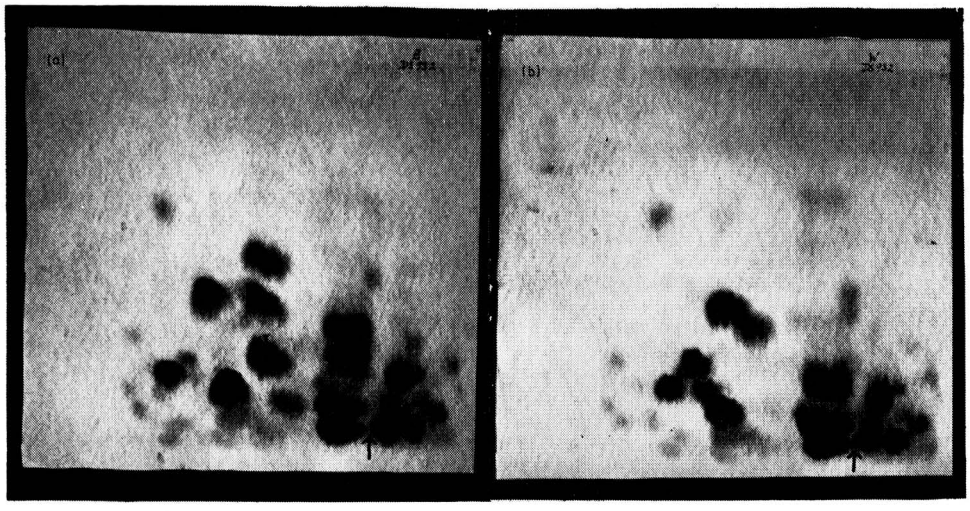


FIG. 5. *Peptide maps of tryptic hydrolysates of* (a) *performic acid-oxidised hordothionin α and* (b) *purothionin α respectively*

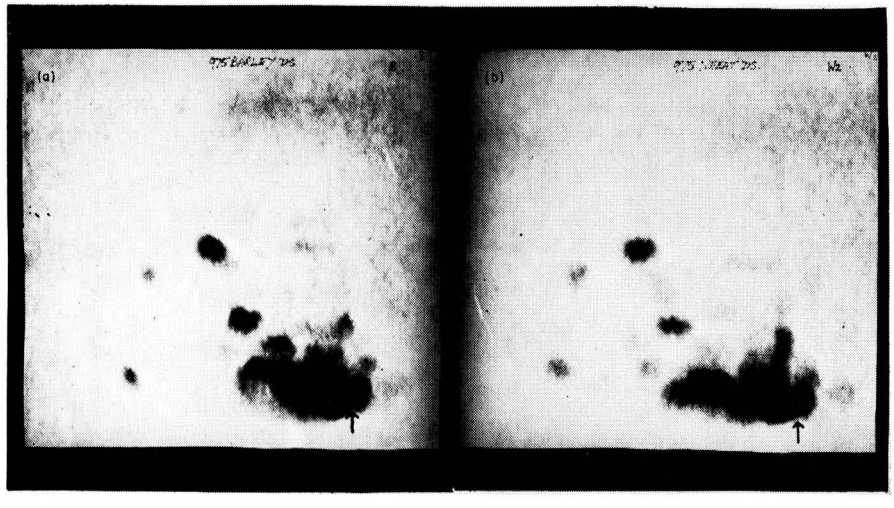


FIG. 6. *Peptide maps of α-chymotryptic hydrolysates of* (a) *performic-acid-oxidised hordothionin α and* (b) *purothionin α respectively*

residues each for lysine and arginine. Four amino acids, methionine, isoleucine, histidine and tryptophan are completely absent in hordothionin *α*. However, the presence of isoleucine in purothionin *α* was confirmed,[4] both qualitatively and quantitatively.

The calculation of the integral number of residues in the molecule was based on the postulates Glu = 3, Phe = 3 and Tyr = 2 residues, respectively, for both barley and wheat products. This gives values for isoleucine and valine which for wheat are more removed from integers than would be expected from the analytical errors, (isoleucine has previously given anomalous values[4]) and it is noteworthy that it is only in respect of these amino acids that purothionin and hordothionin differ significantly (Table I). It is conceivable that microheterogeneity, involving substitution of one amino acid by another, exists in these materials. Perutz & Lehmann[11] state that in a comparison of the amino acid sequences of haemoglobins and myoglobins of different species, only 7 of more than 140 sites remained invariant and also emphasise that many mutations involving neutral residues would remain undetected.

As was previously found for the purothionins, the C-terminal end group of hordothionin α has been shown to be lysine, although the amount found corresponds to a molecular weight of the peptide chain much lower than that calculated from the amino acid composition.

The double-diffusion experiments provide strong evidence that hordothionin α and the α and β purothionins and globulins of wheat possess common antigenic sites, which are usually regarded as being formed by the conjunction of 3–4 amino acids, which are not necessarily adjacent in the same polypeptide chain.

'Fingerprinting' experiments brought to light significant differences between hordothionin α and purothionin α. These differences are small after α-chymotryptic hydrolysis of performic acid-oxidised samples. The time of hydrolysis was kept short to avoid as far as possible complication due to hydrolysis by any contaminating trypsin at the numerous arginyl and lysyl sites. Tryptic hydrolysis of performic acid-oxidised samples caused more extensive hydrolysis than chymotryptic hydrolysis, as was expected, and although 9 major peptide components from the two materials were identical, at least 4–5 other barley components were absent from wheat and *vice versa*, indicating differences in amino acid sequence. Free lysine and arginine were not found in significant quantities on these peptide maps, as demonstrated by comparison with maps of the free amino acids run under the same conditions. Thus, apparently, sequences of three of these amino acids, or two at the C-terminal end of the chain, are not present, although statistically possible for proteins with amino acid compositions of these materials. An additional factor to be considered is the inhibitory action of adjacent prolyl, aspartyl and glutamyl residues on the activity of trypsin (e.g. refs. 12, 13). Comparison of the 'fingerprints' of peptides obtained after tryptic digestion of untreated and performic acid-oxidised samples of hordothionin α or purothionin α showed that there were regions in which cystine was absent, giving rise to identical peptides from both digestions (cf. Figs 4(a) and 5(a), 4(b) and 5(b)). The remaining 'core', held together by unbroken disulphide bonds, in the unoxidised samples, moved further on electrophoresis than the peptides from the oxidised samples containing the negatively charged cystein acid. Work is in progress to characterise the structures more fully, with particular regard to the chemical behaviour of the numerous disulphide bonds.

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References

1. Balls, A. K., Hale, W. S., & Harris, T. H., *Cereal Chem.*, 1942, **19,** 279
2. Fisher, N., Redman, D. G., & Elton, G. A. H., *Cereal Chem.*, 1968, **45,** 48
3. Nimmo, C. C., O'Sullivan, M. T., & Bernardin, J. E., *Cereal Chem.*, 1968, **45,** 28
4. Redman, D. G., & Fisher, N., *J. Sci. Fd Agric.*, 1968, **19,** 651
5. Elton, G. A. H., & Ewart, J. A. D., *J. Sci. Fd Agric.*, 1962, **13,** 62
6. Balls, A. K., Hale, W. S., & Harris, T. H., *Cereal Chem.*, 1942, **19,** 840
7. Goodwin, T. W., & Morton, R. A., *Biochem. J.*, 1946, **40,** 628
8. Spies, J. R., & Chambers, D. C., *Analyt. Chem.*, 1949, **21,** 1249
9. Akabori, S., Ohno, K., & Narita, K., *Bull. chem. Soc. Japan*, 1952, **25,** 214
10. Press, E. M., Piggot, P. J., & Porter, R. R., *Biochem. J.*, 1966, **99,** 356
11. Perutz, M. F., & Lehmann, H., *Nature, Lond.*, 1968, **219,** 902
12. Hirs, C. H. W., Moore, S., & Stein, W. H., *J. biol. Chem.*, 1960, **235,** 633
13. Anderer, F. A., Uhlig, H., Weber, E., & Schramm, G., *Nature, Lond.*, 1960, **186,** 922

SOYABEAN SAPONINS

IX.*—Studies of their effect on birds, mammals and cold-blooded organisms

By I. ISHAAYA, YEHUDITH BIRK, A. BONDI and Y. TENCER

The effect of soyabean saponins on the growth of chicks, mice, rats and *Tribolium castaneum* larvae and on the survival time of tadpoles and guppies was studied. Soyabean saponins did not impair growth of chicks when added at five times the concentration in a normal soyabean-supplemented diet. They had also no effect on the growth response of rats and mice and on the amount of ingested food, but caused slight growth retardation of *Tribolium castaneum* larvae. Soyabean saponins showed a pronounced detrimental effect on tadpoles and guppies.

Introduction

It is commonly believed that the soyabean saponins, which simulate lucerne saponins in their chemical composition, also possess similar 'anti-nutritional' properties such as growth depression in monogastric animals.[1,2] In view of the increasing use of soyabean meal for consumption by humans and farm animals it is important to find out whether soyabeans possess these detrimental properties. The non-specific inhibiting effect of soyabean saponins on various enzymes can be easily counteracted by proteins which accompany the saponins in the meal.[3] It is the aim of the present study to establish the nutritional and physiological effects of soyabean saponins on the growth of chicks, mice and rats and to compare them with the effect of ingested lucerne saponins, under the same conditions, on the development of chicks.[4,5] Since the growth-depressing effect of lucerne saponins could be overcome by addition of cholesterol to the diet[6] the effect of ingested saponins on the blood cholersterol level was determined as well.

In view of the toxicity of saponins, in general, to fish and amphibians and the failure of the common pests of stored products, *Tribolium casteneum* and *Tribolium confusum*, to develop on soyabeans,[7,8] the influence of soyabean saponin on some cold-blooded organisms and insects was also examined.

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Experimental

Soyabean saponin extract (SBSE) was prepared as described by Birk *et al.*[9] and lucerne saponin was prepared by the method of Thompson *et al.*[10]

Growth experiments with chicks

These were carried out on eighteen groups of ten, one-day-old, chicks which had been kept for 4 days, prior to the start of the experiment, on ground maize. Two groups were then put for 9–12 days on each of the following diets which contained different amounts of SBSE, lucerne saponin, cholesterol or lucerne saponin plus cholesterol. The composition of the 9 diets is given in Table I. All nine diets contained 24·5% protein. The minerals consisted of 35·8% $CaHPO_4$, 25% $CaCO_3$, 15% KH_2PO_4, 5% $KHCO_3$, 4·2% $MgSO_4$, 13·3% NaCl and 1·7% Aurofac. The vitamins per 100 g diet were: 0·5 mg thiamine, 0·5 mg riboflavin, 6 mg nicotinic acid, 1·5 mg pantothenic acid, 0·5 mg pyridoxine, 0·4 mg folic acid, 0·02 mg biotin, 0·05 mg vitamin K, 0·02 mg vitamin B_{12}, 150 mg choline chloride, 2000 I.U. vitamin A, 300 I.U. vitamin D, and glucose to make up the mixture to 600 mg. Weight gain per chick was recorded during each day of the experiment. The cholesterol level of the plasma of chicks kept on diets 5–9 was measured at the termination of the experiment by the method of Abell *et al.*[11]

TABLE I

Percentage compositions of the diets supplemented with SBSE, lucerne saponin, and cholesterol for chicks

	Diet No.								
	1	2	3	4	5	6	7	8	9
Soyabean meal	50	50	50		50	50	50	50	50
Soyabean meal residue*				37·5					
Soyabean oil	3	3	3	3	3	3	3	3	3
Pre-mix of vitamins and minerals (4% minerals, 0·6% vitamins, 0·6% methionine and 2·7% Alphacel)	7·9	7·9	7·9	7·9	7·9	7·9	7·9	7·9	7·9
SBSE		0·25	1·0						
Lucerne saponin					0·1	0·3	0·5	0·3	
Cholesterol								1·0	1·0
Carbohydrates	39·1	38·85	38·1	51·6	39·0	38·8	38·6	37·6	37·9

* After removal of 80% ethanolic extract for preparation of SBSE and drying of the residue at 50°C

Growth experiments with mice

Diets supplemented with SBSE, lucerne saponin and cholesterol were given to four groups of white mice, S.W.R. variety. Each group consisted of 4 boxes distributed in the form of a Latin square 4×4. Three mice, 28–31 days old, of approximately the same weight, were put in each box. The boxes were placed in a constant temperature room of 23°±1°. The mice went through a preparation period of one week, being kept on synthetic food and sorghum grains for the first 3 days, and on a fully synthetic diet for the final 4 days. The experiments were started by supplementing the basal synthetic diet with different concentrations of SBSE, lucerne saponin and cholesterol. The percentage composition of the basal diet was: casein (light white soluble, BDH) 18, cornflour 73, olive oil 5, and salt mixture (No. 2 USP NBCo) 4. The vitamins supplied per 100 g diet consisted of: 0·2 mg thiamine, 0·3 mg riboflavin, 0·2 mg pyridoxine, 1·6 mg calcium pantothenate, 100 mg choline chloride 200 I.U. vitamin A, and 40 I.U. vitamin D.

After 12 days of trial the mice, which had been kept on diets supplemented with lucerne saponin and cholesterol, were anaesthetised by chloroform and wrapped in a Saran net and the blood was taken out by cutting the tail. The blood of every three mice from the same cage was pooled in one test tube which had been previously treated with 1% heparin solution and the level of cholesterol was determined.[11]

Growth experiments with rats

These were carried out on two groups of 25–28-day-old rats. Each group consisted of six rats and those in one group were the paired brothers of those in the second. This arrangement was thought to decrease the variability and to enable a smaller number of animals to be used. The first group was kept on a control diet which consisted of: casein (light white soluble, BDH), 18·0%, carbohydrates (starch plus glucose, 1 : 1) 68·4%, salt mixture (No. 2 USP, NBCo) 4%, Alphacel 1·0%, soyabean oil 8·0% and vitamin mixture 0·6%. The vitamins per 100 g diet were: 0·5 mg thiamine, 0·5 mg riboflavin, 6·0 mg nicotinic acid, 1·5 mg pantothenic acid, 0·5 mg pyridoxine, 0·4 mg folic acid, 0·02 mg biotin, 0·05 mg vitamin K, 0·02 mg vitamin B_{12}, 150 mg choline chloride, 2000 I.U. vitamin A, 300 I.U. vitamin D, and glucose to make up the mixture to 600 mg. The experimental group of rats was kept on the control diet supplemented with 1·0% SBSE.

Experiments with tadpoles and fish

The effect of SBSE on the survival time of tadpoles (*Bufo viridis*) and guppies (*Lebistes reticulatus*) was determined as follows: A 50 ml beaker was filled with 3·75 ml water and 1·25 ml of 1% "Tween 80" in 0·1 M phosphate buffer pH 6·7 as a medium for tadpoles or with 7·5 ml water and 2·5 ml of 1% "Tween 80" in 0·1 M phosphate buffer pH 6·7 as a medium for the guppies. The Tween solution was introduced as an emulsifying agent for the saponins and cholesterol used in various concentrations in these experiments. Each treatment was examined in 10 replicates. The survival time of tadpoles or guppies was determined according to Krama & Schantz.[12]

Stock cultures and growth experiments of larvae

Tribolium castaneum Herbst larvae were reared as described by Birk *et al.*[13] Groups of ten newly hatched larvae were placed in plastic vials (4×2·2 cm) on 1 g of wheat flour enriched with 5% dried yeast. Different amounts of SBSE, lucerne saponin and cholesterol were added to the diet of the larvae and each treatment was assayed in 10 replicates and kept at a constant temperature of 28°.

Results and Discussion

The effect of SBSE, compared with lucerne saponins, on the growth of chicks is shown in Fig. 1. The soyabean saponins did not impair growth of chicks even when added at five times the concentration in a diet containing as much as 50% soyabean meal,[14] whereas the detrimental effect of lucerne saponin increases with the concentration of this saponin in the diet, It should also be noted that Group No. 4, which had been deprived of soyabean saponins, did not exhibit any better growth response. It may thus be concluded that soyabean saponin, whether *in situ* or added as an extract to the diet, does not effect the growth of chicks in any way.

Fig. 1 also shows that addition of 1·0% cholesterol to the diet containing 0·3% lucerne saponin abolishes the growth impairment of chicks caused by the saponin, while addition of 1·0% cholesterol to the control has no effect. No differences of statistical significance could be found between the control groups and those kept on diets supplemented with 0·3% saponin+1·0% cholesterol. The reversal of the detrimental effects of saponin by addition of cholesterol to the diet supplemented with lucerne saponin is in agreement with the earlier findings of Peterson.[6] With regard to this result it seemed important to examine whether the affinity between lucerne saponin and cholesterol could be used for binding lucerne saponin to cholesterol in the digestive tract of chicks and thus to prevent the penetration of cholesterol into the blood

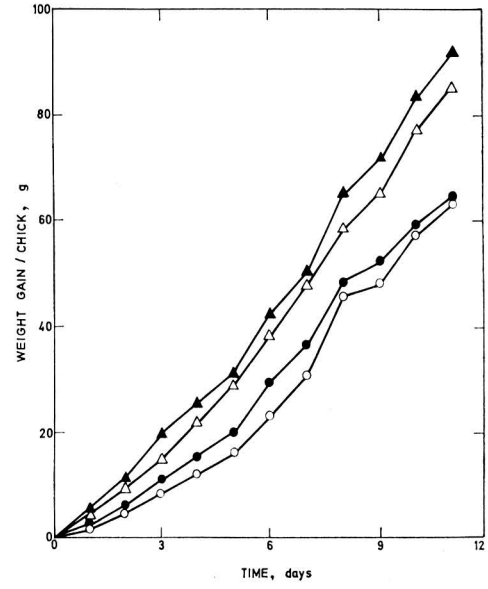

FIG. 1. *Effect of different concentrations of soyabean saponin extract (SBSE), lucerne saponin and cholesterol in the diet on the weight gain of chicks*

▲ Respond to diet No. 1 (control), No. 2 (0·25% SBSE), No. 3, (1% SBSE), No. 4 (soyabean meal residue after removal of SBSE), No. 8 (0·3% lucerne saponin+1% cholesterol) and No. 9 (1% cholesterol)
△ Respond to diet No. 5 (0·1% lucerne saponin)
● Respond to diet No. 6 (0·3% lucerne saponin)
○ Respond to diet No. 7 (0·5% lucerne saponin)

stream. The cholesterol contents of the blood of chicks kept on diets supplemented with various concentrations of lucerne saponin and cholesterol are given in Table II. The figures in Table II suggest that the cholesterol level in plasma has a tendency to fall when lucerne saponins are included in the diet.

The effect of SBSE-supplemented diets on the growth of mice is summarised in Table III. No significant differences have been found between the growth response of mice reared on basic diet and those on SBSE-supplemented diet, as well as between the amounts of feed ingested by the groups. The SBSE, in the concentrations examined, had no detrimental effect on the growth of mice.

The growth experiment with mice provided also additional evidence for the inability of soyabean saponins to complex with cholesterol since ingested SBSE did not decrease the cholesterol level in plasma of mice. Fig. 2 shows that SBSE also had no effect on the growth response of rats.

The effect of SBSE on the survival time of tadpoles and guppies is summarised in Table IV. SBSE had a detrimental effect on both tadpoles and guppies. The organisms seem to be paralysed first, losing their swimming ability, and a short time afterwards they died. Tadpoles with an average weight of more than 200 mg seemed to be more resistant under the same experimental conditions than those of 100 mg. Similar results on the effect of Quillaja saponin on the survival time of tadpoles and fish were obtained by Krama & Schantz[12] and by Wasicky & Wasicky.[15] Since SBSE strongly inhibits the enzymic activity of cholinesterase extracts from tadpoles and guppies,[3] the observed paralytic effect of SBSE on these organisms may perhaps be attributed to the action of SBSE on cholinesterase. SBSE may also effect the breathing mechanisms of the gills by changing their permeability, as has been reported by Vogel[16] for other saponins.

The growth of *Tribolium castaneum* larvae was only slightly affected by SBSE, strongly affected by lucerne saponin and not affected by cholesterol (Table V). The prolongation of time

TABLE II

Cholesterol level in plasma of chicks kept on diets supplemented with lucerne saponin, and cholesterol

Lucerne saponin in diet, %	Cholesterol in diet, %	Cholesterol found in 100 ml plasma, mg
—	—	129
0·1	—	120
0·3	—	99
0·5	—	101
—	1·0	159
0·3	1·0	136

TABLE III

Effect of various concentrations of SBSE on the weight gain and the amount of feed ingested by S.W.R. white mice

Treatment	Average daily weight gain per mouse during 27 days of trial, g	Average daily feed ingested by 3 mice during 27 days of trial, g
Basic diet	0·133	7·97
Basic diet + 0·5% SBSE	0·157	7·76
Basic diet + 1·0% SBSE	0·143	7·42
Basic diet + 3·0% SBSE	0·156	8·06

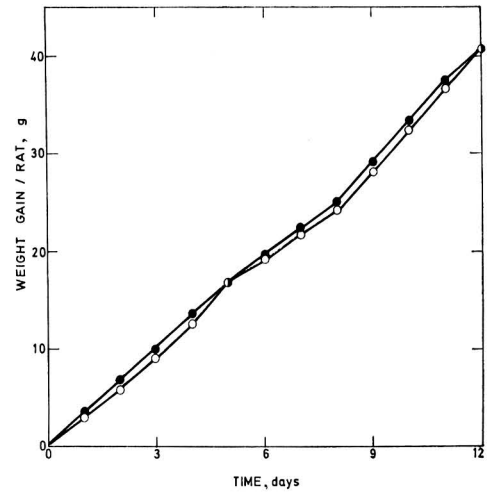

FIG. 2. *Effect of diet supplemented with soyabean saponin on the weight gain of rats*

○ No saponin in the diet
● 1% soyabean saponin extract (SBSE) in the diet

TABLE IV

Effect of SBSE on the longevity of tadpoles (*Bufo viridis*) and guppies (*Lebistes reticulatus*)

SBSE in medium, %	Average life time, min.	
	Tadpoles (100±20 mg body weight)	Guppies (120±20 mg body weight)
0·10	44	41
0·20		23
0·25	25	
0·40		16
0·50	13	

TABLE V

Effect of SBSE, lucerne saponin and cholesterol on the growth rate of *Tribolium castaneum* larvae

SBSE	Lucerne saponin (% in the diet)	Cholesterol	Average weight of *Tribolium castaneum* larvae after 18 days trial, mg
			2·06
0·5			1·81
1·0			1·67
5·0			1·11
10·0			0·82
	0·25		1·49
	0·5		1·16
	1·0		0·90
	0·5	0·5	1·53
	0·5	1·0	1·64
	0·5	2·0	1·84
	1·0	0·5	1·35
		0·5	2·04
		2·0	1·95

of pupation was proportional to the growth impairment, and both effects could be reversed by addition of cholesterol to the diet containing lucerne saponin (Table V). The fact that the growth impairment caused by lucerne saponin is counteracted by cholesterol is probably due to the complex formed between the two. It also indicates that the inhibitory effect of lucerne saponin may arise from complexes formed with larval sterols. This is also supported indirectly by the relative 'harmlessness' of SBSE, which does not combine with cholesterol. On the other hand, soyabean saponins have been proved to be partly responsible for the developmental incompatibility of soyabeans for *Callosobruchus chinensis* L.[17]

It may be concluded that soyabean saponins do not impair the growth of chicks, mice and rats, even when ingested in a 3–5 fold concentration of that present in a common soyabean supplemented diet. This conclusion is also supported by other studies in this laboratory showing that soyabean saponins are hydrolysed by the caecal microflora of rats and chicks and that neither soyabean saponins nor soyabean sapogenins are absorbed into the blood of these animals.[18] The striking differences in cholesterol-binding capacity and biological activities between the chemically similar soyabean and lucerne saponins seem to arise from structural differences between these two groups of leguminous saponins rather than from differences in chemical composition. Further studies on this theme are in progress.

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References

1. Potter, G. C. & Kummerow, F. A., *Science, N.Y.*, 1954, **120,** 224
2. Walter, E. D., Van Atta, G. R., Thompson, C. R. & Maclay, W. D., *J. Am. chem. Soc.*, 1954, **76,** 2271
3. Ishaaya, I. & Birk, Y., *J. Fd Sci. Camb.*, 1965, **30,** 118
4. Heywang, B. W. & Bird, H. R., *Poult. Sci.*, 1954, **33,** 239
5. Anderson, J. O., *Poult. Sci.*, 1957, **36,** 873
6. Peterson, D. W., *J. Nutr.*, 1950, **42,** 597
7. Mickel, C. E., & Standish, J., *Univ. Minn. Agric. exp. Stn Tech. Bull.*, 1946, **175**
8. Mickel, C. E., & Standish, J., *Univ. Minn. Agric. exp. Stn Tech. Bull.*, 1947, **178**
9. Birk, Y., Bondi, A., Gestetner, B., & Ishaaya, I., *Nature, Lond.*, 1963, **197,** 1089
10. Thompson, C. R., Van Atta, G. R., Bickoff, E. M., Walter, E. D., Livingston, A. L., & Guggolz, J., *U.S. Dept Agric. Tech. Bull.*, 1957, **1161,** 63
11. Abell, L. L., Levy, B. B., Brodie, B. B., & Kendall, F. E., *J. biol. Chem.*, 1952, **195,** 357
12. Krama, H., & Schantz, M. V., *Sci. Pharm.*, 1959, **27,** 6
13. Birk, Y., Harpaz, I., Ishaaya, I., & Bondi, A., *J. Insect Physiol.*, 1962, **8,** 417
14. Gestetner, B., Birk, Y., Bondi, A., & Tencer, Y., *Phytochemistry*, 1966, **5,** 803
15. Wasicky, R., & Wasicky, M., *Qualitas Pl. Mater. veg.*, 1961, **8,** 65
16. Vogel, G., *Planta med.*, 1963, **11,** 363
17. Applebaum, S. W., Gestetner, B., & Birk, Y., *J. Insect Physiol.*, 1965, **11,** 611
18. Gestetner, B., Birk, Y., & Tencer, Y., *J. agric. Fd Chem.*, 1968, **16,** 1031

ANALYSIS OF FRUIT JUICE BY ATOMIC ABSORPTION SPECTROPHOTOMETRY

I.—The determination of iron and tin in canned juice

By W. J. PRICE and J. T. H. ROOS

Atomic absorption spectrophotometry has been used to compare the concentrations of iron and tin in canned fruit juice with those in juice not stored in cans. No significant difference was found in the level of iron, but the amount of tin was considerably higher in canned samples. The determination is rapid, and no ashing procedure is required.

Introduction

The determination of traces of contaminants leached from containers in which foodstuffs are stored is of considerable public importance. Foodstuffs which are stored in cans, especially those such as fruit juices which contain a certain residual acidity, are liable to dissolve traces of tin, iron and lead from the container. Iron may be determined readily with *o*-phenanthroline[1] or similar reagents,[2] but the determinations of lead with dithizone[3] and tin with, for example, dithiol reagent[4] are tedious and susceptible to a variety of interferences. For this reason, the possibility of using atomic absorption spectrophotometry for the determination of iron, lead and tin was investigated. However, it soon became apparent that the concentration of lead in the samples analysed was too low for direct determination. Since the atomic absorption technique is both rapid and relatively free from interference, attention was focused on developing a method involving equally rapid sample pre-treatment.

The determination of tin by atomic absorption spectrophotometry has been described by several authors, most of whom have used air–hydrogen flames. The sensitivity of tin absorption in the air–acetylene flame was found by Gatehouse & Willis[5] to be about 5 ppm. Allan[6] used the air–hydrogen flame for increasing tin sensitivity. Amos & Willis[7] used the hotter nitrous oxide–acetylene flame for tin determination and found that the sensitivity was intermediate between that of the air–hydrogen and air–acetylene flames. Amos[8] has recently suggested that interferences which occur in the air–hydrogen flame may be eliminated when the nitrous oxide–acetylene flame is used.

The atomic absorption determination of iron has been extensively investigated and is reported[9] to be virtually interference-free. The determination of iron in plant material and beverages[11] has been described.

Experimental

Apparatus and reagents

A Unicam SP90 Atomic Absorption Spectrophotometer complete with triple lamp turret and nitrous oxide control unit was used throughout this study. The air supply was a Unicam SP93 Air Compressor, and recorder traces were obtained on a Unicam SP22 Recorder. Hollow cathode lamps were supplied by Pye Unicam Ltd.

A Griffin–Christ 'Universal Junior III' centrifuge (supplied by Griffin & George Ltd., London) was used for removal of suspended matter.

Iron stock solution, 1,000 ppm: 1·0 g of high purity iron (B.C.S. 270 : 1) was dissolved in 20 ml of hydrochloric acid (sp. gr. 1·16) and 5 ml of 100 vol. hydrogen peroxide, the solution boiled to remove excess peroxide and diluted to 1 litre in a volumetric flask.

Tin stock solution, 1,000 ppm: 1·0 g of Specpure tin metal was dissolved in 200 ml of hydrochloric acid (sp. gr. 1·16) and diluted to 1 litre in a volumetric flask. This solution was found to be stable for at least six months.

Nitric acid (sp. gr. 1·43), hydrochloric acid (sp. gr. 1·16) and perchloric acid (60% wt./vol.) were all of analytical reagent grade quality and were used without further purification.

Solutions used in investigating possible interferences were prepared from analytical reagent grade chemicals unless otherwise specified.

All solutions were made up in de-ionised water.

Investigation of instrumental conditions

Iron

With a monochromator slit of 0·1 mm (corresponding to a spectrum bandwidth of 0·6 nm) calibration graphs for iron (0–80 ppm) were obtained at different acetylene flow rates between 900 and 1,500 ml/min and different heights (between 0·5 and 1·5 cm) of the light path above the burner top ('burner height' or 'observation height'). The air flow rate was fixed at 5 litre/min. Best sensitivity was obtained with the resonance line at 248·3 nm using an acetylene flow rate of 1,200 ml/min and burner heights of 0·8 to 1·0 cm. The effect of varying the slit width between 0·05 mm and 0·15 mm was also investigated. Predictably, best sensitivity was obtained with the smallest slit width, and sensitivity fell off slightly with wider slits. The sensitivity in a nitrous oxide–acetylene flame was lower than that achieved in an air–acetylene flame by a factor of 2.

Tin

Using a nitrous oxide flow rate of 5 litre/min, the effects of variations in the acetylene flow rate (3,000–5,000 ml/min) and burner height (0·5–1·5 cm) on the tin absorption were investigated at the most sensitive tin wavelength (286·3 nm). Best sensitivity was found when the acetylene flow rate was greater than 4,000 ml/min, with a burner height of 0·5 cm; increasing the acetylene flow rate above 4,000 ml/min produced a negligible increase in sensitivity. The effect of slit width was similar to that for iron.

In an air–acetylene flame, even a highly fuel-rich flame, the sensitivity was lower by a factor of 4 compared with that in nitrous oxide–acetylene, and was not further investigated.

Recommended operating conditions for the determination of both tin and iron are summarised in Table I.

Interferences

Constituents of fruit juices likely to interfere in the estimation of iron and/or tin are potassium ions, citric acid, phosphate ions and sugar. Investigation showed that, of these, both citric acid and sugar interfered seriously in the determination of iron, whilst the determination of tin in the nitrous oxide–acetylene flame was free from interference. The depressive effect of citric acid and sugar on the iron absorption was more noticeable in a fuel-rich air–acetylene flame than in a lean flame; in either case it could be completely removed by the addition of phosphate although very much more phosphate was required when using a fuel-rich flame. Typically, using a lean (therefore hot) flame, the addition of 60 ppm PO_4^{2-} completely eliminated the depressive effect, on 5 ppm of iron, of 10,000 ppm (1% wt./vol.) citric acid. The effect of citric acid could also be eliminated by using a propane–nitrous oxide or an acetylene–nitrous oxide flame, but with a sacrifice of some sensitivity for iron. Because of the necessity to work under conditions of maximum iron sensitivity, and because fruit juices normally contain at least 200 ppm of phosphate ion which is sufficient to overcome the interference from citric acid, a lean air–acetylene flame was used for the determination of iron. The effects of added substances on the iron absorption are summarised in Table II.

Development of the method

Dry ashing of samples of fruit juice was found to be unsatisfactory. Not only was prior evaporation of the sample required, but heating at 500–550° for four hours and longer was insufficient to ash the samples completely. Wet ashing with a mixture of nitric acid and perchloric acid proceeded smoothly and efficiently, but much of the tin in the samples was precipitated as hydrated stannic oxide under these conditions.

The possibility of using a simple dilution of the juice for the direct determination of iron and tin was investigated. In order to ascertain the effect of viscosity on such a method, known quantities of iron were added to successive dilutions of natural orange juice. These were aspirated in the atomic absorption spectrophotometer, and the apparent concentration of iron was determined for each solution. In order to eliminate possible interferences, these determinations were performed using a nitrous oxide–acetylene flame. The results are shown in Table III.

TABLE I

Recommended operating conditions for iron and tin

	Iron	Tin
Wavelength	248·3 nm	286·3 nm
Slit width	0·08–0·1 mm	0·08–0·1 mm
Spectrum bandwidth	0·5–0·6 nm	0·7–0·9 nm
Burner height	0·8–1·0 cm	0·5 cm
Oxidant	Air, 5 l/min	N_2O, 5 l/min
Fuel	Acetylene	Acetylene
Fuel flow rate	1200 ml/min	4000 ml/min
Scale expansion	2–3 times	none
Calibration standards	0–10 ppm	0–50 ppm

TABLE II

Effect of added substances on iron* absorption

Substance	Concentration	Absorbance	Error, %
Nil	—	0·171**	—
Citric acid	50 ppm	0·140**	18
Citric acid	100 ppm	0·118**	31
Sugar	4%	0·164**	4
Nil	—	0·175***	—
Citric acid	50 ppm	0·130***	26
Citric acid	100 ppm	0·088***	50
Sugar	4%	0·142***	19

*30 ppm iron in each case
**lean air–acetylene flame
***fuel-rich air–acetylene flame

Procedure

A 20 ml portion of the sample was transferred to a 100 ml volumetric flask, 10 ml hydrochloric acid was added and the sample was diluted to the mark with water. After thorough mixing a portion of this solution was centrifuged (or, less conveniently, filtered) for analysis. Calibration standards were prepared for iron (0–10 ppm) and tin (0–50 ppm) in 10% (wt./vol.) hydrochloric acid. Using the instrumental settings recommended in Table I, the standard solutions followed by the sample solutions were aspirated.

Accuracy and reproducibility

The accuracy of the proposed method was assessed by analysing samples to which known quantities of iron and tin had been added. The recoveries obtained are given in Table IV. The reproducibilities (expressed as standard deviations) for both iron and tin were calculated on the results of eleven replicate analyses: eleven 20 ml portions of a sample of pineapple juice were treated as outlined under 'Procedure' and aspirated for iron and tin, The results are summarised in Table V. The smallest concentrations of iron and tin in the undiluted juice which could be detected by the proposed procedure were 0·5 ppm and 2·5 ppm respectively. These figures are equivalent to absolute detection limits of 0·1 ppm and 0·5 ppm respectively, but the values will vary somewhat from one hollow cathode lamp to another.

Results and Discussion

The concentrations of iron and tin were determined in both orange juice (fresh, bottled and canned) and pineapple juice (bottled and canned). Each sample was analysed in duplicate according to the proposed method. The results are given in Table VI.

TABLE III

Effect of dilution of fruit juice on iron response

Solution, ml/juice/100 ml	Dilution factor	Iron present, ppm	Apparent iron concentration, ppm	Error, %
0	∞	200	200	—
5	20	200	199	0·5
10	10	200	199	0·5
20	5	200	198	1·0
40	2·5	200	194	3·0
80	1·25	200	187	6·5

TABLE IV

Recoveries of added iron and tin

Iron, ppm			Tin, ppm		
Added	Recovered		Added	Recovered	
	Sample A	Sample B		Sample A	Sample B
0	3·6, 3·7	5·0, 5·1	0	47·0, 47·0	37·7, 34·3
3	6·4, 6·3 6·4	8·1, 8·0	10	56·0, 58·5	46·3, 46·0
6	9·5, 9·5	11·1, 11·3	20	66·0, 66·0	58·5, 58·5

TABLE V

Reproducibility of the method

Sample No.	Iron found, ppm	Tin found, ppm
1	24·0	144
2	24·0	146
3	23·8	144
4	23·8	148
5	23·6	152
6	23·6	148
7	23·8	142
8	23·6	150
9	23·2	150
10	23·8	154
11	23·6	148
Standard deviation	0·21 ppm or 0·9%	3·5 ppm or 2·3%

TABLE VI

Iron and tin in fruit juice

Brand	Description	Iron, ppm	Tin, ppm
—	Fresh orange juice	~0·5, ~0·5	7·5, 7·5
A	Bottled orange juice	2·5, 2·5	30, 25
B	Bottled orange juice	2·2, 2·0	45, 50
C	Bottled pineapple juice	15·5, 15	45, 50
D	Canned orange juice	2·5, 2·5	65, 60
E	Canned orange juice	0·5, 0·5	115, 115
F	Canned orange juice	2·5, 2·5	115, 120
G	Canned pineapple juice	17·5, 17·5	130, 135

As can be seen, very little difference exists in the concentration of iron in canned and bottled juice. However, the concentration of tin appears to be significantly different, with canned juices having predictably a much higher concentration of tin.

An attempt was also made to determine lead in the same solutions, but the level of lead present (if any) was too low to be detected. This indicates a lead content of less than 0·5 ppm in the undiluted samples.

Because of the high sugar content of juice, undiluted samples have a high viscosity. This results in a reduced uptake rate in an atomic absorption spectrophotometer, consequently giving rise to low results. With increasing dilution this effect becomes less and less important, and a 1:5 dilution of the juice is sufficient to minimise the error incurred. A 1:5 dilution is also adequate to prevent blocking of the nitrous oxide burner by the sample solutions. Addition of 1 ml of 1% (by vol.) phosphoric acid to the diluted samples prior to analysis is recommended as a precaution against citrate interference, especially for samples low in phosphate or high in iron.

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References

1. Hummel, F. C., & Willard, H. H., *Ind. Engng Chem., analyt. Edn.*, 1938, **10**, 14
2. Karvanek, M., & Bretschneider, R., *Analyt. Abstr.*, 1963, **10**, 5378
3. Abson, A., & Lipscomb, A. G., *Analyst, Lond.*, 1957, **82**, 152
4. Pearson, D., 'The chemical analysis of food', 5th Edn, 1962 (London: J. & A. Churchill Ltd.)
5. Gatehouse, B. M., & Willis, J. B., *Spectrochim. Acta*, 1961, **17**, 710
6. Allan, J. E., 4th Australian Spectroscopy Conference, Canberra, 1963
7. Amos, M. D., & Willis, J. B., *Spectrochim. Acta*, 1965, **22**, 1325
8. Amos, M. D., *Techtron Element*, 1967, No. 15
9. Allan, J., *Spectrochim. Acta.*, 1958, **10**, 800
10. David, D. J., *Atom. Absorption News*, 1962, **1**, 45
11. Frey, S. W., *Ibid.*, 1964, **3**, 127

EXTRACTION, CHEMICAL NATURE, AND PROPERTIES OF SOIL ORGANIC SULPHUR

By J. R. FRENEY, G. E. MELVILLE and C. H. WILLIAMS

Organic sulphur in soil appeared to be stable in mildly alkaline solutions at 20 , and thus reagents such as bicarbonate or the sodium form of a chelating resin may be used for the partial extraction of organic sulphur in a chemically unmodified form. More complete extraction of organic sulphur from soil could be achieved by the use of sodium hydroxide solutions at pH 12·6, but degradation of humic acid sulphur to fulvic acid sulphur and conversion of organic sulphur to inorganic sulphur occurred in both hot and cold hydroxide solutions at this pH. A comparison of N : S ratios and percentages of reducible sulphur in soils and extracts suggested that none of the reagents studied extracted a sample of organic matter that was fully representative of the organic matter in soil. The results confirm that most of the sulphur in these surface soils is organic and that there is very little inorganic sulphate present.

Sulphur that could be reduced by hydriodic acid occurred in compounds of both high and low molecular weight but data obtained from extraction with bicarbonate and the chelating resin suggested that much of the reducible sulphur occurred in the high molecular weight fraction of soil organic matter.

Introduction

Total sulphur in the surface horizons of many agricultural soils is strongly correlated with organic nitrogen and organic carbon[1,2] and very little inorganic sulphur can be extracted as sulphate or hydrogen sulphide.[2] This, and evidence on N:S ratios[3] has led to assumptions that most of the sulphur in such soils is organic.[4,5]

Few attempts have been made to determine the chemical nature of organic sulphur in soil.[2,6,7] Shorey[8] isolated trithiobenzaldehyde from soil, and sulphur-containing amino acids have been reported to occur free in soil in small quantities[9] and in acid hydrolysates of soil and soil organic matter preparations.[10] Reduction studies with hydriodic acid suggested that a significant fraction of the sulphur in soil was present as organic sulphates, and when boiling sodium hydroxide was used in an attempt to extract this material it was found that most of the reducible sulphur occurred in the fulvic acid fraction.[2]

Little progress in elucidating the exact nature of this sulphur can be expected until a method is found which will separate the organic matter from the inorganic material in soil without change. While a number of reagents have been proposed for this, none has yet proved successful.

Previous work[2] showed that reducible organic sulphur could be converted to inorganic sulphate by heating the soil with 6 N hydrochloric acid and the results suggested that this transformation also occurred, to some extent, in boiling alkali. Although this work suggested that the major part of the reducible sulphur occurred in the fulvic acid (low molecular weight) fraction, this may have been the result of degradation of humic acid in the boiling sodium hydroxide. Again, no information is available on the stability of reducible sulphur in cold alkaline solutions—information which is required before a satisfactory extraction procedure can be devised.

The aim of the present work was to examine the suitability of a number of reagents for the extraction of hydriodic acid reducible sulphur from soil. Attempts were made to assess any changes in sulphur fractions taking place during the extraction of the soil and during the subsequent storage of the extracts, so that the proportion of reducible sulphur occurring in compounds of high and low molecular weight could be assessed.

Experimental

Soils

The 0–4 in layer of surface soil was collected from three different localities in southern New South Wales. It was air-dried and ground to pass a 2 mm mesh sieve. These soils were a yellow podzolic soil derived from siliceous shale, a yellow podzolic soil derived from granodiorite and a chocolate soil derived from basalt.

Extraction of sulphur

Except where otherwise indicated, all extractions were carried out at 20° on a reciprocating shaker.

The following reagents or procedures were tested: (a) chelating resin which appears to have advantages over soluble complexing agents and sodium hydroxide,[11,12] and has been reported to extract a representative portion of the organic matter from European soils[31]; (b) sodium bicarbonate solution at 90° used by Lowe & De Long[14] for the extraction of organic sulphur; (c) sodium pyrophosphate solution at pH 7, and 0·5 N sodium hydroxide solutions which are commonly used for the extraction of soil organic matter; and (d) alkaline extractants in conjunction with an ultra-sonic treatment—alkaline acetylacetone with ultra-sonic disintegration extracted 85–100% of the sulphur from Scottish and Canadian soils.[15]

(*a*) The soil sample (10 g) was shaken with 3 g of wet Chelex 100 resin (100–200 mesh resin, sodium form, as supplied by Bio-Rad Laboratories, Richmond, California, U.S.A.) and 48 ml of water in a 4 oz polyethylene bottle for periods ranging from 1 hour to 12 days. The extracts were centrifuged at 10,600 rev/min (14,350 × *g*) for 15 minutes and filtered through a Whatman No. 42 filter paper. Part of the filtrate was then centrifuged at 30,000 rev/min (100,000 × *g*) for an additional 3 hours.

(*b*) 10 g of soil were shaken with 50 ml of 0·5 N sodium hydroxide solution, 0·2 N sodium bicarbonate–0·3 N sodium carbonate solution at pH 10·2, or with 0·1 M sodium pyrophosphate solution at pH 7 for varying periods. The extracts were filtered through Whatman No. 42 filter papers, and the residues were washed with water. The filtrate and washings were diluted to 100 ml with water.

(*c*) 10 g of soil were extracted for 16 hours with 200 ml of 0·5 M sodium bicarbonate solution, adjusted to pH 10 with

sodium hydroxide.[14] After removal of soil by filtration part of the filtrate was heated at 90° for 16 hours.

(*d*) Soil samples were extracted with alkaline solutions using a Dawe Soniprobe in a manner similar to that described by Halstead *et al.*[15]

Analytical

Total sulphur

This was determined in soil extracts by the sodium bicarbonate–methylene blue method described by Steinbergs *et al.*[16] after appropriate aliquots of the extracts had been evaporated to dryness, in 1·5 × 1·5 cm porcelain crucibles, on a water bath.

Inorganic sulphate

The extract (10 ml), which had been centrifuged at 30,000 rev/min, was acidified to pH 1 with hydrochloric acid, and 50 μg S as potassium sulphate and 0·2 g of sulphur-free charcoal were added. The mixture was stirred, allowed to stand for 15 minutes, and then filtered through a Whatman No. 42 filter paper. A 2 ml aliquot of the colourless filtrate was analysed for inorganic sulphate by the barium sulphate precipitation–hydriodic acid reduction method described by Freney.[17] The potassium sulphate was added to assist in the precipitation of barium sulphate when only small amounts of inorganic sulphate were present.

Humic and fulvic acid sulphur

The pH of the soil extracts was adjusted to 1, by addition of hydrochloric acid, using a glass electrode assembly. The acidified extracts were allowed to stand for several hours and then filtered through a Whatman No. 42 filter paper. The acid-insoluble fraction, 'humic acid', on the filter paper was analysed for total sulphur by the method of Steinbergs *et al.*[16] The acid-soluble fraction, 'fulvic acid', was concentrated by evaporation on a sand bath, transferred to 1·5 × 1·5 cm porcelain crucibles, evaporated to incipient dryness, and total sulphur determined by the method of Steinbergs *et al.*[16]

Reducible sulphur

This was determined by reacting 0·1 g soil, or a suitable aliquot of the extract, with 4 ml of a reducing mixture, consisting of hydriodic acid (sp. gr. 1·7), formic acid (90%) and hypophosphorous acid (50%) in the ratio of 4:2:1 (by vol.), using the apparatus and reagents described by Johnson & Ulrich.[18]

Total organic sulphur

This was determined in soils by a modification of the method outlined by Bardsley & Lancaster,[19] in which phosphate solution (500 ppm P) instead of the acetate solution was used to displace inorganic sulphate, and zinc and hydrochloric acid were used to remove sulphides.

Total nitrogen

This was determined on suitable aliquots of the extracts or on 0·5 g samples of soil by a micro-modification of the Kjeldahl method described by Piper.[20]

Results

Determinations of organic sulphur showed that 164, 260 and 264 ppm S out of the total of 166, 264 and 268 ppm S for soils 1, 2 and 3 respectively was organic sulphur. Thus, it is apparent that most of the sulphur extracted originated from organic forms.

Except for the 12 day extraction with hydroxide, none of these procedures extracted more than 50% of the total sulphur (see Table I). In all cases the amount of sulphur extracted depended upon the alkalinity of the reagent and on the time of extraction. The order of extraction for a set period was $Na_4P_2O_7$ < resin < $NaHCO_3$–Na_2CO_3 < NaOH. The pH of the resin suspension, which was 10·2 at the beginning, fell during the extraction, whereas that of the soluble extractants did not change significantly.

The N:S ratios for the organic matter extracted by the alkaline and neutral reagents were considerably less than the N:S

TABLE I

Total sulphur, total nitrogen, and N:S ratios in soils and soil extracts

ppm S and N

Soil No.	Fraction	In soil	In extract: Sodium pyrophosphate* (1 h)	Chelex 100** (1 h)	Sodium bicarbonate–carbonate** (1 h)	Sodium hydroxide*** (1 h)	Chelex 100 (12 days)	Sodium bicarbonate–carbonate (12 days)	Sodium hydroxide (12 days)
1	Total S	166	29	40	40	68	73	84	130
	Total N	1562	193	332	277	515	588	516	974
	N : S	9·4	6·7	8·3	6·9	7·6	8·1	6·1	7·5
2	Total S	264	42	50	63	113	107	123	182
	Total N	2702	252	350	456	666	858	928	1388
	N : S	10·2	6·0	7·0	7·2	5·9	8·0	7·5	7·6
3	Total S	268	38	35	49	75	76	106	159
	Total N	2524	171	210	298	585	455	661	1190
	N : S	9·4	4·5	6·0	6·1	7·8	6·0	6·2	7·5

* 7·2
** 10·2
*** 12·6
} Initial pH. Final pH of Chelex extracts, 9·4, 9·2, 7·8 for soils 1, 2, 3 respectively

ratios of the original soils. Relatively more sulphur than nitrogen was extracted by these reagents, and the greatest difference was obtained with the pyrophosphate extracts (see Table I).

Reducible sulphur

Hydroxide extracted more hydriodic acid-reducible sulphur than the other extractants and, in general, pyrophosphate extracted less (see Table II). In addition, hydroxide, in one hour, appeared to extract reducible sulphur at the expense of the other sulphur fractions. This can be seen from a comparison of the figures obtained when reducible sulphur is expressed as a percentage of the total sulphur in soils and extracts (see Table II). The percentage reducible sulphur in the extracts more nearly approached that in the original soil when the extractions were continued for 12 days.

Humic and fulvic acid sulphur

The acid-soluble sulphur fraction in all the extracts is termed 'fulvic acid' sulphur and the corresponding insoluble fraction is referred to as 'humic acid' sulphur, in accordance with common usage. However, this should not be interpreted as suggesting that fulvic, or humic, acids obtained from different extracts are, in fact, the same or even similar.

As the relative amounts of these two fractions in soil are unknown, it is difficult to draw firm conclusions from the relative amounts present in the extracts. However, the results (Table II) show that the absolute amounts and relative proportions of these two fractions differed from extract to extract. Of the 1 h extracts, hydroxide contained more humic and fulvic acid sulphur than the other three. The other alkaline extracts, resin and bicarbonate–carbonate, contained similar amounts of humic acid sulphur, and the extracts with the soluble reagents, pyrophosphate and bicarbonate–carbonate, contained similar amounts of fulvic acid sulphur. Pyrophosphate extracts contained small amounts of humic acid sulphur, and resin extracts were very low in fulvic acid sulphur. The humic acid sulphur:fulvic acid sulphur ratios varied widely, being on the average 0·60, 1·17, 1·39 and 3·25 for the 1 h extracts with pyrophosphate, bicarbonate–carbonate, hydroxide and resin respectively (see Table II).

No changes occurred in sulphur fractions when the 1 h extracts with resin, bicarbonate–carbonate, and pyrophosphate were stored at 20° and at the pH of the respective extracts for 12 days. The appropriate figures for the resin extracts are given in Table III. However, changes did take place in these fractions when hydroxide and resin extracts were stored at pH 12·6.

There appeared to be degradation of humic acid sulphur to the low molecular weight fulvic acid sulphur in these highly alkaline solutions. The reducible sulphur content of the fulvic acid fraction also increased when the highly alkaline solutions were stored, but there was no change in the reducible sulphur content of the whole extracts (see Table III).

Inorganic sulphate

Only small amounts of inorganic sulphate were present in the 1 h extracts with pyrophosphate, resin and bicarbonate–carbonate (2, 4, and 1 ppm S for soils 1, 2 and 3 respectively); the amounts present in these extracts were very similar to those present in the original soils. Hydroxide extracts, however, contained at least twice the amount of sulphate present in the other extracts (4, 8, and 6 ppm S respectively).

No changes occurred in sulphate concentrations in the resin, bicarbonate–carbonate or pyrophosphate extracts kept at 20°, and at the pH of the extracts, for 24 h. However, there were significant increases in inorganic sulphate concentrations in the hydroxide extracts when these were stored at pH 12·6 for the same period and at the same temperature (sulphate increased to 6, 10, 7 for soils 1, 2 and 3 respectively).

Added sulphate could be completely recovered by the method used if the extracts were centrifuged at 30,000 rev/min

TABLE II

Reducible sulphur, humic acid sulphur, and fulvic acid sulphur in soil extracts

Fraction	Soil No.	In soil	In extract						
			Sodium pyrophosphate (1 h)	Chelex 100 (1 h)	Sodium bicarbonate–carbonate (1 h)	Sodium hydroxide (1 h)	Chelex 100 (12 days)	Sodium bicarbonate–carbonate (12 days)	Sodium hydroxide (12 days)
Reducible S (ppm)	1	54	10	13	13	29	24	35	45
	2	88	19	24	28	63	40	44	68
	3	102	19	13	26	48	33	44	70
Reducible S (as a % of total S)	1	32·5	34·5	32·5	32·5	42·6	32·9	41·7	34·6
	2	33·3	45·2	48·0	44·4	55·8	37·4	35·8	37·4
	3	38·1	50·0	37·1	53·0	64·0	43·4	41·5	44·0
Humic acid S (ppm)	1		13	32	23	42	48	55	66
	2		17	37	37	67	79	74	101
	3		9	26	21	39	59	55	81
Fulvic acid S (ppm)	1		16	8	17	26	24	29	64
	2		25	13	26	46	28	49	81
	3		29	9	28	36	17	51	78
Humic acid S / Fulvic acid S	1		0·81	4·00	1·35	1·62	2·00	1·90	1·03
	2		0·68	2·85	1·42	1·46	2·82	1·51	1·25
	3		0·31	2·89	0·75	1·08	3·47	1·08	1·04
	Mean		0·60	3·25	1·17	1·39	2·76	1·50	1·11

but not when the extracts were centrifuged at 10,600 rev/min. The high-speed centrifugation markedly reduced the ash contents of the humic acid fraction of all the extracts, with the greatest reduction occurring in the resin extracts (see Table IV). The corresponding changes in total sulphur owing to centrifugation were very small, and it is difficult to determine whether this small amount of sulphur was associated with the clay removed or whether it was in separate high molecular weight organic compounds.

The presence of clay in the sediment after high-speed centrifugation was confirmed by *X*-ray diffraction. This suggested that the presence of clay was responsible for the low sulphate recoveries in extracts prepared by centrifugation at 10,600 rev/min since, during the clarification procedure, when the solution is acidified, sulphate could be absorbed by the clay and removed from solution.

Reducible sulphur in humic and fulvic acid fractions

The results given in Table V show that the 1 h extracts with pyrophosphate and bicarbonate–carbonate, and the 12 day resin extracts contained similar amounts of reducible sulphur in fulvic acid. Very little reducible sulphur in humic acid was present in the pyrophosphate extracts. The 1 h extracts with bicarbonate–carbonate and resin contained amounts of reducible sulphur in humic acid which were similar, and which were considerably larger than those present in the pyrophosphate extracts. The amount extracted was increased by increasing the period of extraction (see Table V).

The 12 day hydroxide extracts contained much more reducible sulphur in fulvic acid than any of the other extracts and less reducible sulphur in humic acid for soils 2 and 3 than the 1 h hydroxide extracts. This suggests that breakdown of humic acid occurred during extraction. Apart from the 1 h hydroxide extracts the 12 day resin extracts contained the largest amount of reducible sulphur in humic acid.

When hydroxide, bicarbonate–carbonate, and bicarbonate extracts obtained at 20° were heated, the reducible-sulphur content of the whole extract increased, as well as the reducible-sulphur content of the fulvic acid fraction (see Table VI).

TABLE III

Stability of a number of sulphur fractions in Chelex 100 extracts at different pH values

ppm S

Sulphur fraction	Soil No.	1 hour extract	1 hour extract stored at 20°c for 12 days*	1 hour extract stored at 20°c and pH 12·6 for 12 days
Fulvic acid S	1	8	8	16
	2	13	14	23
	3	9	9	16
Humic acid S	1	32	32	24
	2	37	36	27
	3	26	26	19
Reducible S in the fulvic acid fraction	1	6	6	10
	2	7	7	13
	3	5	5	7

* pH of the extracts, after removal of soil, was 9·4, 9·2 and 7·8 for soils 1, 2 and 3 respectively.

The reducible sulphur content of the whole extract did not change over the 12-day period at any pH value.

TABLE IV

Effect of centrifugation on total sulphur content of the extract, and ash content of the humic acid

Extractant	Soil No.	Total sulphur, ppm S		Ash in humic acid fraction, %	
		10,600 rev/min	30,000 rev/min	10,600 rev/min	30,000 rev/min
Sodium pyrophosphate	1	29	24	16·0	6·0
	2	42	35	17·2	6·2
	3	38	32	34·5	7·5
Chelex 100	1	40	36	24·3	5·9
	2	50	45	24·4	6·0
	3	35	29	57·6	7·6
Sodium bicarbonate–carbonate	1	40	35	9·6	5·0
	2	63	60	11·0	4·8
	3	49	44	15·3	7·7
Sodium hydroxide	1	68	60	19·2	5·4
	2	113	95	21·6	5·6
	3	75	66	35·5	6·3

TABLE V

Reducible sulphur in the fulvic acid and humic acid fractions of soil organic matter extracted by pyrophosphate, bicarbonate–carbonate, hydroxide and resin

ppm S

Fraction	Soil No.	Extractant						
		Pyrophosphate (1 h)	Bicarbonate–carbonate (1 h)	Bicarbonate–carbonate (12 days)	Chelex 100 (1 h)	Chelex 100 (12 days)	Hydroxide (1 h)	Hydroxide (12 days)
Humic acid	1	0	4	22	7	18	14	17
	2	3	18	27	17	31	37	22
	3	5	11	20	8	20	27	16
Fulvic acid	1	10	9	13	6	6	15	28
	2	16	10	17	7	9	26	46
	3	14	15	24	5	13	21	54

TABLE VI

Effect of heating on the content and distribution of reducible sulphur in 0·5 N sodium hydroxide, 0·2 N sodium bicarbonate–0·3 N sodium carbonate, and 0·5 M sodium bicarbonate* extracts

ppm S

Fraction	Soil No.	Hydroxide extract	Hydroxide extract heated**	Bicarbonate–carbonate extract	Bicarbonate–carbonate extract heated***	Bicarbonate extract	Bicarbonate extract heated***
Whole extract	1	45	58	35	44		
	2	68	82	44	60		
	3	70	86	44	60	37	50
Fulvic acid	1	28	48	13	21		
	2	46	62	17	35		
	3	54	66	24	41	26	42

* Lowe & De Long[11] attempted to extract organic sulphur from soil by heating with 0·5 M sodium bicarbonate at pH 10·0 and 90°C for 16 h
** Heated at 120°C for 4 h
*** Heated at 90°C for 16 h

Ultrasonic treatment

It was found that, although alkali alone did not dissolve any sulphur from the tip of the sonifier, appreciable amounts of sulphur were dissolved from it by alkaline solutions of soil organic matter. The reducible-sulphur contents of soil extracts were increased by the equivalent of 120–200 ppm S in soil during 30 min of extraction, resulting in levels which were greater than the original total sulphur content of the soil. In view of this high level of contamination, the study of this technique was not considered possible.

Discussion

Except for sodium hydroxide, none of the reagents examined extracted more than 50% of the total soil sulphur and, therefore, cannot be regarded as being effective extractants. Because the N:S ratio and the percentage of reducible sulphur in all extracts differed from those of the original soil, it is apparent that none of the reagents extracted a sample that was fully representative of the whole soil organic matter. It is possible, however, that they did extract unchanged discrete fractions with composition different from that of the whole organic matter.

Degradation of humic acid sulphur to fulvic acid sulphur was apparent in the highly alkaline extracts (i.e. at pH 12·6) and, thus, the ratios for humic acid S:fulvic acid S in these extracts cannot be indicative of the proportions of these two fractions in soil. Breakdown of humic acid sulphur did not appear to occur in the cold pyrophosphate, bicarbonate–carbonate or resin extracts in 12 days, and it is therefore possible that no breakdown occurred during the 1 h extraction. The different ratios of humic acid S-fulvic acid S in these three extracts must then reflect differential extraction by the individual reagents. It appears that fulvic acid sulphur was more readily accessible to the soluble reagents than to the insoluble complexing reagent, and the poor extraction of humic acid sulphur by neutral pyrophosphate agrees with the results previously obtained for nitrogen.[21] Therefore, it is not possible to obtain any indication of the relative amounts of these two forms of sulphur in soil from these results.

It is important to note that there was little inorganic sulphate in any of the extracts, and that no increase occurred in this fraction when the mildly alkaline solutions were stored. Organic sulphur was, however, converted to inorganic sulphate when the alkalinity was increased; this again demonstrates the labile nature of organic sulphur in strongly alkaline solutions.

A comparison of the results obtained for the distribution of reducible sulphur between humic and fulvic acids in the 1 h and 12 day hydroxide extracts show that degradation of the humic fraction must have occurred in the highly alkaline solutions. This was confirmed by following the changes in reducible sulphur in resin extracts stored at pH 12·6. As no

changes appeared to occur in the distribution of reducible sulphur between the humic and fulvic fractions in pyrophosphate, bicarbonate–carbonate or resin extracts stored under mild conditions, it must be concluded that pyrophosphate was a good extractant of fulvic acid reducible sulphur but was a poor extractant of humic acid reducible sulphur. The other two reagents appeared to extract both fractions over an extended period and it is possible that the distribution of reducible sulphur in these extracts was more indicative of the distribution in soil than that occurring in either pyrophosphate or hydroxide extracts. The results certainly suggest that much of the reducible sulphur in soil occurs in high molecular weight compounds (humic acid) and that previous observations[2] regarding the distribution of reducible sulphur in hot hydroxide extracts should not be extrapolated to the soils themselves.

Organic sulphur in soil can be divided into two fractions, namely one in which the sulphur is not directly bonded to carbon and can be reduced to hydrogen sulphide by hydriodic acid, and one in which the sulphur is directly bonded to carbon and is not reduced to hydrogen sulphide by hydriodic acid.[17] The increase in reducible sulphur in the whole hydroxide, bicarbonate or bicarbonate–carbonate extracts on heating suggests, therefore, that cleavage of carbon–sulphur bonds had occurred. This type of fission in cystine residues yielding sulphur not bonded to carbon has been reported to occur in proteins and peptides.[22]

The increase in reducible sulphur in fulvic acid in the heated alkaline extracts may also have been due to cleavage of the carbon–sulphur bonds or to degradation of the reducible sulphur compounds in the humic acid fraction. These results demonstrate conclusively that hot alkaline solutions[2,14] cannot be used for the extraction of organic sulphur in unmodified form.

It appears, then, that organic sulphur in soil is stable at 20° in mildly alkaline solutions (at least up to pH 10·2), and that any of the reagents studied, with the exception of hydroxide at pH 12·6, could be used for the extraction of a fraction of the organic sulphur from soil in a chemically unmodified form. If the resin is used for this purpose, then it may be necessary to extend the extraction period to extract a reasonable amount of fulvic acid sulphur.[3] Bicarbonate–carbonate appeared to extract both forms of sulphur without detectable change and, thus, may be a suitable reagent for the extraction of organic sulphur from soil.

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References

1. Donald, C. M., & Williams, C. H., *Aust. J. agric., Res.* 1954, **5,** 664
2. Freney, J. R., *Aust. J. agric. Res.*, 1961, **12,** 424
3. Melville, G. E., Freney, J. R., & Williams, C. H., *J. Sci. Fd Agric.*, 1969, **20,** 203
4. Walker, T. W., *Agrochimica*, 1964, **9,** 1
5. Whitehead, D. C., *Soils Fertil.*, 1964, **27,** 1
6. Lowe, L. E., & De Long, W. A., *Can. J. Soil Sci.*, 1963, **43,** 151
7. Lowe, L. E., *Can. J. Soil Sci.*, 1965, **45,** 297
8. Shorey, E. C., *U.S. Dept. Agric. Bur. Soils*, Bull. No. 88
9. Paul, E. A., & Schmidt, E. L., *Proc. Soil Sci. Soc. Am.*, 1961, **25,** 359
10. Bremner, J. M., in 'Soil Biochemistry', (eds McLaren, A. D., & Peterson, G. H.,) 1967, p. 19 (New York: Marcel Dekker)
11. Bremner, J. M., & Ho, C. L., *Agron. Abstr.*, 1961, p. 15
12. Yuan, T. L., *Soil Sci.*, 1964, **98,** 133
13. Cheng, H. H., & van Hove, J., *Pedologie, Gand*, 1964, **14,** 8
14. Lowe, L. E., & De Long, W. A., *Can. J. Soil Sci.*, 1961, **41,** 141
15. Halstead, R. L., Anderson, G., & Scott, N. M., *Nature, Lond.*, 1966, **211,** 1430
16. Steinbergs, A., Iismaa, O., Freney, J. R., & Barrow, N. J., *Analytica chim. Acta*, 1962, **27,** 158
17. Freney, J. R., *Soil Sci.*, 1958, **86,** 241
18. Johnson, C. M., & Ulrich, A., *Calif. Agric. Expt Stn*, 1959, Bull. No. 766, p. 54
19. Bardsley, C. E., & Lancaster, J. D., 'Methods of Soil Analysis', 1965, Vol. IX, p. 1102 (Madison, Wisconsin: American Society of Agronomy)
20. Piper, C. S., 'Soil and Plant Analysis', 1942 (Adelaide: University of Adelaide)
21. Choudhri, M. B., & Stevenson, F. J., *Proc. Soil Sci. Soc. Am.*, 1957, **21,** 508
22. Savige, W. E., & Maclaren, J. A., in 'The Chemistry of Organic Sulphur Compounds' (eds Kharasch, N., & Meyers, C. Y.), 1966, Vol. II, p. 367 (Oxford: Pergamon Press)

ASSOCIATION BETWEEN LOSS OF VOLATILES AND REDUCED INCIDENCE OF BREAKDOWN IN JONATHAN APPLES ACHIEVED BY WARMING DURING STORAGE

By R. B. H. WILLS and W. B. McGLASSON

The amounts of n-butyl, iso-amyl and n-hexyl alcohols, and their acetate esters, and the ratios of acetate ester:alcohol given off by Jonathan apples during storage at −1° were examined. After 14 weeks' storage, warming of the fruit at 20° for three days resulted in increased emanation of the alcohols and esters, and an increase in the ratios of ester:alcohol given off during subsequent storage at −1°. The increase in ester: alcohol ratios indicated that warming had a differential effect on the metabolism of these compounds. These data support the hypothesis that loss of acetate is involved in controlling the development of low-temperature breakdown in apples.

Introduction

Low-temperature breakdown is a physiological disorder which affects many varieties of apples. Various workers[1-4] have suggested that a reduction in breakdown is achieved by treatments which assist the removal of a toxic volatile substance from the fruit.

Wills[5] studied the volatiles given off by fruit stored under conditions which promoted different rates of water loss and, hence, different susceptibilities to breakdown.[3] He found that, as the rate of water loss increased, the ratios of the loss of n-butyl, iso-amyl and n-hexyl acetates to their corresponding alcohols increased. Wills & Scott[6] studied the relation between the incidence of breakdown in individual apples which showed a range in natural susceptibility when stored under uniform conditions, and the volatiles given off during storage. They found that the three ratios of acetate ester: alcohol decreased as the susceptibility to breakdown increased.

These relations were further studied in this paper using the mid-storage warming treatment,[2,7] a well-known method of reducing breakdown that is totally different from those used previously.[3,5] The fruit were warmed for three days during storage, and the volatiles given off by the fruit were analysed one week after the fruit were returned to low temperature.

Materials and Methods

Jonathan apples from Bilpin, New South Wales were distributed at random into 32 groups, each of 20 fruit. Each group was stored at −1° in a box lined with polyethylene film. After 14 weeks' storage, 20 groups were exposed by unfolding the polyethylene film, and transferred to a temperature of 20° for three days. They were then returned to storage at −1° in the polyethylene-lined boxes. The fruit were transferred to a temperature of 20° after 26 weeks at −1° and examined for breakdown after a further seven days.

The volatiles given off by all units were collected one week after the warmed fruit had been returned to −1°. The methods described by Wills[5] were used for the collection and g.l.c. analysis of esters and alcohols. The amount of acetic acid given off was estimated by g.l.c. on a 3 foot column of 20% Tween 80 on Celite (60–80 mesh, acid-washed) with a flame ionisation detector. The operating conditions were: oven temperature, 120°; nitrogen carrier gas flow rate, 40 ml/min; hydrogen flow rate, 20 ml/min; air flow rate, 300 ml/min; volume injected, 10 μl.

Results and Discussion

Following the warming treatment there was a significant increase ($P < 0.01$ in all cases) in the amounts of the three

TABLE I

Effect of warming on loss of volatiles

Compound	Treatment	Amounts given off (μg/kg per day)		
		butyl	iso-amyl	hexyl
Ester	Warmed (n=20)	36·0 ± 2·4	10·7 ± 0·50	29·3 ± 0·93
	Not warmed (n=12)	10·3 ± 1·2	3·3 ± 0·20	14·0 ± 0·80
Alcohol	Warmed	5·0 ± 0·18	1·8 ± 0·07	4·9 ± 0·24
	Not warmed	2·3 ± 0·08	0·9 ± 0·07	3·0 ± 0·18
Ester:alcohol	Warmed	2·4 ± 0·11	2·0 ± 0·08	2·1 ± 0·12
	Not warmed	1·7 ± 0·16	1·3 ± 0·06	1·6 ± 0·06

acetate esters and the three alcohols given off by the fruit and the ratios of ester:alcohol (Table I). The rate of loss of acetic acid (10·3 ±0·9 and 10·3 ±1·4 μg/kg per day from warmed and control fruit respectively) was not affected.

The incidence of breakdown, after 26 weeks' storage at −1°, in the warmed fruit (15·8% affected) was significantly lower ($P < 0.001$) than the incidence of the disorder in the untreated fruit (47·5% affected).

These effects are consistent with those obtained from the humidity treatments used by Wills[5] as both the rate of loss of ester and the rate of loss of ester relative to that of alcohol increased following the treatment which would be expected to cause the least breakdown. The increased loss of acetate has now been associated with three different conditions which result in reduced breakdown, namely increased water loss, lower natural susceptibility and warming during storage. It therefore seems likely that increased loss of acetate from the fruit plays a positive role in reducing breakdown.

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References

1. Overholser, E. L., Winkler, A. J., & Jacob, H. E., *Bull. No.* 370, *Calif. agric. exp. Stn*, 1923
2. Smith, W. H., *Nature, Lond.*, 1958, **181,** 275
3. Scott, K. J., & Roberts, E. A., *Aust. J. exp. Agric. Anim. Husb.*, 1967, **7,** 87
4. Martin, D., Lewis, T. L., & Cerny, J., *Aust. J. Agric. Res.*, 1967, **18,** 271
5. Wills, R. B. H., *J. Sci. Fd Agric.*, 1968, **19,** 354
6. Wills, R. B. H., & Scott, K. J., *Aust. J. biol. Sci.*, 1968, **21,** 1307
7. Scott, K. J., & Roberts, E. A., *Aust. J. exp. Agric. Anim. Husb.*, 1968, **8,** 377

ERRATA

In the paper by Möttönen *J. Sci. Fd Agric.*, 1969, **20**

Page 281 left hand column line 5
Page 283 right hand column line 5
and Page 284 left hand column line 10

for 'tan α/'*flour*' and tan α/(*c*/*f*)'
read '(tan α)/'*flour*' (tan α)/(*c*/*f*)'

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JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

JULY, 1969

1.—AGRICULTURE AND HORTICULTURE

General: Soils and Fertilisers

Possibilities of palaeopedology and limitations of its use. J. Fink (*Z. PflErnähr. Bodenk.*, 1968, **121**, 19–33).—A review with 15 references. M. LONG.

Formation of chalky bands in soils in warm dry areas. H. Franz and G. Franz (*Z. PflErnähr. Bodenk.*, 1968, **121**, 34–42).—These bands, where still in process of formation, are typical of semi-arid climates, such as parts of Morocco and the Canary Isles. Periodic, slight rain penetrates only to a shallow depth; during the dry periods this water evaporates, pptg. the dissolved solids within the moistened zone. Since no biological mixing takes place, this zone is enriched. The same applies to chalk originating from calcareous siliceous rocks, such as basalt, so that a chalky band forms above this type of rock. Formation of such bands indicates a deterioration in physical properties of soil and facilitates erosion. (13 references.) M. LONG.

Dune catena on the clay plains of the west central Gezira, Republic of the Sudan. M. A. J. Williams (*J. Soil Sci.*, 1968, **19**, 367–378).—The form, distribution, and possible origin of the dunes is discussed, and the dune-swale soil catena is described. A. H. CORNFIELD.

[A] Soils of the semi-arid South West Africa. [B] Soils of the dry South West African savannah. H. Scholz (*Z. PflErnähr. Bodenk.*, 1968, **120**, 105–118; 118–130).—[A] Much of the area surveyed adjoins the Namib desert and has rainfall averaging 100–200 mm, and is characterised by calcareous crusts. Numerous profile data are presented. (20 references.)

[B]. The area examined has an average rainfall of 200–300 mm. It is largely covered with old, red soils of pleistocenic origin, interspersed with more recent brown soils. The brown colour is characteristic of the area. (19 references.) A. G. POLLARD.

Soils of the moist South West African savannah. H. Scholz (*Z. PflErnähr. Bodenk.*, 1968, **120**, 208–221).—Deep layers of loose rock were sedimented during the pleistocene period and deep red brown soils have developed in this material. The red-brown soils are mostly found in the plains, whilst the valley soils and deeper sites are mid-brown in colour. The black soils of the depressions are rich but of only local occurrence. The light brown soils on old dune sands are very impoverished. Soils with hardpans are occasionally found and others with grey sediments rich in clay are found in the backwater zone of the dams. (18 references.) M. LONG.

Characterisation of the inositol penta- and hexa-phosphate fractions of Canadian and Scottish soils. R. B. McKercher and G. Anderson (*J. Soil Sci.*, 1968, **19**, 302–310).—Esters of *myo-* and *scyllo*inositol together constituted more than 90% of the mixed esters extracted from a number of Canadian (*C*) and Scottish (*S*) soils by 3N-NaOH and separated by anion exchange chromatography. Relatively small amounts of *dl*-inositol and neoinositol were also detected in hydrolysates. The ratio of *myo-*+*dl*-inositol hexaphosphates to *scyllo*inositol hexaphosphate ranged from 1·1 to 2·7 in *C* and 1·8 to 4·6 in *S* soils. The ratio of hexa- to pentaphosphates ranged from 0·9 to 2·4 in *C* and 3·0 to 4·3 in *S* soils. There were no consistent relationships between the constitution of the inositol polyphosphate fraction and other soil properties. A. H. CORNFIELD.

Phosphorus reactions with soils high in iron oxides. U. S. Sree Ramulu (*Diss. Abstr., B.*, 1967, **28**, 20)—P fixation in samples of 12 acid soils having relatively high contents of free Fe oxides and low contents of Al oxides is examined. Fe oxides (determined by dithionite method) and the P fixed were significantly correlated but regression analysis indicated the presence of two groups of samples and one lone individual. Kaolinitic samples formed one group and the second comprised those containing kaolinite (*K*) and vermiculite (*V*) and having a lower ratio of P fixed/Fe oxide content. The lone sample contained *K* and gibbsite. The regression, P fixed *vs.* oxalate (darkness)-extractable Fe oxides showed that all samples, except the one containing gibbsite, fell into one group. Differences in correlations between the results suggest the presence of a non-reactive fraction of free Fe oxides in the *V* samples. Other evidence indicated that Fe trapped in the interlayers of *V* was not available for P fixation. A. G. POLLARD.

Distribution of pyrophosphate-extractable iron and organic carbon in soils of various groups. C. L. Bascomb (*J. Soil Sci.*, 1968, **19**, 251–268).—Potassium pyrophosphate (0·1M) removes very little Fe from cryst. Fe oxides at pH 10, but peptises finely divided, hydrous amorphous oxides and org. matter in soils. The Fe and C contents of extracts from each horizon of 26 British soil profiles showed distinctive patterns, independent of the residual dithionite-sol. Fe. Thus extracts of humus Fe podzols have max. Fe and C in the B horizon, whilst peaty gley podzols have max. Fe in the B horizon and max C in the surface horizon. These groups are differentiated from non-podzols, which have max. pyrophosphate-extractable Fe and C in the surface horizon, decreasing with depth. Intermediate patterns help to quantify differences in soils of classes having properties of more than one soil group. A. H. CORNFIELD.

Mica weathering in relation to structural and compositional chemistry. R. A. Leonard (*Diss. Abstr., B.*, 1967, **28**, 19).—Rates of release of K from various micas (particle size, 1–5 μm) were determined, using Na tetraphenylboron. Vermiculites prepared from the micas by exhaustive extraction of interlayer K had much lower layer charges than did the corresponding micas. Oxidation of octahedral Fe^{2+} did not explain this loss of charge (except in biotites) and a proton-incorporating mechanism is suggested. Relationships between measurements of resistance to replacement of K, compositional and structural characteristics, and the *b*-dimension of octahedral layers are discussed. A. G. POLLARD.

Adsorption of amino acids by montmorillonite. F. D. Ovcharenko, N. V. Vdovenko, V. P. Telichkun and Yu. I. Tarasevich (*Ukr. khim. Zh.*, 1969, **35**, 123–128).—The adsorption of norleucine and lysine monohydrochloride on various cationic forms of montmorillonite (**I**) was studied at various pH and amino acid concn. For Na-**I** adsorption increases with acidity and at pH 2 and equal wt. concn. of 250 mg equiv./100g of clay reaches 90–100% of the exchange capacity. Further increase of concn. does not increase the adsorption significantly. It is shown that in acid medium the amino acids are adsorbed on the surface of **I** as cations. Heating the specimen to 45–90° leads to partial conversion to zwitterions. Intermediate products of thermal decomposition of adsorbed amino acids are NH_4^+ cations. Features of the i.r. absorption spectra of adsorbed acids are discussed. (12 references.) (From authors' summary.) R. J. M.

Influence of kaolinite, bentonite and peat on physical properties of sand and a sandy loam. S. Schahabi and U. Schwertmann (*Z. PflErnähr. Bodenk.*, 1968, **120**, 174–190).—The influence of 0–15% additions of kaolin (*K*), Na- and Ca-bentonite (*B*) and peat (*P*) on bulk density (**I**), pore size distribution (**II**), pF moisture curves (**III**), aggregation (**IV**), Atterberg limits, and modulus of rupture (**V**) of a sand and a sandy loam was studied. **I** increased with addition of *K*, but decreased with rise in added *B* and *P*; **II** changed correspondingly. With *K* coarse pores (> 50 μm) decreased, medium pores (10–0·2 μm) increased and fine pores remained unchanged, *B* gave a large increase in fine pores while *P* increased the medium pores. **IV** of sand particles < 20 μm was unaffected by *K*. More than 3% of Na-*B* increased **IV**; Ca-*B* was even more effective. Liquid and plastic limits remained unchanged by *K*, whilst *B* increased them, especially the former. *P* raised the plastic limit in the sandy loam. **V** of sand was slightly increased by *K* and considerably by *B*, exhibiting a max. with increasing *B* up to a

certain value. In sandy loam with high initial V a decrease occurred in all cases. (19 references.) M. LONG.

Advances in soil stabilisation, 1961–1967. O. G. Ingles (*Rev. pure appl. Chem.*, 1968, **18**, 291–310).—Basic research, including permeability (origins, measurement, control), vol. stability and strength (chemical and mechanical aspects) is reviewed, together with considerations arising from the practical application of the results of these investigations. e.g., design criteria for stabilised layers, effects of soil type on stabilisation response, and the solution of field problems in mixing, compaction, curing, etc. (159 references.) J. M. JACOBS.

Tillage for greater crop production. Am. Soc. agric. Engrs, Am. Soc. Agronomy and Soil Conservation Soc. Am. (*ASAE Publication*, 1968?, No. PROC-168, 94 pp.).—Proceedings of a conference held at Detroit, Dec. 11–12, 1967, contain 25 papers on tillage practices and their effects on soils and crop production. P. C. W.

Influence of melioration ploughing on physical and chemical properties of soils formed out of sands. B. Dobrzański and H. Domżal (*Annls Univ. Mariae Curie-Skłodowska, Agric. E*, 1967, **22**, 1–14).—The effects of deep (melioration) ploughing of podzolic soils to 40 cm (against the customary 25 cm) and application of farm manure, peat or clay on the chemical and physical properties of the soils were determined after a 4-year crop rotation. The results were: (1) some distinct changes in the structure of the soil and in some physical and chemical properties occurred; (2) the 25–30 cm thick soil layer was loosened, eliminating the plough sole, and a more compact 'melioration inset' was produced at the depth of 40 cm; (3) the 'melioration inset' had a lower total porosity, air capacity and permeability compared with control plots tilled to 25 cm; (4) only the farm manure was capable of increasing the available P and K in the soil profile; (5) deep ploughing did not change the pH of the soil, but application of low-moor peat reduced the acidity at the 'melioration inset'; and (6) translocation of the part of the humus horizon deeper into the soil profile produced only a short-lived decrease in the content of org. substances in the arable layer. (14 references.) T. M. BARZYKOWSKI.

Particle size analysis of soils compared by sodium metaphosphate and sodium carbonate dispersion methods. J. B. Dixon, R. J. McCracken, T. C. Peele and C. I. Rich (*Proc. Soil Sci. Soc. Am.*, 1968, **32**, 734–735).—Comparison of the Na_2CO_3–centrifuge (Jackson, '*Soil Chemical Analysis*', 1956) and $NaPO_3$–pipette (*Soil Sci.*, 1949, **68**, 15–24) methods for particle size analysis of 13 soils showed that results for % sand gave very similar values by the two methods. Although values for % clay differed significantly by the two methods the differences were too small to be important. A. H. CORNFIELD.

Capillary activity of soil and its removal. O. Dobozy (*Tenside*, 1968, **5**, 340–344).—The physical properties of a number of soils were compared with respect to particle size, solidity, limit of flow, plasticity index, and the effects of added surface-active agents on these functions were studied. Such additions to fine grain soils showed that they can be rendered hydrophobic and consolidated, by spraying with a 6% solution of imidazoline deriv. prepared by reacting sperm oil fatty acids with ethylenediamine, diethylenetriamine or hydroxyethylenediamine. A typical additive is 1-aminoethyl-2-spermyl-2-imidazoline, and its use in aq. solution is recommended for below ground constructions, thereby avoiding the neccessity of removing and replacing the soil. G. R. WHALLEY.

Theoretical analysis of two-dimensional transient flow of water in unsaturated and partly unsaturated soils. J. Rubin (*Proc. Soil Sci. Soc. Am.*, 1968, **32**, 607–615).—The Darcian flow-equation for two-dimensional transient transfer of water in rectangular unsaturated or partly unsaturated soil slabs was solved numerically. A. H. CORNFIELD.

Water flow and hydraulic conductivity in unsaturated soils. K. H. Hartge (*Z. PflErnähr. Bodenk.*, 1968, **121**, 42–57).—A review discussing the conditions of 'no flow' in unsaturated soil, methods, and the invalidity of the Darcy formula under certain conditions. (15 references.) M. LONG.

Permeability measurements with air and water on model samples. H. Hanus (*Z. PflErnähr. Bodenk.*, 1968, **120**, 131–140).—Three soils from different locations and showing different aggregate stabilities were used as model samples, half of each sample being stabilised artificially by sprinkling with a 1% solution of BV 60 (BASF). Permeability tests were made with aggregates of different sizes. Differences between permeability to air and to water were considerable and attributable to differences in water-stability of the aggregates. The ratio of air- and water-permeabilities, measured under comparable conditions, can serve as a measure of relative structural stability of soils. (10 references.) A. G. POLLARD.

Infiltration of water into soils as influenced by surface conditions. W. M. Edwards (*Diss. Abstr., B.*, 1967, **28**, 22).—Infiltration of water into a silt loam was estimated using the water-flow equation and laboratory determinations of the water content–suction–diffusivity relationships. The effect of a developing soil crust on infiltration was studied by exposing 0·5 cm-thick crusts to different periods (30–90 min.) of simulated rainfall. Estimated infiltration was consistently less when this effect was taken into consideration. Near equilibrium, infiltration rates and cumulative infiltration were linearly related to the saturated conductivity of the surface layer of soil, though not consistently to the surface clay or org. matter contents. Infiltration rate always exceeded the saturated conductivity (*SC*) of the crust. With increased exposure to drop-impact the moist bulk *d* of the crust increased and the *SC* diminished. A. G. POLLARD.

Salt movement in soils having no water table. S. H. Abd-el-Malik (*Diss. Abstr., B.*, 1967, **28**, 1).—Soils packed in cylinders were wetted with aq. $CaCl_2$, either at the surface or at a level below it, to test for the movement of water and salt downwards by drainage and for the rise of both due to evaporation at the surface. In each cycle of wetting and drying the downward movement of salt exceeds the upward movement, leaving the max. salt concn. at or near the depth of water penetration. In the reclamation of saline soils, repeated leaching with sufficient water to bring the soil to field moisture capacity moves sol. salts downward, improves the water-holding capacity (*WHC*) and lowers water losses by evaporation. If saline water is used for wetting the soil, the downward movement of liquid continues longer than when fresh water is used. In stratified soils, salts tend to accumulate in finer-textured layers regardless of the positions of such layers in the soil columns. A coarse soil layer at the surface minimises loss of water and limits the upward movement of water and salt. With a subsurface layer coarser than the surface layer movement of both salt and water is restricted and salt may accumulate above the stratified interface unless sufficient water is added to force a downward movement of both salt and water. The presence of salt in a soil lowers the *WHC* at all tensions. Other observations suggest that salt movement by wind is an important factor in the development of desert soils. A. G. POLLARD.

Effects of salts on oxygen diffusion rate measurements in unsaturated soils. R. W. Rickman (*Proc. Soil Sci. Soc. Am.*, 1968, **32**, 618–622).—The effects of ionic species and salt concn. on measurements of O_2 diffusion rates in unsaturated media were investigated, using Pt electrodes partially immersed in solutions to approximate an unsaturated medium. Conditions are described under which the electrodes should operate most satisfactorily or have min. interferences from salt. A. H. CORNFIELD.

Convective diffusion in clay systems. A. R. Overman (*Proc. Soil Sci. Soc. Am.*, 1968, **32**, 616–618).—Convective-diffusion theory is applied to the transport of ions through clay columns, and allows calculation of the relative transfer rates of salt by convection and diffusion. A. H. CORNFIELD.

Effect of soil-mineral weathering on the sodium hazard of irrigation waters. J. D. Rhoades, D. B. Kruger, and M. J. Reed (*Proc. Soil Sci. Soc. Am.*, 1968, **32**, 643–647).—Samples of six arid-land soils (pH 5·8–8·0) were packed in permeameters, leached with MeOH to remove readily sol. org. matter (to minimise subsequent microbial activity) and then equilibrated with irrigation waters. Soil solutions were displaced from the nearly saturated columns and analysed at bi-weekly intervals until a steady composition was attained. Effluent solutions were 3–5 mequiv. per l higher in total salt content, accounted for primarily by Ca^{2+} and Mg^{2+} bicarbonate, than in the applied waters. The net effect of these changes in composition was a 30–90% reduction in sodium adsorption ratio (*SAR*) of the applied solutions. *SAR* reductions were greatest for the waters of low salt concn., but were significant even for water containing 15–20 mequiv. salt per l. Results are discussed in relation to the evaluation of irrigation water quality. A. H. CORNFIELD.

Mineral-weathering correction for estimating the sodium hazard of irrigation waters. J. D. Rhoades (*Proc. Soil Sci. Soc. Am.*, 1968, **32**, 648–652).—An equation previously used to evaluate the Na hazard of irrigation waters was modified to include the effect of soil-mineral weathering on the composition changes which occur when waters are applied to soils. The mineral-weathering coeff. required for this equation can be determined by a simple laboratory procedure. For a calcareous soil there was excellent agreement between calculated sodium adsorption ratio (*SAR*) values and values obtained in a controlled lysimeter experiment. A. H. CORNFIELD.

Leaching requirement for exchangeable sodium control. J. D. Rhoades (*Proc. Soil Sci. Soc. Am.*, 1968, **32**, 652–656).—A method is described for estimating the leaching fraction required to keep the sodium adsorption ratio (*SAR*) at the lower limit of the plant rooting zone below some limiting value. The choice of the limiting *SAR* depends on plant tolerance to Na and on soil permeability. A. H. CORNFIELD.

Influence of ionic strength and ion-pair formation between alkaline earth metals and sulphate on sodium–divalent cation exchange equilibria. T. S. Rao, A. L. Page, and N. T. Coleman (*Proc. Soil Sci. Soc. Am.*, 1968, **32**, 639–643).—The Na^+–Mg^{2+} and Na^+–Ca^{2+} ion exchange equilibria were studied in two soils and in an ion exchange resin by batch equilibration procedures. The apparent equilibria in terms of concn. were different for Cl^- and SO_4^{2-}. The differences were reduced but not eliminated by ion activity corrections based on ionic strength. A more satisfactory agreement between results in Cl^- and SO_4^{2-} systems was obtained when allowance was made for solution phase activities and ion-association of Ca^{2+} and Mg^{2+} with SO_4^{2-}. A. H. CORNFIELD.

Calcium : aluminium exchange equilibria in clay minerals and acid soils. B. S. Coulter and O. Talibudeen (*J. Soil Sci.*, 1968, **19**, 237–250).—Exchange reactions between 0·01N-$AlCl_3$ of different pH and Ca-saturated clays and soils showed that Al^{3+} and $Al(OH)_2^+$ were adsorbed from solutions of pH > 4·0 and Al^{3+} and H^+ from solutions of pH < 3·0. The cation exchange capacity of the materials increased when Al was adsorbed. Al^{3+} was strongly preferred to Ca^{2+} on all soils and clays. The equilibrium const. for Ca : Al exchange (K') was identical for soils before and after oxidising their org. matter and did not vary for any exchanger, with Al-saturation or with the initial pH of the $AlCl_3$ solution. This proved the validity of the procedure used for calculating exchangeable Al^{3+}. K' values for Ca : Al exchange favoured Al^{3+} in the order vermiculite > soils > illite > montmorillonite. The influence of surface-charge densities of the clay minerals on this order is discussed and a method proposed and tested for calculating the K' value of a soil from its mineralogical composition. A. H. CORNFIELD.

Susceptibility of interlayer potassium in illites to exchange. S. J. Smith (*Diss. Abstr., B.*, 1967, **28**, 19–20).—When the illites (**I**) were placed in aq. NaCl the exchange of Na for K was restricted by the accumulated K in the solution, the effect varying with the extent of depletion of K and with the mineral used. In presence of Na tetraphenylboron (**II**) to remove sol. K the max. K replacement in 7 days was 44–94%. The 10Å components in **I** expanded to 12·3 and/or to 15Å as K was replaced by Na. The amounts of extractable K in **I** were increased by heating at 450° or by subjecting them to ultrasonic vibration in presence of **II**. Pretreatment of **I** with NaOBr, and other modifications, did not affect the amount of extractable K. *X*-ray diffraction data obtained at various stages of K depletion are discussed. A. G. POLLARD.

Adsorption of manganese, cobalt, copper and zinc by goethite from dilute solutions. H. Grimme (*Z. PflErnähr. Bodenk.*, 1968, **121**, 58–65).—Synthetic goethite (0·1 g) was shaken with 50 ml 0·1N-KNO_3 containing 10^{-5} mole/l of Mn^{2+}, Co^{2+}, Cu^{2+} or Zn^{2+} as nitrate; pH was varied by adding HNO_3 or NaOH. The pH and the Mn, Co, Cu or Zn content of the equilibrated solution were measured. Increasing amounts of the heavy metals were adsorbed with increasing pH, the adsorption beginning at different pH values, in the order Cu, Zn, Co and Mn at pH 3, 4, 4·6 and 5 respectively. At a given pH the degree of adsorption was in the order Mn < Co < Zn < Cu. A close linear correlation was found between the negative log of the solubility product of metal hydroxide and the pH at which a given amount of cation was adsorbed. In contrast with ion exchange by clay minerals, this form of exchange occurs between protons of the positively charged inner part of the electric double layer and cations in solution. Trace elements are probably concentrated in Fe oxides in soils by first being adsorbed and then being occluded and irreversibly fixed as the particle grows. Haematite and amorphous Fe oxide behaved in the same way as goethite. M. LONG.

Distribution of amino sugars with depth in soil profiles. F. J. Stevenson and O. C. Braida (*Proc. Soil Sci. Soc. Am.*, 1968, **32**, 598–600).—In four silt loams the proportion of amino sugars present as glucosamine increased with depth from the A to the C horizon. Glucosamine accounted for 66–94% of the amino sugars present. A. H. CORNFIELD.

Amino compounds of soil organic matter formed during humification of ^{14}C-labelled glucose. G. H. Wagner and V. K. Mutatkar (*Proc. Soil Sci. Soc. Am.*, 1968, **32**, 683–686).—When soils were incubated for 6 months after addition of ^{14}C-labelled glucose, 17–22% of the labelled C was incorporated into 13 major amino compounds (**I**) separated after hydrolysis with 6N-HCl. Although all the **I** were labelled, the extent of labelling differed. **I** with the highest sp. activity were those which are known to predominate in bacterial and fungal cell walls. A. H. CORNFIELD.

Zinc dust distillation of soil humic compounds. E. H. Hansen and M. Schnitzer (*Fuel, Lond.*, 1969, **48**, 41–46).—Humic and fulvic acids were extracted from soil, and decomposed by 'drastic' Zn dust distillation, in which 0·5 g of org. matter was heated with 17·5 g of Zn dust at 450° for 5 min and then at 520° for 15 min, and 'milder' Zn dust fusion, in which 0·5 g of org. matter was mixed with 3 g of Zn dust, 1 g of NaCl and 5 g of $ZnCl_2$, heated to 300° and maintained at that temp. for 15 min. The main products from both methods were polysubstituted naphthalenes, substituted phenanthrene, and substituted and unsubstituted anthracene, pyrene and perylene. The results indicate that significant amounts of polycyclic aromatic ring structures or structures yielding such systems under the experimental conditions used, occur in the 'nuclei' of soil humic compounds. (14 references.) M. GREENAWAY.

Transformations of nitrogen in solodised solonetz soils with particular reference to denitrification. R. J. K. Myers (*J. Aust. Inst. agric. Sci.*, 1968, **34**, 220).—Leaching of org. matter from the surface soil is the most likely explanation for high potential denitrifying activity in B horizon soils. Denitrification may be a significant factor in the nitrogen economy of solodised solonetz soils. E. G. BRICKELL.

Mineralisation of nitrogen in grass roots. J. F. Power (*Proc. Soil Sci. Soc. Am.*, 1968, **32**, 673–674).—Roots of bromegrass, grown with and without added N, were added to soil and incubated for 10 weeks. Where roots containing 0·84% total N were used there was a net immobilisation of soil N after 10 weeks and the extent of immobilisation increased with level of root application (0·25–0·50% dry matter). Where roots containing 1·44% N were used there was net mineralisation of N after 10 weeks incubation, and the extent of accumulation of mineral N increased with rate of application (0·25–1·0%). A. H. CORNFIELD.

Influence of nitrification suppressants on rate of ammonium oxidation in field soils. B. R. Sabey (*Proc. Soil Sci. Soc. Am.*, 1968, **32**, 675–679).—The oxidation of added NH_4^+-N in silty clay and clay loams, enclosed in plastic bags and buried in field plots during autumn, winter, and spring, was reduced by addition of 1 ppm (soil basis) of N-Serve [2-chloro-6-(trichloromethyl)pyridine] and 3 ppm of AM (2-amino-4-chloro-6-methylpyrimidine). In the spring the normal rapid nitrification was delayed about 4 weeks by the treatments. Treatment of $(NH_4)_2SO_4$ with a resinous polymer coating before application in autumn did not decrease nitrification rate in the following spring. A. H. CORNFIELD.

Production of mineral nitrogen by soil samples, contained in polyethylene bags, under field conditions and in the laboratory. D. A. van Schreven (*Pl. Soil*, 1968, **29**, 170–183).—When incubated in ventilated glass jars at 29° clay polder soils showed slightly less mineral N accumulation than when incubated in polyethylene bags. The amount of mineral N produced during the growing season (5·5 months) in soil held in bags buried at a depth of 15 cm in the field was similar to that produced during 2 months of incubation at 29° in the laboratory. When buried at 30 and 50 cm depths mineral N production corresponded to 1·5 and 1·0 months of laboratory incubation. Soils outside the bags in the field mineralised somewhat less of their org. N than did soils inside the bags. 0·8–1·6% of the total N present in the soils in the bags buried in the field was mineralised during the growing season. A. H. CORNFIELD.

Chemical transformations of nitrite in soils. D. W. Nelson (*Diss. Abstr., B.*, 1967, **28**, 23–24).—In the decomposition and/or fixation of NO_2^- in soil, pH and org. matter contents (*OMC*) are the principal factors, both processes being inversely related to soil pH. In soils of pH 5–7, the amount of NO_2^--N fixed and the amount volatilised at a given pH increased with increasing *OMC*. At pH < 5 the amount of NO_2^--N volatilised diminished and the amount fixed increased with increasing *OMC*. In soils treated with NO_2^--N air-drying increased the decomposition of NO_2^- but had little effect on fixation. The amount of NO_2^- decomposed and the amount fixed on incubation of NO_2^--treated soil increased with NO_2^- level and with time and temp. of incubation. In neutral or acid soil, gaseous products of decomposition of NO_2^- included NO_2 (largely by self-decomposition of HNO_2: $2HNO_2 \rightarrow NO + NO_2 + H_2O$) and N_2, together with small amounts of N_2O. Max. yields of N_2O and N_2 were given by soils of high *OMC*. Production of

NO_2 was inversely proportional to soil pH, although significant amounts were formed at pH > 7. Fixation of NO_2^--N and reduction of NO_2^--N to N_2 or N_2O were largely due to soil org. matter, probably phenols which are nitrosated by NO_2^-. A. G. POLLARD.

Plant treatment in relation to the rhizosphere effect. II. Foliar applications of fertiliser chemicals and antibiotics in relation to the rhizosphere microflora of rice. III. Foliar applications of trace elements and metallic chelates in relation to rhizosphere microflora of rice. T. K. Ramachandra Reddy (*Pl. Soil*, 1968, **29**, 102–113; 114–118).—II. Effects of foliar sprays of urea, NH_4 salts, KCl, gibberellin, actidione and agrimycin on numbers of fungi, bacteria and actinomycetes and types of fungi in waterlogged rice rhizospheres were studied.

III. The numbers of fungi and actinomycetes in the rhizosphere of rice were increased by foliar sprays of Zn, B, Mn, and Mo. Bacterial numbers were unaffected by Zn and B and decreased by Mn and Mo. When foliar sprays of EDTA chelates of Zn, Fe, Cu and Mn were applied, fungal numbers were increased only by Mn, actinomycete numbers by Zn, and bacterial numbers by Zn and Mn. The proportion of different types of fungi was also affected by some of the treatments. A. H. CORNFIELD.

Inter-relations of micro-organisms and mulberry. III. Influence of heterotrophs on nitrifiers in the rhizosphere. V. N. Vasantharajan and J. V. Bhat (*Pl. Soil*, 1968, **29**, 156–169).—Three species of *Pseudomonas* and one each of *Achromobacter* and *Bacillus* capable of degrading methionine (**I**) were shown to be stimulated in the rhizosphere of mulberry. The bacteria were able to reverse the inhibitory effect of **I** on soil nitrification, whilst two of them were able to form NO_2^- from **I**. Mulberry root exudates of **I** may be the cause of the stimulation of nitrifying activity in the rhizosphere. A. H. CORNFIELD.

Influence of trace elements, used as supplementary fertilisers, on soil microflora. D. Bertrand and A. de Wolf (*C.r. hebd. Séanc. Acad. Agric. Fr.*, 1968, **54**, 1130–1133).—Comparative bacterial counts made on fallow and cultivated soils, with and without the supplementary trace elements, showed these elements to have a beneficial effect on the soil bacteria. The flora of fallow soil were also influenced by the cultivation of potatoes. P. S. ARUP.

Effect of organic matter, flooding time, and temperature on dissolution of iron and manganese from soil *in situ*. B. D. Meek, A. J. MacKenzie, and L. B. Grass (*Proc. Soil Sci. Soc. Am.*, 1968, **32**, 634–638).—Measurements *in situ* in a calcareous silty clay showed that flooding decreased E_H (redox potential) only to a small extent at either soil temp. studied (average values of 35° and 19°). When chopped lucerne (18 metric tons per ha) was incorporated into the top 10 cm of soil E_H was decreased to a much greater extent by flooding, at the higher soil temp., and reached a value of −100 mV at 20 and 46 cm depths. Flooding in the absence of added lucerne increased soil solution Mn^{2+} and Fe^{2+} concn. to a relatively small extent compared with flooding plus lucerne treatment at the higher temp. The results show the importance of temp. and org. matter in the dissolution of Fe and Mn and confirm the correlation between sol. Fe and Mn and E_H. The data are discussed in relation to the pptn. of Fe and Mn compounds on tile drains. A. H. CORNFIELD.

Determination of solubility product of soil constituents such as gypsum. J. M. Rance and B. G. Davey (*Proc. Soil Sci. Soc. Am.*, 1968, **32**, 670–672).—The solubility product (S) of gypsum was calculated using a converging cyclic procedure which involved the use of a published value for the dissociation const. of gypsum, an experimentally determined value for total Ca^{2+} concn., and the second approximation of the Debye-Huckel equation. The S of gypsum at 20° was $2{\cdot}46 \times 10^{-5}$. The method is applicable to other sparingly sol. salts. A. H. CORNFIELD.

Soluble aluminium in soils and some factors affecting its magnitude. L. M. Lavkulich (*Diss. Abstr., B.*, 1967, **28**, 19).—Amounts of Al in soil solution under different conditions were examined by equilibrating soil or the pure mineral materials with water without agitation but with aeration to control pH. Small concn. of Al present in the soil solution were not controlled by the solubility of gibbsite (**I**), which is, however, closely in agreement with the sol. Al in highly acid soils. The presence of **I** in soils having [Al] consistent with that of **I** could not be detected by d.t.a. Equilibrium diagrams indicated that the level of Al in soil solution did not conform with the stability of **I**. It is concluded that the soils examined are actively weathering and that amorphous aluminosilicates detectable in the soils may serve as a 'sink' for the retention of Al and, together with Al-interlayered clay minerals, may be the main sources of Al maintaining the small [Al] in soil solution. A. G. POLLARD.

Influence of some soil-forming factors on the content and distribution of trace elements in soil profiles. I. Occurrence of Cu and Mn in relation to the ground-surface relief. II. Distribution of trace elements in the soil in relation to its utilisation. J. Gliński (*Annls Univ. Mariae Curie-Sklodowska, Agric. E.* 1967, **22**, 21–35; 37–68).—Samples of Polish soils from slopes of varying inclination were taken from tops, slopes and from the foot of slopes and analysed for total and sol. Cu and Mn. Cu and Mn contents in the soils of the areas with medium and deep relief were different; the lowest contents of Cu and Mn were found in soils taken from slopes compared with contents in soils taken from the top-land or from the foot of slopes. Distinct differences in total Cu and Mn contents occurred in different soil types. The highest Cu content was found in chernozem, medium content in rendzinas and pseudopodzolic soils formed from loess, and the lowest content in brown acidic soils. The highest Mn content was found in brown acidic soils and the lowest in rendzinas. (11 references.)

II. The distribution of 11 microelements (Cu, Mn, Cr, Ti, Sr, Ba, V, Ni, Co, Pb and Zn) and 3 macroelements (Fe, Al and Mg) in various forest and arable soils was determined by spectral emission analysis. The variations in the distributions of the elements when a forest soil was converted into an arable one are discussed. (50 references.) T. M. BARZYKOWSKI.

Processes in the root environment [in relation to transfer of nutrients from soil to crop]. P. H. Nye (*J. Soil Sci.*, 1968, **19**, 205–215).—An address with 15 references. A. H. CORNFIELD.

Effect of soil properties on the uptake of radioactive strontium by plants. II. Experiments with model soils having systematically varied characteristics. J. Günther and D. Schroeder (*Z. PflErnähr. Bodenk.*, 1968, **120**, 78–89; cf. *Idem, ibid.*, 1968, in press).—White clover was grown in a slightly acid Brown Earth soil modified by addition of varied proportions of clay, peat and sand or of H_2SO_4, NaOH or $CaCl_2$. Carrier-free ^{89}Sr was added to all cultures. The Sr content and the Sr/Ca ratio in the plants were lowered by increasing the Ca and/or clay contents, the pH or the base-exchange capacity of the soil. With very high levels of water-sol. Ca, e.g., > 10% of the exchangeable Ca in the soil, the Sr content of the plants increased in spite of a decline in Sr/Ca ratio. The discrimination factors $Sr_{pl}/Sr_{soil} . Ca_{pl}/Ca_{soil}$ were lowered by increasing the pH or clay content of the soil but were not affected significantly by the org. matter content. (14 references.) A. G. POLLARD.

Silicon, an element useful to plants. F. Fidanovski (*Z. Pfl-Ernähr. Bodenk.*, 1968, **120**, 191–207).—A review with 75 references.

Effects of irrigation and fertilisers on soil fertility. M. T. Vittum, D. J. Lathwell, and G. H. Gibbs (*Agron. J.*, 1968, **60**, 563–565).—The use of irrigation (average of 10·7 cm water each year) on silt loams, cropped with different rotations of vegetable crops, for 13 years had no significant effect on soil pH, org. matter, or available (chemically extractable) P, Ca and Mg, but slightly decreased available K in comparison with non-irrigated plots. Application of 30 kg P and 54 kg K per ha annually for 14 years decreased soil pH, increased available P and K and had no effect on org. matter, Ca and Mg. A. H. CORNFIELD.

Nutrient uptake by lowland rice under flooded and non-flooded soil conditions. E. C. Cherian, G. M. Paulsen, and L. S. Murphy (*Agron. J.*, 1968, **60**, 554–557).—The levels of P and Mn were higher whilst those of Ca and Mg were lower in the foliage of rice grown under flood compared with unflooded conditions. Flooding sometimes decreased foliar K and Zn, but had no consistent effect on Fe and Cu. Superphosphate and liquid ammonium polyphosphate were more effective as sources of P for flooded rice than was solid, but under non-flooded conditions there was little difference among the sources. Under flooding 100 ppm applied P was needed for optimum top growth, whilst without flooding 50 ppm P was adequate for max. growth. Application of 10–100 ppm Mo (as EDTA salt) or 10 ppm Fe (EDTA) did not affect top growth, although foliar Mn was increased by the higher Mn application. A. H. CORNFIELD.

Predicting potassium uptake by grain sorghum. B. W. Hipp and G. W. Thomas (*Agron. J.*, 1968, **60**, 467–469).—The leaf K level at full bloom for max. growth of sorghum plants was ≮ 1·5% (dry basis). Studies with eight soils showed that K uptake by sorghum was related to both soil solution K (K_{ss}) and exchangeable K (K_{ex}). A correlation coeff. of 0·890 was obtained between K% in leaves and $K_{ss} + \log (1 + K_{ex})$. A. H. CORNFIELD.

Indices of availability of soil calcium to plants. Muhammad Ramzan (*Pl. Soil*, 1968, **29**, 18–26).—Alluvial, calcareous, red and black soils were equilibrated with solutions having salinities of 30

and 120 mequiv. per l and sodium adsorption ratio (*SAR*) of 7 to 40. Uptake of Ca by rye seedlings (modified Neubauer method) decreased with increasing salinity and with increasing *SAR*, particularly at the lower salinity. A Ca adsorption ratio value $\left[a_{Ca}^{0.5} \Big/ \left(a_{Na}^{0.5} + a_{Mg}^{0.5}\right)\right]$ was generally better correlated with uptake of Ca by ryegrass than were either *SAR* or exchangeable Ca. A. H. CORNFIELD.

Diffusion of fertiliser salts in soils. H. Abratis, P. Hammerschmid, O. Knacke and E. Merz (*Z. PflErnähr. Bodenk.*, 1968, **120**, 159–167).—When placed on the surface of soil KCl spreads by vol. diffusion, the K^+ lagging behind the Cl^- due to the stronger sorption of K^+ by soil. With highly sol. phosphates hydrolysis and pptn. occur, leading to large local pH changes, changes in the composition of the phosphates and a decrease in the soil free moisture. This is especially marked with clays. The P of basic slag behaves differently. It is mainly bound in the soil and although diffusion is slight it is very rapid and extends to a considerable depth. M. LONG.

Tests on micro soil samples for pH, organic carbon, total nitrogen and total phosphorus. G. Möller (*Z. PflErnähr. Bodenk.*, 1968, **121**, 11–19).—Micro determinations of pH. org. C, total N and P, based on macro procedures, are described. For pH measurement, 100 mg of soil was mixed with 5 ml of N-KCl; org. C was determined on 50–100 mg, after CrO_3 oxidation, and N and P were measured colorimetrically after Kjeldahl digestion of a 100-mg sample. M. LONG.

Direct determination of iron in soil extracts by atomic absorption spectrophotometry. F. R. Khan and A. H. Cornfield (*Pl. Soil*, 1968, **29**, 189–191).—Fe could be determined by direct aspiration (without preliminary treatment except dilution where necessary) of the filtrates of dry and water-logged soils extracted with N-NH_4OAc (pH 7·0), N-NH_4OAc (pH 4·0), and Morgan's reagent (0·5 N-AcOH–0·75 N-NaOAc, pH 4·8). Results obtained agreed closely with those obtained colorimetrically (with 2,2′-dipyridyl). A. H. CORNFIELD.

Direct titration of ammonium acetate extract of soils by versenate for the estimation of exchangeable calcium and magnesium. S. P. Behal (*J. Instn Chem. India*, 1968, **40**, 212–216).—The method of Cheng and Bray (*Soil Sci.*, 1951, **72**, 449–458) is rapid, simple, and satisfactory, Ca and Mg can be determined (together) in the same aliquot, the acetate ion does not interfere, and the end-point is sharp provided the solution is not highly coloured. E. G. BRICKELL.

Application of microprobe analysis to the study of phosphorus in soils. R. H. Qureshi, D. A. Jenkins, R. I. Davies and J. A. Rees (*Nature, Lond.*, 1969, **221**, 1142–1143).—Describes how soil samples (20–30 μm thick), vac.-impregnated with polystyrene resin and then suitably polished and coated, can be submitted to the electron microprobe. Results permit spatial distribution of P within distinctive features to be determined qual. and its mode of occurrence to be inferred from its association with, e.g., Ca, Al, Fe, Y and Ce. Examples described are (*i*) a decomposing root, (*ii*) a complex Fe–Mn concretion and (*iii*) a clayey cutan lining two circular voids. W. J. BAKER.

Use of the diphenylamine test for detection of nitrates in plants. E. Rauterberg (*Z. PflErnähr. Bodenk.*, 1968, **121**, 4–11).—A drop of a 1% solution of diphenylamine in conc. H_2SO_4 is placed on a 1-mm thick section of leaf or petiole and the depth of the blue coloration is estimated. The effect of increasing N fertiliser applications to tomatoes could be demonstrated by this method. The beneficial effect of lupin admixed with a straw base dressing for turnips was demonstrated, the effect persisting in the following rye crop. Potatoes given a green manure had higher NO_3^- levels than did those not given the dressing. Turnips given an artificial manure made from straw and $CaCN_2$ were less well supplied with N than those given the same amount of N, with or without straw supplied separately. The test is of use in determining the need for N top-dressings. M. LONG.

Soils and fertilisers. G. W. Cooke (*Jl R. agric. Soc.*, 1968, **129**, 128–148).—A review is given, covering cultivation, cropping systems, soil structure, nutrient cations, K, Mg, Na, trace elements, soil and plant analysis, nitrates in water and crops, N in crops, fertilisers used in the U.S.A., K manuring and nutrition of glasshouse and horticultural crops. (57 references.) C. J. R.

Sorption and elutriation of nutrients by ion exchange resins. J. Soukup (*Z. PflErnähr. Bodenk.*, 1968, **120**, 167–174).—The elutriation of NO_3^-, NH_4^+, HPO_4^{3-} and K^+ from an ion exchange resin was studied in various substrates and compared with the leaching out of org. and mineral fertilisers. Ion exchanger-sorbed nutrients were very much more resistant to elutriation than were mineral fertilisers. NO^{3-}-N, particularly, benefited from sorption on the ion exchange resins. (11 references.) M. LONG.

Solubility of P_2O_5 in superphosphates. V. G. Logomerac (*Kemija Ind.*, 1968, **17**, 791–793).—Investigations showed that the nutritive value of P_2O_5 in various P fertilisers cannot be evaluated by determination of their solubilities in water, neutral or alkaline NH_4 citrate or in citric acid. As the chemicals of various soils react specifically with P_2O_5 in fertilisers, the evaluation of their nutritive values based only on their solubilities may give a completely false picture; and the evaluation of phosphoric fertilisers should thus be based on different criteria. (11 references.) T. M. BARZYKOWSKI.

Gaseous loss of fertiliser nitrogen in relation to depth of placement of ammonium sulphate in submerged rice soils. Z. Aleksic, H. Broeshart, and V. Middelboe (*Pl. Soil*, 1968, **29**, 338–342).—Pot tests with waterlogged rice using ^{15}N-labelled $(NH_4)_2SO_4$ applied to soil from six tropical countries showed that gaseous loss of N was twice as high where $(NH_4)_2SO_4$ (200 kg per ha) was applied to the surface than when applied at a 7-cm depth. Loss of applied N ranged from 17 to 64% of that applied on the surface and from 7 to 42% of that applied at depth. Of the N in the whole plant, that derived from the fertiliser was 1–17% higher with depth than with surface placement. The difference in utilisation of fertiliser N between depth and surface application was accounted for, almost entirely, by difference in loss of gaseous N. A. H. CORNFIELD.

Nitrogen fertilising of grassland in spring. P. F. J. van Burg (*Neth. Nitrogen Tech. Bull.*, 1968, No. 6, 45 pp.).—Results of 16 field trials during 1959–1965 are reported. N should be applied from mid Feb. to early April, depending on the growth stage at which the herbage is to be harvested, and the more N applied the earlier should be the application date, though some may be lost due to leaching by rain into deeper horizons. Use of $(NH_4)_2SO_4$ with early applications results in lower N losses than with NH_4NO_3-limestone or $Ca(NO_3)_2$, but it has serious drawbacks such as a sharp decline in soil pH and an adverse effect on herbage quality from the hypomagnesaemia standpoint; leaf scorch can also occur, particularly in frosty weather. (15 references.) E. G. BRICKELL.

Phosphorus fertilisation of soils in depth. IV. E. M. Bastisse (*C.r. hebd. Séanc. Acad. Agric. Fr.*, 1968, **54**, 1094–1102).—In percolation experiments as in the previous parts, results obtained with Ca hypophosphite (**I**) plus phosphite were almost the same as those obtained with phosphite alone. During the manufacture of **I** for agricultural use involving fertilisation in depth, it would be advantageous not to separate the by-product Ca phosphite from the **I**. P. S. ARUP.

Metal contamination of soil in the Woburn Market-Garden experiment resulting from application of sewage sludge. H. H. Le Riche (*J. agric. Sci., Camb.*, 1968, **71**, 205–207).—In 1959, soil samples from two plots which had received a total of 568 tons of sludge per acre were extracted with 0·5-N AcOH and the extracts analysed for Cr, Cu, Ni, Pb and Zn; in all cases the amounts of these elements had increased, compared with control plots. Leeks grown on treated plots had significantly higher concn. of Cu, Ni and Zn; beets and potatoes contained more Ni and Zn but not more Cu or Pb. The five elements were still present in appreciably greater amounts in treated plots than in untreated, even when ~ 6 years had elapsed after treatment. M. LONG.

Sampling and analysis of fertiliser-quality anhydrous ammonia. R. A. Hoodless and R. E. Weston (*Chemy Ind.*, 1969, 374–376).—The procedure described avoids the difficulties inherent in the Institute of Petroleum and A.O.A.C. methods; provided no neutral volatile matter, e.g., gaseous N_2, is present, it gives reliable results and an indication of the degree of contamination by mineral oil or water. The sampling apparatus (mainly of steel) comprises an injection head fitted with needle valves, bleeding tube and a nylon injection tube; the head is connected to the source by a flexible coupling having a non-return valve. Samples are collected in 1-l polyethylene bottles containing a known wt. of water, and aliquots of the solution are taken, quickly weighed, and then (after dilution) titrated with H_2SO_4. The necessary precautions are detailed. W. J. BAKER.

Plant Physiology, Nutrition and Biochemistry

Relationship between growth and evapotranspiration. F. Lemaire (*C.r. hebd. Séanc. Acad. Agric. Fr.*, 1968, **54**, 1056–1063).—In pot

experiments with ryegrass in which growth was varied by nutritional factors, the nearer nutrition approached the optimum, the lower became the degree of evapotranspiration for the production of vegetable solid matter per unit of wt. P. S. ARUP.

[A]. Effect of soil moisture regimes in sugar-cane plant. [B]. Measurement of water stress in sugar-cane plant. Abdul Hamid Khan and R. Samaniego (*W. Pakistan J. agric. Res.*, 1968, **6**, No. 2, 19–31; 32–40).—[A] Reduction in growth and yield, and morphological changes connected with responses to moisture stress are described. (31 references.)

[B] In comparative trials, more reliable results were obtained by the method of cutting the leaf discs and soaking them in water in test tubes at room temp. for 5 h than by floating the discs on water in a Petri dish, by placing the discs on moist foam, or by placing the whole leaf with cut petiole in water in a beaker. (15 references.) P. S. ARUP.

Linear programme for determining nutrient culture mixtures. D. R. Jensen and P. Sutton (*Agron. J.*, 1968, **60**, 571–576).—Linear programming techniques are described for determining the quantities of compounds to be used in preparing nutrient cultures which satisfy the requirements of quant. plant nutrition experiments. A. H. CORNFIELD.

Relationships between soil volume used by roots and nutrient accessibility. I. S. Cornforth (*J. Soil Sci.*, 1968, **19**, 291–301).—Increasing the vol. of soil available to plants growing in pots increased yields of oats, ryegrass, kale and tomatoes and decreased the intensity of rooting. N uptake per unit vol. of soil without added N was independent of both the total vol. of soil and intensity of rooting, whilst P uptake per unit vol. decreased with increasing soil vol. Responses to applied P increased, whilst response to applied N decreased, with increasing soil vol. The value of either P or N in compensating for decreasing soil vol. depends on how much the supply of the other nutrient is limiting growth. A. H. CORNFIELD.

Translocation of rubidium, caesium and potassium to new leaf growth in bush beans. A. Wallace (*Pl. Soil*, 1968, **29**, 184–188).—Nutrient solution studies using labelled Rb^+, Cs^+, and K^+ showed that translocation of Cs^+ to new leaves of bush beans was slower whilst that of Rb^+ was quicker than that of K^+. Considerably more Rb^+ than Cs^+ was absorbed by the plants. A. H. CORNFIELD.

Nitrate-stimulated uptake and transport of strontium and other cations. W. A. Jackson and D. C. Williams (*Proc. Soil Sci. Soc. Am.*, 1968, **32**, 698–704).—Supplying NO_3^- to N-depleted wheat seedlings stimulated the uptake of Sr^{2+}, Mn^{2+}, Mg^{2+}, Cs^+, Na^+ and K^+. When NO_3^- was supplied before or during uptake of Sr^{2+}, the transport of Sr^{2+} to the shoots was increased more than total uptake. Transport to shoots of previously absorbed Sr^{2+} was enhanced by subsequent NO_3^- treatment, more so when nitrates of Ca^{2+}, Mg^{2+} or Na^+ than of K^+ or NH_4^+ were supplied. A. H. CORNFIELD.

Zinc nutrition of alfalfa [lucerne]. Shitao Yie Lo and H. M. Reisenauer (*Agron. J.*, 1968, **60**, 464–466).—Studies in solution culture showed that N source (addition of NO_3^- or use of nodulated plants) had no effect on the relationship between relative yield and leaf Zn %. In the winter, 0·5 μM of Zn in the nutrient solution was required for max. yield, while in the autumn (low growth rate) 0·25 μM was adequate. However, rate of growth did not influence the leaf Zn% (≮ 6 ppm, dry basis) associated with max. yields. The value is ~ 50% or less of that required by most other crops and explains the infrequent response of lucerne to Zn fertilisation. There was a negative correlation between leaf P% and leaf Zn%, and visual Zn deficiency symptoms occurred when leaf P was abnormally high. Normal leaves had a P/Zn ratio < 800. A. H. CORNFIELD.

Factors influencing foliar uptake of phosphorus by grape vines. A. J. Bester and J. T. Meynhardt (*S. Afr. J. agric. Sci.*, 1968, **11**, 477–481).—Uptake of P from solutions of H_3PO_4 adjusted to pH 2·5 with KOH was better than uptake from solutions at pH 3·5 or 5·0. Solutions at pH 1·5 caused severe leaf injury. The use of surfactants (Teepol, Carbowax or Triton X-100) diminished uptake, but glycerol had no such effect. P. S. ARUP.

Distribution of soluble proteins and enzymes during early development of *Pisum sativum*. A. K. Mills and R. K. Crowden (*Aust. J. biol. Sci.*, 1968, **21**, 1131–1141).—Seedlings of a dwarf early flowering variety of *P. sativum* were grown on Hoagland's solution and protein was extracted from various parts of the plant at different stages of growth. Polyacrylamide gel electrophoresis was carried out in slotted trays which allowed comparison of a number of samples under identical conditions. In the developed gels, protein was located by amido black staining, peroxidase activity by incubating with *o*-dianisidine and H_2O_2 at pH 4·4, esterase activity by incubating with α-naphthyl acetate and diazo blue B at pH 6·4 and amylase activity was detected by the absence of colour when the gel incorporating starch was treated with I_2. Gels were photographed and quant. comparison was made by densitometry. (22 references.) J. B. WOOF.

Inhibition of protein synthesis and cation uptake in beetroot tissue by cycloheximide and cryptopleurine. G. M. Polya (*Aust. J. biol. Sci.*, 1968, **21**, 1107–1118).—Washed and aerated discs cut from the parenchyma of fresh beetroot were incubated in Krebs–Ringer solution with a range of amino acids and L-^{14}C-leucine. Discs were removed at intervals and extracted with $CCl_3 \cdot CO_2H$ followed by HCO_2H. The activity in the extracts gave a measure of absorbed protein and non-protein leucine, and the effects of cycloheximide (**I**) and cryptopleurine (**II**) on this process were determined. Both **I** (at 3×10^{-4} M) and **II** (at $1{\cdot}35 \times 10^{-5}$ M) completely inhibited incorporation of leucine into protein and at the same level rapidly inhibited Tris-induced cation uptake, as demonstrated using ^{22}Na. **I** caused a net leakage of K^+ and Cl^-. In aged discs the cytoplasmic potential (−108 ± 27 mV) was hyperpolarised by −50 to −95 mV on exposure to Tris chloride–KCl under conditions of net K^+ uptake, and this was completely reversed by the addition of **I**. **II** at $2{\cdot}2 \times 10^{-5}$ M did not prevent stimulation of mitocondrial respiration by ADP. Since inhibition of protein synthesis inhibits cation uptake, care is required when interpreting the effects of inhibitors on ion uptake. In aged tissue the relation between the two effects is probably indirect. (38 references.) J. B. WOOF.

Streptomycin inhibition of protein synthesis in peas reversed by bivalent cations. M. A. Venis (*Nature, Lond.*, 1969, **221**, 1147–1148).—Experiments with ^{14}C-labelled material show that the inhibition is overcome by presence of Mg^{2+}, Ca^{2+}, Mn^{2+}, Zn^{2+}, Fe^{2+} or Co^{2+} and that the antagonism is probably the result of mutual competition between streptomycin and, e.g., Ca, for intracellular binding sites. Thus, the presence of 5–10 mM-Mg results in only 20% inhibition, 20 μM-Mn or -Ca is highly effective, 200 μM-Mn or 20 μM-Zn causes complete reversal of inhibition, whilst Fe or Co (100–500 μM) produces partial reversal. But Cu^{2+} either has no effect or is toxic between 0·01 mM and 5 mM; K^+ and Na^+ have no effect, apart from that due to the presence of ~ 6 mM-K in the buffer solution at pH 5·5. W. J. BAKER.

Effect of micronutrient and other deficiencies on yield and mineral composition of forage crops. H. C. Harris (*Proc. Xth int. Grassld Congr.* [1966], 1968, 175–178).—Various fertiliser treatments were applied to forage crops grown in glass containers on soils of low fertility. Major elements usually increased yields and plant content of the element concerned. Micronutrient deficiencies decreased yields but generally increased the N and mineral content of the forage. Cutting affected the treatments. Except for N in legumes, yield was negatively correlated with % of an element in the forage. M. LONG.

Effect of nitrogen nutrition and clipping frequency on regrowth of perennial ryegrass [pot trials]. K. Dilz (*Proc. Xth int. Grassld Congr.* [1966], 1968, 160–164).—Regrowth of turves grown in pots was loosely related to the amount of carbohydrate present in the turf and closely related to the amount of crude protein. At the end of the trials the N taken up from fertiliser top-dressings and from the N reserve in the turf was distributed between the aerial part of the plant and the turf in the ratio 2:1. M. LONG.

Genetic and biochemical aspects of *o*-hydroxycinnamic acid synthesis in *Melilotus alba*. H. J. Gorz, F. A. Haskins and A. Kleinhofs (*Proc. Xth int. Grassld Congr.* [1966], 1968, 704–708).—The *cis* and *trans* isomers of *o*-hydroxycinnamic acid in *Melilotus alba* Desr. occur mainly as the β-D-glucosides. These are formed via the following pathway: phenylalanine (from shikimic acid) → *trans*-cinnamic acid → *o*-coumaric acid → *o*-coumaryl glucoside (*trans*-D-glucosyl-*o*-hydroxycinnamic acid) → coumarinyl glucoside (*cis*-β-D-glucosyl-*o*-hydroxycinnamic acid) (**I**). In disrupted tissue **I** is rapidly hydrolysed by endogenous β-glucosidase to coumarinic acid which then lactonises to coumarin. The *cu* gene influences the content of the glucosides by controlling the *o*-hydroxylation of cinnamic acid, while the *b* gene is associated with the loss of β-glucosidase activity. This work is connected with the breeding of low-coumarin varieties of sweet clover. (28 references.) M. LONG.

Influence of reserve substances [non-structural carbohydrate] on dry-matter production [by grass] after defoliation. Th. Alberda (*Proc. Xth int. Grassld Congr.* [1966], 1968, 140–147).—Actively growing clonal material of perennial ryegrass was subjected to either a short period at 30° in darkness, in nutrient solution or to a similar period at 15° in full light, in water. The first treatment gave lower contents of water-sol. carbohydrate (**I**). Leaf production with high values of **I** was greater by a final ratio of 4:1. Respiration was greater with high **I**, but photosynthesis was lower. New leaves were formed at the expense of the reserves with high **I**, whilst with low **I**, tillers tended to die. It is concluded that high **I** is important for regrowth and maintenance of a sward after cutting.
M. LONG.

Relationship between carbohydrate accumulation and growth of grasses under different microclimates. R. E. Blaser, R. H. Brown and H. T. Bryant (*Proc. Xth int. Grassld Congr.* [1966], 1968, 147–150).—Low temp., N and moisture limited growth and led to the increased formation of sol. carbohydrates (*C*). Accumulation of *C* involved a dynamic system of energy balance; there is a net energy loss when growth demands for *C* exceed photosynthesis, whilst *C* accumulates when growth demands are low. (20 references.)
M. LONG.

Physiology of regeneration of lucerne (*Medicago sativa* L.). K. C. Hodgkinson (*J. Aust. Inst. agric. Sci.*, 1968, **34**, 221).—Org. compounds in the roots, labelled with ^{14}C before complete herbage removal, were used as respiratory substrates and to a lesser extent were translocated into new shoots for ~ 20 days during regeneration. Most of the compounds used were derived from the tap root. Studies of the distribution of photosynthate from leaves confirmed that carbohydrate 'reserves' in the tap root contribute to new short growth only until the first formed leaves begin to export photosynthate.
E. G. BRICKELL.

Presence of starch and α-amylase in the leaves of plants. J. W. Gates and G. M. Simpson (*Can. J. Bot.*, 1968, **46**, 1459–1462).—α-Amylase was found to be present in the leaves of 79 plant species from 30 families. Starch was present in most leaves; where absent it could be induced in all but 2 species by floating leaves on 2% sucrose in light (600 ft candles for 24 h). In such plants, α-amylase may function to prevent accumulation of starch. (10 references.)
C. J. R.

Effect of varied nitrogen- and potash-nutrition on the content of soluble amino compounds and on the yield of spring wheat. M. Helal and K. Mengel (*Z. PflErnähr. Bodenk.*, 1968, **120**, 89–98; cf., *Idem., ibid.*, **120**, 12–20).—In pot culture experiments, two levels of K supply were supplemented by increasing proportions of N. The total sol. N content of the wheat plants, notably of glutamic acid (**I**), alanine, aspartic acid (**II**), serine and γ-aminobutyric acid, increased with the N supply, the effect being most marked during shoot formation and ear emergence; at the tillering stage only **I** responded to increasing N. Increase in the K supply had no effect on the **I** content but lowered the glutamine, asparagine, **II** and glycine and the total sol. amino acid contents of the plants particularly during tillering. More inorg. N was utilised in the synthesis of these amino acids as the external N supply was increased, the K supply being an influential factor in the synthesis. (17 references.)
A. G. POLLARD.

Effect of temperature on tocopherol content of seed fats. H. Beringer and N. P. Saxena (*Z. PflErnähr. Bodenk.*, 1968, **120**, 71–78).—Sunflower, flax and oat plants were grown in pots and a day temp. of 12° or 28° was maintained during seed development. In all three species the lower temp. resulted in increased lipid contents but in decreased tocopherol contents in the seed oil. A secondary effect of low-temp. growth was a decrease in the ratio of vitamin E-active tocopherols to polyunsaturated fatty acids, this being an important factor in the nutrition of man and animals. (18 references.)
A. G. POLLARD.

Physiological studies on the genus *Trifolium* with special reference to the South African species. IV. Effect of calcium and pH on growth and nodulation. J. G. C. Small (*S. Afr. J. agric. Sci.*, 1968, **11**, 441–457).—In water or sand cultures at pH 6·5, supplied with combined N, but not inoculated with rhizobium, yields from three S. African indigenous spp. were much less dependent on the Ca content of the medium (5–100 ppm) than were the yields from three European spp. Yields from all the spp. increased with increasing pH (4·0–6·5). Nodule formation was promoted in all spp. by increases in Ca concn. (0–8 ppm); in some cases it was further promoted by further Ca increases. (25 references.)
P. S. ARUP.

Nodulation of *Medicago sativa* in solution culture. III. Effects of nitrate on root hairs and infection. IV. Effects of indole-3-acetate in relation to acidity and nitrate. D. N. Munns (*Pl. Soil*, 1968, **29**, 33–47; 257–262).—III. When NO_3 was maintained in continuous supply in solution cultures at 0·02–2·0 mM it reduced the numbers of curled root hairs and of nodules on *M. sativa*. Presence of indole-3-acetate (> 10^{-8}M) overcame the NO_3^--inhibition of curling without affecting nodulation. Nitrate reduced nodule numbers somewhat even when present in the nutrient only before inoculation, or when present on the 1st to 3rd day after inoculation; NO_3^- also delayed nodulation and this delay corresponded with delay in appearance of infection threads.

IV. Indole-3-acetate (IAA) added to solution cultures alleviated but did not eliminate inhibition of nodulation of *M. sativa* by acidity and nitrate. Nitrate inhibition was not alleviated when IAA was added at 5×10^{-7}M only at inoculation, but this addition augmented the stimulation of nodulation by later, smaller additions, of IAA. When addition of IAA was delayed until infection threads appeared, 10^{-9} or 10^{-8}M IAA approx. doubled the number of threads that reached the root cortex and the number of nodules that formed subsequently. In a medium without NO_3^- (normally inhibitory to nodulation) at pH 4·9, IAA added at inoculation stimulated nodulation, particularly at 10^{-9}M, whilst root hair curling was increased only at concn. of 10^{-8} or 10^{-7}M IAA.
A. H. CORNFIELD.

Root-nodule bacteria for some cultivated species of *Trifolium*. J. Brockwell and A. H. Gibson (*J. Aust. Inst. agric. Sci.*, 1968, **34**, 224–227).—Experiments with *Trifolium cherleri* L., *T. hirtum* All., *T. incarnatum* L., *T. repens* L. and *T. subterraneum* in association with *Rhizobium trifolii* showed that the strains used for clover inoculants were not necessarily the most effective available for any particular clover species nor were they the most consistently effective in association with a no. of species. A strong case therefore exists for the production of specialised inoculants containing rhizobia with specific characteristics appropriate for particular species. (16 references.)
E. G. BRICKELL.

Ecological studies of root-nodule bacteria introduced into field environments. I. Survey of field performance of clover inoculants by gel immune diffusion serology. II. Initial competition between seed inocula in nodulation of *Trifolium subterraneum* L. seedlings. W. F. Dudman and J. Brockwell (*Aust. J. agric. Res.*, 1968, **19**, 739–747; 749–757).—I. The serological procedure was used for the identification of strains of *Rhizobium trifolii* originating from inoculated seeds at intervals during 3–42 months after sowing. No evidence was obtained that the strains introduced would become permanently established in the soil. (19 references.)

II. The same technique was used to test the persistence of clover rhizobia in a soil naturally rich in the rhizobia. Of the 860 isolates found in the soil during Apr. to Sept., 405 came from treatments in which three inoculation strains had been used for seed coating with a broth and peat mixture. The bearing of the results on the persistence of the inoculant strains is discussed. (11 references.)
P. S. ARUP.

Alkalinity of plant ash as a measure of the excess cations in plants. A. Jungk (*Z. PflErnähr. Bodenk.*, 1968, **120**, 99–105).—The excess of cations over non-volatile inorg. anions in plants showed agreement with the alkalinity of the plant ash only when the excess was > 100 mequiv./100 g of dry matter. At lower levels there was a progressively increasing difference between the two sets of values, agreement between which was reached if the plant material was mixed with NaOH before ashing. The error is explained by loss of Cl^- during ashing in absence of NaOH, at low levels of excess of cations.
A. G. POLLARD.

Carotenoids of the marigold, *Tagetes erecta*. A. U. Alam, J. R. Couch and C. R. Creger (*Can. J. Bot.*, 1968, **46**, 1539–1541).—Pigments of *Tagetes erecta* were separated using multiple fractional extractions and t.l.c. The petals showed a predominance of xanthophylls (98·7%) and only a small amount of carotenes. By absorption studies these were characterised as lutein (64·1%), antheraxanthin (31·1%), γ-cryptoxanthin (3·1%), β-carotene (0·6%) phytofluene (0·4%) and α-carotene (0·15%). (11 references.)
E. G. BRICKELL.

Oxidation and carotenogenesis regulating factors in mangoes. A. K. Mattoo, V. V. Modi and V. V. R. Reddy (*Indian J. Biochem.*, 1968, **5**, 111–114).—Ethylene occurs to the extent of 0·02–0·18 ppm in ripening mango slices. During the climacteric rise, catalase and peroxidase activities increased due to the disappearance of a heat labile and non-dialysable inhibitor present in unripe fruits. In the ripe fruit, carotenogenesis was regulated by phosphatase which

appeared to dephosphorylate the intermediates; β-carotene stimulated the activity of this enzyme. (17 references.)
E. G. BRICKELL.

Germination of seeds of wheat varieties in salinised soils. M. Afzal and Muhammad Rashid (*W. Pakistan J. agric. Res.*, 1968, **6**, No. 2, 1–5).—In laboratory and in pot tests for germination, 12 out of 16 wheat varieties were tolerant to salinities up to 1·25%; of the other four, three were described as 'medium tolerant', and one as 'medium susceptible'. (10 references.) P. S. ARUP.

Germination of maize seed produced in the spring season. Abdul Ghafoor Bhati and Muhammad Ismail Zaffar (*W. Pakistan J. agric. Res.*, 1968, **6**, No. 2, 10–18).—Seeds of early, medium and late varieties of maize and seeds of other hybrids obtained in the spring season showed the same capacity for germination as did seeds produced during the normal season, but the germination of the spring seed was a little slower, and was improved by allowing the seed to dry to a moisture content of ~ 12%. P. S. ARUP.

Method for sterile germination of maize seed. J. J. Du Toit (*S. Afr. J. agric. Sci.*, 1968, **11**, 615–616).—Available methods are not entirely satisfactory. The proposed method involves the cutting away of any loose parts of the pericarp, of the blackish aleurone layer at the point of attachment, and of the part of the seed coat covering the embryo, followed by steeping for 1 min in 0·1% aq. $HgCl_2$ plus 5 ml of Teepol per l and by washing in sterile water. The seeds are then removed individually to test tubes each containing 5 ml of sterile water and, after 72 h, transferred to potato dextrose agar in Petri dishes for germination. The method gives a 100% yield of sterile seed, free of plant pathogens. P. S. ARUP.

Effect of temperature on the growth of wheat. II. 'Grass-clump' dwarfs and dwarf varieties. III. Chemical constituents of Marquillo, Kenya Farmer and two dwarf progeny grown at three temperatures. Y. Yao and D. T. Canvin (*Can. J. Bot.*, 1969, **47**, 1–8, 35–45).—II. All 'grass-clump' hybrids and dwarf selections differed from normal varieties in that they were sterile when grown at low temp. but became essentially 'normal' above a threshold temp. (21, 26 or 30°). If the precise temp. is not known, then elongation and seed production can be induced by growing under continuous light at 26–30°. (17 references.)

III. Marquillo, Kenya Farmer and Marquillo × Kenya Farmer wheat dwarfs 1 and 2 were grown at 16, 21, and 26°. There was no deficiency of sol. carbohydrates, amino acids, org. acids or chlorophyll between 16 and 26°, if anything there was a marked accumulation of most of these substances in the non-growing plants at 16°. It appears that during the low temp. treatment an inhibitor is accumulated that blocks growth. (20 references.)
E. G. BRICKELL.

Proportional reduction in shoot growth of grapevines with root systems maintained at constant relative volumes by repeated pruning. M. S. Buttrose and M. G. Mullins (*Aust. J. biol. Sci.*, 1968, **21**, 1095–1101).—Cuttings of *Vitis vinifera* L. cv. Muscat Gordo Blanco, syn. Muscat of Alexandria were grown in water culture after being established in vermiculite. The root vol. was assessed at intervals by water displacement and groups of plants were pruned to maintain the root vol. at 75, 50 or 25% of the control group. Dry wt. of shoots, parent cane and roots, together with shoot length and leaf no., were recorded during the controlled growth. At the two most severe pruning levels, the shoot length was already retarded after 1 week and subsequent growth was related to the level of pruning. Shoot wt. was also diminished and it is concluded that roots are a source of a growth substance required for normal growth of shoots. (15 references.) J. B. WOOF.

Growth responses of Marquillo × Kenya Farmer wheat dwarf 1 and 2 to gibberellic acid, kinetin and indolebutyric acid under controlled environmental conditions. Y. Yao and D. T. Canvin (*Can. J. Bot.*, 1969, **47**, 53–58).—Indolebutyric acid and kinetin had no effect on plant height or development, and did not alter the response to gibberellic acid. Gibberellic acid treatment increased culm height and induced plants to flower earlier but suppressed final yields of shoot and seed. Continuous light decreased plant height, time to ear emergence and tiller no., but did not alter shoot or seed yields. E. G. BRICKELL.

Gibberellin and growth in stone fruits: Induction of parthenocarpy in plum. D. I. Jackson (*Aust. J. biol. Sci.*, 1968, **21**, 1103–1106).—Flowers were restricted to 50 on each of 10 branches on 5 mature plum trees. Pollination was prevented by excising the distal end of flowers containing stigmas and anthers two days before full bloom. Hormone solutions were applied to the cup formed by emasculation, to the sepals and petals on non-emasculated flowers or to shoots from vegetative buds using a camel-hair brush. The rates of increase in the dia. of four fruits per branch were determined. GA_3 and a mixture of gibberellins A_4 and A_7 each induced seedless fruit development, and whilst the parthenocarpic fruit grew more rapidly early in the season, the final dia. was only 60% of the control. Application only to vegetative shoots did not induce parthenocarpy. (10 references.) J. B. WOOF.

Preparation of low concentrations of sulphur dioxide for experimental gas treatment of biological materials [plants]. N. Faller and W. Höfner (*Z. PflErnähr. Bodenk.*, 1968, **120**, 155–159).—Sulphur dioxide is produced by acidifying $Na_2S_2O_5$ with H_2SO_4 and the $[SO_2]$ is adjusted to the required level (0·05–6·0 mg/m³ of air) by diffusion through rubber, polyvinyl chloride or polyethylene. The temp. dependence of the diffusion process was studied between 0 and 50°. M. LONG.

Crops and Cropping

Photosynthesis of wheat under field conditions. I. Interaction of photosynthetic organs. D. W. Puckridge (*Aust. J. agric. Res.*, 1968, **19**, 711–719).—Four wheat varieties were examined to determine differences in grain production in response to ear shading and defoliation treatments under field conditions. Responses to both were affected by the level of competition and by the no. of tillers on the plants, and there was a movement of assimilates between shoots. Compensation, in production of assimilate by other organs, was very pronounced when parts of the plant were shaded or leaves removed. The N content of the grain was not affected by ear shading. (16 references.) P. C. W.

Sprouting problem in soft white wheats. H. A. Ibrahim (*Diss. Abstr., B.*, 1967, **28**, 16–17).—Environmental and genetical factors influencing the sprouting of wheat grain in the ear are examined in numerous varieties and lines. Varieties having red grains sprouted less than did those with white grains; few white varieties showed any appreciable degree of dormancy. Comparative tests of sprouting under conditions comparable with those in the field were made by placing wheat heads on a wire mesh and spraying with water. Sprouting was influenced by the amount of moisture in the grain, this, in turn, being a measure of physical maturity; in white varieties, the higher the moisture the greater was the resistance to sprouting. Erect heads and those tilted to 135° sprouted less than did those tilted to 45° or 90° although varietal differences in sprouting were more important than differences in position. Seeds from basal spikelets of both red and white varieties germinated more slowly than did those from other positions on the spike. Sprouting in crosses of red and white varieties was examined and genetical explanations of sprouting resistance are advanced.
A. G. POLLARD.

Effects on winter wheat of ammonium sulphate, with and without a nitrification inhibitor, and of calcium nitrate. J. K. R. Gasser and F. G. Hamlyn (*J. agric. Sci., Camb.*, 1968, **71**, 243–249).—Winter wheat grown on a sandy-loam and on a clay-loam was given an autumn dressing of $(NH_4)_2SO_4$ (**I**), with or without 2-chloro-6-(trichloromethyl)pyridine (**II**). Dressings of 75 or 150 lb/N acre were applied either as **I**, treated or untreated with **II** to the light soil and 50 or 100 lb to the heavy soil, or as $5Ca(NO_3)_2 \cdot NH_4NO_3 \cdot 10H_2O$ (**III**) in the spring, On the no-N plots yields were 17 and 46 cwt/acre on the sandy-loam and clay-loam respectively and on the high-N plots 52 and 56 cwt/acre. On the light soil **I** with **II** raised yields by 3·5 cwt/acre with the low dressing and 5·4 cwt with the high. **II** had no effect on yield on the heavy soil. At 50 lb/acre spring and autumn dressings had equal effect, but at 100 lb the spring dressing was slightly more effective. **III** at 75 lb was significantly better than **I** on the sandy loam. **II** had no effect on rate of uptake nor on total N uptake from spring-applied fertiliser.
M. LONG.

Effect of controlled atmosphere on the heats of respiration of fresh produce and high moisture field corn [maize]. R. T. Toledo (*Diss. Abstr., B.*, 1968, **28**, 5073).—Heats of respiration (*HR*) of fresh peas, lima beans, sweet-corn, apples and high moisture (21%) field maize were determined during storage in air and in controlled atm. The *HR* increased with time of storage, but the rate of increase was not as great in the controlled atm. as in air, and the *HR* of samples treated with sorbate (fungicidal agent) were lower than for untreated samples. The results indicate that mould activity contributes a large part of the *HR*, and that heating of grain can be avoided by suppressing mould growth. The use of a mould inhibitor in controlled atm. shows promise in prolonging the storage life of damp grain. F. C. SUTTON.

European Brewery Convention. 17th Report of the Barley Committee: Trials [1966]. W. Wilten (1968, 230 pp.).—Includes reports of trials ranged in alphabetical order of country. P. P. R.

Association between grain yield and several other plant characteristics in rice. Chen-Seng Huang (*Diss. Abstr., B.*, 1967, **28**, 22–23).—Pairs of sub-line strains derived from two varieties of rice were utilised in a study of grain yield in relation to date of maturity. In each pair of strains one member was made up by bulking seed from at least five early-maturing plants in an F_3 line and the other was from bulked seed of five late-maturing plants of the same line. In nearly all cases the sub-line from the early-maturing strain matured significantly earlier and with heavier yields than did the sub-line derived from the late-maturing types. In a similar experiment differences in grain yield between sub-lines within lines were not significant. A. G. POLLARD.

Improving the rice plant and its culture. R. F. Chandler, jun. (*Nature, Lond.*, 1969, **221**, 1007–1010).—Methods of culture and the development of new varieties by the International Rice Research Institute are described. Low yields of rice in the tropics are due to the plant being tall and leafy and subject to lodging (fall over), especially when heavily fertilised and intensely controlled. The new stiff strawed variety, IR8, is resistant to weather, lodging and most diseases, has a grain/straw ratio of $\sim 1 \cdot 2$, and (mainly due to increased photosynthesis) regularly produces an average yield of 6000–8000 kg/hectare in S.E. Asia, S. America and Africa. Such dwarf varieties, generally the result of crossing tall Indonesian with short Chinese, are early maturing and insensitive to photoperiod, thus permitting planting in any month and up to three crops annually. Even better strains are being developed to improve grain quality and resistance to certain diseases. The associated researches reviewed include variety/fertiliser interactions, relation between solar energy and yields, chemical control of weeds in lowland transplanted rice, insect control with lindane and diazinon and varietal resistance to insects, especially the green leafhopper and brown planthopper. W. J. BAKER.

Effect of gamma irradiation on potatoes. C. Boffi, L. Ferrari and G. Ferrara (*Chimica Ind., Milano*, 1969, **51**, 173–175).—'Majestic' seed potatoes collected in Sept.–Oct. and kept at 4° were irradiated in the following Jan. on a rotating disc so that all sides were exposed to the radiation for such times as to give an absorption of 5000, 7500, 10,000, 17,000 and 30,000 rad. Part of each lot was examined immediately and part after 4 months storage. With 7500 rad, sprouting was almost completely inhibited but it was unaffected by 5000 rad. Determinations were made of substances sol. in 70% EtOH, of sugars, vitamin C and amino acids. Fructose, glucose and galactose were increased by irradiation to a max. at 10,000 rad, decreasing with higher doses. The glucose was doubled and the fructose trebled. Di- and tri-saccharides were hydrolysed; the behaviour of saccharose was anomalous, decreasing up to 10,000 rad and then increasing considerably with higher doses. Ascorbic acid was unaffected at all doses. Amino acids in general increased; methionine was an exception, being completely destroyed at 30,000 rad. Total amino acids showed a 50% increase after 30,000 rad irradiation, with intermediate increases at lower dosages. J. I. M. JONES.

Analysis of yields of grasses in mixed and pure stands. J. P. van den Bergh (*Versl. landbouwk Onderz. Ned.*, 1968, No. 714, 71 pp.).—Observations on the behaviour of mixed cultures showed that better results would be obtained by sowing the grass giving the best yield under the prevailing conditions rather than by sowing the best yielding mixture of species, the advantages of which may be only temporary. (32 references.) (In English.) P. S. ARUP.

Aqueous ammonia compared with other nitrogenous fertilisers as solids and solutions on grass. D. R. Hodgson and A. P. Draycott (*J. agric. Sci. Camb.*, 1968, **71**, 195–203).—Italian ryegrass was given (*i*) single or split dressings of injected aq. NH_3, (*ii*) solid $(NH_4)_2SO_4$ and (*iii*) injected aq. $(NH_4)_2SO_4$. A single injection of aq. NH_3 and a split application of solid gave little difference in total dry matter (*DM*) yield. (*iii*) eliminated scorch problems caused by (*ii*) at $\leqslant$ 448 lb N/acre and gave greater *DM* yields. A single injection of aq. NH_3 was also compared with application of NH_4NO_3/urea as solid or solution. At 560 and 840 lb N/acre, total *DM* yields were similar but at 280 lb/acre a split dressing of solid gave more *DM*. Recoveries of N by the treatments are compared; least N was recovered from surface-applied solutions of NH_4NO_3/urea. Single injections of aq. NH_3 were found to be as efficient as other fertilisers tested. M. LONG.

Variation of carbohydrates in various species of grasses and legumes. K. Ojima and T. Isawa (*Can. J. Bot.*, 1968, **46**, 1507–1511).—Several grasses of both northern and southern Japanese types and a few species of legumes were grown in N. Japan and analysed for carbohydrates. Results indicated a correlation between the type of reserve carbohydrates and the region of origin of the species. (14 references.) E. G. BRICKELL.

Effects of competitive stress on the growth and development of some grass species and populations. I. Rhodes (*J. Aust. Inst. agric. Sci.*, 1968, **34**, 223).—Rate of leaf appearance, individual leaf size and especially tiller production in seedlings were all affected by competitive stress. In a mixture of *Festuca arundinacea* and HI ryegrass (**I**), root competition from the latter was mainly responsible for suppression of the former. With **I** and *Phalaris coerulescens* (**II**), suppression of the latter was due to root and shoot competition. *Lolium rigidum*, **I** and **II** differed in their abilities to develop from seed in established swards of the same three species, and the established swards differed in their aggressiveness towards the seedlings. E. G. BRICKELL.

Yield stability as an aid to the preliminary assessment of introduced forage plants. R. L. Burt and K. P. Haydock (*J. Aust. Inst. agric. Sci.*, 1968, **34**, 228–231).—The data of Stilt (*Agron. J.*, 1958, **50**, 136) were reanalysed to determine plant × year interactions. For each species/strain/mixture of perennial forage, a linear regression of yield on the mean yield of all plants for each season was computed to measure plant adaptation. E. G. BRICKELL.

Influence of nitrogen fertilisation and drying method on yield and chemical composition of *Dactylis glomerata*, *Bromus inermis* and *Phleum pratense*. C. L. Rhykerd, J. E. Dillon, C. H. Noller and J. C. Burns (*Proc. Xth int. Grassld Congr.* [1966], 1968, 214–218).—*Dactylis glomerata* (**I**), *Bromus inermis* and *Phleum pratense* were fertilised with N at rates of 0, 75, 150, 300, 600, and 1200 lb/acre. Dry matter yield increased with increasing N except for the top level, while total N% increased continuously. The sol. carbohydrate content of **I** was inversely related to N level, whilst the other two grasses were unaffected. Lyophilised samples were substantially higher in water-sol. N and sol. carbohydrate, but lower in total N, than oven-dried material. (13 references.) M. LONG.

Response of herbage yields and quality to a wide range of nitrogen application rates. D. Reid (*Proc. Xth int. Grassld Congr.* [1966], 1968, 209–213).—Various rates of fertiliser N, ranging from 0 to 800 lb/acre, were applied to a nearly pure grass (*Lolium perenne*) sward. The relationship between herbage yields and N application was linear up to 300 lb/acre, further increases in N giving only slight increases in dry matter yield. Crude protein continued to rise up to the 600 lb level, although above 400 lb/acre the increase was mainly due to increases in NO_3^--N. M. LONG.

Response of grass swards to nitrogenous fertiliser. D. W. Cowling (*Proc. Xth int. Grassld Congr.* [1966], 1968, 204–209).—Dry matter yield was determined by the level of N in a series of trials on pure grass swards, increasing levels of N giving increasing recovery of N and increasing yields of dry matter. Differences in yield at higher levels of N occurred because of variation in (*a*) N recovery and also in (*b*) the dry matter : N ratio. In defoliation trials differences arose at the highest N level due mainly to variation in (*b*). The herbage production curve showed that N recovery was progressive, but that differences in dry matter yield between two levels of N became greater as (*b*) increased with time. (10 references.) M. LONG.

Nitrogen effect on herbage production of grasslands on different sites. Th. A. de Boer (*Proc. Xth int. Grassld Congr.* [1966], 1968, 199–204).—With intensive grassland husbandry in the Netherlands many growth factors are constant. Two factors, N fertilisation and water supply status of the soil, still vary within a wide range. The interaction of these with climate is discussed in relation to other N response investigations. (12 references.) M. LONG.

Photosynthesis and respiration of four pasture grasses as affected by moisture conditions and salinity. V. N. Schroder (*Proc. Xth int. Grassld Congr.* [1966], 1968, 181–184).—Pangola, Bahia, Bermuda (*B*) and St Augustine (*A*) grasses are tolerant to levels of NaCl as high as 4500 ppm in irrigation water and to 36 days of continuous flooding. Salt levels > 9000 ppm greatly reduce photosynthesis and transpiration, with less effect on respiration. *B* and *A* are the most tolerant; *B* absorbs less Na while *A* is the more tolerant of high levels within the plant. M. LONG.

Use of plant analysis for determining P and K needs of grasslands. N. Knauer (*Proc. Xth int. Grassld Congr.* [1966], 1968, 171–174).—The P and K supply may be accurately determined by plant analysis. Limiting values of P and K can be obtained from the high correlations between the plant contents and manuring levels. Limiting

values grade with crude protein contents so that use of young and old plants is possible. M. LONG.

Carbohydrate reserves in orchardgrass. W. G. Colby, M. Drake, Hisatomo Oohara and Norihito Yoshida (*Proc. Xth int. Grassld Congr.* [1966], 1968, 151–155).—Seasonal fluctuations in the sol. carbohydrate reserves of orchardgrass are largely influenced by climate, rising in periods of cool moist weather and falling in hot and dry conditions. Levels fall sharply after N fertilisation. When heavy N fertilisation is followed by hot dry weather, reserves are often reduced to dangerously low levels, with resultant poor recovery growth, and plant injury. (10 references.) M. LONG.

Moisture use of pasture plants in a desert environment. N. H. Tadmor, O. P. Cohen, L. Shanan and M. Evenari (*Proc. Xth int. Grassld Congr.* [1966], 1968, 897–906).—*Agropyrum elongatum* (**I**), *Hordeum bulbosum*, *Medicago sativa* (**II**), *Oryzopsis holciformis*, *O. miliacea* (**III**), *Phalaris tuberosa* (**IV**) and *Avena sterilis* (**V**) were grown in the 100-mm rainfall area of the Negev desert. Water use was low (1–2 mm/day) during winter and drought years and high (3–5 mm/day) in wet springs. Dry perennial plants during summer dormancy used only 0·1–0·3 mm/day. **I**, **II**, **III**, **IV** and **V** had low requirements. Differences in water use were related to length of growing season and rooting depth. Even in the 100-mm rainfall desert some useful annual and perennial plants are able to prosper by using the surface runoff from desert slopes. (24 references.) M. LONG.

Intra-seasonal changes in height growth of plants in response to nitrogen and phosphorus. G. M. Van Dyne and I. Thorsteinsson (*Proc. Xth int. Grassld Congr.* [1966], 1968, 924–929).—Height growth of *Agropyron spicatum*, *Stipa comata* and *Oxytropis serecia* were related to the effects of fertilisers on production and basal cover and to soil moisture. N, but not P, caused the grasses to attain their max. growth rate while they were shorter. M. LONG.

Reserve carbohydrate and regrowth potential of tropical grasses. A. Adegbola (*Proc. Xth int. Grassld Congr.* [1966], 1968, 933–936).—Regrowth potentials of Guinea grass (**I**) and giant star grass (**II**) pastures, fertilised with 0, 100, 200 and 400 lb N/acre, were evaluated in a dark chamber on the basis of dry matter produced per 6 in. dia. sod plug. Amount and % of carbohydrate in the stem bases and roots of **I** were positively correlated with observed regrowth potential as was the carbohydrate content of the stolons and rhizomes of **II**. There was no correlation between regrowth potential and current dry matter yields in either species. (14 references.) M. LONG.

Influence of defoliation on carbohydrate status and nutritive value of perennial veld grasses. B. R. Roberts and D. P. J. Opperman (*Proc. Xth int. Grassld Congr.* [1966], 1968, 940–944).—Data for dry matter, crude protein and P yields and the total available carbohydrate, crude protein and P in the form of root and stubble reserves are presented for *Themeda triandra*, *Cymbopogon plurinodis* and *Eragrostis lehmanniana*. Both glasshouse and field trials indicate the importance of early summer rest periods for max. herbage and nutrient yields and max. growth reserve status in climax grass species. M. LONG.

Light transmission and chlorophyll amount in a sward as substitutes for leaf-area index [as a measure of dry-matter production]. Shuichi Nishimura, Tadakatsu Okubo and Masao Hoshino (*Proc. Xth int. Grassld Congr.* [1966], 1968, 117–120).—Relative light intensity (*RLI*) was measured by two CdS photoconductive cells, one in the sward and the other in the full sun. *RLI* and leaf area index (*LAI*) were highly correlated, as were chlorophyll content and *LAI*. The optimal value of chlorophyll content in a unit area of sward corresponds to the optimal *LAI*. In Ladino clover regrowth it is between 1·2 and 2·3 g/m^2. M. LONG.

Output of pastures under a clover nitrogen regime in Northern Ireland. J. Lowe (*Proc. Xth int. Grassld Congr.* [1966], 1968, 187–191).—N fertiliser reduced and K_2O increased the clover to grass ratio, but at higher levels of N, K_2O was ineffective in maintaining clover. Where N was not applied yield and quality of herbage improved as the clover ratio rose. (12 references.) M. LONG.

Changes in swards of natural grasslands under influence of fertilisation and utilisation. J. G. Liiv (*Proc. Xth int. Grassld Congr.* [1966], 1968, 839–842).—Application of 54 kg of P_2O_5 and 60 kg of K_2O per ha gave an increase in both sward density and in legume growth in the first 2–3 years; 54 kg P_2O_5, 60 kg K_2O and 34 kg N per ha after 4–5 years led to an increase in the short grasses, especially *Festuca rubra*, and a decrease in legumes. The first treatment plus 20 tons of compost after the 5–7th years led to a decrease in short and an increase in the tall grasses and some increase in legumes. When grazed, fertilised pastures improved, their productivity increasing and exceeding considerably that of the hay-lands. M. LONG.

Pasture establishment on unploughable hill country in New Zealand. Factors influencing establishment of oversown grasses and clovers. N. A. Cullen (*Proc. Xth int. Grassld Congr.* [1966], 1968, 851–855).—Successful establishment depended on reducing competitive effects by grazing and by use of paraquat. CaO, P and N had little effect on grass establishment, although P helped clover growth and survival. Close grazing and use of paraquat aids establishment in dense browntop, but does not do so with sparse vegetation. Oversowing is most successful in early spring. M. LONG.

Problems of grassland establishment and maintenance on hill-country in Victoria. R. J. Newman (*Proc. Xth int. Grassld Congr.* [1966], 1968, 875–878).—Large tracts of land in this area, previously considered unsuitable for grassland, can be made productive by sowing clovers and grasses. Mo, P and S deficiencies can be made good by application of superphosphate (2–4 cwt per acre) and of a Mo compound (2 oz per acre). Where the soil is especially acid, establishment of clover is achieved by drilling lime with the seed or coating the seed with lime. M. LONG.

Growing red clover. Anon. (*Leafl. U.S. Dep. Agric.*, 1968, No. 531, 8 pp.).—Areas for growth in the U.S.A., varieties, companion crops, soil requirements, seeding, stand management, uses (hay and silage, pasture and in soil improvement), diseases, and seed production are described. E. G. BRICKELL.

Moisture movement in the alfalfa [lucerne] stem during drying. L. O. Bagnall (*Diss. Abstr., B.*, 1967, **28**, 151).—Effects of epidermal and internal resistance to the movement of water out of lucerne stems and the consequent changes in wt. of stems were examined, and drying constants were determined from an exponential regression on the sequence of wt. Effective diffusivities were calculated and the result of removal of the epidermis, cortex and phloem in respect of drying rate was examined. Immersion of the stems for 3–20 sec in water at 140°F or for 20 sec. or more at 200°F increased the drying rate. Other temp. and times in the range 120–200°F and 1 sec–1 min had no significant effect on drying rates. A. G. POLLARD.

Experimental cultivation of some essential oil-bearing plants in saline soils. V. Chandra, A. Singh and L. D. Kapoor (*Perfum. essent. Oil Rec.*, 1968, **59**, 869–873).—The growth of essential oil-bearing plants in Lucknow with soils having pH values of 8·5–10 was compared with similar plants grown in other parts of India, and the yields of oils were compared. The plants included *Mentha piperita* L., *M. arvensis* Linn., *Matricaria chamomilla* Linn., *Vetiveria zizanioides* Staf., *Cymbopogon nardus* L., *C. winterianus*, *Ocimum kilimandscharicum* Guerke, *Tagetes minuta* L. and *Anethum graveolens* L. The yields of essential oil from all these plants are comparable to those obtained from plants grown in other parts of India with normal soils. G. R. WHALLEY.

Effect of inoculation on yield of groundnut (*Arachis hypogea*). Manzoor Elahi Raja, Dilshad Muhammad Khan and Gulzar Ahmad (*W. Pakistan J. agric. Res.*, 1968, **6**, No. 2, 79–84).—Both seed and field inoculation (the latter by watering with an aq. inoculant suspension) gave increases in yields, but seed inoculation gave yields exceeding those from field inoculation by 2·9–8·5% over 3 years. (17 references.) P. S. ARUP.

Effect of CCC on cotton plants. M. M. El-Fouly, J. G. Salib and F. K. El-Baz (*Z. PflErnähr. Bodenk.*, 1968, **121**, 66–76).—Two trials were carried out in different parts of Egypt to find the effect of CCC on the growth and yield of two varieties of cotton plants. CCC as a spray was more effective in shortening the plants than soaking the seed in a CCC solution or mixing it with a CCC powder. Early spraying about 6 weeks after planting results in a marked reduction in internodal distance without adversely affecting yield. Leaves of the treated plants were darker green than the untreated due to a higher chlorophyll content, the main roots of the treated plants were longer and the secondary roots were more numerous. (In English.) (16 references.) M. LONG.

Use of 2-chloroethyltrimethylammonium chloride (Cycocel or CCC) as growth retardant on cotton. M. Siraj-ud-Din Khan and Waheed Sultan (*W. Pakistan J. agric. Res.*, 1968, **6**, No. 2, 52–59).—In order to check excessive cotton growth in Pakistan, cotton trees were sprayed with CCC at 0·1 or 0·2 lb/acre, with the result that growth was suppressed, chiefly by shortened internodes. The leaves became darker and more fleshy, but yields of cotton per plant were reduced. P. S. ARUP.

Control of bitter pit and breakdown by calcium in the apples Cox's Orange Pippin and Jonathan. J. van der Boon, A. Das and A. C. van Schreven (*Versl. landbouwk. Onderz. Ned.*, 1968, No. 711, 43 pp.).—Bitter pit incidence was checked by high leaf- and fruit-Ca, and promoted by high leaf- and fruit-K and by high ratios of K:Ca, K:(Mg + Ca) and Mg:Ca. The relationship held for sprayings with $Ca(NO_3)_2$ but not for gypsum dressings; the latter were effective on sandy soils only. Control of bitter pit with Ca was often accompanied by diminished incidence of breakdown. (50 references.) (In English.) P. S. ARUP.

Distribution of pips and ripening of Golden Delicious apples. J.-C. Pech and J. Fallot (*Fruits d'outre mer*, 1968, **23**, 573–579).—In fruits with seeds in only two of the five cavities of the ovary, ripening of the tissues, as judged by penetrometric determinations and by the contents of starch and sugars, was retarded in the zones of tissue immediately facing the seedless cavities. In contrast, ripening was more rapid in the zones facing the cavities which contained seeds. (20 references.) P. S. ARUP.

Effect of gibberellic acid on size and quality of seedless grapes (Shundokhani) in Quetta/Kalat region. Muhammad Saeed, Abdul Haq and Abdul Hameed Khan (*W. Pakistan J. agric. Res.*, 1968, **6**, 85–97).—Sprayings with the acid in increasing concn. (5–50 ppm) gave increasingly larger grapes, showing increasingly lower sugar contents and higher acid contents. Concn. of 40–50 ppm of acid interfered with the taste; the optimum concn. was 35 ppm. P. S. ARUP.

Effects of soil flooding on ethanol content of tomato plants related to certain environmental conditions. E. F. Bolton (*Diss. Abstr., B.*, 1967, **28**, 1–2).—Metabolic changes in tomato plants are examined in relation to soil flooding. Ethanol (**I**), as an index of cellular O_2-deficiency, served as a parameter and its concn. responded to environmental factors under the anaerobic conditions caused by flooding. In plants grown in controlled environments, **I** was determined in xylem exudates, in the condensates in a transpiration chamber and in plant tissues. High air temp. resulted in high **I** concn. in the exudate, max. being reached after 24 h soil flooding. With intermediate air temp., the concn. of **I** reached max. in 12 h after flooding. In flooded soils soil temp. had little effect on **I** concn. at low air temp. and low light intensity but with medium air temp. and high light intensity, a decline in soil temp. delayed the accumulation of **I**. Losses of **I** by transpiration were small but proportional to exudate concn. for each flooding period. Root excretions contained a significant concn. of **I**, 12 h after flooding. In anaerobic plants **I** concn. was greater in the lower sections of stems and in the upper roots; in CO_2 (20·1%) with O_2 (20·1%) **I** concn. did not exceed that of aerobic plants. Plants flooded in large vol. of soil contained less **I** than did those in smaller soil vol. The **I** contents of plants flooded in the field serve as useful indices of the effects of environmental conditions. A. G. POLLARD.

Factors affecting plant growth on sodic soils. J. T. Thorup (*Diss. Abstr., B.*, 1967, **28**, 20–21).—Failure of tomato plants in high-Na soils is not necessarily due to low $[Ca^{2+}]$ in the soil nutrient. Sufficient Ca^{2+} remains available to meet plant requirements even when the soil Na_2CO_3 is high enough to ppt. $CaCO_3$. Failure of the plants to grow, as shown experimentally, could not be ascribed to Na toxicity or to excessive osmotic pressure in the nutrient but resulted from direct injury to the plants' roots. In media of high Na_2CO_3 content (pH ≮ 9) plants wilted in a few minutes, root tips were blackened and unable to absorb sufficient water, lateral roots were distorted and no new roots were formed. When the nutrient pH decreased to ≯ 8 new adventitious roots appeared, the plants recovered and growth was resumed within a few days. The main effect of high $[CO_3^{2-}]$ appeared to be that of buffering the nutrient at high pH and thus preventing formation of new adventitious roots. A. G. POLLARD.

Relation of nitrogen nutrition to magnesium deficiency in celery. L. A. Montoya (*Diss. Abstr., B.*, 1967, **28**, 13).—Some inter-relationships between the effects of different levels of N and Mg supplies to celery are discussed. Plants were grown with an optimal and constant Mg level but with various N sources. Analysis of field samples of chlorotic and non-chlorotic plants suggested that in soils of pH 4·8–4·9 and having low NO_3^-/NH_3 ratios, both the Mg content of the plants and the occurrence of chlorosis, were related more closely to the NH_4^+-N than to soil-Mg. Rates of development of chlorosis varied with the sources and concn. of N applied, as also did the rate of recovery when the plants were transferred to NO_3^- media or exposed to foliar sprays of $Mg(NO_3)_2$. Chlorosis was associated with higher [Mg] in the tops than in the roots or with $[NH_4^+\text{-N}] > 2$ mequiv./l in the nutrient. Frequent applications of high [Mg] as foliar sprays diminished symptoms of chlorosis temporarily but were less effective for control than was an all-NO_3^- nutrient. Varietal differences in response of the plants to the treatments were noted in regard to NH_4^+ sensitivity, growth, Mg content and general chemical composition. Best growth was obtained with relatively high root temp. which in soil, favours nitrification and thus restricts NH_4^+ accumulation. A. G. POLLARD.

Effect of nitrification inhibitor on yield and nitrate content of spinach. B. L. Bengtsson (*Z. PflErnähr. Bodenk.*, 1968, **121**, 1–4).—Addition of 2-chloro-6-(trichloromethyl)pyridine (N-serve), as a nitrification inhibitor, to $(NH_4)_2SO_4$ fertiliser reduced the NO_3^--N content of the crop, However, the crop yield was also reduced, so that application of heavier N dressings to restore the yield gave a crop with the same undesirably high NO_3^--N content. (In English.) (10 references.) M. LONG.

Pith discoloration and breakdown in cauliflower (*Brassica oleracea* var. *botrytis*) J. J. Lauber (*Diss. Abstr., B.*, 1967, **28**, 12).—The discoloration resembled that in cauliflowers grown under conditions of B deficiency but no other indications of such deficiency were apparent. No sign of pathogenic micro-organisms was found. Supplementary furrow irrigation increased the discoloration and breakdown of the pith. Neither a complete fertiliser, a side-dressing of N, nor supplementary B, Ca or Fe, separately or together, as soil or spray applications, affected the disorder. In field experiments stem hollow appeared with or just before the discoloration and was initiated during a period of high air temp. In two seasons the phenolic content of the stem-tip pith tissue was inversely related to tissue moisture and, in turn, to the previous soil moisture conditions. During early development of the discoloration the moisture content of the stem-tip pith was decreasing. In sand cultures a low level of B (0·05 ppm), alone or with a low level of Fe increased stem hollow and discoloration. In sand-cultured plants symptoms of B deficiency were apparent in leaves when a low-B nutrient was used whereas in field-grown plants these symptoms were not found even when discoloration was present. A. G. POLLARD.

Influence of spacing and fertiliser on flower yield and pyrethrins content of pyrethrum. J. E. Parlevliet, S. N. Muturi and J. G. Brewer (*Pyrethrum Post*, 1968, **9**, No. 4, 28–30).—Flower yields increased with closer spacing but the pyrethrins content was affected neither by spacing nor by the application of triple superphosphate above 200 lb/acre. E. G. BRICKELL.

Forestry. C. E. Hart (*Jl R. agric. Soc.*, 1968, **129**, 169–182).—A survey of the current needs and trends in forestry. (27 references.) C. J. R.

Inhibition of seedling hoop pine (*Araucaria cunninghamii*) on forest soils by phytotoxic substances from the root zones of *Pinus*, *Araucaria* and *Flindersia*. D. I. Bevege (*Pl. Soil*, 1968, **29**, 263–273).—Non-mycorrhizal hoop pine seedlings were watered for 10 months with leachates prepared by passing complete nutrient solution through sand in which were growing 2–3-year-old trees of hoop pine, slash pine (*Pinus elliottii*), or crown ash (*Flindersia australis*). Leachates from all three species caused some mortality and reduced dry-matter production of hoop pine. Survivors growing on sand exhibited necrosis and chlorosis, whilst those growing on soil exhibited stunting and browning. The treatments had no significant effect on shoot N or K%, but decreased shoot P% and, in particular, total uptake of P. A. H. CORNFIELD.

Pest Control

Organic pesticides. Reactions of dialkylamidomethylphosphonic chlorides with amines and hydrazines. A. F. Grapov, L. V. Razvodovskaya and N. N. Mel'nikov (*Zh. obshch. Khim.*, 1967, **37**, 828–831).—When *N,N*-dialkyl-methylphosphonic chlorides were reacted with primary or secondary amines, or phenylhydrazine, the corresponding *N,N'*-trialkyl- or tetra-alkyl-methylphosphonic diamides or *N,N*-dialkyl-methylphosphonamidic hydrazides were formed. The compounds possessed fungicide activity. *N,N*-diethyl-*P*-methyl-2-phenylphosphonic hydrazide in concn. of 0·003% brought about suppression in growth of mycelium of *Botrytis cinerea*, *Fusarium moniliforme*, *Venturia inaequalis* and *Aspergillus niger*. A. L. B.

One-electron transfer properties of dipyrido[1,2-*a*:2′,1′-*c*]pyrazinium dibromide. A. L. Black and L. A. Summers. (*J. chem. Soc., C*, 1969, 610–611).—The behaviour of the title compound (**I**) and 6,7-dihydro-6-hydroxydipyrido[1,2-*a*:2′,1′-*c*]pyrazinium dibromide (**II**) on reduction in aq. solution at pH 3·5 with Zn dust was studied by

u.v. absorption spectroscopy and polarography. **II** is the 6-hydroxy deriv. of the herbicide diquat. Both **I** and **II** were reduced by a one-electron transfer to give stable radical cations, but in the case of **I** this transfer was largely reversed by atm. oxidation. **II** was stable in aq. solution at pH < 4, but at pH > 4 it was present substantially in an aldehyde form. In post-emergent herbicide tests, **II** was slightly active at a rate equivalent to 8 lb/acre, whereas **I** was very active at rates as low as 8 oz/acre against several plant species. (12 references.) J. I. M. JONES.

Stability of pyrethroid concentrates and pure esters. P. J. Godin (*Pyrethrum Post*, 1968, **9**, No. 4, 17, 21).—As pyrethroids approach 100% purity, the stability decreases rapidly, and the pyrethrins are much more unstable than the cinerins and jasmolins. It is recommended that all samples of pyrethroids and concentrates should be stored in glass containers in the dark at −20°, and extracts similarly but in a normal refrigerator; dilute solutions should be freshly prepared and never be left standing on benches unstoppered and exposed to light; evaporation and weighing of samples before dilution requires special care. E. G. BRICKELL.

Modified laboratory scheme for the preparation of (+)-pyrethrolone from crude pyrethrum extract. D. R. Maciver (*Pyrethrum Post*, 1968, **9**, No. 4, 41–43).—The process of Staudinger and Ruzicka (*Helv. chim. Acta.*, 1924, **7**, 177) is followed up to the isolation of the mixed ketolones as their semicarbazones. These are separated by fractional crystallisation and the fraction with the higher m.p., enriched with pyrethrolone semicarbazone, is then subjected to fission and semicarbazone exchange with pyruvic acid. The isolation of pyrethrolone in good yield as its hydrate then follows smoothly according to Elliot (*Chemy Ind.*, 1958, 685).
E. G. BRICKELL.

Pyrethrin II and related esters obtained by reconstitution. M. Elliott and N. F. Janes (*Chemy Ind.*, 1969, 270–271).—Pyrethrins I and II are prepared by reconstitution from pure (+)-pyrethrolone and the chlorides of (+)-*trans*-chrysanthemic (**I**) and (+)-*trans*-pyrethric acids. The possible isomerisation reactions occurring during the prep. of **I** by alkaline hydrolysis of Me_2 chrysanthemum dicarboxylate are discussed. Large amounts (> 500 mg) of pyrethrin II and other pyrethrolone esters can be prepared, without thermal isomerisation, by esterification of anhyd. (+)-pyrethrolone first at 0° and then at ~ 22° for 16 h with (+)-*trans*-pyrethric acid chloride and pyridine in benzene, followed by transference (with benzene) of the entire mixture to a 5-in column of Al_2O_3 in benzene. The processed product is eluted with benzene and, when striations appear, an 80-ml fraction of pure pyrethrin II is collected. Inert atm. and semi-darkness are necessary for the operations. (13 references.) W. J. BAKER.

New insecticidal isomer of hexachlorocyclohexane (BHC). K. Visweswariah and S. K. Majumder (*Chemy Ind.*, 1969, 379–381).—Separation and detection of components in the mother liquor (still insecticidally toxic) after removal of lindane from commercial BHC are described. As well as α-, β-, γ-, δ- and ε-BHC, four other components were obtained by t.l.c., one of which (7·5–8% concn.) was isolated by column chromatography on SiO_2-gel with n-hexane as solvent. Its chemical and physical properties indicate a new isomer (m.p. 75–76°) of BHC having good toxicity *vs. Tribolium castaneum*. W. J. BAKER.

Influence of siduron and its degradation products on soil-microflora. M. L. Fields and D. D. Hemphill (*Weed Sci.*, 1968, **16**, 417–420).—The effects of siduron [*N*-(2-methylcyclohexyl)-*N'*-phenylurea] (**I**), and its degradation products [2-methylcyclohexylamine (**II**) and aniline] on micro-organisms in turfgrass soil treated annually for 3 years with 10 lb of **I** per acre and in soil treated in the laboratory with 10 ppm of the materials were studied. **I** reduced the counts of *Azotobacter* sp. and *Chlorella vulgaris* but had no effect on actinomycetes, filamentous fungi, soil ciliates and other algae; **II** suppressed *Azotobacter chroococcum* and soil ciliates. Aniline (another degradation product) depressed growth of *Aspergillus versicolor* and *A. fischeri*, but had no effect on a number of other fungi. A. H. CORNFIELD.

Effect of DCPA (dimethyl-2,3,5,6-tetrachloroterephthalate) on soil micro-organisms. B. G. Tweedy, N. Turner, and M. Achituv (*Weed Sci.*, 1968, **16**, 470–473).—The numbers of actinomycetes were increased, whilst bacterial numbers were unaffected, by treating soil with DCPA (19 lb per acre) annually for 5 years. Addition of DCPA to soils and pure cultures had no adverse effects on microbial growth, and several micro-organisms utilised the herbicide as a source of C. Degradation products of DCPA were methyl-2,3,5,6-tetrachloroterephthalate and 2,3,5,6-tetrachloroterephthalic acid.
A. H. CORNFIELD.

Influence of water flux and porous material on movement of substituted [urea] herbicides. J. M. Davidson, C. E. Rieck, and P. W. Santelmann (*Proc. Soil Sci. Soc. Am.*, 1968, **32**, 629–633).—The rate at which fluometuron [*N'*-(3-trifluoromethylphenyl)-*N*,*N*-dimethylurea] (**I**) and diuron moved through a saturated porous material depended on the water flux or average pore velocity. Effluent samples derived from a known vol. of 0·01 N-$CaSO_4$ or $CaCl_2$ containing 29 ppm of herbicide, followed by further leaching with 0·01 N-$CaSO_4$, showed that **I** and Cl^- mixed in a similar manner at the two rates studied. Experimental and calculated effluent concn. distributions did not agree when **I** was retained by the porous material. The results are of importance in relation to frequency and application rate of herbicides to irrigated soils.
A. H. CORNFIELD.

Chemistry and toxicology of agricultural chemicals: A four-year summary report [1965–1968]. 1968, 212 pp. (Univ. California, Fd Prot. Toxicology Center).—Papers are included on a large no. of research projects, under the following main headings: (*i*) Analytical methods and instrumentation (*ii*) Biological methods for toxicity measurement (*iii*) Biological manifestations of toxicity (anatomical, biochemical, nutritional and physiological) and (*iv*) Environmental fate of pesticides, and their effects. P. P. R.

Herbicides. S. A. Evans (*Jl R. agric. Soc.*, 1968, **129**, 149–168).—A review, including cereals, sugar-beet, crops for processing, couch, weed populations and competition with crops, susceptibility of weeds to herbicides, herbicide application and min. cultivations (direct-drilling technique). (84 references.) C. J. R.

Vapour pressures of low-volatile esters of 2,4-D. G. W. Flint, J. J. Alexander, and O. P. Funderburk (*Weed Sci.*, 1968, **16**, 541–544).—The v.p. (mm Hg) of esters at 187° was: for iso-octyl 2·7, 2-ethylhexyl 3·0, butoxyethanol 3·9, propylene glycol butyl ether 3·9, and isopropyl 16·7. Extrapolation to 25° supports this ranking at working temp. A. H. CORNFIELD.

Translocation of two organic arsenicals in purple nutsedge, *Cyperus rotundus*. R. L. Duble, E. C. Holt, and G. G. McBee (*Weed Sci.*, 1968, **16**, 421–424).—Studies with ^{14}C-labelled Na_2 methanearsonate (DSMA) and amine methanarsonate (AMA) applied to purple nutsedge shoots, combined with chemical analysis for As suggested apoplastic and symplastic movement of both arsenicals in the plant. Most of the applied As was found in the treated shoot, although there was some movement to terminal tubers, roots and rhizomes. The C-As bond remained relatively intact, although there was some ^{14}C activity in respiratory CO_2 several days after treatment. Acropetal and basipetal movement of the materials was not affected by leaf age. A. H. CORNFIELD.

Response of purple nutsedge [*Cyperus rotundus*] and yellow nutsedge, [*C. esculentus*, to dichlobenil. W. S. Hardcastle and R. E. Wilkinson (*Weed Sci.*, 1968, **16**, 339–340).—Purple and yellow nutsedge tubers were stored at 5° for 2–12 weeks in soil treated with dichlobenil (**I**) (2–10 lb per acre). Subsequent respiration of tubers was higher for purple than for yellow nutsedge, but was not affected by time of storage or level of **I** in the soil. Subsequent sprouting and shoot production (at 25°) decreased somewhat with increasing level of **I** during the storage treatment. Sprouting was no different between the two varieties, but average shoot production was higher for purple than for yellow nutsedge. A. H. CORNFIELD.

Influence of some herbicides on photosynthesis and respiration of crop plants and weeds. II. Correlation between the action of atrazine and external conditions. III. The action of Afalon [linuron] and Tenoran [chloroxuron]. IV. Action of Burex [pyrazon]. K. Olech (*Annls Univ. Mariae Curie-Sklodowska, Agric. E*, 1967, **22**, 149–166; 167–183; 185–195).—II. The influence of atrazine (**I**) applied to the leaves and roots of plants on photosynthesis was studied under varying conditions of light, temp., R.H. and wind, using beans (*Phaseolus vulgaris*), sunflower (*Helianthus annuus*), cockle (*Agrostemma githago*) and barley (*Hordeum sativum*). When **I** was administered to the roots, increased temp. and decreased R.H., wind and light hastened the inhibition of photosynthesis. When **I** was applied to the leaves, increased temp. also produced a faster decrease in photosynthesis, but decreased R.H. and wind delayed the inactivation of photosynthesis by **I**. **I** did not affect the respiration of 5–7 day-old barley seedlings grown in the dark. (14 references.)

III. Linuron (**II**) and chloroxuron (**III**) were applied to the leaves and roots of 11 species of crop plants and weeds. **II** restrained photosynthesis and its action was much faster when it was administered to the under- than to the top-side of leaves and at a higher ambient temp. and R.H. **III** was less active in hindering the photo-

synthesis when applied to leaves, but was much more efficient when applied to roots. (24 references.)

IV. Pyrazon was applied to the leaves and roots of seven plants, and in all except sugar-beet decrease or complete inhibition of photosynthesis occurred. The results indicated that pyrazon does not act directly on the respiration of plants. (10 references.)

T. M. BARZYKOWSKI.

Inhibition of degradation of monuron in cotton leaf tissue by carbamate insecticides. C. R. Swanson and H. R. Swanson (*Weed Sci.*, 1968, **16**, 481–484).—When cotton leaf discs were treated simultaneously with monuron (**I**) and carbamate insecticides, **I** degradation was strongly inhibited by 1-naphthyl methylcarbamate and 4-benzothiophene-*N*-methylcarbamate. The carbamates did not prevent the metabolism of **I** to 1-(*p*-chlorophenyl)-3-methylurea, but they prevented subsequent degradation of this compound.

A. H. CORNFIELD.

Introduction of *Ophiobolus graminis* into new polders and its decline. M. Gerlagh (*Versl. landbouwk. Onderz. Ned.*, 1968, No. 713, 97 pp.).—The sources of the fungal infection, and the course of the disease which appears in the first cereal polder crops after reeds are described. The decline of the disease was due to an antibiotic substance produced by the soil micro-organisms that developed during repeated cereal cropping. (168 references.) (In English.)

P. S. ARUP.

Root rot of young maize plants. The causal fungi. J. J. Du Toit (*S. Afr. J. agric. Sci.*, 1968, **11**, 595–604).—*Helminthosporium pedicellatum* and *Fusarium* spp., especially the former, were the most virulent of the fungi isolated from lesions; they acted by penetration and colonisation. Different isolates of the same pathogen varied in virulence. A *Pythium* sp. was associated with root lesions in one type of soil only. Cases were found in which spp. of *Penicillium*, *Aspergillus* and/or *Trichoderma* contributed to rot. (14 references.)

P. S. ARUP.

Silver scurf disease (*Helminthosporium solani*) of potato. J. C. Mooi (*Versl. landbouwk. Onderz. Ned.*, 1968, No. 716, 62 pp.).—Conditions for infection by and damage caused by *H. solani* and possible methods of control are examined. Infection occurs mainly through seed potatoes, and not through the soil. Growth of the fungus is promoted by high soil moisture and prevented by ventilation during storage. Infection is less prevalent in early- than in late-lifted potatoes. Several fungicides were applied to the soil or to the tubers without much success. (26 references.)

P. S. ARUP.

Effect of relative humidity and temperature on survival and growth of *Botrytis cinerea* and *Sclerotinia sclerotiorum*. L. van den Berg and C. P. Lentz (*Can. J. Bot.*, 1968, **46**, 1477–1481).—Survival and growth of both organisms depended on R.H., temp. and type and strain. Mycelium and conidia of *B. cinerea* survived under the conditions at which vegetables are usually stored but the mycelium of *S. sclerotiorum*, in most instances, did not. (18 references.)

E. G. BRICKELL.

Occurrence of *Bemisia tabaci* (Genn.) in field and its relation to leaf curl disease of tobacco. B. G. Hill (*S. Afr. J. agric. Sci.*, 1968, **11**, 583–593).—Max. infestation occurred during the mid-season (Dec.–Feb.). and then declined. Adults lived during the winter on ratoon tobacco, where breeding continued slowly. During the season the no. of infected plants increased from 3 to 23%. The flies were more numerous on the sides of the field than in the centre. The numerical connection between the no. of infected plants and the no. of flies present was not very clear. The weed *Sida cordifolia* L. showed symptoms of leaf curl resembling those of tobacco, but the identity of the virus concerned in the two cases was not established. (19 references.)

P. S. ARUP.

Replant diseases of apple in the Netherlands. H. Hoestra (*Meded. Lab. Phytopath. Wageningen*, 1968, No. 240, 105 pp.; *Meded. Landbouwhogesch. Wageningen*, 1968, No. 68-13).—This disease, specific to replanted trees, occurs on some heavy orchard soils, and is not caused by nematodes. The disease can be controlled by broad spectrum treatments against soil organisms such as heating the soil at 60° for 30 min, treatments with chloropicrin, MeBr, propylene oxide or thiram, or by lowering the pH of the soil. A pot test, using apple seedlings, was devised for testing whether a soil needs treatment or not. Theories as to the cause of the disease, based on these and other observations, are discussed; it appears that some of the soil bacteria and/or actinomycetes are involved. (164 references.) (In English.)

P. S. ARUP.

Effects of photoperiod, temperature and humidity on uptake, translocation and metabolism of a systemic phosphonate insecticide. A. W. P. Colcby (*Diss. Abstr., B.*, 1967, **28,** 2).—The influence of environmental conditions on uptake of ^{32}P-labelled *O*-methyl *O*-*p*-methylthiophenyl methyl phosphonothionate (**I**) by cotton plants is examined. An increase in photoperiod from 10 to 14 h increased plant growth and the rate and total uptake of **I** and its translocation, and improved its insecticidal action; its degradation rate was also increased. In continuous light these trends were reversed although, at 80°F there was a slightly greater intake of **I**, a larger proportion of $CHCl_3$-sol. matter (**II**) in the plant and a greater mortality of aphids than in a 10-h photoperiod. High humidity reduced the rates of uptake and translocation of **I**, the growth of the plants and the **II** fraction after 24 h. After 3 days the radioactivity of the **II** fraction had increased. High R.H. lowered the rate of degradation of the **II** fraction to extents which were greater at 65° than at 80°. An increase in temp. accelerated plant growth, the uptake and translocation of **I** and also its degradation rate.

A. G. POLLARD.

Flour beetles. (*Econ. Leafl., Brit. Mus.* [*Nat. History*], 1968, No. 17, 4 pp.).—A brief description is given of the beetle (*Tribolium*) and its habits.

P .C. W.

Effect of pre-harvest spray of α-naphthaleneacetic acid and *p*-chlorophenoxyacetic acid on control of berry drop in Anab-e-Shah, grapes (*Vitis vinifera* Linn.). M. Madalagatti Rao, P. Narasimhami N. Nagaraja and B. Anandaswami (*J. Fd Sci. Technol.*, 1968, **5**, 127–128).—Berry drop was reduced by spraying with either of the chemicals at concn. of 50, 100 or 250 ppm, in 4% Waxol-O or water containing 0·5% of Tween-80. Optimum results were obtained with α-naphthaleneacetic acid at 100 ppm.

P. S. ARUP.

Hygroscopic additive to phenoxy herbicides for control of salt-cedar, *Tamarix pentandra*. E. E. Hughes (*Weed Sci.*, 1968, **16**, 486–488).—Addition of 5% (by vol.) of polypropylenediol to sprays of ester formulations of 2,4-D, 2,4,5-T and silvex [fenoprop] increased the extent of injury to saltcedar.

A. H. CORNFIELD.

Effects of herbicides on capacity of spring parsley [*Cymopterus watsonii*], to photosensitise chickens. M. Coburn Williams (*Weed Sci.*, 1968, **16**, 350–352).—The effect of treating spring parsley with herbicides on the production of 'phototoxic' substances was measured by determining the amount of parsley required by chicks to produce visible erythema (redness) on the combs within 24 h. Phototoxicity of spring parsley to chicks was reduced 4 weeks after treatment with 2,4-D and 2,4,5-T amine salts and esters (2–4 lb per acre) or with picloram (0·5 lb) alone or with 2,4-D. (10 references).

A. H. CORNFIELD.

Control of quackgrass (*Agropyron repens*) with Pyriclor (2,3,5-trichloro-4-pyridinol). K. P. Buchholtz (*Weed Sci.*, 1968, **16**, 439–441).—Quackgrass was controlled by application of Pyriclor (1–4 lb per acre) 1–8 months before planting maize. Maize on treated plots grew normally and yielded as well as on plots given the standard pre-ploughing treatment with atrazine (2 lb per acre). Oats were injured by soil residues of Pyriclor from a 2 lb per acre application made 20 months previously, whilst lucerne was hardly affected.

A. H. CORNFIELD.

Influence of paraquat on seed germination. A. P. Appleby and R. G. Brenchley (*Weed Sci.*, 1968, **16**, 484–485).—Germination of lucerne and red clover seed on the soil surface was not affected by application of paraquat (**I**) spray (1 lb in 50 gal per acre), but germination of Kentucky bluegrass and perennial ryegrass was severely reduced. A layer of soil approx. 0·25 in. thick over the seed completely protected all seed from the effects of **I**. Tall fescue, red fescue, and orchardgrass germination was also reduced severely by direct application of 0·5 lb of **I** in 50 gal per acre, whilst germination of bentgrass, smooth brome, oatgrass and timothy was moderately reduced.

A. H. CORNFIELD.

Assessing compatibility of herbicides and nitrogen fertiliser solutions. A. D. Klosterboer and C. E. Bardsley (*Weed Sci.* 1968, **16**, 468–470).—The stability of herbicides suspended in solutions of urea and NH_4NO_3 of varying concn. was determined. The dispersion stability (*D* value) is the difference in sp. gr. between the salt solution+herbicide and the salt solution alone. The *D* value decreased with increasing concn. of salt. The lower the content of active ingredients in the herbicide the more effectively did the surfactants, and possibly other components of the formulation, offset the adverse effect of the salts on *D*.

A. H. CORNFIELD.

Effect of herbicides on smooth bromegrass (*Bromus inermis*) in relation to time of application and nitrogen fertilisation. M. K. McCarty and C. J. Scifres (*Weed Sci.*, 1968, **16**, 443–446).—Treatment of smooth bromegrass with 2,4-D, 2,4,5-T, picloram (**I**) and

dicamba (**II**) for control of weeds resulted in more damage to the grass when the materials were applied in the autumn than in the spring. Application of N (55 lb per acre) partly overcame the effects of the herbicides in reducing grass yields, except where **I** was applied at 1 lb per acre. Phytotoxicity increased in the order 2,4,5-T, 2,4-D, **II**, **I**. A. H. CORNFIELD.

Control of weeds in Japanese holly, *Ilex crenata*. S. W. Bingham (*Weed Sci.*, 1968, **16**, 478–481).—Weeds in Japanese holly were considerably reduced by application of simazine (3 lb)+aminotriazole (1 lb per acre), DCPA (15 lb), diphenamid (5 lb), and dichlobenil (4 lb) without any phytotoxic effects to the holly. Considerable saving in labour requirements for hand weeding resulted. A. H. CORNFIELD.

Chemical renovation of roughland pastures. J. E. Winch, G. W. Anderson and T. L. Collins (*Proc. Xth int. Grassld Congr.* [1966], 1968, 982–987).—For birdsfoot trefoil to be successfully established the native Canada bluegrass must be severely suppressed. Granular dalapon is as effective as sol. powder in controlling the sod and allowing the trefoil to produce higher yields than did untreated areas. Cultivation is not necessary and seed may be sown in Nov., March or April. (15 references.) M. LONG.

Insect pheromones. F. E. Regnier and J. H. Law (*J. Lipid Res.*, 1968, **9**, 541–555).—A review in which sex, alarm, recruitment, primer and other pheromone systems are examined. Tables are provided showing the insect concerned, the chemical compounds (and their structural formulae) together with the response threshold. (83 references.) C. V.

Insect hormone activity of *p*-(1,5-dimethylhexyl)benzoic acid derivatives of *Dysdercus* species. M. Suchý, K. Sláma and F. Šorm (*Science, N.Y.*, 1968, **162**, 582–583).—Deriv. of this acid are juvenile hormone analogues with selective action on the hemipteran insects of the family Pyrrhocoridae, this activity is constant on five species of *Dysdercus* (*D*) and is about 10 times lower on *Pyrrhocoris*; there is no activity on hemipterans of some other families. Absence of profound species-specific variation on the activity suggests that the most active compounds of this type can be used as selective pesticides against all species of *D*. C. V.

Assay of activity of soil herbicides using *Chlorella vulgaris*. D. A. Addison and C. E. Bardsley (*Weed Sci.*, 1968, **16**, 427–429).—A bioassay method (6 days duration) using *Chlorella vulgaris* was effective in showing the presence of low concn. of monuron, diuron (**I**) and prometryne (**II**). Water extracts of **I** from soil assayed in this way were highly correlated with the amount of **I** added to the soil. Assay for **I** in soil treated with varying levels of the herbicide was highly correlated with reduction in dry matter yields of oats (15 days growth). This bioassay method detected residues of **I** and **II** (2·24 kg per ha) 150 days after application to a sandy loam. A. H. CORNFIELD.

Quantitative determination of 'pyrethrins' by gas-liquid chromatography. III. Through chromatography of the methyl esters of chrysanthemic acid and chrysanthemum dicarboxylic acid. S. W. Head (*Pyrethrum Post*, 1969, **9**, No. 4, 31–36).—Isolation of these acids followed by g.l.c. determination as their Me esters is described. The results were about 10% lower than those obtained by the A.O.A.C. method. (16 references.) E. G. BRICKELL.

Spectrophotometric method for the determination of malathion residues. M. S. Žigić (*Kemija Ind.*, 1968, **17**, 810–811).—In the determination of malathion residues, the stability of the developed colour of the Cu dimethyldithiophosphate complex was investigated at room temp. at 20 min intervals. The spectrometric method for determination of pesticide residues in wheat was examined and the absorption of the yellow colour was measured within 5 min of its development as this is the limit of its stability. T. M. BARZYKOWSKI.

Supplementary interpretations of the n.m.r. spectra of phosphorus pesticides. L. H. Keith and A. L. Alford (*Analytica Chim. Acta*, 1969, **44**, 447–448).—100 MHz spectra were recorded with a Varian HA-100 n.m.r. spectrometer. A 2–3% solution of $SiMe_4$ was used as an internal standard and $CDCl_3$ and CCl_4 were used as solvents. Spectra were recorded using the frequency sweep mode at a sweep time of 500 sec. Analytical standards of commercial organo-P pesticides and some experimental compounds were used at a concn. of 300 mg/ml. J. KORKISCH.

Fluorometric determination of pesticides. G. G. Guilbault and M. H. Sadar (*Analyt. Chem.*, 1969, **41**, 366–368).—A highly sensitive fluorometric method is used to measure the rate of formation of the fluorescent 4-methylumbelliferone from its non-fluorescent heptanoate, which is cleaved even by 10^{-5} units of lipase. Enzyme concn. of 0·006 units/ml is used for inhibition by the pesticide, concn. of which are obtained by reference to the calibration curves of % inhibition *vs.* concn. These curves are rectilinear for Sevin (0·1–2), heptachlor (0·1–2), aldrin (0·1–5), lindane (0·8–10) and DDT or 2,4-D (10–1000 μg/ml); the error over these ranges is 2–3%. Organo-P compounds, e.g., Sarin and Systox, interfere, and it is advisable to remove inorg. ions by an initial extraction of the pesticide with Me Cellosolve. Mixtures of pesticides can be separated by t.l.c. and each inhibitor determined separately. W. J. BAKER.

Analytical applications of the phosphatase enzyme system. Determination of bismuth, beryllium and pesticides. G. G. Guilbault, M. H. Sadar and M. Zimmer (*Analytica Chim. Acta*, 1969, **44**, 361–367).—A fluorometric method is described for the determination of Bi and Be, and the pesticides methyl parathion, aldrin and heptachlor. The technique is based on the inhibition of acid and alkaline phosphatase by these substances. The substrate umbelliferone phosphate which is used, is cleaved by phosphatase to the highly fluorescent umbelliferone (λ_{ex}=346 nm at pH 5, and λ_{ex}=365 nm at pH 8; λ_{em}=450 nm). Be (0·01–0·3), Bi (1–70), aldrin (5–100) and heptachlor (50–700 μg/ml) inhibit the hydrolysis catalysed by alkaline phosphatase, and methyl parathion (5–500 μg/ml) inhibits that catalysed by acid phosphatase, causing a decrease in the slopes of the fluorescence–time curves. This decrease is a direct measure of the concn. of inhibitor. 4-Methylumbelliferone nonanoate was found to be a good substrate for acid phosphatase in the presence of large amounts of phosphate (1·0 M). As little as 0·00006 units of acid phosphatase can be assayed with an accuracy of 2%. J. KORKISCH.

Small laboratory apparatus for measuring accelerated loss rates of pesticide deposits by 'rainwashing'. F. T. Phillips (*Chemy Ind.*, 1969, 414–415).—The apparatus permits determination of rates at which pesticides are progressively removed from the dry surface residues after spraying. Fractions of the water washings are collected over a period of ~ 120 sec and the pesticide is extracted therefrom and determined by g.l.c. The residue left finally on the surface is also removed by solvent and analysed by g.l.c. Results for such continuous rainwashing of different DDT formulations on glass and cotton-leaf surfaces, respectively, showed satisfactory reproducibility. W. J. BAKER.

Chlorinated biphenyls in fish, mussels and birds from the River Rhine and the Netherlands coastal area. J. H. Koeman, M. C. ten Noever de Brauw and R. H. De Vos (*Nature, Lond.*, 1969, **221**, 1126–1128).—Based on g.l.c. and mass spectrometric identifications, the occurrence and distribution of the chlorinated biphenyls (**I**) are reported. Separations were effected on a 5-ft column of 10% DC200 on Gaschrom Q at 200° with He or N_2 as carrier gas; apolar compounds, including **I**, were first eluted with hexane, and then dieldrin and endrin were eluted with 10% Et_2O in hexane. Retention times increased with no. of Cl atoms per mol. Most of the **I** in fish and sea birds in the Wadden Sea originated in the Rhine; lower chlorinated **I** seemed to be less persistent. Concn. are not yet fatal to wild life but regular monitoring to ascertain future trends is recommended. Apart from their toxicity, **I** can interfere in the detection of DDE, *o,p'*-DDT, *p,p'*-DDT and DDD. (12 references.) W. J. BAKER.

Animal Husbandry

Assessment of herbage legume varieties. III. Annual variation in chemical composition of eight varieties. W. E. Davies, T. A. Thomas and N. R. Young (*J. agric. Sci., Camb.*, 1968, **71**, 233–241). The % N, P, K, Ca, Mg, Na and *in vitro* dry-matter digestibility (*D*) were determined at 14-day intervals over a 2-year period on the primary growth of eight legumes. Highly significant differences were found between species, date of sampling and years for all constituents. Clover differed from lucerne for all constituents except P and white clover from red for all except Ca. N, P, K, Na and *D* decreased with maturity. Ca decreased in the second year, while N, P and Na increased. These legumes supplied adequate amounts of Ca, Mg, N, K and Na (white clover, and the first two cuts of red clover and lucerne) for milk production and growth, but while P was adequate for growth it was not so for lactation at the 20 kg/day rate. (12 references.) M. LONG.

Digestibility and feeding value of long, ground and pelleted early- and late-cut hay. B. V. Tro (*Diss. Abstr., B.*, 1967, **28**, 25–26).—The hay (grass+lucerne), prepared in the various ways, was fed to dairy heifers, milch cows and steers. The heifers received an all-hay

ration *ad lib.* twice daily and free-choice minerals. Grinding (hammer-mill) early- and late-cut hay increased feed consumption and gain in wt. and pelleting the ground material further increased the effect. Processing was more effective with early- than with late-cut material. Similar trials were made with cows just past lactation peaks, the *ad lib.* ration being supplemented with grain. Feed consumption, milk production and composition were unaffected by the physical form of the processed hays, but milk yields were significantly greater when early- than when late-cut hay was used. When hay was fed *ad lib.* as the sole ration twice daily to steers, grinding and pelleting increased the dry matter (*DM*) intake but lowered the digestibility of the *DM*, total carbohydrates and gross energy without affecting that of protein. Pelleting increased the digestibility and *DM* intake of the ground hay and also the digestibility of the ether extract. On the basis of *DM* intake and digestibility (except ether extract) early- was superior to late-cut hay whether fed long, ground or pelleted. Dustiness and poor palatability of ground hay may detract from its value. A. G. POLLARD.

Digestibility of forage and its relationship to lignification. R. J. Wilkins (*J. Aust. Inst. agric. Sci.*, 1968, **34**, 221–222).—In 13 forages, including *Chloris gayana*, *Dactylis glomerata* and *Lolium perenne*, potential cellulose (**I**) digestibility exceeded digestibility *in vivo* by 2·8–8·4%. Potential digestibility of **I** in faeces was not significantly correlated with digestibility *in vivo* or with the content of **I**, N or total available carbohydrate in the forage. It did, however, correlate more closely with the proportion of lignified tissue, determined by staining with safranin and fast green, than with chemical estimates of lignin content. E. G. BRICKELL.

Utilisation of nitrogen from legumes on cultivated pastures in the central regions of the forest zone of the U.S.S.R. A. A. Kutuzova (*Proc. Xth int. Grassld Congr.*, [1966], 1968, 191–194).—Cultivated pastures rich in legumes (30–50 wt.-%) yield, without N fertilisers, about 200 centners/ha of green fodder (centner = 100 kg) and 820 kg/ha of protein, an increase of 80–100 kg per ha in N content. Both red and white clovers are incorporated under more continental conditions, reducing the period of use to 4–5 years. (10 references.) M. LONG.

Effect of different rates of fertilisers on yields and chemical composition of *Bromus inermis* leys. N. G. Andreyev and V. A. Savitskaya (*Proc. Xth int. Grassld Congr.*, [1966], 1968, 279–282).—Application of N 120 kg, P_2O_5 60 kg and K_2O 60 kg/ha raised yields of *Bromus inermis* (bromegrass) to 144 centners/ha over a 2-year period, compared with a control yield of 45·6 centners. Crude protein also rose from 10·6 to 16·5%. M. LONG.

Effect of high rates of nitrogenous fertilisers on yield and chemical composition of grasses. R. I. Toomre (*Proc. Xth int. Grassld Congr.*, [1966], 1968, 227–230).—Grasses can utilise very large amounts of N and application of 510 kg N/ha was found insufficient. Grass yields of > 200 centners/ha and crude protein yields of up to 40 centners/ha were possible with high rates of N application. Cocksfoot and perennial ryegrass utilise high rates of N more successfully than timothy, meadow fescue or smooth-stalked meadow grass. M. LONG.

Nitrogen accumulated by white clover as a means of raising yields of cultivated pastures in the Baltic Republics of the Soviet Union. A. Sau (*Proc. Xth int. Grassld Congr.*, [1966], 1968, 195–199).—White clover may accumulate 150–200 kg of N/ha per year; of this 80–120 kg is in the crop. This replaces 120–150 kg/ha of mineral N and ensures 45–60 metric centners (mc)/ha of dry matter and 8–12 mc/ha of crude protein in years with moderate rainfall. The optimal % of white clover is 30–40% of the dry matter yield. In dry years the fall in yield can be stabilised by periodic N fertilisation or by sprinkler irrigation. M. LONG.

Pasture establishment and maintenance on blanket-peat soil. E. J. Grennan and M. A. O'Toole (*Proc. Xth int. Grassld Congr.*, [1966], 1968, 842–846).—Ground limestone (20 cwt), 36 lb P, 1 cwt K, 20 lb $CuSO_4$, and 2 lb $CoSO_4$ per acre at sowing and yearly dressings of 27 lb P (as basic slag) and 56 lb K are recommended. A *Lolium perenne–Trifolium repens* mixture is satisfactory. With this management the stocking rate was increased from 0·2 to 3 ewes per acre, and an output of 25·2–27·4 cwt utilised starch equivalent was achieved with sheep. (16 references.) M. LONG.

Comparison of saponins from Du Puits, Lahontan, Ranger, and Uinta alfalfas [lucernes]. M. W. Pedersen, D. E. Zimmer, J. O. Anderson and C. F. McGuire (*Proc. Xth Int. Grassld Congr.*, [1966], 1968, 693–698).—Correlations between chick growth rate and wt. of germinating lettuce seed or *Trichoderma* sp. were positive but low, while *Trichoderma* and lettuce seed assays were closely related. The plant assays for saponins showed that Du Puits lucerne was the most toxic and Lahontan the least. Chick assay placed Du Puits also as the most toxic but did not confirm the low toxicity of Lahontan. The saponins of these two lucernes, separated by t.l.c., gave different colour reactions with $SbCl_3$/vanillin reagent and different degrees of toxicity to *Trichoderma*. (18 references.) M. LONG.

Effect of environmental factors on alkaloids in *Phalaris tuberosa*. R. M. Moore, J. D. Williams and J. Chia (*Proc. Xth int. Grassld Congr.*, [1966], 1968, 524–527).— The principal alkaloids in *Phalaris tuberosa*, *N,N*-dimethyl-, 5-methoxy-*N,N*-dimethyl- and 5-hydroxy-*N,N*-dimethyl-tryptamine, considered to be the cause of neurological disorders and sudden death in sheep grazing these pastures, are affected by temp., light intensity and NO_3^--N. Day length has no effect. Levels rose with increasing temp. and amounts of NO_3^--N and with shading. M. LONG.

Response of plant reserves to systems and intensities of grazing on mountain rangeland in northwest U.S.A. G. A. Garrison (*Proc. Xth int. Grassld Congr.*, [1966], 1968, 937–940).—The dry wt. of basal and underground plant regions of carbohydrate accumulations were obtained from soil cores. Both the % carbohydrate and wt. of root-stem base wt. for two forage species were significantly higher under moderate and light grazing than under heavy grazing, and were significantly higher under a deferred rotation system than under a season-long system. M. LONG.

Productivity and nutritive value of tropical pastures at Ibadan. V. A. Oyenuga and F. O. Olubajo (*Proc. Xth int. Grassld Congr.*, [1966], 1968, 962–969).—The faecal index technique was used to evaluate the productivity and intake of three tropical grass/legume pastures with grazing by N'Dama and Zebu steers. The addition of *Stylosanthes gracilis* to *Cynodon plectostachyum*/*Centrosema pubescens* pastures did not result in higher herbage dry matter yields or intakes but did lead to a higher rate of live-wt. gain per acre per annum and per animal per day. M. LONG.

Influence of natural inocula on the dynamics of the growth of lactic bacteria and yeasts in ensiled potatoes. A. Szember and W. Słupczyński (*Annals Univ. Mariae Curie-Sklodowska, Agric. E*, 1967, **22**, 247–255).—An essential condition for obtaining a good quality silage is a rapid development in the silo of lactic bacteria. In order to establish optimum conditions for proper fermentation, some natural inocula, e.g., 1% of clover hay or 0·2% of sour milk, were added to steamed potatoes and the growth of the no. of lactic bacteria and yeasts and variations in pH and sugar content were determined. Steamed Jerusalem artichokes and potatoes acted as controls. Even a small addition of natural inocula accelerated rapid multiplication of lactic bacteria and depression of pH. Clover hay was more effective than sour milk and contributed to a better utilisation of sugars by ensiling micro-organisms. (16 references.) T. M. BARZYKOWSKI.

Mineral supplementation of grass for silage in prevention of hypomagnesaemia. W. J. M. Black (*Proc. Xth int. Grassld Congr.*, [1966], 1968, 556–560).—To overcome hypomagnesaemia commonly found with hill cows and ewes in winter, addition of 1·5–2 kg of calcined magnesite (90% MgO) per 1000 kg of green herbage at the time of ensiling proved beneficial without having any undesirable effects on either silage or animal. Losses of added Mg were very small during herbage preservation. M. LONG.

Methods of increasing magnesium contents of herbage, with particular reference to prevention of hypomagnesaemic tetany in ruminants. J. R. Todd (*Proc. Xth int. Grassld Congr.*, [1966], 1968, 178–180).—The incorporation of species naturally rich in Mg into mixed herbage is not feasible due to the reduction of yield of the sward as a whole. Addition of 300 lb/acre of Mg is effective on light or sandy soils but is unlikely to be economic on heavy soils. Dusting with MgO at the rate of 15 lb of Mg/acre is effective and is independent of soil type. (18 references.) M. LONG.

Nutritive value of silage as influenced by silage fermentation and ration supplementation. M. E. Mc Cullough (*Proc. Xth int. Grassld Congr.*, [1966], 1968, 581–584).—Addition of buffers to silage during the ensiling process to increase lactic acid production, caused a reduction in silage intake and milk production. Milk production was improved by supplementation with beet pulp molasses when the molar proportion of rumen AcOH was 58–65%. Rations which caused the % of AcOH to fall outside this range reduced milk production. M. LONG.

Amines and ammonia as products of protein decomposition in silage. N. Voss (*Proc. Xth int. Grassld Congr.*, [1966], 1968, 540–546).—Before ensiling, ryegrass, a grass mixture and lucerne contained no tyramine (**I**), phenylethylamine (**II**) or tryptamine (**III**).

The ensiled material contained from < 10 up to 826 mg-% of **I**; trace amounts of **II** and **III** were sometimes found. The [NH_3] varied from 71 to 3388 mg-%; the histamine content of lucerne silage varied from 50 to 240 mg-%. All figures were based on dry matter. Production of **I** and NH_3 decreased with increasing degrees of pre-wilting and maturity and with decreasing crude protein. Lucerne silage contained more **I** and NH_3 than did grass silage. N fertiliser increased **I** and NH_3 in ryegrass silage. Addition of sugar inhibited the breakdown of protein when the plant itself did not have a favourable sugar/crude protein ratio.
M. LONG.

Influence of nitrate on silage fermentation. G. W. Wieringa (*Proc. Xth int. Grassld Congr.*, [1966], 1968, 537–540).—Nitrite-N, an intermediate in the reduction of NO_3^--N, inhibits butyric acid fermentation, so that a silage, high in NO_3^-, which is known to be of poor quality from its pH and NH_3 content, may nevertheless have a low butyric acid content. Although a grass containing 0·6–1% of NO_3^- in dry matter may give a better quality silage than expected from its chemical composition, it is better to utilise it at a more mature stage. (10 references.) M. LONG.

Effect of chemical preservatives in silage making. W. Laube, F. Weissbach and H. H. Budzier (*Proc. Xth int. Grassld Congr.*, [1966], 1968, 546–551).—Kofasalz (87% Ca formate and 13% $NaNO_2$) (**I**) and $Na_2S_2O_5$ were compared as additives to silages made in plastic bags from green rye, meadow grass, red clover and lucerne. **I** was superior in stimulating lactic acid formation and in inhibiting alcohol formation and protein breakdown. Fermentation losses were lower with **I**, while **II** exhibited desirable effects late in the fermentation only. Microbiological studies confirmed the chemical data. (28 references.) M. LONG.

Comparative effects of wilting and of sodium metabisulphite on quality and nutritive value of alfalfa [lucerne] silage. N. Fatianoff, M. Durand, J. L. Tisserand and S. Z. Zelter (*Proc. Xth int. Grassld Congr.*, [1966], 1968, 551–555).—Over a 5-year period it was shown that the determining factor inhibiting butyric fermentation and NH_3 formation is the dry matter content of the initial material. This must be < 21% for metabisulphite (**I**) silages and > 33% for wilted ones. Compared with green forage, org. matter digestibility is reduced by conservation, but no systematic difference results from the treatment. N retention is lower for poor quality **I** silage, but similar for a 35% wilted silage. M. LONG.

Relative efficency of silage making in polythene-covered bunkers and vacuum-compressed stacks. R. J. Lancaster (*Proc. Xth int. Grassld Congr.*, [1966], 1968, 560–564).—High moisture grass silage was made (*i*) in stacks covered by polyethylene film (*PF*), (*ii*) in stacks covered by *PF* and vac. compressed and (*iii*) in concrete bunkers covered with *PF*. Losses were similar with all three methods; dry matter losses were ~ 11% after 6 months and 30% after 11 months storage. Quality differences were small, treatment *ii* being the best, followed by *iii*. M. LONG.

Voluntary intake of silage. C. E. Harris, W. F. Raymond and R. F. Wilson (*Proc. Xth int. Grassld Congr.*, [1966], 1968, 564–568).—Intake of silage can be increased by wilting, although mature sheep appeared to be less sensitive to factors which affected intake by lambs. As the length of feeding period is extended, intake is reduced, possibly due to the low pH, associated with high levels of org. acids. (16 references.) M. LONG.

Comparison of dry matter intake of silage and hay by cattle. U. Wellmann (*Proc. Xth int. Grassld Congr.*, [1966], 1968, 568–570).—Hay is preferred to silage, although the feeds come from the same herbage. Where hay and silage are fed separately, live wt. gains and nutrient utilisation are best with hay. Where the two are fed together, intake of dry matter is highest when hay is fed *ad lib* and silage is restricted. Intake increases with increasing dry matter content of the grass. M. LONG.

Effect of direct-cut silage, compared to hay, on intake, digestibility, nitrogen utilisation, heifer growth and rumen retention. D. R. Waldo, R. W. Miller, L. W. Smith, M. Okamoto and L. A. Moore (*Proc. Xth int. Grassld Congr.*, [1966], 1968, 570–574).—Dairy heifers fed on unwilted legume silage grew at a slower rate and consumed less dry matter than did others fed on hay from the same source. This could not be explained either on the basis of greater reticulo-rumen fill, longer rumen retention time or limited capacity of the lower gastrointestinal tract. With hay, N retention was greater and rumen NH_3 was lower. M. LONG.

Utilisation of hay and/or silage by dairy cows. S. Nordfeldt (*Proc. Xth int. Grassld Congr.*, [1966], 1968, 588–593).—Roughage made from clover and grass was fed to dairy cows either as (*i*), hay, (*ii*), equal parts of hay and silage or (*iii*), silage. With a concentrate consisting of 15% dried beet pulp, *i* was a good roughage; *ii* also gave good results but *iii* was inferior due to changes in quality which occurred over the experimental period. M. LONG.

Feeding value of high dry matter corn [maize] silage for dairy cattle. M. J. Owens, N. A. Jorgensen and H. H. Voelker (*J. Dairy Sci.*, 1968, **51**, 1942–1945).—Feeding trials with cows showed that the feeding value of high dry matter maize silage was nearly equal to that of maize silage of medium dry matter content. However, due to large field losses, the necessity for re-chopping before ensiling, lower energy yields and lower carotene content, it is concluded that a general recommendation for delaying harvesting of maize for the production of high dry matter silage is not warranted, except in emergency situations. (14 references.) M. O'LEARY.

Comparative value of a hay and of a lucerne silage as sole roughage during premature weaning of calves. M. Candau (*C. r. hebd. Séanc. Acad. Agric. Fr.*, 1968, **54**, 1047–1056).—Silage made from lucerne hay (at 47% solids) proved a suitable roughage feed for 3–20-week-old calves, giving the same wt. gains as did a diet based on lucerne hay as roughage. (10 references.) P. S. ARUP.

Utilisation of home-grown roughages by dairy cows. II. Use of different roughages during summer season. S. J. Daniel, M. R. Bhosrekar and D. N. Mullick (*Indian J. Dairy Sci.*, 1968, **21**, 43–49).—Data from feeding trials with Sahiwal and Tharparkar cows, grazed on summer roughages and fed 1 kg of concentrates per 3 kg of milk, are presented. M. O'LEARY.

Performance of Holstein-Friesian steers fed on all-concentrate ration diluted with ground hay. E. E. Lister, D. P. Heaney and W. J. Pigden (*J. Dairy Sci.*, 1968, **51**, 1946–1949).—The effect of diluting a barley-based, all-concentrate diet with 20% and 40% of ground hay on the performance of Holstein-Friesian steers during fattening to 385 kg from 200 kg live wt. was investigated. 20% dilution did not affect average daily gains, but 40% dilution significantly lowered them. Feed efficiency was significantly depressed by both levels of dilution. The apparent digestibility of dry matter, N-free extract, and gross energy significantly decreased with increasing dilution. There were no significant differences between levels of digestible energy intake, but the efficiency of energy utilisation decreased with increasing dilution. (11 references.) M. O'LEARY.

Feed intake by grazing ruminants. I. Chromic oxide and polyethylene as added faecal markers and chromogen as an endogenous marker for estimating grass intake. A. Kemmink and N. D. Dijkstra (*Versl. landbouwk. Onderz. Ned.*, 1968, No. 717, 29 pp.).—In trials with wethers, polyethylene powder of fine quality proved to be the most suitable marker, travelling at the same rate as the feed, without retention or effects on digestibility. Average annual recoveries were 99·9–100·4 ± 1·6%. Chromic oxide (impregnated into paper) showed recoveries of 93·4 ± 0·6%, some retention in the gut, and other disadvantages. Recoveries of chromogen were 101·1 ± 2·0%. (78 references.) P. S. ARUP.

Effect of method of grass conservation and herbage maturity on performance and body composition of beef cattle. R. B. McCarrick (*Proc. Xth int. Grassld Congr.*, [1966], 1968, 575–580).—Daily live wt. gains of animals fed on simultaneously harvested unwilted silage, partially wilted silage, barn-dried hay and tetrapod hay were similar although the dry matter intakes of the hays were 20–30% higher than those of the unwilted silage. Intakes and wt. gains were 12 and 100% greater, respectively, for forage cut at ear emergence than for that cut two weeks later. Although dry matter yield/acre was 60% higher for the late cut, live wt. gain and carcass gain/acre were higher on the early cut. 'Gut fill' was not affected by herbage maturity. Carcass gain per acre and per 100 lb live wt. gain and energy storage were greater in silage-fed animals and silage favoured a more rapid fat deposition. M. LONG.

Photogrammetric measurements on live steers for predicting carcass characteristics. F. Hagan (*Diss. Abstr., B.*, 1967, **28**, 4–5).—Numerous steers (Angus, Herefords, crossbreeds) all born within one month (spring) were separated randomly into three groups at weaning and fed by three systems (*i*) full-fed one month after weaning, (*ii*) hay+silage in winter and full-fed in the following summer, (*iii*) pasture in the second summer and full-fed during the second autumn. The animals were slaughtered when judged to be graded as 'low choice'. Stereophotographs were taken 1–2 days before slaughter to obtain external measurements for comparison with carcass data. Correlation coeff. (live wt. held constant) and the % variance between the various parameters are examined. Of nine carcass factors measured, treatment effect was not significant for

any one of them and the breed effect was significant only for % lean, % fat and wt. of round. A. G. POLLARD.

Relationship of some linear and physical measurements to beef carcass composition. D. M. Allen (*Diss. Abstr., B.*, 1967, **28**, 3–4).—Two groups of 40 steer carcasses each were selected on a wt. basis (500–550 and 700–750 lb, respectively) and within each group a further selection was made according to rib fat thickness (0·26–0·50, 0·51–0·75, 0·76–1·00, and 1·01–1·25 in.). External fat thicknesses were measured by probes at specified distances from the dorsal mid-line. One side of each carcass was divided into wholesale cuts, each being separated into muscle, fat and bone, and the other side divided into boneless, closely-trimmed retail cuts. Inter-relationships between these various factors are examined. A. G. POLLARD.

Herbage consumption and milk production in grazing dairy cows. J. F. D. Greenhalgh (*Proc. Xth int. Grassld Congr.*, [1966], 1968, 351–355).—Herbage allowances for strip-grazed cows amounting to 11·4, 15·9, 20·4 or 25·0 kg of dry matter per head per day, gave rise to daily intakes of 10·9, 11·9, 12·6 and 12·6 kg of org. matter. The corresponding yields of fat-corrected milk (*FCM*) were 15·2, 15·0, 15·9 and 15·6 kg. In trials comparing S24 ryegrass and S37 cocksfoot, the two grasses reached ear emergence at the same time and gave equal yields, but the S24 was more digestible than the S37 by 5 units. Cows on S24 had intakes 8% higher than those on S37 and produced 4% more *FCM*. The proportion of AcOH in the rumen volatile fatty acids of sheep increased from 60 to 62% during the 4-week trial with S24 and from 60 to 68% with S37. (10 references.) M. LONG.

Mineral balance in dairy cows fed on grass, with special reference to magnesium and sodium. A. Kemp (*Proc. Xth int. Grassld Congr.*, [1966], 1968, 411–415).—Data from a series of balance trials carried out with dairy cows fed fresh herbage showed that, whilst Na had a high availability, Mg availability was low. The requirements of Mg for availabilities ranging from 10 to 25% was from 49 to 20 g/day for a cow yielding 20 kg of milk/day. Urinary Mg was a better criterion of Mg status than serum Mg, [Mg] < 50 mg/l indicating deficiency. For a similar yielding cow the Na requirement ranged from 18 to 14 g/day with availability ranging from 75 to 95%. Salivary Na and K afforded a better index of Na status than did urinary Na; the latter varied from day to day. Salivary [Na] of > 3·0 g/l and K of < 0·5 g/l indicated a satisfactory condition. M. LONG.

Effect of lactation on intake of dry matter, magnesium, calcium and potassium by grazing cows. A. C. Field (*Proc. Xth int. Grassld Congr.*, [1966], 1968, 355–359).—Comparison of voluntary intakes by lactating and non-lactating twins indicated that lactation increases Mg and Ca intakes by 25–30% merely as a result of increased dry matter intake, whilst intake of K increases to 43% due to selection of herbage richer in K. There was no relation between these increases and milk yield. Selection of herbage richer in Mg, Ca and K occurred except during the latter part of the season when the trend for K was reversed. (10 references.) M. LONG.

Statistical research on fate of dietary mineral elements in dry and lactating cows. I. Calcium. II. Magnesium. F. Lomba, R. Paquay, V. Bienfet and A. Lousse (*J. agric. Sci., Camb.*, 1968, **71**, 173–180; 181–188).—I. A study of the calculated correlations between faecal and urinary Ca, Ca excretion in milk, digestible Ca and Ca balance, and 75 other factors, showed that Ca metabolism is regulated in the digestive tract. This is confirmed by the fact that lactation Ca requirements increase at the same time as the amounts of digestible Ca and the Ca balance. Apart from this factor, the fate of dietary Ca is independent of the ingested amounts, but depends on the nature of the diet. Cereals, especially barley, are particularly beneficial in this respect. The Ca balance in cows producing 11–20 kg milk daily, is not inevitably negative and could be equilibrated by diet adjustment. The other nutritive factors do not effect Ca utilisation, but a close parallel exists between digestibility, urinary excretion and balance of both Ca and Mg. (10 references.)

II. A similar study of correlations between Mg urinary and faecal losses, milk Mg, digestible Mg and Mg balance was carried out as in I. Mg absorption is enhanced by increasing Mg and Ca intake; it is decreased by increasing N and less so by fat. Lactating requirements have no effects at the level used. Urinary losses are unrelated to Mg intake and digestibility, so that the balance is directly proportional to the digestible amounts. Faecal endogenous losses of Mg can be highly variable. As with Ca the Mg balance is not inevitably negative in cows producing 11–20 kg of milk daily. (20 references.) M. LONG.

Urea supplementation of roughage-based diets for cattle. P. B. O'Donovan (*J. agric. Sci., Camb.*, 1968, **71**, 137–144).—In contrast to trials in the tropics, urea (**I**) supplementation did not increase the rate of gain or intake of barley straw by growing bullocks. A response to **I** was found when a barley–soyabean meal was fed with the straw in contrast to the nil response with rolled barley. Blood content of **I** rose to a max. after the third week of feeding **I** and then declined. Concn. of **I** were higher in blood when **I**+meal was fed instead of **I**+molasses. N retention from **I** was not increased when barley was added to the all-straw diet. A hay–barley ration gave gains not significantly different from those of animals fed extra N as soyabean meal or **I**. (15 references.) M. LONG.

Milk production on a protein-free feed, using urea and ammonium salts as sole source of nitrogen. A. I. Virtanen (*Proc. Xth int. Grassld Congr.*, [1966], 1968, 19–29).—Feeding trials with ^{15}N-labelled NH_3 and urea showed that all essential amino acids can be synthesised in the rumen. The efficiency of the process increased when the animal became adapted to the diet. Potato starch was used in the trials in combination with cellulose, sucrose, urea, NH_4^+ salts, plant oils, vitamins and minerals. The quantity of urea was eventually raised to 600 g per day for a 450 kg cow. There was no build-up of ammonia in the rumen or other adverse effects. The free amino acid composition of blood from the urea-fed animals was lower than that of normally fed animals, histidine being ~ 20% of the value normally found, although the composition of the whole blood protein was similar. The composition of milk from cows fed urea corresponded with normal fat-rich milk as far as fat and protein were concerned, although when the level of urea feeding was raised the protein level rose considerably. The amino acid composition of the protein was indistinguishable from that of normal milk. In the fat the palmitic acid content was particularly high, that of oleic acid was lower and amounts of lower unsaturated fatty acids and branched-chain fatty acids were somewhat higher than normal. Generally vitamin content was similar to that of normal milk. Milk flavour was unaffected by urea feeding and the technique is suggested as a means of investigating milk flavour problems. M. LONG.

Effect of unsaturated oils on rumen fermentation, blood components, and milk composition. P. N. Varman, L. H. Schultz and R. E. Nichols (*J. Dairy Sci.*, 1968, **51**, 1956–1963).—The addition of 250 ml/day of either safflower or cod-liver oil to the diet of lactating cows significantly depressed the fat content of the milk, but milk yield, protein and *SNF* contents were not affected. A significant decrease occurred in the acetate content of the rumen fluid, while its contents of propionate, butyrate, isovalerate and valerate increased. Rumen fluid pH was not significantly affected. Levels of jugular blood plasma acetate increased significantly. The concn. of plasma free cholesterol and free fatty acids increased, whereas those of total plasma triglycerides decreased. (33 references.) M. O'LEARY.

Effects of diet, time after feeding, and position sampled on numbers of viable bacteria in the bovine rumen. M. P. Bryant and I. M. Robinson (*J. Dairy Sci.*, 1968, **51**, 1950–1955).—Bacterial counts were conducted on samples of digesta from different rumen positions obtained at 1, 2·5, 5·5, and 10 h after feeding heifers either chopped lucerne, pelleted lucerne, equal weights of lucerne hay and grain, or hay crop silage. Bacterial numbers were lowest at 1 h and increased significantly between 1, 2·5, and 5·5 h. With all rations, bacterial numbers were significantly lower in the ventral than in the dorsal rumen. Numbers in the reticulum were similar to those in the ventral rumen when hay or hay-grain was fed, but were higher ($P < 0·01$) when pellets were fed and lower ($P < 0·05$) when silage was fed. (21 references.) M. O'LEARY.

Free amino and volatile fatty acids in bovine rumen fluid on a ration including a urea-containing concentrate. V. V. Sharma, J. D. Donker and D. E. Otterby (*Indian J. Dairy Sci.*, 1968, **21**, 21–26).—18 amino acids were identified in the rumen liquor of cattle fed a concentrate containing 2·4% urea, 85·5% ground maize and 11·8% soyabean meal, fed 2 h after feeding lucerne hay in the morning and maize silage in the evening. Arginine, histidine, cystine and leucine were found in lower proportions than estimated in the feed, while lysine (+ornithine) was found in higher proportion. Lysine (+ornithine) accounted for ~ 25% of amino-N. Phenylalanine, methionine, cystine, histidine and arginine usually occurred in amounts < 1 mg/100 ml. The combined molar concn. of acetic, propionic and butyric acid was not affected by changing from hay to silage. The acetic content was lowest after silage, that of butyric was highest, while propionic acid content was not affected by ration change. (25 references.) M. O'LEARY.

Feed processing. IV. Effect of feeding expanded grain and ground hay on concentrations of rumen ammonia. V. F. Colenbrander, E. E. Bartley, J. L. Morrill, C. W. Deyoe, R. M. Meyer and H. B. Pfost (*J. Dairy Sci.*, 1968, **51**, 1974–1977).—Experiments with rumen-fistulated, identical twin cattle showed that feeding of either expanded sorghum grain or finely ground hay reduced rumen pH and the AcOH : $EtCO_2H$ ratio. Both types of feed increased total rumen volatile fatty acid concn. but the effect was greater with expanded grain. Rumen NH_3-N was not materially reduced by the ground hay but was significantly reduced by the expanded grain. (14 references.) M. O'LEARY.

Pasture bloat and the rôle of 18-S protein. J. M. McArthur and J. E. Miltimore (*Proc. Xth int. Grassld Congr.*, [1966], 1968, 518–521).—The agent causing bloat in cattle is a protein (**I**) of sedimentation velocity 18-S (18 Svedbergs). Foams of high shear strength are formed, thus immobilising rumen gas. Forages causing bloat contained ~ 4·5% of the **I**, whilst those which did not contained < 1%. **I** content of lucerne increased with age to a max. and then decreased; the range of **I** content in varieties of lucerne was 4·5 to 5·2%. (16 references.) M. LONG.

Relationship between vitamin B_{12} and propionate metabolism with special reference to stress of lactation. M. M. Mathias (*Diss. Abstr., B.*, 1967, **28**, 48).—The rate of incorporation of labelled propionate or succinate into methyl malonate (*M*) and Krebs cycle intermediates was investigated, using a nuclear-free prep.; the mitochondria were disrupted to uncouple the Krebs cycle. Essential coenzymes were added at optimum levels. After incubation at 37° for 15 min the products were examined chromatographically. Liver samples were obtained from cows representing various stages of lactation and levels of milk production. Ability to metabolise propionate varied considerably. Incorporation of ^{14}C from propionate into metabolic intermediates beyond *M* was correlated significantly with liver-vitamin B_{12} levels and was associated with liver-methylmalonyl-CoA isomerase activity. Variation in the latter could be accounted for by differences in lactation characteristics. As the degree of stress of lactation increased the incorporation of propionate beyond *M* diminished. The effect of high levels of *M* precursors, with or without supplements of vitamin B_{12}, on growth, lactation and utilisation of propionate was examined with rats fed purified diets. Reversal of the growth-depressing effect of propionate by vitamin B_{12} was confirmed and extended to lactation performance. Supplementation with biotin alone did not give this response. A. G. POLLARD.

Composition of cows' milk. I. Environmental and managerial influences. II. Genetic influences. S. Loganathan and N. R. Thompson (*J. Dairy Sci.*, 1968, **51**, 1928–1932; 1933–1935).—I. Means and standard deviations for 4664 age-adjusted 305-day lactation records on 3472 Holstein-Friesian cows were as follows: milk, 6820 ± 1384 kg; fat, 3·67 ± 0·41% and 245 ± 50 kg; and *SNF*, 8·51 ± 0·37% and 560 ± 112 kg. Age at calving was responsible for 10·0–14·3% of the total variance in milk, fat, and *SNF* yields. Days milked accounted for 14·0–18·0% of the variance in these constituents. Month of calving accounted for 0·5–5·8%, length of pregnancy 0·1–1·2%, and body wt. 0–0·8% of the total variance in yields. Age at calving and length of pregnancy affected *SNF* % significantly, accounting for 12·4 and 6·4%, respectively, of the total variance. None of the factors investigated affected fat % significantly. It is suggested that lactational records of milk, fat and *SNF* yields should be adjusted for age at calving, days milked and month of calving prior to being used in genetic analyses. (23 references.)

II. Heritabilities and genetic correlations were estimated from 4552 records on 3472 Holstein-Friesian cows by 630 sires. Heritabilities by paternal half-sib correlation of deviations from herd mates were: milk yield, 0·23; fat yield, 0·09; *SNF* yield, 0·22; fat %, 0·45; and *SNF* %, 0·47. Heritabilities by regression of daughter on dam with 438 pairs of records were: milk yield 0·37; fat yield 0·22; *SNF* yield 0·27; fat %, 0·55; and *SNF* %, 0·25. Genetic correlations among yields were: milk fat, 0·79, milk *SNF*, 0·95; and fat *SNF*, 0·85. It is concluded that selection for milk yield should give 94% as much gain in *SNF* yield as would direct selection for this trait. (19 references.) M. O'LEARY.

Accuracy of test interval method of calculating Dairy Herd Improvement Association records. R. W. Everett and H. W. Carter (*J. Dairy Sci.*, 1968, **51**, 1936–1941).—The accuracy of the test interval method of calculating production credits for official Dairy Herd Improvement Association production records was significantly improved by the incorporation of correction factors for adjusting production in the first and last test periods. M. O'LEARY.

Levels of first winter feeding in relation to performance of Cheviot ewes. VI. Life-time production from the hill. R. G. Gunn (*J. agric Sci., Camb.*, 1968, **71**, 161–166).—No significant treatment differences were found in life-time production (a measure calculated from lamb production). However, higher production in early life following higher levels of first winter feeding was associated with less efficient later production and poorer ewe survival in a hard environment. (14 references.) M. LONG.

Feed intake of grazing sheep differing in age, breed, previous nutrition and live weight. J. P. Langlands (*J. agric. Sci., Camb.*, 1968, **71**, 167–172).—The intake of a flock of Merino wethers initially fed on a poor pasture was greater than that of another initially fed on a good quality pasture when the two flocks were combined and grazed on a succession of four pastures. Differences in the mean digestibility of six pastures for Dorset Horns, Merinos, Southdowns and Border Leicesters estimated by faecal N concn. were significant, as were differences in faecal output. In both trials, faecal output of sheep of similar breed and receiving similar diets increased with age up to about 3 years and then declined. (10 references.) M. LONG.

Production and absorption of volatile fatty acids from rumen of sheep. G. J. Faichney (*Aust. J. agric. Res.*, 1968, **19**, 791–802).—In digestibility trials with lucerne, straw and high concentrate diets, the proportions of the digested energy absorbed as volatile fatty acids were 33·6, 42·4 and 33·2%, respectively. On the lucerne diet, the difference between the mean molar proportions of the acids absorbed and those in the rumen approached significance for acetic ($P < 0·10$) and was highly significant for butyric acid ($P < 0·01$), but the differences were not significant for the other diets. (42 references.) P. S. ARUP.

[A] Effect of urea on absorption of volatile fatty acids from the rumen of sheep fed on oat straw. [B] Effect of frequency of feeding on utilisation of roughage diets by sheep. G. J. Faichney (*Aust. J. agric. Res.*, 1968, **19**, 803–811; 813–819).—[A] The addition of 2% of urea to pellets of poor oat straw increased dry matter and energy intake, the N balance, and the rate of passage of the food through the alimentary canal. The increased intake caused a significant increase in the volatile fatty acids absorbed from the rumen, but not in their composition. The urea supplementation appeared unlikely to do more than to permit bare maintenance. (24 references.)

[B] The feeding of sheep with 800 g/day of lucerne in eight portions of 100 g at 3 h intervals instead of all in one daily portion caused no differences in digestibility or N balance. No substantial benefits were obtained by the more frequent feeding. (19 references.) P. S. ARUP.

Effect of histamine combined with formic or acetic acid on food intake and rumen motility, when infused into the omasum of a ram. H. Neumark and A. Tadmor (*J. agric. Sci., Camb.*, 1968, **71**, 267–270).—Histamine and formic acid introduced separately directly into the omasum had no effect on food intake but together stopped feeding and rumen motility. Acetic acid had a less marked effect. M. LONG.

Influence of protein digestion on plasma amino acid levels in sheep. J. P. Hogan, R. H. Weston and J. R. Lindsay (*Aust. J. biol. Sci.*, 1968, **21**, 1263–1275).—Three Merino sheep were fed on a basal diet of wheaten hay supplemented during successive 14-day periods by Ca caseinate infused at 50, 100 and 150 g/day. Five other sheep were fed on dried ryegrass which supplied 7–43 g/day of N. The essential amino acids in the plasma increased with each infusion but the increase was less marked in the third than in the preceding applications. Levels of valine, leucine and phenylalanine rose relative to the total, S-containing acids remained steady, whilst isoleucine, lysine, histidine and arginine fell. Ryegrass diets had a similar effect but the increase in phenylalanine was small. There was little change in the non-essential amino acids. The variations observed in the ratios of valine, leucine, histidine, lysine and glycine may provide a method for predicting protein digestion in the intestines. (28 references.) J. B. WOOF.

Effect of dietary chlortetracycline on rate of growth of wool in sheep. M. K. Hill and J. R. Wythes (*Nature, Lond.*, 1969, **221**, 1057).—In comparison with an unsupplemented diet, the wool growth rate of 16 mature Merino ewes of similar live wt. was increased by 10–38% when their low- and high-protein rations, respectively, were supplemented by chlortetracycline (10 mg per 450 g ration). The increase was max. for sheep fed the high protein ration, and there were small but non-significant improvements in live wt. gain. Alternative mechanisms are suggested; it is most probable that the antibiotic primarily retards the proliferation of deaminase-producing bacteria so that the increased availability of

amino acids is sufficient to accelerate the rate of synthesis of wool without adding appreciably to the net energy value of the ration. W. J. BAKER.

Studies in depilation. VIII. Structural changes in the wool follicle during depilation with acid and enzyme systems. J. R. Yates (*Aust. J. biol. Sci.*, 1968, **21**, 1249–1261).—A histological investigation was carried out on the structural changes in the wool follicle during depilation with HOAc, trypsin and Pancrozyme C_1A. There is an early separation of epidermal and outer root sheath from underlying tissue and physical obstruction of the outer root sheath (*ORS*) material causes resistance to fibre withdrawal at this stage. As the initially tightly packed cylindrical *ORS* is digested, resistance to fibre removal disappears and separation occurs in the lower part of the prekeratinous zone. (18 references.) J. B. WOOF.

Pig farming in the United Kingdom—its development and future trends. D. B. Bellis (*Jl R. agric. Soc.*, 1968, **129**, 24–42).—(16 references.) C. J. R.

Effects of simplifying feeding methods for growing pigs by using a single ration throughout and by providing cereal and supplement components unmixed. R. Braude and J. G. Rowell (*J. agric. Sci., Camb.*, 1968, **71**, 271–275).—Three feeding methods were compared: (*i*) sow and weaner type meal up to 120 lb live wt., then a finisher type meal to slaughter; (*ii*) sow and weaner type meal up to slaughter; (*iii*) as *ii* but with the cereal and supplements given separately. A small, but uneconomic, gain was given by *ii*. Non-mixing of the food constituents had no harmful effect. (11 references.) M. LONG.

Utilisation of wet feed by bacon pigs with special reference to pipe-line feeding. T. J. Forbes and N. Walker (*J. agric. Sci., Camb.*, 1968, **71**, 145–151).—No differences between wet and dry feeding of pigs was found in respect to growth rate, although food conversion ratios tended to be lower with wet feeding. Temp. of 5, 13 and 35° had no effect. Carcass characteristics were not affected by any of the treatments nor were digestibilities of dry matter and N. (15 references.) M. LONG.

Influence of the addition of some herb mixtures [to the food] on weight gains, food utilisation and quality of butchery products in fattening of swine. T. Soroka and K. Wideński (*Annls Univ. Mariae Curie-Skłodowska, Agric. E*, 1967, **22**, 257–274).—Feedstuffs containing 1·5, 3·0 and 4·5% of herbs were fed to groups of swine during fattening, and the influence of the herbs on digestibility of food, wt. gain, food utilisation and the dressing and consumption value of butchery products were determined. The control animals digested the food slightly better than those fed with food containing herbs. The herbs did not contribute to the daily wt. gain or to a better utilisation of food. Admixture of herbs to the food had no apparent influence on pigs dressing value, but in some groups slightly less fatness was observed. Canned ham and sirloin from pigs given the food with herbs had a higher consumption value than the same products processed from the control animals. (10 references.) T. M. BARZYKOWSKI.

Influence of intake of dietary energy in pregnancy and lactation upon sow productivity. F. W. H. Elsley, R. M. MacPherson and I. McDonald (*J. agric. Sci., Camb.*, 1968, **71**, 215–222).—Large White gilts (52) were divided into four treatment groups at mating. The groups were arranged as a 2 × 2 factorial with daily energy intakes during pregnancy of 8·3 (**I**) or 5·2 Mcal (**II**) and during lactation of 20 (**III**) or 13·8 Mcal (**IV**). Daily intakes of protein, minerals and vitamins were similar for all treatments. **I** led to a net pregnancy gain of 22 kg and **III** led to reduced wt. loss during lactation. **II** caused significantly larger litters at birth in the first litter. Combinations of **I** and **IV** and **II** and **III** led to similar sow live wt. at the end of the third litter and to similar total wt. of weaned pigs. M. LONG.

Variation between and within birds in the estimation of metabolisable energy content of diets for laying hens. W. H. Foster (*J. agric. Sci., Camb.*, 1968, **71**, 153–159).—Consistent breed differences and significant differences within strains were found in classical metabolisable energy (*ME*) estimates. Estimates were obtained of the within-bird variation in these determinations and standard errors of the mean classical *ME* value predicted from trials using various numbers of pullets of one breed and various numbers of measurements per pullet. (12 references.) M. LONG.

Photoperiodism and age as factors affecting the protein requirements of laying pullets. D. J. Bray (*Poult. Sci.*, 1968, **47**, 1005–1013).—When the output potential of laying hens was lowered due either to inadequate photostimulation or to a declining rate of lay associated with longevity of production, higher dietary levels and intakes of protein were required despite the decreased output. Results are discussed in relation to a concept that the dietary level and intake of protein may be adjusted by a factor directly related to level of egg output. A. H. CORNFIELD.

Evaluation of four cereal grain and three protein level combinations for layer performance. R. J. Lillie and C. A. Denton (*Poult. Sci.*, 1968, **47**, 1000–1004).—Egg production, body wt. gains and feed efficiency with respect to egg production increased with level of protein (10–15%) in the feed irrespective of the type of cereal grain used. The effectiveness of type of grain for egg production decreased in the order oats, maize, wheat, barley. Oats also produced greater body wt. gains and feed efficiency and lower mortality than did the other grains. Oats were superior to the other grains for egg production on a 10% protein diet and for body wt. gains on 10% and 12·5% protein diets. There were no differences in egg production due to type of grain on 12·5% and 15% protein diets or on body wt. on the 15% protein diet. A. H. CORNFIELD.

Effect of the ratio of maize to soyabean protein in layer diets upon the response to supplemental amino acids. D. J. Bray (*Poult. Sci.*, 1968, **47**, 815–821).—Young laying pullets were fed 8·5% protein diets derived from various combinations of maize and soyabean and supplemented with amino acids. The response to 0·2% added methionine increased as soyabean protein replaced maize protein. A mixture of tryptophan, lysine, isoleucine, and valine gave decreasing responses as soyabean protein replaced maize protein. Where maize provided more than half the protein, deletion of isoleucine from the feed caused greater depression in egg yields than did deletion of tryptophan or lysine. At intermediate combinations of maize and soyabean protein there were small, but consistent, responses to methionine and to the other mixed amino acids. A. H. CORNFIELD.

Comparison of the nutritive values of opaque-2, floury-2, and normal maize for the chick. G. L. Cromwell, J. C. Rogler, W. R. Featherston and T. R. Cline (*Poult. Sci.*, 1968, **47**, 840–847).—The protein qualities of the opaque-2 and floury-2 mutant genes of maize were compared with that of normal maize in maize–soyabean meal diets. Floury-2 maize was superior to normal maize for chick growth to 21 days of age primarily because of its higher concn. of lysine and methionine. Opaque-2 maize appeared to be inferior to normal maize, but with methionine supplementation it was superior, because of its higher lysine content. A. H. CORNFIELD.

Keratins as sources of protein for the growing chick. V. Feather and hog hair meals in broiler diets. E. T. Moran, jun. and J. D. Summers (*Poult. Sci.*, 1968, **47**, 940–945).—Broiler cockerels fed diets with low levels of feather meal (replacing 18% of the soyabean protein) showed wt. gains and carcass quality comparable with those fed the control rations. High levels of either feather or hog hair meal (replacing 40–50% of soyabean protein) reduced wt. gains and produced poorer carcass quality. This was shown to be due to inadequate lysine and methionine. Neither low nor high levels of feather or hog hair meals affected the performance of females. A. H. CONRFIELD.

Feed pigments for hens. II. Influence of feeding single and combined sources of red and yellow pigments on egg yolk colour. T. S. Nelson and J. N. Baptist (*Poult. Sci.*, 1968, **47**, 924–931).—Carotenoid pigments extracted from anaerobic mud, algae, tomato paste, lobster shells and bacteria and fed to hens were deposited as yellow, brown and red colours in the egg yolk. A combination of a small amount of a red pigment with lutein produced deep yellow-coloured yolks, similar to those obtained by feeding high levels of lutein. Astaxanthin was 30–50 times more effective than lutein in increasing yolk colour. A. H. CORNFIELD.

Egg yolk pigmentation. M. L. Scott, I. Ascarelli and G. Olson (*Poult. Sci.*, 1968, **47**, 863–872).—Yolk pigment deposition by White Leghorn hens fed synthetic carotenoids, β-apo-8′-carotenal or canthaxanthin, and natural sources of xanthophylls (*X*) was studied with a number of basal diets. Ability to absorb and deposit *X* in the egg varied among individual hens within a strain. The extent of deposition of chemically-determined *X* varied with source of the material. Carotenoids varied in their efficiency as yolk pigmenters. The apparent *X* content of the yolk varied considerably depending on method of extraction. Some *X* produced erroneously high pigmentation values by visual scoring as compared with spectrophotometric measurements. Determination of *X* extracts at 440 mμ gave a good measure of the desirable yellow colour required commercially. A. H. CORNFIELD.

Effect of cottonseed products and feed additives on egg yolk discoloration. J. G. Berry, G. W. Newell, D. P. Holder, G. V. Odell

and D. E. Bee (*Poult. Sci.*, 1968, **47**, 783–794).—Effects of dietary additions of cottonseed meal and oil, piperazine, dibutyltin dilaurate, beef tallow, vegetable fat, and pure gossypol on the incidence of egg yolk abnormalities during 30 days of storage at 2° and 25° are reported. A. H. CORNFIELD.

Shell strength and carbonic anhydrase activity of the shell gland of the domestic fowl. P. J. Heald, D. Pohlman and E. G. Martin (*Poult. Sci.*, 1968, **47**, 858–862).—There was no significant correlation between shell strength and carbonic anhydrase activity in extracts of shell gland epithelium in two egg-producing and one broiler strain of chicken. A. H. CORNFIELD.

Phosphorus assay techniques. I. Assay of soft phosphate with chicks. I. Motzok (*Poult. Sci.*, 1968, **47**, 967–974).—Availability of P in soft phosphate to chicks was studied with different ratios of Ca/inorg. P (Ca/P) in the diets by comparison with $CaHPO_4$. With a dietary Ca/P ratio of 2·5 the availability of P from soft phosphate was 71%, whilst with a ratio of 2·2 the availability was 63%. With a ratio of 3·1 there was no response in bone ash values to addition of soft phosphate although there was a response to $CaHPO_4$. Low values for the availability of P in soft phosphate reported by other workers are probably due to the use of too wide or too narrow Ca/P ratios. A. H. CORNFIELD.

Radioactive iron absorption and retention by chicks fed different levels of dietary iron. W. R. Featherston, T. J. Pockat and J. Wallace (*Poult. Sci.*, 1968, **47**, 946–950).—Chicks receiving ^{59}Fe ($FeCl_2$) by intraperitoneal injection retained most of the injected Fe regardless of the level of dietary Fe (10–96 ppm). Chicks receiving diets containing deficient (10 ppm) or marginal (31 ppm) levels of Fe retained higher proportions of the original dose of orally administered ^{59}Fe than did those receiving excess dietary Fe (96 ppm). The proportions of the supplied isotope present in liver, blood and intestine decreased with increasing dietary Fe level. Chicks fed the Fe-deficient diet exhibited lower haematocrit, haemoglobin and liver-Fe levels compared with those receiving marginal or excess Fe; in those receiving marginal Fe, normal levels were maintained. A. H. CORNFIELD.

Magnesium requirement of the laying hen. H. M. Edwards, jun. and D. Nugara (*Poult. Sci.*, 1968, **47**, 963–966).—Egg production fell to low levels within 15 days of putting hens on Mg-deficient diets containing high levels of P. When the hens were fed a purified diet containing 0·8% of P (0·6% supplemental inorg. P and 0·2% from isolated soyabean protein) their Mg requirement was between 490 and 900 ppm in the diet for normal egg production.
A. H. CORNFIELD.

Influence of EDTA on performance of chicks fed maize–soyabean meal diets with and without trace mineral supplementation. P. W. Waldroup, T. E. Bowen, H. L. Morrison, S. J. Hull and V. E. Tollett (*Poult. Sci.*, 1968, **47**, 956–960).—Addition of 100–1600 ppm EDTA to a maize–soyabean meal diet (22% protein) had no effect on wt. gains and efficiency of feed utilisation of chicks to 28 days of age or on toe and hock conditions, irrespective of whether trace elements (100 ppm Fe^{2+}, Mn^{2+} and Cu^{2+} and 10 ppm Zn^{2+}) were added. A. H. CORNFIELD.

Zinc, copper and manganese toxicities in turkey poults and their alleviation by EDTA. P. Vohra and F. H. Kratzer (*Poult. Sci.*, 1968, **47**, 699–704).—Turkey poults tolerated 2000 ppm Zn^{2+}, 676 ppm Cu^{2+}, and 4080 ppm Mn^{2+} in their diets from 5 to 21 days of age without any deleterious effects. 4000 ppm Zn^{2+} in the diet depressed wt. gains, but this was overcome by addition of 15·4–30·8 mmole of EDTA per kg of diet. EDTA did not overcome growth depression due to 8000 ppm Zn^{2+} in the diet. 30·8 mmole EDTA per kg of diet prevented the growth depression due to 810 ppm Cu^{2+}, but not that due to higher levels. 3240 ppm Cu was lethal without EDTA, and severely growth-depressing even in its presence.
A. H. CORNFIELD.

Measuring ^{65}Zn absorption and biological half-life in the chicken. F. A. Suso and H. M. Edwards, jun. (*Poult. Sci.*, 1968, **47**, 991–999).—The levels of ^{65}Zn in the blood of chickens were higher when the isotope was injected than when supplied in the feed or given orally. Absorption of Zn decreased whilst biological half-life increased with increasing age of the bird (1 day to 18 weeks). There were significant differences due to breed in the ability to absorb Zn; New Hampshires fell into two groups in this respect, indicating the possibility of a heritable factor determining Zn absorption.
A. H. CORNFIELD.

Effect of manganese upon the epiphyseal growth plate in the young chick. R. M. Leach, jun. (*Poult. Sci.*, 1968, **47**, 828–830).—Reduction in the width of the epiphyseal plate associated with Mn deficiency in chicks is the result of an impairment in matrix production rather than in cell proliferation. A. H. CORNFIELD.

Effect of embryonic administration of ^{131}I upon reproductive performance of the White Leghorn. F. R. Mraz (*Poult. Sci.*, 1968, **47**, 916–919).—Injection of 16-day-old embryos with 5 μCi ^{131}I (NaI) had no effect on subsequent livability or egg production, but injection with 25 μCi or higher levels reduced both. Injection of 50 μCi ^{131}I into 16-day-old male embryos had no effect on their fertility. A. H. CORNFIELD.

Toe abnormality in chicks fed propylene glycol. T. E. Bowen and P. W. Waldroup (*Poult. Sci.*, 1968, **47**, 1036).—A toe abnormality (turning outwards of toes at each joint), the incidence of which increased with level (2·5–10·0%) of propylene glycol in the diet, is described and illustrated. A. H. CORNFIELD.

Influence of the concentration of saccharose in solutions used for autumn feeding of bee colonies on the quantity and quality of food stored by them and on the condition of bees and their hibernation. J. Woźnica (*Annls Univ. Mariae Curie-Skłodowska, Agric. E*, 1967, **22**, 275–299).—Extensive research on application of saccharose solution as a substitute food source for autumn feeding of bee-colonies is described. The observations were carried out for 2 years on 60 bee-colonies in 2 bee-yards, situated in different localities and environments. Results of feeding trials with saccharose solutions showed that a solution of suitable concn. and given at the right time can be converted into the correct bee food necessary for safe hibernation of the colonies. A 60% solution was the most suitable, and solutions of up to 66·7% might be useful when wax production for sealing the honeycombs is needed. Saccharose feeding should be completed (in Poland) by late Aug./early Sept. (31 references.) T. M. BARZYKOWSKI.

Metabolism of 3,3′-dichloro-5,5′-dinitro-*O,O′*-biphenol (Bayer 9015) anti-fluke agent in a lactating cow. W. H. Gutenman and D. J. Lisk (*J. Dairy Sci.*, 1968, **51**, 1977–1979).—No residues of the anti-fluke agent, Bayer 9015, were detected in milk or urine up to nine days after oral administration of a single dose of 3 mg/kg to a 635 kg Holstein cow. 0·17 ppm of the intact compound was detected in the faeces two days after dosing. The compound disappeared within 6 min when incubated at a concn. of 5 ppm with fresh rumen fluid. M. O'LEARY.

Influence of antibiotic supplementation of pigs in tropical climates or resistance of their enterobacteria towards antibiotics. L. Delcambe and R. Remacle (*Revue Ferment. Ind. aliment.*, 1968, **23**, 212–219).—Average gains in wt. by pigs receiving 15 g of aureomycin per ton of feed were 84 kg whilst the gains for the controls were 50 kg. From observations on the reactions of various bacteria towards aureomycin and other antibiotics, it is concluded that no resistance, in the true sense, was developed; erroneous conclusions had arisen through over-dosing. (50 references.) P. S. ARUP.

Nucleic and free amino acid response in avian tissues during tuberculosis infection. R. L. Squibb, H. Siegel, M. Solotorovsky and M. M. Lyons (*Poult. Sci.*, 1968, **47**, 519–524).—When chicks were infected with avian tuberculosis, muscle RNA was depressed and this correlated with a high rate of catabolism. Serum valine, leucine and isoleucine increased, whilst alanine, histidine and lysine decreased. During the latter stages all the free amino acids were depressed in the liver. A. H. CORNFIELD.

Effect of coumaphos ('Baymix') on poultry. D. L. Nelson, J. O. Mozier, R. G. White and A. D. Allen (*Poult. Sci.*, 1968, **47**, 960–962).—Addition of coumaphos, (used in chicken diets for control of faecal fly larvae) at up to 80 ppm to the diet of hens for 4 weeks had little effect on egg production, mortality, feed consumption, or feed efficiency, but decreased plasma cholinesterase. 160 ppm of coumaphos decreased body wt., feed consumption and egg production. A. H. CORNFIELD.

Mortality among chinook salmon associated with the fungus *Dermocystidium*. R. L. Allen, T. K. Meekin, G. B. Pauley and M. P. Fujihara (*J. Fish. Res. Bd Can.*, 1968, **25**, 2467–2475).—Serious outbreaks of a pernicious disease caused by *Dermocystidium* are reported in both adult and juvenile salmon in the Columbia river and its tributaries. The disease, which causes death in a large % of the infected fish, appears to be most virulent at water temp. < 60°F. Diquat treatment at a dilution of 1 : 500,000 for 1 h gives some prophylactic relief. (11 references.) E. G. BRICKELL.

Rapid determination of diazinon and its oxygen analogue in animal tissues by gas chromatography. A. F. Machin and M. P. Quick (*Analyst, Lond.*, 1969, **94**, 221–225).—Diazinon (**I**) and diazoxon (**II**) are determined simultaneously, without the need for clean-up, by use of a selective thermionic P detector. Sample prep. consists

of trituration with sand and Na_2SO_4, elution with MeOH or Et_2O and then concn., before injection into a column of 1·5–2% XE-60 on Chromosorb W at 170°. The internal standard is $Bu^t_3PO_4$; the detector responds linearly to both compounds and is more than three times as sensitive to **I** as to **II**. Recoveries from fortified tissues are satisfactory for 0·05–1 ppm of **I** and 0·2–2 ppm of **II** in a 0·1 g sample, and a working range of 0·02–10 ppm for **I** is possible. Results for blood, fat, liver, muscle and brain are listed.
W. J. BAKER.

Growth and resistance to stress in brook trout fed sublethal levels of DDT. K. J. Macek (*J. Fish. Res. Bd Can.*, 1968, **25**, 2443–2451).—DDT at 2·0 mg/kg per week for 31 weeks increased the wt. gain of underyearling brook trout (*Salvelinus fontinalis*). At different rates of DDT for 26 weeks followed by starving, or feeding at 10% of normal, the cumulative mortality was 96·2% for fish exposed to 3·0 mg, 88·6% for those at 2·0 mg/kg per week and 1·2% for untreated fish. Mortality appears to be due to the interaction of DDT residues with a combination of environmental stresses, viz., starvation, decreasing water temp., and possibly the physiological stress associated with spawning. (16 references.) E. G. BRICKELL.

Degradation of DDT in Atlantic Salmon (*Salmo salar*). G. L. Greer and U. Paim (*J. Fish. Res. Bd Can.*, 1968, **25**, 2321–2326).—T.l.c. showed that hatchery-reared Atlantic salmon parr degraded *p,p'*-DDT, absorbed from aq. suspensions, to 1,1-dichloro-2,2-bis(*p*-chlorophenyl)-ethylene (*p,p'*-DDE) and -ethane (*p,p'*-TDE) within 9 h. DDT adsorbed on external surfaces of the salmon was not degraded. (20 references.) E. G. BRICKELL.

2.—FOODS

Carbohydrate Materials

Cereals, flours, starches, baking

Expansion and swelling of raw and parboiled rice during cooking. M. Mahadevappa and H. S. R. Desikachar (*J. Fd Sci. Technol.*, 1968, **5**, 59–62).—Expansion in length, breadth and lateral thickness and the rate of increase in wt. and vol. at different stages of cooking were determined in milled raw (**I**) and parboiled rice (**II**) from *Coimbatore sanna* and *Taichung*-65(*T*) varieties of paddy. **II** showed lower values than **I**. The time required for cooking to the same degree of softness was greater for **II** than for **I**. The increase in wt. and vol. was more for **I** than for **II**. Expansion characteristics of brown rice were atypical and parboiled brown rice had higher expansion than raw rice along the breadth, but not along the length. With progressive cooking, the kernels opened along the dorsal and ventral lines of fusion. I. DICKINSON.

Some flavour components of wheat. M. A. E. McWilliams (*Diss. Abstr., B.*, 1968, **28**, 5072).—The volatile flavour fraction of lightly milled wheat was isolated by steam distillation under vac. and also at atm. pressure. The sample obtained under vac. was ether extracted and then evaporated to yield the desired wheat essence. Steam distillation at atm. pressure provided headspace vapours that were used for a portion of the g.c. analysis. 18 compounds identified are listed. F. C. SUTTON.

I. Isolation and characterisation of a soluble wheat flour protein. II. Protein changes in the wheat kernel during maturation. W. W. Fish (*Diss. Abstr., B.*, 1968, **28**, 4867–4868).—A combination of ion exchange chromatography on CMC and gel filtration on Sephadex G-75 was used to isolate water-sol. wheat flour proteins. Analytical techniques used in isolating particular proteins are described. Changes in the lactic acid extractable proteins of developing wheat endosperms were also studied. The proportion of higher mol. wt. endosperm proteins increased with maturation of the kernel. No correlation was found between fluctuations in the levels of the free amino acids and changes in the endosperm proteins.
F. C. SUTTON.

Testing of wheat flour for presence of nitrite and nitrosamines. B. H. Thewlis (*Fd Cosmet. Toxicol.*, 1967, **5**, 333–337).—Neither NO_2^- nor nitrosamines could be detected in different varieties of flour and bread. Exposure of the flour to NO_2 gas or to motor exhaust fumes gradually increased the NO_2^- content but did not give rise to any detectable nitrosamines. Only after the addition of Et_2NH to the exposed flour and heating at 70° could any detectable amount of diethylnitrosamine be found. These results are in disagreement with those of other workers. (16 references.)
P. S. ARUP.

Colorimetric determination of protein in wheat flour by use of 'Orange G' and 'Amido black 10 B' dyes. M. Grüner, J. Jurić and M. Filajdić (*Kemija Ind.*, 1968, **17**, 803–809).—A colorimetric method for determining the protein content of wheat flour is described. On the basis of statistical analysis, regression equations were calculated. The method can be used for conventional analytical work.
T. M. BARZYKOWSKI.

Industrial use of wheat flour for thickening purposes. P. Meredith (*N.Z. Jl Sci.*, 1968, **11**, 720–721).—Inactivation of amylases in flour so as to greatly increase its thickening power is effected in a few min by treatment of the slurry at ~20° with enough HCl to decrease the pH to ~2·5. After neutralisation with NaOH, the slurry is ready for normal use. The process gives max. of 800–1100 Brabender Units. W. J. BAKER.

[A] Flour, bread and wheat grain fractions in decalcification tests. [B] Wheat bran factors in decalcification tests. [C] Phytates in decalcification tests *in vitro*. T. H. Grenby (*Archs oral Biol.*, 1967, **12**, 513–521; 523–529; 531–537).—[A] In mixtures of dental enamel or Ca phosphate with saliva (or acid buffers) and bread or cooked flour, incubated at 37·4° for 24 h, considerably more Ca and PO_4^{3-} were dissolved when the mixture contained white than when it contained wholemeal bread or flour. Experiments with uncooked flour, wheat-bran and -germ and phytase indicated the presence in wheat of a decalcification inhibitor (possibly wheat phytates) which, however, could be destroyed by natural wheat enzymes with loss of activity and liberation of phosphate. (13 references.)

[B] In similar tests, wheat germ had no effect on decalcification, but the aleurone of bran contained inhibitors that responded to tests for phytates. (10 references.)

[C] Material rich in phytates containing mainly Ca and Mg as cations was isolated from bran and shown to inhibit decalcification by acid buffers. Na phytate had similar effects. The importance of the phytate-P was confirmed. The influence of the anion on the decalcification inhibition mechanism is discussed. The relation of this work to the control of dental caries *in vivo* is considered. (16 references.) P. S. ARUP.

Mixing of wheat flour doughs. Work input as a process parameter. P. Wade (*Fd Process. Mktg*, 1968, **37**, 471–474).—The importance of work input as a parameter of the mixing process in bread dough and in two types of biscuit dough is compared. All three types of dough possess the elastic and plastic rheological properties normally attributed to the presence of a well developed gluten network. Work inputs are calculated from the power consumption of the mixer motor corrected for the power required to drive the mixer when empty. The level of work input at the mixing stage was shown to have a marked effect on the dough consistency in the three types of dough which were of very different compositions. However, the effect on the finished product depends on the nature and extent of the processing applied to the dough between the end of mixing and its entry into the oven. (15 references.)
I. DICKINSON.

Hydrogenated corn [maize] oils. Effect on bread baked from flours of single wheat varieties. Y. Pomeranz and E. R. Hayes (*Fd Technol., Champaign*, 1968, **22**, 1446–1448).—In trials on the effects of 3 g per 100 g of flour of widely differing wheat varieties (16), oils of I.V. 40–60 had the best effect on most hard winter or spring wheats. Flours from weak wheats benefited from additions of unhydrogenated maize oil as much as from additions of the hydrogenated oil. Adding the oils to flours improved crumb colour, grain and texture of the bread produced, and the intensity of crust colour was highest in bread baked from hard red spring flours and decreased as the m.p. of the hydrogenated oils increased.
P. S. ARUP.

Factors affecting the yield of bread. L. Milatović (*Kemija Ind.*, 1968, **17**, 559–563).—Factors are discussed which influence both the quality and quantity of bread produced from wheat flour.
T. M. BARZYKOWSKI.

Edible wool products. F. B. Shorland and K. W. Bentley (*N.Z. Jl Sci.*, 1968, **11**, 722).—Scones and sponge cakes have been successfully made with wool protein prepared by enzymic digestion with proteolytic enzymes. Binding properties of this protein are similar to those of flour and the baked products scarcely differ in appearance and taste from those made with flour. W. J. BAKER.

Sugars and confectionery

Bacteriological properties of some confectionery products and some raw materials used for their production. M. Kovačević (*Kemija Ind.*, 1968, **17**, 567–571).—Data are presented on the microflora of chocolate, candies and confectionery products, the

deterioration of cocoa beans due to fermentation and also on the inhibitory influence of chocolate. The bacteriological examination of 558 samples of indigeneous raw materials, such as eggs, milk powder, sugar, wheat and flour used in manufacture of confectionery products, is described. T. M. BARZYKOWSKI.

Fermentation and Alcoholic Beverages

Chemistry of hop constituents. XXXIII. Reactions of β-acids. J. P. Regan and J. A. Elvidge (*J. Inst. Brew.*, 1969, **75**, 10–14).—The degradation products of colupulone produced by exposure to air for several weeks were studied by boiling in wort and by acid and alkali treatment to determine whether they can play any part in the production of characteristic aromas. Atm. oxidation yielded 2-methylbut-3-en-2-ol and 3-methylbut-2-en-1-ol, and the same products were obtained from wort boiled with colupulone. To enhance the changes, pure colupulone was heated under reflux with 2N-HCl with continuous extraction with ether in a Likens apparatus. The same products were formed (2% yield) and analysis of the non-volatile residue by t.l.c. indicated the presence of 85% unchanged colupulone and a complex mixture of products including acylphloroglucinol. Treatment of colupulone with dil. alkali gave a compound similar to *p*-isohumulone and its structure was elucidated by n.m.r. spectroscopy. This compound was shown to contribute to after bitterness in beer and may be provided by isomerised hop extracts. J. B. WOOF.

4-desoxyhumulones in hops. R. O. V. Lloyd, P. V. R. Shannon and S. J. Shaw (*J. Inst. Brew.*, 1968, **75**, 32–36).—4-Desoxyhumulones have been reported to be present in Hallertau hops to the extent of 0·05%. 4-Desoxyhumulone (I) and 4-desoxycohumulone were detected directly, and simultaneously with the α- and β-acids, in a commercial extract of Fuggles hops by g.l.c. of their Me_3Si ethers, and the identification was confirmed by mass spectrometry. A standard 4-desoxycohumulone was prepared by u.v. irradiation of colupulone and purified by t.l.c., and using this the concn. of **I** in mature hops was found to be 0·3%. J. B. WOOF.

Chromatographic properties of barley and malt β-amylases. D. E. LaBerge and W. O. S. Meredith (*J. Inst. Brew.*, 1969, **75**, 19–25).—In Conquest barley, β-amylase occurs as a heterogeneous polydisperse enzyme. During malting, four distinct enzymes (β-amylase I–IV) are formed which can be separated on columns of carboxymethyl cellulose and assayed automatically using an Auto-Analyzer with dinitrosalicylic acid as substrate. If thioglycerol is present in the extract, β-amylase I and small amounts of II are detectable. During the early stages of germination, I disappears and in malt III is the major component accompanied by smaller amounts of IV. Barley II and malt III have similar chromatographic properties but it is not known whether they are identical enzymes. (20 references.) J. B. WOOF.

Origin of carbohydrate in beer sediments. R. Letters (*J. Inst. Brew.*, 1969, **75**, 54–60).—Beer sediments may be of two types, one containing polysaccharide associated with protein and polyphenols and the other consisting almost entirely of polysaccharide and forming as a gel. An enzymic procedure is described for determining whether the carbohydrates are derived from an α- or a β-glucan. Addition of EtOH to the beer pptd. a mixture of both, but the polysaccharide which became associated with protein and polyphenol appeared to be exclusively α-glucan. α-Glucans readily give cyclodextrins which form inclusion complexes, and such complexes may be involved in protein–polyphenol–polysaccharide sediments and may modify the rates of polymerisation reactions. (30 references.) J. B. WOOF.

Anthocyanogens and headspace air in relation to colloidal stability of beer. J. Posada (*J. Inst. Brew.*, 1969, **75**, 50–54).—Investigations on a number of commercially prepared beers showed that reduction of anthocyanogen content from 25 to 6 ppm caused a considerable improvement in colloidal stability of the bottled beer and that at the lower levels the sensitivity to O_2 in the headspace was less marked. Since it is difficult to achieve low headspace air, it is preferable to produce beers with a low anthocyanogen content since these can have reasonable stability at headspace air levels of 4 ml. (11 references.) J. B. WOOF.

Phenolic constituents of beer and brewing materials. IV. Further observations on anthocyanogens and catechins as haze precursors in beer. J. W. Gramshaw (*J. Inst. Brew.*, 1969, **75**, 61–83).—Development of chill and permanent haze in beer as a result of addition of various polyphenols is described. Catechins influenced stability favourably since they copolymerised with flavan-3,4-diols. D-(+)-Catechin also acted as a haze precursor in the presence of air because it readily undergoes oxidative polymerisation. A biflavan anthocyanogen isolated from barley was shown to behave slightly differently in its haze-forming potential from a similar anthocyanogen from beer. Traces of 5, 7, 3′, 4′-tetrahydroxyflavan-3,4-diol, a stereoisomer of a beer component and behaving very much like it as a haze precursor, was identified in barley and polyamide adsorbates. J. B. WOOF.

Amino acid composition of the beer haze components retained by inorganic adsorbents. G. Préaux, P. Holemans, M. Van Der Vurst and R. Lontie (*J. Inst. Brew.*, 1969, **75**, 42–49).—The amino acid compositions of the substances retained by bentonite (**I**), Stabifix (**II**) and Stabiquik (**III**) on addition to filtered Pilsner beers was determined by ion-exchange chromatography. There were considerable differences between the three adsorbents; **II** was the most selective since the amino acid composition of the adsorbate resembled most closely that of chill haze. **III** adsorbed less prolamin than did **II**, but more than **I**, whilst the **I** removed more salt-sol. protein. This was confirmed by determining the amount of nitrogenous material desorbed from each adsorbent with the prolamin solvents 60% EtOH or Pr^iOH. Chromatography on Sephadex G-25 and G-50 of beer before and after treatment confirmed that material other than protein was adsorbed. (30 references.) J. B. WOOF.

Chemical prediction of shelf life. C. C. Thompson and E. Forward (*J. Inst. Brew.*, 1969, **75**, 37–42).—Rapid chemical methods are described for determining the levels of 'oxidisable polyphenols' using cinchonine sulphate and 'sensitive proteins' by reaction with gallic tannic acid. In mixtures containing different proportions of stabilised and unstabilised beers, the cinchonine sulphate haze produced was inversely proportional to the shelf life. (14 references.) J. B. WOOF.

Browning in white wines. I. Time and temperature effects upon tannin and leucoanthocyanidin uptake by musts from seeds and husks. C. S. Du Plessis and P. De Wet (*S. Afr. J. Agric. Sci.*, 1968, **11**, 459–467).—The uptake of tannins and leucoanthocyanidin by the must from seeds and husks of various white cultivars was studied, and the coloration was shown to originate mainly from the husks, the intensity varying with the time (6–24 h) and temp. (15–35°) of contact. The contribution to the colour by the seeds was very small. (21 references.) P. S. ARUP.

[A] Influence of ester and fusel alcohol content upon the quality of dry white wine. [B] Identification of *cis*-3-hexenol in dry wine made from Pedro grapes. W. W. D. Wagener and G. W. W. Wagener (*S. Afr. J. agric. Sci.*, 1968, **11**, 469–476; 605–606).—[A] Results of organoleptic and g.l.c. examination of 9 wines showed that good bouquet and flavour were significantly correlated with high ester and low fusel alcohol content. An exceptional case is discussed. (12 references.)

[B] In the above g.l.c. analysis of Pedro wine, a peak appeared near but clearly separated from that of n-hexanol. The alcohol forming the peak was trapped, rechromatographed and identified by i.r. spectroscopy as *cis*-3-hexenol. The amount present was ~0·8 mg/l. P. S. ARUP.

Detection of histamine in wine by paper and thin-layer electrophoresis using Pauly's reagent. H. G. Maier (*Z. analyt. Chem.*, 1969, **244**, 256).—The electrophoretic determination of histamine after concentrating the wine is described. Detection limits are 0·3–0·8 μg, depending on the substrate (paper, glass 'paper', or silica gel–cellulose). R. WASPE.

Reduction of microbial population of apple cider by ultra-violet irradiation. W. O. Harrington and C. H. Hills (*Fd Technol., Champaign*, 1968, **22**, 1451–1454).—The apparatus, consisting of three u.v. lamps connected in series, used for the irradiation of a continuous thin film of flowing cider is described. A total microbial count reduction of 99% was achieved by clarifying with Pectinol and u.v. exposure for 40 sec. The storage life of the cider was increased without any alteration in flavour. (14 references.) P. S. ARUP.

Determination of propionic acid by gas chromatography. A. Motquin (*Revue Ferment. Ind. aliment.*, 1968, **23**, 226–229).—Aq. solutions (e.g., dil. vinegar) containing propionic acid (**I**) are neutralised with 0·5N-NaOH (and a min. of phenolphthalein as indicator), and then evaporated to dryness. To the residue, dissolved in 0·1 ml of water, are added 0·5N-tartaric acid equiv. to the 0·5N-NaOH previously added, plus a slight excess, and 2 ml of a 1·5% solution of isovaleric acid (**II**) to function as an internal standard in the g.l.c. determination of **I**. The graph representing mg of **I** against the ratio (height of peak × retention time of **I**) : (height of peak × retention time of **II**) is rectilinear. P. S. ARUP.

Fruits, Vegetables, etc.

Relation between low temperature breakdown and the volatiles given off by individual apples. R. B. H. Wills and K. J. Scott (*Aust. J. biol. Sci.*, 1968, **21**, 1307–1309).—80 graded apples were stored for 6 months at 30°F in boxes lined with polyethylene film and individual apples were then placed in jars with air of 75% R.H. Headspace analysis was carried out by g.l.c. The ratios of n-butyl, isoamyl and n-hexyl esters to the corresponding alcohols showed very significant negative correlations with the % of apples with breakdown. J. B. WOOF.

The influence of HMF [hydroxymethylfurfural] and furfural on the degradation of anthocyanin in cherries. T. Lovrić, J. Debicki, D. Ševčik and G. Skitarelić (*Kemija Ind.*, 1968, **17**, 799–802).—Experiments with marasca cherry juice and pigments extracted by means of model solutions showed that HMF and furfural (which can develop in fruit juices) cause the degradation of anthocyanin and consequently some discoloration of the juices. This phenomenon was more evident when treating an aq. solution of anthocyanin with HMF of higher concn. T. M. BARZYKOWSKI.

Effect of pre-harvest spray of sodium orthophenylphenate and captan on the control of decay in Anab-e-Shahi grapes (*Vitis vinifera* Linn.). P. Narasimham, M. Madalagatti Rao, N. Nagaraja and B. Anandaswamy (*J. Fd Sci. Technol.*, 1968, **5**, 63–64).—The relative efficacies of the two sprays were assessed when applied four days prior to harvest for the control of decay during transit and storage. Both sprays reduced decay considerably, and captan was superior to sodium orthophenylphenate. Neither fungicide impaired the quality or composition of the grapes. I. DICKINSON.

[A] Sulphur dioxide absorbed by sultanas and influenced by time of sulphuring. [B] Changes in total sulphur dioxide of sulphured sultanas during storage and influence of losses of sulphur dioxide on colour. K. D. Exarchos, A. A. Moüsidis and G. K. Katsouras (A) (*Delt. Ergasion Inst. Tekhnol. Phytikon Proionton*, 1968, No. 4, 139–147; 149–157).—[A] The total SO_2 retained by Thomson seedless raisins was not affected by the length of time between harvesting and sulphuring, but the colour was correlated with this period and with storage conditions; delay in sulphuring prevented colour alteration, even after prolonged treatment with SO_2.

[B] Losses of SO_2 during storage were not much affected during 125 days by the storage temp. or by the initial SO_2 content. The losses, occurring mostly during the first days, did not have much effect on the initial colour of the sulphured raisins. Changes in moisture content occurred. (From English summaries.)
P. S. ARUP.

[A] Preservation of figs. [B] Preservation of fruit and utilisation of waste products. [C] 'Gla'ce' products. Drying of water melon rinds, apricots and plums. Sher Ahmad Khan, Saifullah Khan and Rafiullah [Khan] (*W. Pakistan J. agric. Res.*, 1968, **6**, No. 2, 141–143; 144–145; 146–148).—[A]. Directions are given for the prep. of 'canned figs-Bannu English', fig jam, and the drying of 'figs dried gla'ce product'.

[B]. Directions are given for making jam from the peels and cores obtained from the canning of pears, and of date jelly from low quality dates.

[C] Directions are given for the manufacture of 'gla'ce' products by the drying of apricots, plums and water melon rinds.
P. S. ARUP.

Precursors of typical and atypical roasted peanut [groundnut] flavour. J. A. Newell (*Diss. Abstr., B.*, 1968, **28**, 5072).—Raw and roasted groundnuts and heated and unheated sol. extracts of raw groundnuts were analysed for various compounds, and those which decreased in concn. during heating were implicated as precursors of groundnut flavour. Only amino acids and carbohydrates decreased in concn. during roasting of groundnuts, and studies indicated that aspartic acid, glutamic acid, glutamine, asparagine, histidine and phenylalanine were associated with the production of typical groundnut flavour, and threonine, tyrosine, lysine and an unidentified amino acid were considered to be precursors of atypical flavour.
P.C.W.

Factors that affect quality in peanut [groundnut] products as determined by organoleptic evaluation. M. C. Thomas, C. M. Lyman, B. C. Langley and V. J. Senn (*Fd Technol., Champaign*, 1968, **22**, 1442–1446).—Evaluation as fresh roasted and fried nuts and nut butter showed that products of the best quality were obtained from mature nuts grown on soil that had been treated with pentachloronitrobenzene as a pre-emergence fungicide, and afterwards irrigated. Bag- or field-cured nuts were superior to nuts that had been cured at 49° for 24 h. (11 references.) P. S. ARUP.

Metabolism of yeasts isolated from brines of pickled Spanish-type green olives. I. Assimilation of lactic, acetic and citric acids. J. Ruiz Cruz and F. González Cancho (*Grasas aceit.*, 1969, **20**, 6–11).—The assimilation of the acids under aerobic and under anaerobic conditions by 16 yeasts isolated from the brines was examined. Six of the yeasts, most representative of the brines, were able to utilise all the acids under aerobic conditions, and the other yeasts one or two of the acids. None of the yeasts could utilise the acids under anaerobic conditions. L. A. O'NEILL.

[A] Methods of preparation and pickling of ripe black olives for table use. [B] Discoloration (Galazoma) of black table olives during pickling. [C] Changes in sugar content in ripening of green table olives and during treatment for preparation of green Spanish olives. [D] Sorbic acid as preservative for fermented green olives. I. Action of ascorbic acid on development of acidity during the last phase of lactic acid fermentation of green olives. K. D. Exarchos (A, B) and Ph. A. Legakis (*Delt. Ergasion Inst. Tekhnol. Phytikon Proionton*, 1968, No. 4, 43–70; 71–83; 85–102; 103–112).—[A] In pickling experiments in 1·2 kg glass jars, lactic acid fermentation was retarded in concn. of ⩾8–9% of NaCl. Products of greatly improved quality were obtained by promoting early lactic acid fermentation by gradually increasing the concn. of NaCl to 7–7·5% during the first 60–90 days instead of using concn. of 10–15%. (11 references.)

[B] Discoloration was avoided by ensuring the absence of traces of Fe in the brine containing 6–7% of NaCl, even when the olives were stored at 30–32°.

[C] Considerable decreases in sugar content occurred in olives from irrigated trees during June–Oct. Treatment with 2·25% aq. NaOH for 8 h and washing caused further losses of ~55%. The eventual low sugar content of these olives seriously retarded the lactic acid fermentation during pickling. (14 references.)

[D] The addition of 0·1% of sorbic acid to the pickling brine after the first phase of the lactic acid fermentation had slowed down (at pH 4·5) checked the development of yeasts, and so allowed the fermentation to proceed to a higher degree of acidity. The addition of glucose at this stage encouraged the growth of the yeasts, but not that of the lactic acid bacteria. The addition of sorbic acid (0·1%) caused the development of a special taste in the olives after 40–50 days, and turned the pickling brine brown. (From French summaries.) P. S. ARUP.

Selected metabolites of *Erwinia carotovora* as indicators of quality for peas, carrots and tomatoes. M. L. Fields and A. de Guzman (*Fd Technol., Champaign*, 1968, **22**, 1457–1459).—Three strains of *E. carotovora* produced more volatile and non-volatile acids, butan-2-ol-3-one and much more butane-2,3-diol than were present in the control samples of the vegetable extracts. (16 references.)
P. S. ARUP.

Non-alcoholic beverages

Recovery and utilisation of natural apple aroma. K. D. Exarchos and G. K. Katsouras (*Delt. Ergasion Inst. Tekhnol. Phytikon Proionton*, 1968, No. 4, 159–170).—Results obtained for various methods of obtaining apple juice aroma are compared. For the satisfactory improvement of fruit juices, it is necessary to add ~3 times the normal amount of aroma concentrate, based on the aroma present in the fresh apple juice. (From English summary.)
P. S. ARUP.

Method for measuring water-soluble volatile constituents of citrus juices and products. M. H. Dougherty (*Fd Technol., Champaign*, 1968, **22**, 1455–1456).—Samples are distilled, and the aq. portion of the distillate is separately collected by means of an oil trap. The volatiles are oxidised by $K_2Cr_2O_7/H_2SO_4$, giving a C.O.D. value for the volatile content of the juice or product being analysed which was used to calculate the amount of conc. volatile compounds to be added to conc. citrus juice to obtain the desired level of volatiles in the reconstituted juice. P. S. ARUP.

Oxygen and ascorbic acid effect on the relative stability of four anthocyanin pigments in cranberry juice. M. S. Starr and F. J. Francis (*Fd Technol., Champaign*, 1968, **22**, 1293–1295).—Three concn. of ascorbic acid and three levels of headspace O_2 were used that would encompass current manufacturing practices for the commercial prep. of cranberry cocktail. Pigment losses increased with increasing levels of these variables and were greatest when the highest levels of both O_2 and ascorbic acid were present in the same sample. The total pigment decreased on storage, but the % of peonidin and cyanidin galactoside increased whereas the % of their arabinosides decreased. A degradation index increased linearly with storage time. (19 references.) I. DICKINSON.

Preparation of liquid fruits by enzymic processing. K. R. Sreekantiah, S. A. Jaleel and T. N. Ramachandra Rao (*J. Fd Sci. Technol.*, 1968, **5**, 129–132).—Yields of juice from guavas were increased to 85% by heating the pulp sufficiently to destroy only the natural enzymes, and then treating it with 0·5% of a pectinolytic enzyme for 18 h at 40°. An enzyme prepared from a culture of *Aspergillus niger* gave the same results as did commercial pectinolytic enzymes. On filtration, the treated pulp yielded a clear juice with the natural fruit flavour and containing only 35–55 mg of tannin per 100 ml of juice, if prepared from ripe fruits. (13 references.) P. S. ARUP.

Consistency of tomato products. IV. Improvement of acidified hot break process. J. R. Wagner, J. C. Miers, D. W. Sanshuck and R. Becker (*Fd Technol., Champaign*, 1968, **22**, 1484–1488).—Rapid heating of the acidified juice by blending with water or tomato juice at 200°F, instead of slowly heating during blending, enabled max. consistency to be attained at pH 2·5 instead of 1·5, thus effecting a saving of acid. Citric acid, tartaric acid, or HCl was better for this purpose than AcOH. Other benefits were increased serum viscosities, decreased pectin degradation, and less risk of blender corrosion. (13 references.) P. S. ARUP.

Effect of heat processing on starch, sugars, proteins, amino acids and organic acids of tomato juice. S. S. El Miladi (*Diss. Abstr., B.*, 1968, **28**, 5069–5070).—With tomato juice, heat of sterilisation caused denaturation and partial hydrolysis of protein to amino acids and gelatinisation and hydrolysis of starch to glucose. G.l.c. was used to investigate free sugar content, and isomers of tomato sugars were determined. Amino acids were qual. and quant. analysed using an automated amino acids analyser. 19 amino acids were separated, and the major component was glutamic acid (~48% of total acids in fresh and 64% in processed juice). 8 org. acids were separated, and the pyrollidone carboxylic acid concn. was found to be directly dependent on processing, probably as a result of formation from glutamine and asparagine. F. C. SUTTON.

Tea, coffee, cocoa

Analysis of tea flavanols by gas chromatography of their trimethylsilyl derivatives. A. R. Pierce, H. N. Graham, S. Glassner, H. Madlin and J. G. Gonzalez (*Analyt. Chem.*, 1969, **41**, 298–302).—The flavanols in the pretreated extract are submitted to silylation with bis(trimethylsilyl)acetamide in pyridine solution at ~22° and the reaction mixture is injected directly on to an 8-ft column of 3%OV-1 on Gas Chrom Q (60–80 mesh). The column is operated first at 250° for separation of catechin, epicatechin and epigallocatechin (triphenylbenzene as internal standard), and then at 300° for separation of epicatechin gallate and epigallocatechin gallate (coronene as internal standard); the carrier gas is He. Because pure calibration standards of the Me_3Si ethers were unavailable, it was necessary to use a gas balance density at 260° to determine the efficiency of the silylation. Results for prepared mixtures and for samples of tea show good accuracy and reproducibility. (23 references.) W. J. BAKER.

Milk, Dairy Products, Eggs

Relation between micellar and serum casein in bovine milk. D. Rose (*J. Dairy Sci.*, 1968, **51**, 1897–1902).—In a study of the serum–micellar casein system in bovine milk, evidence was obtained which indicates that micellar and serum casein do not form an equilibrium system controlled by the solubilities of the various caseins. The results also suggest that, at a fixed level of Ca caseinate, the Ca phosphate (**I**) content of the micelles and the degree of polymerisation of temp.-sensitive casein components (**II**) (mainly β-casein) are the major factors controlling the proportion of casein present in micellar form. Loss of either **I** or **II** from the micelle releases additional casein to the serum; loss of both has a more than additive effect. (17 references.) M. O'LEARY.

Accounting for protein in dairy manufacturing processes. A. LeBaron and R. Brog (*J. Dairy Sci.*, 1968, **51**, 1980–1985).—Measurement of milk protein by dye-binding methods is advocated as being a convenient way of reconciling purchased protein with that actually present in finished dairy products. An analysis of data obtained under various manufacturing and laboratory conditions indicated that a protein accountability of at least 99% can be achieved, provided care is exercised in recording weights and ingredients and in testing. M. O'LEARY.

Purification of protease from the fungus *Endothia parasitica*. K. Hagemeyer, I. Fawwal and J. R. Whitaker (*J. Dairy Sci.*, 1968, **51**, 1916–1922).—Two methods for purifying protease from *Endothia parasitica* are described. The enzyme was most stable at pH 4–5, but quite unstable at pH 7. Gel filtration on Sephadex G-100 indicated its mol. wt. to be 37,500. The activity of the enzyme was not affected by iodoacetamide, *N*-ethylmaleimide, *p*-chloromercuribenzoate, Na tetrathionate, $HgCl_2$, α-*N*-tosyl-L-phenylalanine chloromethyl ketone or α-*N*-tosyl-L-lysine chloromethyl ketone. The isolectric point of the enzyme is below pH 4·6. (17 references.) M. O'LEARY.

Free, bound and microsomal phospholipids in milk. M. M. A. Al-Shabibi (*Diss. Abstr., B.*, 1968, **28**, 5069).—The bulk of the phospholipids (**I**) in milk were bound with proteins and often with sugar. The composition of the dialysable **I** suggested the presence of a free **I**. The composition of the dialysable phosphatidylcholine (**II**) differed from that obtained from the whole milk or the milk microsomes. Agitation, such as cream separation or the continuous mild stirring during the prep. of the natural protein free milk system, increased the amount of the dialysable **II**. The preferential labelling of the phosphatidylethanolamine in *in vitro* incubation may be explained by the hypothesis that this fraction is so oriented on the fat globule membrane as to be readily available for interesterification. F. C. SUTTON.

Simple and rapid turbidimetric method for estimation of proteose–peptone, proteose and peptone in milk. V. K. Joshi and N. C. Ganguli (*Indian J. Dairy Sci.*, 1968, **21**, 15–20).—A description is given of a rapid procedure for estimating proteose–peptone, proteose and peptone in milk, based on the development of the turbidity which follows the addition of $CCl_3 \cdot CO_2H$ for proteose–peptone and $(NH_4)_2SO_4$ for proteose to the casein-whey protein-free filtrate obtained from heated milk. M. O'LEARY.

Factors influencing the oxidation of fat in dry whole milk. J. J. Ritchie (*Diss. Abstr., B.*, 1968, **28**, 5072–5073).—A continuous free fat extraction procedure was developed in which the solvent flow rate through a 20-g powder sample was controlled at 5 ml/min. Using benzene as the solvent, the peroxide value could be determined directly on the extract using an $Fe(SCN)_3$ procedure. The amount of free fat can be reduced by decreasing the total fat content of the milk powder, homogenisation of the condensed milk, increasing the mean dia. of the powder particles, avoidance of excessive dryer exit air temp. and maintaining a liquid feed temp. above the m.p. of the fat. F. C. SUTTON.

Volatile components of milk fat steam distillates identified by gas chromatography and mass spectrometry. T. J. Siek and R. C. Lindsay (*J. Dairy Sci.*, 1968, **51**, 1887–1896).—Over 100 volatile compounds were detected in vac. steam distillates of butteroil, fresh raw cream, fresh pasteurised cream and pasteurised stored cream, including >30 volatiles not previously reported in milk products. Some aromatic compounds and aliphatic hydrocarbons were detected for the first time in fresh raw cream. (33 references.) M. O'LEARY.

Turbidimetric determination of fat in milk. P. Walstra (*J. Dairy Sci.*, 1968, **51**, 1964).—The author defends his previous criticisms of the accuracy of the photometric method of determining fat in milk described by Haugaard (*ibid.*, 1966, **49**, 1185). M. O'LEARY.

Application of Gerber test for estimation of fat in buffalo milk. T. D. Pruthi and V. R. Bhalerao (*Indian J. Dairy Sci.*, 1968, **21**, 6–9).—A description is given of a procedure for applying the Gerber fat test to buffalo milk, involving the use of a 10·75 ml pipette. (19 references.) M. O'LEARY.

Factors influencing post-dormant activity of *Bacillus cereus* spores in milk and model systems. M. Koka (*Diss. Abstr., B.*, 1968, **28**, 4863).—The influence of heat treatments of the spore and the milk on the post-dorminant activity of the spore, the effects of activators on spore germination, and the nature of the macro-mol. changes in milk proteins resulting from the proteolytic activity of the organism were determined. The effects of pH and L-alanine (**I**) concn. on spore germination were studied and inhibition of **I**-induced germination of spores was demonstrated by the use of the sulphydryl group inhibitor *N*-ethylmaleimide. Of the various spore germinants studied, only L-cysteine and heated glutathione stimulated germination, but not to the level exhibited by **I**. A compound tentatively identified as tryptophan was obtained on degradation of α_S-casein, thus supporting the observation that the protease enzyme of *B. cereus* 7 is a carboxypeptidase of the general class of exopeptidases. F. C. SUTTON.

Some cultural and physiological characteristics of *Staphylococcus aureus* isolated from milk and cheese. A. K. N. Al-Dulaimi (*Diss. Abstr., B.*, 1968, **28**, 4861).—The effectiveness of H_2O_2–catalase

treatments on the destruction of *S. aureus* native to raw milk and on selected cultures added to milk under laboratory conditions and during cheese making were determined. Only a small percentage of the cultures were resistant to antibiotics; the majority were resistant to penicillin. Generally, the more heat resistant the culture, the more it resisted the action of H_2O_2. *S. aureus* cultures lost their ability to grow on a medium containing 7·5% NaCl after heating at temp. as low as 120°F in the absence of H_2O_2 and as low as 105°F in the presence of H_2O_2. F. C. SUTTON.

Low temperature irradiation of milk. R. A. Scanlan and R. C. Lindsay (*J. Dairy Sci.*, 1968, **51**, 1967–1968).—Irradiation of milk to 4·5 Mrad at 5° resulted in extreme browning and caramelisation. Irradiation at −80 and −185° caused the development of an extremely bitter flavour. Evidence was obtained which indicated that the bitter component was a protein or a non-dialysable protein fragment. M. O'LEARY.

Elimination of a flavour defect in milk treated for iodine-131 removal by ion exchange. W. H. Stroup, A. L. Reyes, R. B. Read, jun., R. W. Dickerson, jun. and G. K. Murthy (*J. Dairy Sci.*, 1968, **51**, 1964–1966).—A procedure is described for eliminating a flavour defect in milk treated for ^{131}I removal without using reagent grade HCl and without discarding any milk. M. O'LEARY.

Mixed salt solution used to strip iodine-131 from anion resin for removing radionuclides from milk. I. B. Brooks, J. P. Walker and B. F. Rehnberg (*J. Dairy Sci.*, 1968, **51**, 1923–1925).—The use of waste cationic regenerating solution from a milk decontamination process for stripping ^{131}I from the anion resin is described. M. O'LEARY.

Desalting milk and whey by ion retardation and gel filtration. S. Nakai, W. A. Blair, A. Helmersen and B. A. Eagles (*J. Dairy Sci.*, 1968, **51**, 1909–1911).—Cottage cheese whey was desalted by gel filtration on Sephadex G-10 and Bio-Gel P-2 and ion retardation on Resin AG 11A8. The principal limitation of the method is the low capacity of the resin which would necessitate the use of several columns alternately in practical applications of the procedure. (10 references.) M. O'LEARY.

Keeping quality of pasteurised milk. R. N. Sinha, I. P. Singh and V. K. N. Nambudripad (*Indian J. Dairy Sci.*, 1968, **21**, 1–5).—The effects of storing commercially pasteurised cow and buffalo milk at 5–10° for 5 h followed by 6 h at 37° on their keeping and bacteriological qualities were investigated. Keeping quality, as determined by the clot-on-boiling test, was consistently longer for cows' milk than for buffalo milk. With both milks, storage at 5–10° had little effect on quality, but subsequent storage at 37° caused a loss of 40% in keeping quality and a 55% reduction in methylene blue reduction time. Both total and coliform counts increased on storage at 37°, and samples that initially had a negative coliform test showed high coliform counts after storage. (11 references.) M. O'LEARY.

Comparison of water vapour sorption by milk powder components. E. Berlin, B. A. Anderson and M. J. Pallansch (*J. Dairy Sci.*, 1968, **51**, 1912–1915).—Water vapour sorption by milk powder components was dependent on the relative pressure of the atm. to which the powder was exposed. At low relative pressure, water was preferentially bound by casein. As the relative pressure increased towards 0·5, lactose bound water more strongly and acquired sufficient moisture to undergo transition from the glass to the crystalline hydrate state. At relative pressures > 0·5 the salts absorbed sufficient water to set the conditions necessary for protein destabilisation. (12 references.) M. O'LEARY.

Cherry-milk beverage stabilisation and its measurement. S. H. Schanderl and T. I. Hedrick (*Fd Technol., Champaign*, 1968, **22**, 1429–1432).—The adverse effects of the cherry acids on the stability of the milk were overcome by the addition of 0·25% of K_2HPO_4 which enabled the cherry-milk to be pasteurised at 145°F in 30 min with the production of a satisfactory beverage with a shelf life of ≮14 days. Incipient destabilisation of the casein in samples was indicated by abrupt changes in reflectance or viscosity. P. S. ARUP.

Effect of heat treatment and storage temperature on phosphatase reactivation in butter. T. R. Freeman, J. L. Bucy and A. W. Rudnick, jun. (*J. Dairy Sci.*, 1968, **51**, 1926–1927).—Phosphatase tests were carried out on butters made from cream pasteurised at 77, 85, 93 and 101·7° with holding times of 15 sec, 10 and 15 sec, 5, 10 and 15 sec, and 1, 5, 10 and 15 sec, respectively. The butters were stored at 4·4 and 10° for 24 h, one, two and four weeks. Positive test reactions were obtained most frequently under the following conditions: pasteurisation at 93° for 10 sec, storage at 10°, four weeks storage, and during the Sept.–Nov. period. Positive reactions were not obtained with butter made from cream pasteurised at 77°. It is suggested that phosphatase reactivation problems in butter may be minimised by pasteurisation of cream at low temp. and by storage of the butter at 1° or lower. M. O'LEARY.

Changes in ovomucin concentration during thinning of thick white in eggs. B. R. Baliga, S. B. Kadkol and N. L. Lahiry (*J. Fd Sci. Technol.*, 1968, **5**, 71).—The quant. isolation of ovomucin (**I**) from thick and thin white from shell eggs stored for different lengths of time at room temp. was studied. There was no change in concn. of **I** in thin white up to the 7th day but there was a fall in concn. on further storage. There was no change in concn. of **I** in thick white up to the 7th day, there was an increase in concn. on further storage up to the 15th day, and storage beyond this led to a decrease in concn. (14 references.) I. DICKINSON.

Guar gum and triacetin in meringues and a meringue product cooked by microwaves. R. Upchurch and R. E. Baldwin (*Fd Technol., Champaign*, 1968, **22**, 1309–1310).—The combination of guar gum (0·6%) with 0·1 or 0·3% of triacetin resulted in significantly (5% level) greater reduction of whipping time than when the gum alone was used. The additive treatments reduced the amount of drip lost from meringues up to 1 h after whipping. The mixture was cooked for 105 sec in an electronic range and the meringue dessert produced was stable, did not release liquid, and three months storage in a household-type freezer did not affect the quality. I. DICKINSON.

Comparison of frozen, foam-spray-dried, freeze-dried and spray-dried eggs. II. Gels made with milk and albumen or yolk containing corn [maize] syrup solids. III. Baked custards prepared from eggs with added corn [maize] syrup solids. N. J. Wolfe (III) and M. E. Zabik (*Fd Technol., Champaign*, 1968, **22**, 1465–1469; 1470–1476).—II. The three dehydration processes, especially foam-spray drying, had adverse effects on the colour of the baked coagula prepared with whole milk and the dried products, when these were compared with products obtained from frozen eggs. Foam spraying also produced albumen coagula of low gel strength, but foam-dried yolks gave gels of high strength.

III. In comparison with custards made from frozen eggs or with those from eggs dried by the other processes, custards from freeze-dried eggs had the toughest crusts. The colour of the baked custards from the dehydrated products was not as good as that obtained using frozen eggs. Products of smoother texture resulted from freeze-dried than from foam-dried eggs. Custards baked at 85–87° were firmer and of better flavour than those baked at 81–83°. (22 references.) P. S. ARUP.

Edible Oils and Fats

Decolorisation of olive oil. I. Variation of some physicochemical characteristics as a function of the operating conditions. A. Amati, A. Minguzzi and G. Losi (*Riv. ital. Sostanze grasse*, 1969, **46**, 73–79).—Changes in characteristics of sansa olive oil on bleaching with different earths at 40–110° were investigated, especially chemical constants, u.v. spectrophotometric characteristics, colour, and elaidic acid content. (61 references.) L. A. O'NEILL.

Changes in some properties of fats during deodorisation. J. Zajíc, L. Forman, and J. Kozma (*Fette Seifen AnstrMittel*, 1968, **70**, 860–863).—The influence of steam deodorisation of a semi-refined sunflower seed oil was evaluated using a laboratory-scale apparatus, using 2–2·3 kg of steam per kg of oil. At temp. > 200°, significant changes occurred in the consistency and glyceride configuration, which affected margarine production. Changes that occurred at 180–240° were assessed by taste, odour, dilatometric and penetration tests, and crystallising properties were determined. The results were also confirmed by d.t.a. (29 references.) G. R. WHALLEY.

Determination of vegetable oils added to preserved fish, especially sardines in oil. J. Wurziger and G. Hensel (*Fette Seifen Anstr-Mittel*, 1968, **70**, 973–978).—A method is described for the approx. determination of olive oil present in sardine oil, in which the total sterols are pptd. by digitonin. The Liebermann–Burchard reaction is then carried out on the pptd. sterols, using $NaHSO_4$ instead of H_2SO_4 and the resultant colour is determined spectrophotometrically after 30 sec. Using an appropriate filter and observing the difference in the colour obtained, sitosterols can be readily differentiated from cholesterol. The amount of olive oil present is estimated from the average sterol content. G. R. WHALLEY.

Volumetric determination of soyabean oil in seeds by indirect complexometry with Mg(II). R. García-Villanova and M. T.

Marín-Aznar (*Grasas aceit*, 1969, **20**, 1–4).—The pulverised seeds are saponified with ethanolic KOH solution (8%), and after dilution with EtOH, the fatty acids are pptd. with 0·05 or 0·02 M-aq. $MgSO_4$ in the presence of an NH_3/NH_4Cl buffer. After filtration of the Mg soaps the excess Mg^{II} is determined by titration with 0·05 or 0·02M-EDTA solution, using Eriochrome Black T indicator, from which the oil content can be indirectly calculated. The method is very reproducible but the results for the oil content of seeds are appreciably lower (5–6%) than those obtained by direct Soxhlet extraction, suggesting that material other than oil is extracted in this procedure. Previous drying of the seeds is unnecessary.
L. A. O'NEILL.

Comparison of analytical methods for the estimation of fatty acid composition of vegetable and animal fats and oils. Z. E. Shoeb (*Grasas aceit.*, 1969, **20**, 4–6).—The fatty acid compositions of cacao butter, sunflower oil and beef tallow were determined by (1) g.l.c. on a column of polyethylene glycol adipate (15%) on Celite at 180°, (2) paper chromatography using a stationary phase of undecane and mobile phase of acetic acid/acetonitrile, (3) u.v. spectrophotometry after alkali-isomerisation and (4) determination of I_2 and CNS values and unsaponifiable content. The best results were given by g.l.c., which was the only technique giving the minor constituents, but paper chromatography gave identical results to g.l.c. for the major components of the vegetable oils. (10 references.) (In English.) L. A. O'NEILL.

Influence of the Al_2O_3–CrO_3 system on the kinetics of autoxidation of soyabean oil. II. J. Śliwiok and J. Siechowski (*Fette Seifen AnstrMittel*, 1968, **70**, 934–937).—The various phase transformations that took place when an Al_2O_3–CrO_3 catalyst system was heated were investigated, and it was shown that the Cr ions could occur in different oxidation states. Cr^{5+} appeared to exert the greatest catalytic effect on the autoxidation kinetics of soyabean oil. Another catalytic rate-determining factor was the degree of dispersion of the Cr ions on the carrier. G. R. WHALLEY.

Meat and Poultry

Physical and chemical properties of bovine striated muscle. H. K. Herring (*Diss. Abstr., B.*, 1968, **28**, 5071).—Studies were conducted on the contraction state of the myofibril and its associated physical, histological, and chemical characteristics. Biochemical and physicochemical aspects of natural actomyosin from muscles varying in tenderness, contraction state, and *post mortem* age were investigated. Ageing muscle *post mortem* resulted in the appearance of an additional dissociable component in natural actomyosin as shown by analytical ultracentrifigation. F. C. SUTTON.

Freezer storage effects on beef prepared by an interrupted cooking procedure. R. E. Baldwin and B. M. Korschgen (*Fd Technol., Champaign*, 1968, **22**, 1261–1265).—Boneless chuck rolls are pre-roasted to an internal temp. of 43°, chilled overnight in a refrigerator, sliced and broiled quickly just before serving. This 'roasteak' procedure is recommended as a convenience form of cooked beef. The quality can be protected by dipping the slices in a solution of antioxidant such as Na tripolyphosphate and Na ascorbate before freezing. (14 references.) I. DICKINSON.

Rate of temperature rise and physical and chemical properties of ground beef cylinders fabricated from selected muscles of the round. III. Effect of surface fat. K. Funk, P. J. Aldrich and T. F. Irmiter (*Fd Technol., Champaign*, 1968, **22**, 1285–1289).—The effects of an external layer of fat of predetermined size and thickness on the properties of ground beef cylinders containing 2·39, 10·12, 19·96 and 29·07% fat were determined and compared with data for plain beef cylinders. No significant differences attributable to the fat contents of the uncooked meat cylinders were noted in the total cooking time of fat-wrapped cylinders. Significant differences ($P \leqslant 0{\cdot}01$) attributable to the fat contents of the uncooked fat-wrapped cylinders existed in the % of moisture, fat, protein and ash of the cooked cylinders, total, volatile and drip losses and the % change in height incurred during the cooking process. An inverse relationship existed between total cooking losses and fat content of the raw meat as indicated by a significant correlation ($P \leqslant 0{\cdot}05$) between the two. (11 references.) I. DICKINSON.

Extension of bacteriological examination of foodstuffs to soup concentrates. M. Kovačević and A. Janeš (*Kemija Ind.*, 1968, **17**, 565–566).—237 samples of beef and chicken soup concentrate were examined bacteriologically, and only 90% conformed with the health regulations. Consequently it is suggested that existing regulations should be extended to soup concentrates.
T. M. BARZYKOWSKI.

Various acid effects on the peeling performance, shelf life, colour and flavour of frankfurters. J. R. Chipley and R. J. Saffle (*Fd Technol., Champaign*, 1968, **22**, 1462–1464).—Immersion of the sausages in 5% aq. H_3PO_4 for 30 sec, instead of in liquid smoke solution, improved their peeling performance and colour without adverse effects on flavour or on the bacterial or yeast counts after 5–16 days. Citric and acetic acid were also effective.
P. S. ARUP.

Quality of aged hams as affected by alternating ageing temperatures. J. D. Kemp, R. H. Smith and W. G. Moody (*Fd Technol., Champaign*, 1968, **22**, 1315–1316).—Hams aged for 5 months at weekly alternating temp. of 65 and 95°F were significantly more tender than those aged at 65°F. Hams aged for 2 months at 75°F and 2 months at 95°F were significantly more tender than hams aged for 4 months at 75°F. An increase in wt. loss occurred at the higher temp. Flavour and overall satisfaction scores were improved in one trial but not in another by alternating ageing temp. Free fatty acid values developed at a faster rate where alternating temp. were used. I. DICKINSON.

Physical properties important for freeze drying poultry meat. C. J. King, W. K. Lam and O. C. Sandall (*Fd Technol., Champaign*, 1968, **22**, 1302–1308).—The amount of water frozen during freezing, N_2 surface areas, moisture sorption isotherms, porosity, bulk *d*, thermal conductivity and mass transport parameters of the freeze dried meat were determined. These properties depended on such processing parameters as the temp., pressure, humidity and grain orientation, as well as the freezing rate before drying. Product shrinkage and rehydration ratios were also determined as functions of processing conditions during drying. (27 references.)
I. DICKINSON.

Degradation of inosinic acid in poultry meat during frozen storage. J. Davidek and A. W. Khan (*Fd Technol., Champaign*, 1968, **22**, 1317–1318).—The breakdown of inosinic acid increased progressively with storage time and the rate of breakdown increased markedly with elevation of storage temp. The accumulation of inosine and hypoxanthine also increased with storage time and increasing temp. between −10 and −80°, while in poultry stored at −5°, the amount of inosine as well as hypoxanthine increased to a max. value during 5–10 weeks of storage and decreased thereafter. Since the level of inosinic acid in fresh muscle from birds raised and processed under similar conditions is fairly constant and related to flavour, the results suggest that an objective method of quality assessment of poultry meat based on inosinic acid measurement may be feasible. I. DICKINSON.

Fish

Factors affecting quality of fresh fish and its retention by chilling. R. B. Nair and N. L. Lahiry (*J. Fd Sci. Technol.*, 1968, **5**, 107–116).—A review. (128 references.) P. S. ARUP.

Seasonal variation in the amount and characteristics of the oil of oil-sardine (*Sardinella longiceps*) fish. D. P. Sen and G. L. Chaluvaiah (*J. Fd Sci. Technol.*, 1968, **5**, 117–122).—Over one year the fish had a max. fat content of 11·0–15·7% during Sept.–Dec., and a min. of 2·7–4·4% during June and July. The I.V. of the oil showed max. in Aug. (171) and Dec. (161), and min. in Oct. (158) and March (149). The characteristics of the oil from different parts of the body, and relationships between the oil content and length and wt. of the fish and between the fat and moisture contents are discussed. P. S. ARUP.

Artificial drying of salted mackerel. S. V. Suryanarayana Rao and V. S. Khabade (*J. Fd Sci. Technol.*, 1968, **5**, 123–126).—Drying on trays with a cross-current of air at 45° and R. H. 50% for 12 h gave satisfactory results for pre-salted fish. Good results were also obtained by artificial drying for 6 h, followed by sun-drying, under proper sanitary conditions. P. S. ARUP.

Use of pyrethrum and other insecticides for the control of the blowfly, *Calliphora terraenovae* (Macq.), infesting light-salted cod fish in Newfoundland. R. F. Morris and D. Andrews (*Pyrethrum Post*, 1968, **9**, No. 4, 9–12, 36).—Blowflies that attack light-salted fish during the first few days of the drying process can be adequately controlled by immersing the fish in a solution containing pyrethrum at as low a concn. as 0·062% pyrethrins. Gardona EC 1%, Vapona 0·5%, Dylox 0·25%, Co-Ral 0·25% and Dursban 0·005% were all effective as dip treatments; Gardona WP 1% and Korlan 0·125% were ineffective. E. G. BRICKELL.

Spices, Flavours, etc.

Maturity, regional variations and retention of green colour of cardamom. C. P. Natarajan, S. Kuppuswamy and M. N. Krishna-

murthy (*J. Fd Sci. Technol.*, 1968, **5**, 65–68).—Data on regional variations in maturity of harvested berries are reported. Soaking of green cardamom in 2% Na_2CO_3 solutions for 10 min fixed the green colour during drying and subsequent storage.
I. DICKINSON.

Routine analysis of volatile oils and other materials in spices. I. Pepper, paprika, mace and pimento. U. Gerhardt (*Fleischwirtschaft*, 1968, **48**, 1207–1212).—Steam distillation by Moritz's method gives reproducible results, and lower limits for the volatile oil contents of pepper, mace and pimento are suggested. The contents of capsanthin pigment present in various types of paprika are tabulated; ash content and ether extract give the best criteria of quality of this product. Ash content, moisture, cold water- and ether-extract values are also given for the other spices.
P. P. R.

Preservatives

Determination of antioxidants. D. Viličić, B. Lui and F. Mihelić (*Kemija Ind.*, 1968, **17**, 731–734).—T.l.c. and spectrophotometry were used for the identification, separation and determination in refined sunflower oil of antioxidants such as propyl, octyl and dodecyl gallate, and butylhydroxy-anisole and -toluene.
T. M. BARZYKOWSKI.

Inhibition of fat oxidation in herring semi-preserves by means of antioxidants. O. E. Nikkilä, M. Kiesvaara and K. Westergren (*Valt. tek. Tutkimusl. Tied.*, 1968, Series IV, No. 97, 41 pp.).—Butylated hydroxyanisole, especially together with butylated hydroxytoluene, gave satisfactory results over ~ 40 days when applied at 0·02% of the total wt. of herring in sauce, which was packed in jars (of capacity ~ 450 g) and kept at 4 or 20°. The antioxidant effects were judged by determinations on the fat of the peroxide value, the *TBA* no. of Tarladgis *et al.* (*J. Am. Oil Chem. Soc.*, 1960, **37**, 44) and the determination of carbonyl compounds in the herring fat; the latter method was unsuitable for routine work. (52 references.) P. S. ARUP.

Pesticides in Foods

DDT and its derivatives in apples as affected by preparation procedures. A pilot study. R. E. Baldwin, K. G. Sides and D. D. Hemphill (*Fd Technol., Champaign*, 1968, **22**, 1460–1462).—*o,p'*- and *p,p'*-DDT and -DDD and *p,p'*-DDE were determined by electron-capture g.l.c. in apples at different stages of processing. Effective reductions in residues of these compounds were obtained by washing, paring and coring, and further reductions occurred during various cooking processes. Washing alone was ineffective. There was no evidence of conversion of *p,p'*-DDT to *p,p'*-DDD or -DDE. P. S. ARUP.

Determination of DDT residues and metabolites in pasteurised frozen yolks by gas-liquid chromatography. A. Cieśliczak, W. Martinek and J. Zerbe (*Chemia analit.*, 1968, **13**, 1133–1140).—The pesticide residues are extracted from the yolks with acetone, which is then removed under reduced pressure, and the residue is dissolved in hexane. The solution is purified on 2 Florosil columns and the pesticides in a benzene eluate are separated on the 150 cm-long column of the Pye Panchromatograph filled with Silicone Oil DC200 deposited at 10% on Gas Chrom P support, operated at 175° with N_2 as carrier gas and using the tritium source electron capture detector. Good separation of aldrin, *p,p'*-DDE, *o,p*- and *p,p'*-DDT is obtained. The response in the 1–10 ppm range of *p,p'*-DDT is linear and the recovery of 2 and 4 ppm of added *p,p'*-DDT is 86·4 ± 5·0% and 79·9 ± 2·5%, respectively.
P. BRYCH.

Food Processing, Refrigeration

Dehydration processes for convenience [instant] foods. R. Noyes (*Fd Processing Rev.*, 1968, No. 2, 367 pp.).—A review is presented of dehydration techniques for producing dried milk products, cheese, yoghurt, eggs, fruit and vegetable juices, dried fruits, potatoes and vegetables, coffee and tea, based on 236 U.S. patents issued between Jan. 1960 and May 1968. P. C. W.

Osmotic dehydration [of vegetables and fruits] with sodium chloride and other agents. D. Hartal (*Diss. Abstr., B.*, 1968, **28**, 5070–5071).—Potato, onion and apple slices were dehydrated with salt solutions and dry salt–starch mixtures. The variables studied were solution concn., temp. and agitation, variety, slice thickness and blanching. 4% salt concn. gave max. dehydration without imparting a salty taste. Osmotic dehydration with NaCl as the osmotic agent can serve as a powerful technique in various areas of food processing. F. C. SUTTON.

The physical state of water in biological systems. G. N. Ling (*Fd Technol., Champaign*, 1968, **22**, 1254–1258).—A review is given covering aspects such as the classical view of cell water, an alternative to the membrane theory, the physical state of water in living cells, osmosis, cell permeability, and a mol. basis for the irreversibility of dehydration of a colloidal protein gel. It is concluded that in living cells the protein and water in close association constitute a co-operative assembly which renders the hydration and dehydration of fresh food a much more intricate process than hydration or dehydration of simple small mol. (32 references.)
I. DICKINSON.

Freeze drying of foods and biologicals. R. Noyes (*Fd Processing Rev.*, 1968, No. 1, 313 pp.).—A detailed review is given, based on 105 U.S. patents issued between Jan. 1960 and May 1968.
P. C. W.

Convective heat transfer for freeze drying of foods. C. J. King and J. P. Clark (*Fd Technol., Champaign*, 1968, **22**, 1235–1239).—A review of freeze drying processes and a description of the improved Wurcal process are given. Mixed bed freeze drying was carried out at a pressure < 1 mm with no gas circulation, at 30 mm of N_2 with no gas circulation, and at 16 mm of 80% He and 20% N_2 with the gas being circulated through the bed. Final moisture contents reached after 3 h are tabulated for the different methods. Only the new process with circulating He/N_2 at 16 mm reached acceptable moisture levels after 3 h. (11 references.)
I. DICKINSON.

Packaging

Mechanism of sulphide staining in tin foodpacks. B. C. Seiler (*Fd Technol., Champaign*, 1968, **22**, 1425–1429).—The material responsible for staining was shown to be SnS. In observations on the staining of tin surfaces produced by hot-dipping or electroplating, caused by grape fruit or prune juice, it was found that the staining could not be traced to the action of impurities, but to an electrochemical process in which the (220) and (321) crystal planes of Sn were attacked in preference to the (112) planes. (15 references.)
P. S. ARUP.

Inhibition of nitrate-induced corrosion of tinplate cans. P. W. Board and R. V. Holland (*Br. Corros. J.*, 1969, **4**, 162–163).—Procedures for inhibiting nitrate-induced detinning of tinplate cans are suggested. Adjustment of pH to > 6 should be effective for foods of low acidity but could not be used for more acidic foods. Several 'soft' compounds were found to inhibit detinning in acid foods and their inhibitory power was correlated with their ability to reduce the hydrogen discharge potential of tinplate. A possible mode of action of the inhibitors is discussed and the properties required in inhibitors are listed. (11 references.) P. C. W.

Miscellaneous

Nutrition, proteins, amino acids, vitamins

Cultivation of the yeast *Candida lipolytica* on hydrocarbons. II. One-stage continuous cultivation on gas oil. M. Dostálek, V. Munk, O. Volfová, Z. Fencl and K. Pecka (*Biotechnol. Bioengng.*, 1968, **10**, 865–875).—Biomass production and deparaffination of the gas oil substrate (by means of urea adduct formation and g.l.c.) were followed in continuous culture. Under optimum conditions with a dilution rate of 0·16–0·19 h^{-1}, biomass was produced at 1·7 g/h with a yield coeff. of 0·92. With the same extent of deparaffination, the yield in continuous culture was double that obtained by batch cultivation. Individual n-alkanes in the substrate were degraded at different rates and differences in substrate composition will thus affect optimum growth conditions. Adequate control can be achieved by varying oil concn. and dilution rate.
J. B. WOOF.

Free amino acid analysis of *Phaseolus* seeds. R. Pant and D. R. P. Tulsiani (*J. Fd Sci. Technol.*, 1968, **5**, 138–139).—A procedure is described involving the exhaustive extraction of the acids from the dried, defatted seed powders with 70% EtOH and the two-dimensional chromatography of the extracts, the separated acids being identified using special spray reagents. The total amino acids were determined in the extracts from seeds of different bean varieties by means of the ninhydrin colour reaction. The qual. composition of the free amino acids of 10 seeds are tabulated. All the seeds contained 11–15 free acids and all were incomplete in essential

acids. The bean seeds contained only 3–5 amino acids. The contents of the free acids were 0·15–0·36 g (in terms of glycine) per 100 g of dry seed powder. P. S. ARUP.

Thermal and 2450 MHz microwave energy effect on the destruction of thiamine. S. A. Goldblith, S. R. Tannenbaum and D. I. C. Wang (*Fd Technol., Champaign*, 1968, **22**, 1266–1268).—Conventional heating of thiamine (**I**) was conducted at 101·5 and 108·5° for 50 and 25 min, respectively. Microwave heating of **I** was conducted at 102·8, 33 and 0° for 50, 30 and 45 min, respectively. The degree of destruction of **I** by microwave energy at 102·8° was due solely to the temp. Exposing **I** solution at 0° for 45 min and 33° for 30 min to microwaves caused no destruction. It is concluded that microwave energy *per se* has no effect on the destruction of **I**. I. DICKINSON.

Unclassified

Trials with an infant food supplement based on groundnut flour in diets of young children. S. M. Pereira, G. Jesudian, R. Sambamurthy and V. Benjamin (*J. Fd Sci. Technol.*, 1968, **5**, 133–137).—A spray dried test milk containing 26% of protein of which 60% came from groundnut flour, 30% from buffalo milk solids, and 10% from cereals was used for feeding trials with children aged 6–18 months, in comparison with a prep. from buffalo milk containing equiv. amounts of fat and proteins, with mineral and vitamin supplements. Growth increases obtained with the test food were equiv. to those obtained with the buffalo milk prep. P. S. ARUP.

Epidemiological importance of the presence of salmonella in jellied frogs' legs. L. Desmet-Paix, R. Lambion, E. van Oye and A. Veulemans (*Revue Ferment. Ind. aliment.*, 1968, **23**, 220–225).—Out of 15 spp. of salmonella detected in these products, five were of oriental origin, seven from other sources, and three were of wide distribution. The importance of hygienic precautions during prep. is emphasised. P. S. ARUP.

Paraffin oil for extraction and collection of volatile microconstituents in food. P. E. Nelson and J. E. Hoff (*Fd Technol., Champaign*, 1968, **22**, 1395–1398).—The food sample is blended with paraffin oil and anhyd. Na_2SO_4, and the mixture is separated by centrifuging. The volatiles are stripped from the decanted and filtered paraffin layer with He at 40° and condensed in a cold trap. Recoveries at various stages of the process were checked by g.l.c. determinations of peak-heights obtained from the food volatiles and from known amounts of added volatile compounds. The close agreement between similar peaks in chromatograms obtained from a replicate sample of tomato juice extracted using the paraffin oil technique illustrated the reproducibility of the method. (15 references.) P. S. ARUP.

3.—SANITATION, WATER, etc.

Reproduction inhibition in houseflies with triphenyltin acetate and triphenyltin chloride alone and in combination with other compounds. S. B. Hays (*J. econ. Ent.*, 1968, **61**, 1154–1157).—Feeding of diets containing > 0·25 mg/g of either Sn compound resulted in excessive fly mortality. Both sexes were sterilised by 0·25 mg/g, but the female to a greater extent. When fed to ovipositing females very few more viable eggs were deposited. The acetate and chloride compounds were as effective as tepa and all were more effective than metepa or apholate at the concn. tested. When tepa and triphenyltin chloride were offered free choice with non-treated diets to a large population, all were completely eliminated in 35 days. C. M. HARDWICK.

Metabolism of carbonyl-^{14}C-labelled Imidan, *N*-(mercaptomethyl) phthalimide-*S*-(*O*,*O*-dimethylphosphorodithioate), in rats and cockroaches. J. B. McBain, J. J. Menn and J. E. Casida (*J. agric. Fd Chem.*, 1968, **16**, 813–820).—Metabolites excreted by rats in the urine after oral administration were chiefly phthalamic acid and phthalic acid (representing 40·7% and 10·9% of the Imidan) with smaller amounts of five minor metabolites, and $\gtrless$ 0·04% as Imidan and its thiol analogue (Imidoxon). The two acids were excreted unchanged after oral administration. In cockroaches, the major metabolite was phthalamic acid. These results support other evidence that Imidan is rapidly metabolised in mammals, mainly to innocuous, water-sol. metabolites. (23 references.) P. S. ARUP.

Ground equipment and insecticides for mosquito control. Am. Mosquito Control Association (*AMCA Bull.*, 1968, No. 2 (revised), 101 pp.).—The principles of mosquito control are outlined, and different types of insecticides are surveyed (solutions, emulsions, dusts, suspensions, granulars and resins). Principles of dispersal of insecticides are outlined. Hand- and power-operated equipment for applying insecticides is surveyed, and the control of larvae and adult mosquitoes is discussed. Insecticide resistance and precautions in the use of pesticides (toxicity, and treatment of poisoning) are described. P. C. W.

Quantitative comparison of the smoke of mosquito coils prepared from extracts of different 'pyrethrins' content. D. J. Webley (*Pyrethrum Post*, 1968, **9**, No. 4, 4–8).—The pyrethrins composition of the smoke and the clonal extracts of two standard coils and five coils prepared from extracts of widely different compositions were determined by g.l.c. Coils prepared by dipping were more active against adult *Aedes aegypti* mosquitoes than the standard coils prepared from powder, and coils prepared from extracts with high pyrethrin I : pyrethrin II ratios were much more active than coils with a low ratio. E. G. BRICKELL.

Germicidal [detergent] compositions. Lennig Chemicals Ltd. (Inventor: M. D. Beach) (B.P. 1,120,230, 16.11.64).—The compositions comprise I_2 and a water-sol. amphoteric surfactant which is a mixture of compounds $R_3C{\cdot}NH{\cdot}[CH_2{\cdot}C(R^1)HO]_mSO_3X^+$ in which R is alkyl of 7–24 total C, R^1 is H or alkyl of 1–4C, *m* is an integer of average value 6–100 (12–50) and X is H or an alkali metal (Na), the R_3CNH residues being derived from an isomeric mixture of amines of 12–15C. The iodophor, which has a wt. ratio of surfactant to I_2 of 1–50 : 1 (2–10 : 1) is mixed with 2–50 wt.-% of 85% H_3PO_4, for dilution with water to form a germicidal detergent, useful in the dairy and poultry industries. J. M. JACOBS.

4.—APPARATUS AND UNCLASSIFIED

Recording micro-penetrometer: design and application. M. Jacobson and G. Armbruster (*Fd Technol., Champaign*, 1968, **22**, 1007–1009).—The instrument consists of a motor-driven probe attached by a lever arm to a transducer converter, the electrical output of which is recorded. The results obtained in testing small fruits, sections of large fruits and foodstuffs agreed well with results obtained with a pressure gauge system. P. S. ARUP.

Rapid polarographic method for determination of zinc in soil extracts and plant ash solutions. D. A. Stanton and R. du T. Burger (*Geoderma*, 1967, **1**, 7–11).—The procedure for preliminary purification of the material is described, this achieving the degree of precision demanded by Barrows and Drosdoff (*Proc. Soil Sci. Soc. Am.*, 1960, **26**, 169) and enabling routine analysis of large numbers of soil and plant samples to be carried out. No significant difference between the mean Zn values obtained by two totally different methods is found and reproducibility is excellent. C. V.

Precise fluorometric microdetermination of selenium in agricultural materials. I. Hoffman, R. J. Westerby and M. Hidiroglou (*J. Ass. off. analyt. Chem.*, 1968, **51**, 1039–1042).—The sample is digested with HNO_3, H_2SO_4 and $HClO_4$ until colourless; H_2O_2 is added, followed, after dilution, by Na_2 EDTA and 2,3-diaminonaphthalene. An extract in cyclohexane is then examined fluorometrically with the aid of standard graphs. A. A. ELDRIDGE.

Studies on the mineral contents of plant materials by radioactivation analysis. A. Fourcy (*Rapp. CEA*, 1968, No. R-3386, 95 pp.).—General principles are reviewed and advantages of this method include sensitivity, accuracy of γ-spectrometry, selectivity, speed, ability to determine the contents of several elements simultaneously and small amounts of materials required. Practical aspects discussed include specimen prep., conservation of fresh specimens, drying and ashing, irradiation procedures, radiochemical steps and counting, using for radioactivation swimming-pool reactor neutrons and 14 MeV neutrons from a generator. Applications in nutrition, physiology and element distribution in plants, genetics, parasitology, toxicology, control of manufactured agricultural and pharmaceutical products and pesticide residues, ecology, radio-ecology, cytology, enzymology and biochemistry are discussed. (57 references.) J. W. TAYLOR.

Physico-chemical behaviour and radio-ecology of ruthenium in hydrobiological systems. R. Bittel (*Série Biblphies, Commiss. Énerg. atom.*, 1968, No. 123, 66 pp.).—This annotated bibliography covers (*i*) the physicochemical properties of Ru and its compounds, especially those, e.g., RuO_4 and salts of $Ru(NO)^{3+}$, concerned in resolving radio-ecological problems, e.g., contamination of foods; analytical procedures for separation and concn. of Ru and for identification of its different species are summarised, (*ii*) the behaviour of Ru (from radioactive effluents) in waters and soils and the mechanisms of its transfer from these media into aquatic organisms and terrestrial flora and fauna. Necessary lines of research in radio-ecology of Ru are indicated. (143 references.) W. J. BAKER.

JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

JULY, 1969

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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