STUDIES ON COMPOSTS PREPARED FROM WASTE MATERIALS. II.*—The Fractionation of Organic Nitrogen

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1. The effect of acid concentration and time of hydrolysis on the amount of nitrogen dissolved from sewage sludge and composts was investigated. The amounts of α-amino-nitrogen and ammonia-nitrogen in solution were determined.

2. The amount of total nitrogen dissolved by 22% (w/v) hydrochloric acid in 16 hours at 100° was 83–88%, and this did not increase appreciably in 48 hours.

3. α-Amino-nitrogen in this solution reached a maximum in 16 hours and then decreased; ammonia-nitrogen continued to increase up to 48 hours. The increase in ammonia was attributed to deamination.

4. The α-amino-nitrogen and amide-nitrogen contents of composts from wheat straw and sewage sludge, and wheat straw and ammonium sulphate, and the solubility of the nitrogen in 2% and 22% (w/v) hydrochloric acid were determined at different stages of decomposition.

5. The changes in the percentage of total nitrogen present as α-amino-nitrogen and amide-nitrogen were greatest during the first few weeks of composting.

6. The percentage composition of the insoluble organic nitrogen present, defined as the nitrogen not soluble in cold 0·1N-hydrochloric acid, appeared to be almost independent after 3 months' decomposition of the initial source of nitrogen used in composting.

Introduction

When cereal straw is composted under aerobic conditions with a soluble form of nitrogen, such as ammonium salts or urea, water-insoluble nitrogen compounds are synthesized as a result of microbial processes.[1–3] When straw is composted with insoluble nitrogen compounds, such as animal faeces, dried blood or sewage sludge, some of the complex nitrogen compounds present are first broken down to simpler water-soluble forms of organic and inorganic nitrogen; these are subsequently largely resynthesized, though losses of nitrogen may also occur during composting.[1, 4, 5]

There is some presumptive evidence[1, 3] that the greater part of the organic nitrogen in composts is microbial protein, but few attempts have been made to extract or determine protein directly. Waksman & Gerretsen[3] adopted the scheme of proximate analysis developed by Waksman & Stevens[6] to follow changes in organic nitrogen in straw composted with ammonium salts, and used the conventional factor 6·25 × insoluble nitrogen to estimate protein; other workers have used closely similar methods.[1] This calculation will give erroneous results in the presence of water-insoluble nitrogenous constituents of non-protein origin, for example nucleic acids and chitin, which are known to be present in microbial tissue.[7]

Russell & Richards,[8] in an extensive study of the effect of storage conditions on nitrogen changes in farmyard manures, determined the percentage of amide-nitrogen in the manure and found it changed little on storage; they did not determine amino-nitrogen.

Mattingly,[9] in preliminary experiments, extracted 80–90% of the nitrogen from sewage sludge and straw–sludge composts by refluxing with 4% hydrochloric acid for 48 hours. Miller[10] used these conditions for the extraction of nitrogen from forage grasses with minimum contamination by carbohydrate. He hydrolysed the nitrogen extracted for a further 24 hours with 6N-hydrochloric acid and fractionated the nitrogen into basic and non-basic fractions by using phosphotungstic acid. With this procedure, Mattingly[9] found 45–50% of the total nitrogen was present as α-amino-nitrogen and 10–30% as volatile bases, estimated as ammonia. The basic and non-basic nitrogen constituted about 11–18% and 33–43% respectively of the total. During composting, the ratio of non-basic to basic nitrogen increased from about 2·5 to 3·0 in 5 months, and in one experiment it was shown that nitrate production in a compost prepared in an incubator was accompanied by decreases in both total non-basic and basic nitrogen.

When this work was completed it came to the writer's attention that Bremner had recently determined nitrogen changes in composts prepared in an incubator from straw and ammonium

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carbonate. He found[11] that about 80% of the total organic nitrogen was soluble in 6N-hydrochloric acid under reflux in 18 hours. He also determined the proportion of amide- and α-amino-nitrogen present and found that these fractions did not change significantly in 100 days. He reported that about 4% of the organic nitrogen was present in his composts in the form of amino-sugars. There appear to be no other accounts in the literature of direct estimation of and changes in α-amino-nitrogen and amide-nitrogen in composts.

This paper describes the hydrolysis and fractionation of the organic nitrogen in composts prepared from wheat straw and sewage sludge, and wheat straw and ammonium sulphate, and some changes in several nitrogen fractions during decomposition. The nitrogen compounds in many composts are very resistant to breakdown in soil and this work was undertaken to obtain more precise information about their chemical composition, with particular reference to α-amino- and amide-nitrogen. A brief note on the methods used, with some preliminary results, has been published elsewhere.[12]

Experimental

Nomenclature used:

'*Soluble nitrogen*' denotes the nitrogen extracted overnight by cold 0·1N-hydrochloric acid. Changes in the soluble nitrogen in the composts used in this work have been described previously.[5]

'*Insoluble organic nitrogen*' denotes the nitrogen in the residue after extracting soluble nitrogen.

'*Dilute-acid-extracted nitrogen*' is that soluble in 2% (w/v) hydrochloric acid in 5 hours at 100°.

'*Concentrated-acid-extracted nitrogen*' is that soluble in 22% (w/v) hydrochloric acid (6N) in 16 hours at 100°.

Methods of analysis

The methods used for determining total nitrogen, ammonia-nitrogen, nitrate-nitrogen and soluble-nitrogen have been described in an earlier paper.[5]

Amide-nitrogen was estimated from the ammonia liberated by hydrolysis with dilute hydrochloric acid. The ammonia was determined by the method of Pucher, Vickery & Leavenworth.[13]

α-Amino-nitrogen was determined by the titrimetric ninhydrin method of Van Slyke, MacFadyen & Hamilton.[14]

Optimum conditions of hydrolysis for estimating amide-nitrogen and α-amino-nitrogen were investigated in detail in preliminary work,[9] and are described in outline below.

Methods of extraction and hydrolysis

About 2 g. of material (40 mesh) was extracted with 50 ml. of acid of appropriate concentration in resistance-glass boiling tubes (32 mm. × 200 mm.) fitted with a short air condenser. All hydrolyses were carried out in a constant-level water bath at 100° and the tubes were weighed after hydrolysis to correct for small losses of water. The tubes were cooled rapidly and the contents filtered through No. 541 Whatman papers.

Total nitrogen was determined on an aliquot (5–10 ml.) of each hydrolysate. For the determination of α-amino-nitrogen a further aliquot (30 ml.) was evaporated to dryness *in vacuo* to remove hydrochloric acid. The residue was dissolved in water (20 ml.) and distilled for 30 min. at 42–44° with 10 ml. of 10% magnesium oxide suspension to remove ammonia. The resulting humin was removed by centrifuging and washed three times with water. The supernatant solution and washings were made up to 100 ml. and α-amino-nitrogen was determined on a 1- or 2-ml. aliquot. For determination of amide-nitrogen an aliquot of the acid extract was neutralized with 3N-sodium hydroxide solution to the first perceptible pink colour with phenolphthalein; the colour was discharged by adding rapidly 10 ml. of phosphate–borate buffer at pH 6·5. The solution was then made alkaline with 3 ml. of the alkaline borax reagent of Pucher, Vickery & Leavenworth, and distilled under the conditions described by these workers.

Soluble nitrogen was removed from all samples of the composts but not from the sewage sludge and wheat straw. The composts used contained nitrate-nitrogen, constituting in some cases 15% of the total nitrogen, and the possibility of reduction of some or all of the nitrate-nitrogen to ammonia-nitrogen during hydrolysis was considered a possible source of error. Bremner[11] has subsequently shown that such reduction does occur. The sewage sludge and wheat straw contained no nitrate-nitrogen.

Materials

The composts used were: (A) straw–sludge, initial nitrogen 1·97% (dry-matter basis), (B) straw–sludge, initial nitrogen 2·44% (dry-matter basis), (C) straw–sludge–ammonium sulphate, initial nitrogen 1·63% (dry-matter basis), (D) straw–ammonium sulphate, initial nitrogen 1·55% (dry-matter basis). Details of the preparation of these composts have been given in an earlier paper.[5]

In addition, samples of the sludge and straw (Table II) used in the preparation of composts A and B were used in some extraction experiments.

Results

1. *Effect of acid concentration and duration of hydrolysis on the total nitrogen, amide-nitrogen, α-amino-nitrogen and humin-nitrogen in sewage sludge and compost B*

Amino-acids are deaminated by hydrolysis in the presence of carbohydrates and the duration of hydrolysis will affect the amount of amino-nitrogen found.[15] Bremner[16] reported the deamination of amino-acids in hydrolysates of soil, and it was expected that deamination would be greater in the presence of the larger amounts of carbohydrate found in composts. The amount of amide-nitrogen estimated by hydrolysis of pure proteins also depends on the time of hydrolysis and concentration of acid used.[17, 18] These considerations suggested it would be essential to study in some detail the effect of different hydrolysis conditions on the estimation of both α-amino-nitrogen and amide-nitrogen in the materials used.

Hydrochloric acid, 4% (w/v) and 22% (w/v) (6N), was employed because (*a*) 4% hydrochloric acid has been used to extract nitrogen from forage grasses[10] and compost, straw and sewage sludge;[9] acid of this concentration has also been widely used for hydrolysing amide groups in proteins;[17, 18] (*b*) 22% (w/v) hydrochloric acid (6N) has been extensively used for hydrolysing pure proteins and for dissolving and hydrolysing the nitrogen in soil organic matter.[16, 19, 20]

A summary of some of these preliminary experiments is given in Table I and the main conclusions from them are given below.

(*a*) *Extraction of total nitrogen.*—It is apparent that in all cases 22% hydrochloric acid extracted more nitrogen than 4% hydrochloric acid, though differences between the two acids became progressively less as the time of extraction increased. Maximum extraction was achieved between 16 and 48 hours with the concentrated acid.

(*b*) *α-Amino-nitrogen in acid hydrolysates.*—The α-amino-nitrogen in hydrolysates with 22% hydrochloric acid increased with time of hydrolysis up to 16 hours for both compost and sludge. After 16 hours, there was a steady decrease from the maximum value recorded. With 4% hydrochloric acid, α-amino-nitrogen increased steadily with time of hydrolysis, but did not reach in 48 hours the maximum value obtained with 22% hydrochloric acid. It is clear that extending the time of hydrolysis beyond 16 hours with the concentrated acid introduces errors owing to deamination.

(*c*) *Amide-nitrogen in acid hydrolysates.*—The ammonia released by acid hydrolysis increased in all cases with the length of hydrolysis and concentration of acid used. The initial rate of release was rapid.[9] After about 8 hours the increase in ammonia appeared to be nearly linearly related to the time of hydrolysis. This is probably due, at least in part, to deamination during the later stages of hydrolysis, as the increase after 16 hours in amide-nitrogen was approximately the same as the decrease in α-amino-nitrogen for hydrolysis with 22% hydrochloric acid (Table I).

(*d*) *Acid-soluble humin.*—When magnesium oxide was used to remove ammonia from hydrolysates, after removal of acid *in vacuo*, a dark-brown or black precipitate was formed in the alkaline solution. On being centrifuged, this acid-soluble humin showed a stratified structure, the lower layer being unchanged magnesium oxide, very faintly brown, grading into a black slimy layer on the surface; the whole precipitate was soluble in 2N-sulphuric acid.

Table I

Summary of the effect of time of hydrolysis at 100° with hydrochloric acid on the extraction of total nitrogen, α-amino-nitrogen, humin-nitrogen and amide-nitrogen from sewage sludge and straw–sludge compost; results as percentage of total nitrogen

Material	Concn. of HCl (w/v)	Nitrogen fraction estimated	Time of hydrolysis, h. 5	8	16	24	48
Sewage sludge	4	Total N	64·5	73·5	82·3	82·3	80·7
		α-Amino-N	16·5	19·0	26·0	31·9	37·2
		Humin-N	8·9	7·0	7·2	7·7	7·0
		Amide-N	11·1	11·3	12·3	12·8	14·2
	22	Total N	79·3	84·3	84·1	88·5	87·9
		α-Amino-N	35·4	40·4	49·4	45·8	44·6
		Humin-N	7·4	8·5	8·3	7·5	7·2
		Amide-N	12·2	12·9	13·8	14·7	16·1
Straw–sludge compost*	4	Total N	55·8	67·2	73·4	75·5	79·7
		α-Amino-N	11·5	15·2	23·2	27·1	33·4
		Humin-N	10·9	9·3	8·7	9·4	8·8
		Amide-N	8·7	9·5	10·5	11·5	12·6
	22	Total N	76·5	80·3	87·6	87·6	87·1
		α-Amino-N	28·5	40·0	46·1	45·1	43·1
		Humin-N	7·7	8·7	8·7	9·8	9·5
		Amide-N	10·8	11·3	12·3	13·4	15·8

* Compost B, final sample after 471 days; soluble nitrogen had been removed with 0·1N-HCl

The humin-nitrogen, as a percentage of total nitrogen, did not change appreciably during acid hydrolysis, nor were there any significant differences between the values obtained on hydrolysis with 4 and 22% hydrochloric acid (Table I). Results of humin-nitrogen estimations were more erratic than those of total nitrogen, α-amino-nitrogen and amide-nitrogen. As the chemical nature of this material is not well defined it was not determined in the fractionation of composts A, B, C and D described below, although the total nitrogen in the fraction is about the same as the amide-nitrogen.

As a result of the extraction experiments described in outline above it was decided to estimate amide-nitrogen after hydrolysis for 10 hours with 4% hydrochloric acid at 100°, and total acid-extracted nitrogen and α-amino-nitrogen after hydrolysis for 16 hours with 22% hydrochloric acid at 100°.

The nitrogen soluble in 2% hydrochloric acid in 5 hours at 100° was also determined.

Table II

Distribution of nitrogen in wheat straw and sewage sludge used in the preparation of composts A, B, C and D

Material used	Fractions as percentage of total nitrogen: Total soluble N	Ammonia-N	Soluble organic N	Conc.-acid-extracted N (a)	Dilute-acid-extracted N (a)	Humin-N	α-Amino-N	Amide-N
Wheat straw	21·8	trace	21·8	82·9	64·0	n.d.	53·6	10·1
Sewage sludge SS 4 + 5 (b)	20·9	3·0	17·9	84·1	58·7	8·3	49·4	11·8
Sewage sludge SS 11 (c)	3·3	2·4	0·9	85·4	57·9	n.d.	48·8	11·8

(*a*) Includes total soluble nitrogen; (*b*) sludge used in preparation of composts A and B; (*c*) sludge used in preparation of compost C; n.d. = not determined.

Table III

Changes in the distribution of nitrogen in straw–sludge compost B during composting; results as a percentage of insoluble organic nitrogen, except in column 2

Days from start	Insoluble organic N (% of total N)	Percentage of insoluble organic nitrogen			
		Conc.-acid-extracted N	Dilute-acid-extracted N	α-Amino-N	Amide-N
0	80·1	(*a*)	(*a*)	(*a*)	(*a*)
9	78·6	73·9	47·6	47·4	9·5
44	97·7	78·1	56·0	48·0	10·9
66	93·0	80·8	55·3	42·8	10·8
114	94·1	80·6	56·5	43·4	11·1
160	93·0	83·2	56·5	44·2	10·4
255	91·3	81·5	55·4	43·7	10·6
471	87·0	87·6	46·4	46·1	9·8

(*a*) Determined only as percentage of total nitrogen

Changes in this fraction have been followed in composts[4, 21] and in farmyard manure[22] by several workers, and it was considered useful to determine them here to provide a comparison between this and other published work.

The nitrogen distribution in the wheat straw and two sludges used in the preparation of composts A, B, C and D is given in Table II.

The very close agreement between the nitrogen distribution in the two sludges, which were taken from drying beds at the Maidenhead Sewage Works at an interval of 14 months, may be fortuitous.

2. *Fractionation of nitrogen in two straw–sludge composts*

The distribution of nitrogen in eight samples of compost B is given in Table III, where results are expressed as percentages of the insoluble organic nitrogen. Fig. 1 shows the changes in the nitrogen distribution of each fraction expressed as a percentage of the total nitrogen.

Table IV

Changes in the distribution of nitrogen in straw–sludge compost A during composting; results as percentage of total nitrogen, and percentage of insoluble organic nitrogen

Days from start	Percentage of total nitrogen					Percentage of insoluble organic nitrogen			
	Insoluble organic N	Conc.-acid-extracted N (*b*)	Dilute-acid-extracted N (*b*)	α-Amino-N	Amide-N	Conc.-acid-extracted N	Dilute-acid-extracted N	α-Amino-N	Amide-N
0	79·8	62·5	41·2	51·5	10·7	(*a*)	(*a*)	(*a*)	(*a*)
114	93·0	71·6	53·5	42·0	8·9	77·1	57·6	45·2	9·6
765	79·0	57·0	39·1	33·2	6·6	72·3	49·5	42·0	8·4

(*a*) Determined only as percentage of total nitrogen; (*b*) excluding 0·1N-HCl-soluble nitrogen

Table IV gives the results of the fractionation of nitrogen in three samples only of compost A; these samples were taken after 0, 114 and 765 days' decomposition to provide some confirmation of the results obtained with compost B.

Interpretation of results from such fractionations is complicated by the circumstance that there are dry-matter and nitrogen losses during composting,[5] which alter the relative composition from one sample to another. Further, changes appear to be different when expressed as percentages of either insoluble organic nitrogen or total nitrogen.

These results are therefore described briefly under three headings: (*a*) organic nitrogen, (*b*) total nitrogen, (*c*) absolute quantities of nitrogen present.

(*a*) *Changes as percentages of the insoluble organic nitrogen.*—The insoluble organic nitrogen in compost B reached a maximum of about 98% of the total nitrogen in about 44 days (Fig. 1). At this time soluble nitrogen was naturally at a minimum. The percentage of organic nitrogen then decreased during the next 22 days, becoming more or less constant at 93–94% of the total nitrogen for a further 100 days. Between 160 days and the end of the experiment

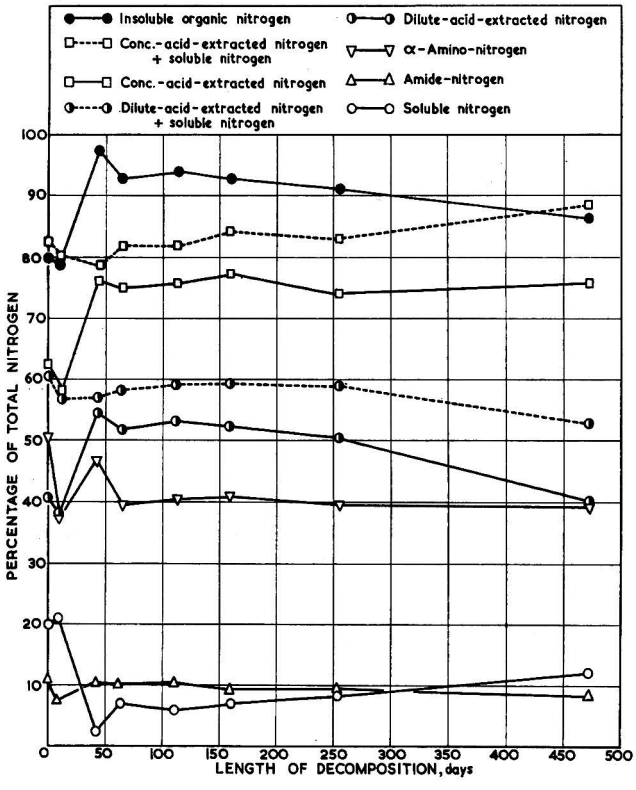

FIG. 1.—*Changes during composting in the nitrogen distribution in straw–sludge compost B*

(471 days) it decreased steadily to 87% of the total nitrogen. In compost A the insoluble organic nitrogen constituted about 80% of the total nitrogen (Table IV) after 765 days, the longest period for which experiments were continued.

The changes in the fractions of the organic nitrogen (Tables III and IV), even over a period of 26 months, were not very pronounced. Concentrated-acid-extracted nitrogen was at a minimum after 9 days in compost B; it then rose slowly for the next 60 days and then remained approximately constant at 80–87% of the organic nitrogen for the remainder of the experiment. Dilute-acid-extracted nitrogen showed closely parallel changes up to 250 days, though the amount of nitrogen extracted was smaller. This fraction decreased to less than 50% of the organic nitrogen in the final samples of both composts A and B.

α-Amino-nitrogen decreased in compost B during the first 66 days to a minimum of 42·8% of the organic nitrogen; it subsequently remained appreciably constant at about 43–46% until the end of the experiment. The organic nitrogen of the final sample of compost A contained 42% of α-amino-nitrogen.

The amide-nitrogen changed little during decomposition, though there was some indication that it decreased in composts that were decomposed for long periods (Tables III and IV).

(*b*) *Changes as percentages of the total nitrogen.*—Many of the changes in nitrogen distribution described above become more apparent when each fraction is expressed as a percentage of the total nitrogen at any sampling. This is done in Fig. 1, which is largely self-explanatory. It is convenient to divide the duration of composting into four periods: (i) 0–9 days, (ii) 9–44 days, (iii) 44–160 days and (iv) 160 days until the end of the experiment.

During the first period there was a marked decrease in α-amino-nitrogen and amide-

nitrogen, but during the second period, when insoluble organic nitrogen reached its maximum value, the α-amino-nitrogen and amide-nitrogen both increased considerably. In the third period the insoluble organic nitrogen, α-amino-nitrogen and dilute-acid-extracted nitrogen all decreased slightly, the amide-nitrogen remaining almost constant in value. In the last period changes were still comparatively small, especially in α-amino-nitrogen, but there were indications that dilute-acid-extracted nitrogen and amide-nitrogen decreased during prolonged decomposition.

(*c*) *Changes in the absolute amount of nitrogen present in each fraction.*—Composts A and B were weighed after 114 days and losses of nitrogen determined;[5] this makes it possible to estimate the absolute amount of each nitrogen fraction present after 114 days (Table V). During this period there was degradation and synthesis of organic nitrogen (Table III and Fig. 1), and the changes in composition at the end of 114 days are the net result of both processes. The results in Table V show clearly that the greatest redistribution of nitrogen during the first 3–4 months of composting was in total soluble nitrogen and α-amino-nitrogen.

Table V

The absolute amount of nitrogen in each fraction of straw–sludge composts A and B at start and after 114 days; initial N = 100

Compost	Days from start	Total N present	Total soluble N	Soluble organic N	Insoluble organic N	Conc.-acid-extracted N (*a*)	Dilute-acid-extracted N (*a*)	α-Amino-N	Amide-N
A	0	100·0	20·2	18·3	79·8	62·5	41·2	51·5	10·7
	114	80·7	5·7	2·1	75·0	57·8	43·2	33·9	7·2
B	0	100·0	19·9	17·8	80·1	62·6	40·6	50·8	11·0
	114	73·5	4·3	1·3	69·2	55·7	39·0	30·0	7·6

(*a*) Excluding 0·1N-HCl-soluble nitrogen

In Fig. 1 and Table V the initial α-amino-nitrogen content of the composts is based on analyses of the straw and sludge used in their preparation (Table II). These materials were not extracted with 0·1N-hydrochloric acid before hydrolysis and the α-amino-nitrogen and amide-nitrogen fractions may include some soluble as well as insoluble organic nitrogen. This circumstance, together with the large losses of total nitrogen from these composts, makes it impossible to draw up a detailed balance sheet for all the fractions individually. The results, however, show unequivocally that in both composts there was (*a*) a net loss of α-amino-nitrogen and amide-nitrogen in 114 days and (*b*) a net loss of soluble nitrogen in the same period.

3. *Fractionation of nitrogen in straw–ammonium sulphate–sludge and straw–ammonium sulphate composts*

Three samples from each of these composts were fractionated by the methods described above. These composts differed only in the form of the nitrogen present initially; in one (C) this was provided by sludge and ammonium sulphate and in the other (D) by ammonium sulphate alone.

Results are given in Table VI as percentages of the total nitrogen and insoluble organic nitrogen, and in Table VII as the absolute amounts present initially and after 97 days.

The redistribution of nitrogen between fractions in both composts was greater than that found for straw–sludge composts. Insoluble organic nitrogen increased between the start and 97 days owing to synthesis from soluble nitrogen, and all the fractions except soluble nitrogen were greater after 97 days than initially (Table VI). At the final sampling these results were reversed owing to the increase in soluble nitrogen between 97 and 341 days, and all fractions, as percentages of the total nitrogen, had decreased. The results in Table VI further show that between 97 and 341 days the dilute-acid-extracted nitrogen and α-amino-nitrogen had both decreased as percentages of the insoluble organic nitrogen.

The most informative changes were again those in the absolute amount of each fraction present (Table VII).

In compost C there was a loss of nitrogen in 3 months of 21·5%. There was no net gain

Table VI

Changes in the distribution of nitrogen in straw–sludge–ammonium sulphate compost C and straw–ammonium sulphate compost D during composting. Results as percentage of total nitrogen and percentage of insoluble organic nitrogen

Compost	Days from start	Percentage of total nitrogen					Percentage of insoluble organic nitrogen			
		Insoluble organic N	Conc.-acid-extracted N (b)	Dilute-acid-extracted N (b)	α-Amino-N	Amide-N	Conc.-acid-extracted N	Dilute-acid-extracted N	α-Amino-N	Amide-N
C	0	55·3	44·2	29·2	31·7	6·8	(a)	(a)	(a)	(a)
	97	91·2	68·5	48·6	41·4	8·6	74·0	53·3	45·0	9·4
	341	82·1	58·6	40·6	36·0	7·2	71·5	49·5	43·9	8·7
D	0	25·1	19·9	13·6	18·0	3·2	(a)	(a)	(a)	(a)
	97	74·4	50·0	37·2	33·6	5·7	67·3	50·0	45·1	7·6
	341	63·6	42·8	29·5	26·5	5·0	67·4	46·4	41·7	7·8

(*a*) Determined only as a percentage of total nitrogen; (*b*) excluding 0·1N-HCl-soluble nitrogen

of α-amino-nitrogen or amide-nitrogen in this compost and a very marked decrease in soluble nitrogen.

In compost D, however, loss of nitrogen was small, and nearly all the soluble nitrogen originally present was either synthesized into insoluble organic nitrogen or remained in the compost in a soluble form. In consequence, there was a net increase in α-amino-nitrogen and amide-nitrogen in 3 months.

Table VII

The absolute amount of nitrogen in each fraction of straw–sludge–ammonium sulphate compost C and straw–ammonium sulphate compost D at start and after 97 days; initial N = 100

Compost	Days from start	Total N present	Total soluble N	Soluble organic N	Insoluble organic N	Conc.-acid-extracted N (a)	Dilute-acid-extracted N (a)	α-Amino-N	Amide-N
C	0	100·0	44·7	6·4	55·3	44·2	29·2	31·7	6·8
	97	78·5	7·0	2·1	71·5	53·8	38·2	32·9	6·8
D	0	100·0	74·9	7·8	25·1	19·9	13·6	18·0	3·2
	97	94·0	24·0	1·1	70·0	47·0	35·0	31·6	5·4

(*a*) Excluding 0·1N-HCl-soluble nitrogen

Discussion

(*a*) *Methods of extraction and fractionation*

(i) *Acid-extracted nitrogen.*—About 83–88% of the nitrogen in the wheat straw, sludge and compost was dissolved by 22% hydrochloric acid at 100° in 16 hours. The percentage dissolved did not increase appreciably in 48 hours (Tables I and II). Bremner[16] found too that the amount of nitrogen in soil dissolved by 6N-hydrochloric acid did not increase after boiling for 12 hours with 6N-hydrochloric acid at the boiling point of the acid, 109°; less nitrogen was dissolved from soil than from the composts described here. He has recently reported very similar results, however, for composts from oat straw.[11]

Less nitrogen was soluble in 4% hydrochloric acid in 48 hours than in 22% hydrochloric acid in 16 hours (Table I). The percentages of the total organic matter soluble in 22% hydrochloric acid in 16 hours and in 4% hydrochloric acid in 48 hours were found in preliminary work to be 68·6 and 64·5% respectively;[9] there seems, therefore, little advantage in using the dilute acid to reduce the solution of soluble or hydrolysed carbohydrates. Moreover, the total α-amino-nitrogen found in solution after further hydrolysis of the 4% hydrochloric acid extract with 22% hydrochloric acid was less than that brought into solution by a single hydrolysis with the more concentrated acid.[9]

(ii) *α-Amino-nitrogen.*—There was a decrease in the α-amino-nitrogen in solution when sludge and compost were hydrolysed for more than 16 hours (Table I), which was presumably due to decomposition of α-amino-nitrogen when hydrolysis was extended to 24 or 48 hours.

Similar results have been observed during the hydrolysis of soils.[16] The estimation of α-amino-nitrogen after hydrolysis therefore provides only a minimum value as there is no simple method of estimating any destruction of α-amino-nitrogen up to 16 hours.

Although the ninhydrin method is highly specific for determining α-amino-nitrogen, one further source of error in the hydrolysis of nitrogen complexes containing both protein and other nitrogenous substances is the formation of glycine from purines during acid hydrolysis.[23] The α-amino-nitrogen values may therefore be low, owing to deamination, or high, owing to the decomposition of purines. Microbial tissue formed during the decomposition of composts contains purines, both free and in the nucleic acids, but the total amount is not likely to exceed 10% of the fungal nitrogen ;[7] the error introduced into α-amino-nitrogen estimations will be considerably smaller, as only part of the purine nitrogen is hydrolysed to glycine.

(iii) *Amide-nitrogen.*—Many nitrogenous substances (simple amides, ureides, allantoin), beside the amide groups of proteins, give ammonia on acid hydrolysis ;[13] if present, such compounds would be removed as soluble nitrogen by extraction with cold 0·1N-hydrochloric acid. Partition of the nitrogen in the straw and sludge into soluble and insoluble organic nitrogen, and separate hydrolysis and study of these fractions, would have given more complete information on the changes in nitrogen distribution during the early stages of composting.

It was considered possible that volatile bases other than ammonia might be formed on the hydrolysis of the insoluble nitrogen in composts, but in preliminary experiments[9] the author detected substances that gave anomalous colours and precipitates with Nessler's reagent only in the hydrolysates of wheat straw. After straw had been composted with sludge for nine days all the volatile bases were present as ammonia.

It has been shown [Table I, Section 1 (*c*)] that the increase in amide-nitrogen after 16 hours can be accounted for by deamination. Although there is no *a priori* reason to assume that nitrogen compounds in composts are closely related to soil organic nitrogen, it is interesting that this result differs from recent work on soil organic nitrogen. Bremner[16] found that, after acid hydrolysis, ammonia increased much more rapidly than α-amino-nitrogen decreased. His values for the ammonia-nitrogen (' amide-nitrogen ') after hydrolysis of soils with 6N-hydrochloric acid for 12 hours were considerably higher than those reported here, but agreed with estimations by other workers on soil nitrogen.[19, 20]

Amide-nitrogen was estimated here by the ammonia released on hydrolysis with 4% hydrochloric acid for 10 hours at 100°. The amount of ammonia formed under these conditions was about 10–15% less (Table I) than by hydrolysis with 6N-hydrochloric acid in 6 hours, which Shore, Wilson & Stueck[18] recommended for the estimation of amide-nitrogen in proteins. The estimates of amide-nitrogen agree closely with those of Russell & Richards for farmyard manure[8] and of Bremner for oat straw composted with ammonium carbonate.[11]

(iv) *Humin-nitrogen.*—Solutions after acid hydrolysis and filtration varied from pale yellow to dark brown and much, though not all, of this colour was removed by magnesium oxide as a dark-brown or black precipitate containing 7–10% of the total nitrogen. This humin appeared to resemble that formed on hydrolysis of most pure proteins more closely than the humin from hydrolysis of soils, which Bremner[16] states contains no black material. The amount formed did not decrease appreciably on acid hydrolysis (Table I). When, however, an acid hydrolysate from the final sample of compost B was distilled[9] (after removal of hydrochloric acid *in vacuo*) with magnesium oxide at 42–44° the amount of humin decreased with time of distillation from 9·6% of the total nitrogen after 60 min. to 6·9% after 200 min. The α-amino-nitrogen in the filtrate increased by 2·2% and ammonia-nitrogen by 0·7% of the total nitrogen. This suggests that humin contains some protein not hydrolysed completely by 22% hydrochloric acid at 100° for 16 hours.

(*b*) *Changes during composting*

(i) *Compost from straw and sludge.*—In composts A and B, 86 and 92% of the nitrogen present initially was derived from the sludge ; about 20% of the total nitrogen was soluble in 0·1N-hydrochloric acid. The total nitrogen content, 1·97 and 2·44% of the dry matter, was high and more than enough for active decomposition.[5] There were marked changes in

the distribution of nitrogen between fractions up to about 100 days of decomposition, but all subsequent changes were smaller and very slow (Fig. 1; Tables III and IV)

The most significant results are those in Table V, which show that in each compost about 40% of the α-amino-nitrogen originally present had been lost after 114 days, and that the soluble nitrogen had decreased during this period from 20% to about 7% of the total nitrogen. Ammonification is almost universal in composts that contain initially some organic nitrogen,[24] and much ammonia was present in composts A and B after 9 days.[5] The results indicate that this ammonia arises by ammonification of the soluble organic nitrogen, which did not contain much α-amino-nitrogen,[5] or by deamination of α-amino-nitrogen present in the insoluble organic nitrogen fraction. The total loss of nitrogen from composts A and B in 114 days was 19·3 and 26·5% of the total present initially. Much of this loss is probably due to ammonia produced in one or both of the ways outlined above in excess of that required by the microbial flora during the period of synthesis of organic nitrogen [Fig. 1; Section 2 (*b*)].

The synthesis of α-amino-nitrogen between 9 and 44 days (Fig. 1; Table III) was accompanied by almost parallel changes in the dilute-acid-extracted nitrogen, which constituted only 47·6% of the insoluble organic nitrogen after 9 days but 56·0% after 44 days. Changes in the solubility of nitrogen compounds in 2% hydrochloric acid during composting have been observed before. Ashworth[4] found, however, that, in a compost of straw and dried blood, prepared in the open and containing about the same percentage of nitrogen initially (2·5%) as compost B, a much higher percentage (about 76%) of the total nitrogen at the start was soluble in 2% hydrochloric acid than was observed in the experiments described here. The nitrogen soluble in 2% hydrochloric acid fell to about 40% in 5 months in this compost.

Conversely, Ashworth found only about 36% of the nitrogen in a grass–dried blood compost of the same nitrogen content was soluble at the start in 2% hydrochloric acid, and that there was little change in this fraction on composting for 5 months. During the decomposition of farmyard manure in heaps, Maiwald & Steigmiller[22] showed that hot-water-soluble nitrogen decreased from 18·1 to 9·4% of the total nitrogen in the first 4½ months of composting and nitrogen soluble in 2% hydrochloric acid also decreased from 46·5 to 42·9%. These changes do not follow closely those described in this paper, although there is some general agreement that nitrogen soluble in 2% hydrochloric acid tends to become stabilized at about 40–50% of the total nitrogen after 4–5 months, and to be independent of the initial amount of nitrogen soluble in this acid.

It is probable that the solubility of nitrogen in 2% hydrochloric acid does reflect in some qualitative way the amount of nitrogen that can be easily ammonified and therefore either utilized or lost during composting. Dried blood is much more easily decomposed in soil than the nitrogen in sludge,[25] and it appears to be more readily utilized as a source of nitrogen in composting.

Changes in amide-nitrogen during the decomposition of composts A and B were comparatively small and followed closely the changes in the α-amino-nitrogen (Fig. 1). There was a definite indication that the amide-nitrogen content of the insoluble organic nitrogen decreased during composting (Tables III and IV).

(ii) *Compost from straw–ammonium sulphate–sludge and straw–ammonium sulphate.*—In composts C and D, 44 and 75% respectively of the nitrogen was initially present in a water-soluble form, largely as ammonium sulphate. The total nitrogen content, 1·63 and 1·56% of the dry matter, was lower than in the straw–sludge composts but well above the minimum necessary for rapid decomposition;[1, 2, 5] the changes in nitrogen distribution during composting were different up to 3 months from those in straw–sludge composts. In both composts (Tables VI and VII) the percentage of the total nitrogen present as acid-extracted nitrogen, α-amino-nitrogen and amide-nitrogen had increased at 97 days owing to synthesis of microbial nitrogen from the soluble nitrogen compounds.

There was no net increase at 97 days in the absolute amount of α-amino-nitrogen or amide-nitrogen present in compost C, but there were marked increases in these fractions in the same time in compost D. These differences are probably related to the different amounts of nitrogen lost in 97 days from the two composts—21·5% in compost C and only 6% in compost D. It appears from the results in Table VII that, in compost D, 50·9 g. of soluble

nitrogen was transformed into insoluble organic nitrogen or lost in 97 days, but only 13·6 g. can be accounted for as synthesized α-amino-nitrogen and 2·2 g. as synthesized amide-nitrogen; only 6·0 g. was lost. The nature of the rest of the synthesized organic nitrogen was not investigated, but some of it is undoubtedly in the form of nucleic acids and amino-sugars.[7, 11]

No direct evidence can be given to explain why the compost from straw, sludge and ammonium sulphate lost so much more nitrogen in 3 months than the compost from straw and ammonium sulphate. It seems likely, however, that when two alternative sources of nitrogen are available in a compost the micro-flora that break down insoluble organic nitrogen do not simultaneously synthesize much inorganic nitrogen.

The results observed with the straw–sludge composts, especially compost A, are consistent with the behaviour of compost C. Both composts A and C contained initially soluble and insoluble nitrogen, both lost about 20% of the initial nitrogen in 3 months, and the composition of both after 3 months, despite wide differences in the initial nitrogen distribution, was closely similar (compare Tables IV and VI). Further, on prolonged decomposition, soluble nitrogen increased in both composts, whereas the percentage of nitrogen in all other fractions decreased.

There was, too, further evidence from composts C and D that the percentage of the insoluble nitrogen extracted by 2% hydrochloric acid decreased as soluble nitrogen increased (Table VI). Kaila[21] recently determined the percentage of nitrogen soluble in 2% hydrochloric acid in composts of straw and inorganic nitrogen prepared in an incubator. Recalculation of her results shows that the amount of nitrogen soluble in 2% hydrochloric acid in two of her composts decreased from about 50% of the total nitrogen after 6 weeks to 35–40% after 4 months. These results, though somewhat higher than the comparable figures for compost D, agree generally in the direction of the changes observed.

Conclusions

Several generalizations about the results of the fractionation of these four composts appear justified if attention is confined to the insoluble organic nitrogen of composts decomposed for at least 3 months. By this stage of decomposition soluble nitrogen is increasing[5] and the composition of the insoluble organic nitrogen is much more uniform than in the starting materials, owing to the net results of synthesis and degradation.

The results collected below from Tables III, IV and VI indicate how the composition of the insoluble organic nitrogen differs in those composts whose soluble nitrogen is (i) less than 15% and (ii) greater than 15% of the total nitrogen.

Nitrogen soluble in cold 0·1N-*HCl as percentage of total nitrogen*

	Less than 15%	Greater than 15%
Conc.-acid-extracted nitrogen (% of insoluble organic N)	74–88	67–72
Dilute-acid-extracted nitrogen (,,)	46–58	46–50
α-Amino-nitrogen (,,)	43–46	42–45
Amide-nitrogen (,,)	9·4–11·1	7·7–8·8

It appears that as soluble nitrogen increases during composting there is a decrease in the amount of insoluble organic nitrogen that can be extracted by concentrated and dilute acids. The amide-nitrogen content of the organic nitrogen also decreases and the content of α-amino-nitrogen remains almost constant. These general conclusions appear to be independent of the initial nitrogen distribution for composts older than about 3 months.

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DETERMINATION OF NITROGEN IN AGRICULTURAL MATERIALS BY THE NESSLER REAGENT.
II.*—Micro-determinations in Plant Tissue and in Soil Extracts

By S. H. YUEN and A. G. POLLARD

The total nitrogen content of plant tissue may be determined by direct nesslerization of sulphuric acid–hydrogen peroxide digests with accuracy up to 5%. Similar application to soil extracts involves somewhat greater errors.

In a spectrophotometric study of the Nessler colour, a filter showing maximum transmission at 420 mμ absorbs the greatest proportion of the colour.

In the sulphuric acid–hydrogen peroxide digestion of plant tissue, added ammonia is fully recovered but added nitrate suffers a loss of 24–47%, the percentage loss decreasing as the amount of nitrate present increases.

Bromothymol blue is a suitable internal indicator for the neutralization of test solutions before nesslerization. The blue colour of the indicator does not affect the absorptiometric measurement of the Nessler colour.

Soil solutions and soil extracts may be decolorized satisfactorily by a specially treated activated charcoal. This charcoal does not absorb appreciable amounts of ammonia from soil extracts made with Morgan's reagent, 2·5% acetic acid or water. For more accurate determinations of ammonia in soil extracts etc., distillation of the ammonia into acid in the presence of bromothymol blue, followed by nesslerization, is preferable.

A modified Nessler reagent that showed diminished susceptibility to interfering factors and could be used immediately after preparation has been described in a previous paper.[1] The present communication deals with the use of this reagent in the micro-determination of total nitrogen in plant tissue and of ammonia-nitrogen in soil extracts and soil solutions by direct nesslerization. Some observations on the effective use of light filters in the spectrophotometric measurement of the Nessler colour are included.

Spectrophotometric measurement of Nessler colour

In previous work it was shown that for the measurement of the Nessler colour a dark-blue filter OB1 (approximate maximum transmission at 435 mμ) afforded about three times the sensitivity of the light-blue filter OB2 (480 mμ).[1] A Unicam spectrophotometer, which has

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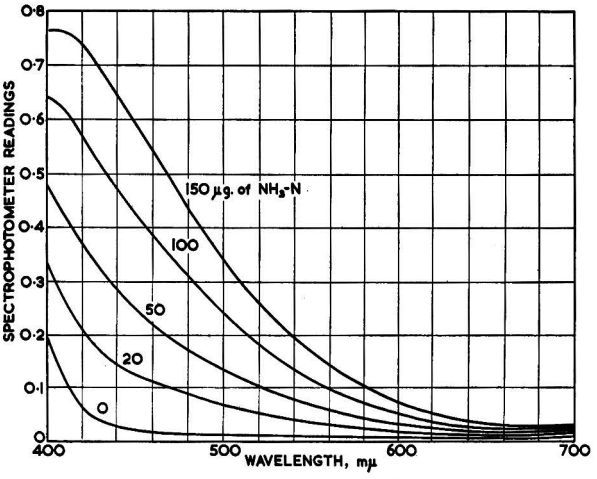

FIG. 1.—*Spectrophotometric study of Nessler colour*

since become available in this Laboratory, made possible a more detailed examination of the character of the Nessler colour with respect to the visible spectrum. To each of a series of 50-ml. graduated flasks, containing respectively 0, 20, 50, 100 or 150 µg. of ammonia-nitrogen, were added 1 ml. of sodium potassium tartrate solution and 2 ml. of Nessler reagent.[1] The readings at different wavelengths, recorded with the Unicam spectrophotometer, are shown in Fig. 1.

It was evident that the spectrophotometer readings for the Nessler colour increased steadily with decrease of wavelength from 600 to 400 mµ. With light transmissions between 600 and 700 mµ, the absorption of Nessler colour was apparently small. Owing to the high 'blank' obtained with wavelengths approaching 400 mµ, the absolute maximum readings for various concentrations of ammonia were actually recorded at about 420 mµ. This observation was subsequently confirmed by using an Ilford spectrum filter 601 (425 mµ) with a Hilger Biochem absorptiometer. This filter proved to be about 25% more sensitive than the dark-blue filter OB1 (435 mµ). Unfortunately it is too dense to give a full-scale deflection of the galvanometer.

Although the spectrophotometer was particularly useful for an investigation of this nature, the readings recorded at a specific wavelength were not strictly a linear function of the ammonia concentration.

Total nitrogen in plant tissue

Digestion of plant tissue.—Digestion of tissue with sulphuric acid and hydrogen peroxide followed by direct nesslerization has been used widely for determination of total nitrogen. Although a colourless solution is obtained rapidly by such digestion, the objection has often been raised that oxidation by peroxide causes a complete loss of nitrate, and under some conditions may possibly involve some loss of ammonia. In order to examine this possibility solutions containing various amounts of ammonia and nitrate (as ammonium sulphate and potassium nitrate) were transferred to 50-ml. conical flasks. The solutions were evaporated to dryness and known weights of plant tissue were added. The modified digestion for phosphate using hydrogen peroxide and sulphuric acid[2] was at first adopted (the whole procedure with additional operation is described below). The ammonia-nitrogen in the digests was subsequently determined by distillation by using the 1% boric acid method.[3] The results are given in Table I.

Generally, the digests with hydrogen peroxide–sulphuric acid showed consistent results for ammonia-nitrogen (1–2% error) by the distillation method. The added ammonia at both rates was satisfactorily recovered, with an error, which consisted of the combined errors of two determinations, of − 2 to + 5%. The added nitrate showed a loss of 23–50% after digestion, the relative loss increasing with the amount of nitrate added. Since the addition

Table I

Recovery of the added nitrogen in the H_2O_2–H_2SO_4 digestion of plant tissue (distillation method)

N added, mg.	Ammonia-nitrogen in 0·110 g. of plant tissue, mg.		
	Wheat straw	Rye-grass 1	Rye-grass 2
Ammoniacal (A)			
0	0·38	1·60	5·12
1·0	1·40 (102)	2·64 (104)	6·17 (105)
2·0	2·34 (98)	3·56 (98)	7·13 (100)
Nitrate (A)			
0	0·37	1·60	5·08
0·5	0·73 (72)	1·94 (68)	5·39 (62)
1·0	0·98 (61)	2·10 (50)	5·68 (60)
Nitrate (B)			
0	0·38	1·61	5·05
0·5	0·76 (76)	1·96 (70)	5·40 (70)
1·0	1·01 (63)	2·14 (53)	5·67 (62)

Figures in brackets are percentages of nitrogen recovered
A Once heating before oxidation
B Twice heating before oxidation

of nitrate in the absence of plant tissue showed a complete loss, a partial reduction of nitrate by organic carbon before the introduction of peroxide was clearly indicated. An attempt to reduce the loss further was made by twice heating the plant–sulphuric acid mixture until dense fumes were given off before adding hydrogen peroxide (B in Table I). This additional operation affected the recovery of nitrate only slightly, the loss of added nitrate ranging from 24 to 47%. However, no complete reduction of the added nitrate was achieved even by prolonged heating before oxidation. Since the nitrate content in plants, except in some young tissues, is negligible in comparison with that of organic nitrogen, and since most of it is reduced to ammonia by preliminary digestion, the loss of some of the nitrate may be disregarded for routine work.

Indicator for use in neutralization of acid digests.—In testing the neutralization of the digests litmus paper is commonly used. Tests were made, however, with indicators that gave a blue colour when alkaline on the assumption that the blue colour due to the indicator would not affect the measurement of yellow Nessler colour but would improve the sensitivity in neutralization. The following B.D.H. indicators were examined: bromocresol green (pH 3·6–5·2), azolitmin (5·0–8·0), nitrazine yellow (6·0–7·2), bromothymol blue (6·0–7·6), α-naphtholphthalein (7·3–8·7), thymol blue (8·0–9·6) and thymolphthalein (9·3–10·5). The tests were carried out as follows: to a 50-ml. flask, 2 ml. of sulphuric acid (1 : 49) was added followed by 1 drop of indicator, both in the absence and presence of ammonia. The solution was neutralized with 1% sodium hydroxide solution and subsequently nesslerized. It was observed that azolitmin, nitrazine yellow and thymolphthalein interfered with the Nessler reaction, but bromocresol green, bromothymol blue, α-naphtholphthalein and thymol blue gave promising results. An extensive comparison of these four showed that thymol blue gave a slightly greater blank, the end-point of α-naphtholphthalein was not very sensitive and there was practically no difference between bromocresol green and bromothymol blue, both of which have a sharp end-point and show no appreciable blank.

Comparison of the titration and absorptiometric methods.—A comparison was made of the distillation method[3] and nesslerization for determining total nitrogen in hydrogen peroxide–sulphuric acid digests of a number of plant tissues. The results are given in Table II.

Table II

Comparison of the distillation method and nesslerization; nitrogen, %

	Distillation method	Direct nesslerization
Wheat straw	0·33	0·38
Rye-grass 1	1·41	1·50
Rye-grass 2	4·57	4·50
Dutch bean	3·79	3·86
Dutch bean leaf	3·28	3·18
Potato leaf	3·35	3·52
Cabbage leaf	3·78	3·84
Swede leaf	3·77	3·61

Results from the two methods showed fair agreement, the deviation of Nessler values from those of the distillation method being ± 3·7%. This error includes that due to the method itself as well as that due to sampling. In the aliquot of the digest used for nesslerization (see Analytical procedure) cations other than ammonium (e.g. Fe and Mn) are insufficient to interfere with the determination. Although the upper limit of tolerance for sulphate is 500 p.p.m. in the determination, sulphate ions introduced in

2-ml. aliquots of the acid digest do not appear to affect the results appreciably. Sulphate may be added to standard solutions for calibration purposes to compensate for its effect on accuracy if present in test solutions in excessive amounts.

Analytical procedure

The preparation of ammonia-free water, sodium potassium tartrate solutions, Nessler reagent and standard solutions for calibration is described in the earlier paper.[1]

Finely ground dry plant tissue (100 mg.) is placed in a 50-ml. conical flask and 1 ml. of concentrated sulphuric acid is added. The flask is warmed over a low flame and, after being set aside for 10 minutes, is heated for a few minutes until dense fumes appear. This preliminary heating is repeated. The flask is cooled, 0·5 ml. of 30% hydrogen peroxide is added and the mixture is boiled gently for about 5 minutes. After cooling again the procedure is repeated, a further 0·2 ml. of hydrogen peroxide being added. The process is repeated several times if necessary. Usually, after a third heating the digestion is completed; the solution is then clear and colourless. When cooled, the solution is washed into a 50-ml. graduated flask and diluted to the mark.

An aliquot (0·5–2·0 ml.) is pipetted into a 50-ml. graduated flask, 1 drop of bromothymol blue (or bromocresol green) is added and the mixture is neutralized with 1% sodium hydroxide solution until slightly blue and then diluted to about 45 ml. Sodium potassium tartrate solution (1 ml.) is added with shaking and the flask is placed in a thermostat at $25 \pm 0{\cdot}5°$. When the contents of the flask have reached the temperature of the bath, 2 ml. of Nessler reagent is added. The mixture is made up to volume and shaken and the flask is then returned to the thermostat. The reading of the colour is taken 20–30 minutes after mixing, with a OB1 filter (435 mμ) and a 4-cm. cell in a Biochem absorptiometer.

The calibration curve is made by preparing a series of 50-ml. flasks containing 0–100 μg. of ammonia-nitrogen and 1 drop of bromothymol blue. After the solution is neutralized with 1% sodium hydroxide solution, the colour is measured, using the blank for comparisons.

Ammonia-nitrogen in soil extracts and soil solutions

Decolorization of the test solutions.—Owing to contamination with organic matter, both soil extracts and soil solutions generally show a yellowish coloration that renders direct nesslerization difficult. Decolorization may be effected by means of purified B.D.H. activated charcoal treated with sodium chloride.[4] The purified charcoal successfully removes colour from filtered Morgan reagent, 2·5% acetic acid and aqueous extracts, but does not affect the ammonia, as indicated in Table III.

The adsorption by the purified charcoal of ammonia-nitrogen from Morgan extracts and from 2·5% acetic acid is negligible. From soil solutions, the purified charcoal in amounts exceeding 0·2 g./25 ml. appears to adsorb an appreciable quantity of ammonia. The use of charcoal in proportion less than 0·1 g./25 ml. is advised in all cases.

Neutralization of decolorized extracts and soil solutions.—Morgan's reagent and 2·5% acetic acid have a considerable effect on the Nessler reaction chiefly owing to the increase of acidity. Soil extracts should therefore be neutralized before nesslerization. It was observed that for neutralization of this kind by 1% sodium hydroxide solution bromocresol green was not effective but bromothymol blue gave a sensitive end-point. The presence of neutralized Morgan reagent or 2·5% acetic acid up to 1·5 and 2·0 ml. respectively had no adverse effect on the determination.

Table III

Adsorption of ammonia-nitrogen from Morgan reagent, 2·5% acetic acid and distilled water by the NaCl-treated purified activated charcoal; recovery, %

Charcoal added,		Morgan reagent		2·5% Acetic acid		Water*	
g./25 ml.	NH_3-N, p.p.m.	10	50	10	50	10	50
0·1		101	101	100	100	98	100
0·2		100	100	100	99	96	100
0·3		99	100	99	101	96	99
0·5		96	99	99	100	95	98

* Reproduced from Yuen & Pollard[4]

Table IV

Ammonia-nitrogen in Morgan extracts, 2·5% acetic acid extracts and soil solutions

Plots		Original	Original plus added ammonia		
			Calculated	Found	Error, %
		Morgan extracts, N in dry soil, p.p.m.			
3	Dung	1·2	21·2	23·6	+ 11·3
4	NPK	24·6	44·6	44·0	− 1·3
8	Check	4·0	24·0	24·6	+ 2·5
9	Lime	3·8	23·8	24·0	+ 0·8
		2·5% Acetic acid extracts, N in dry soil, p.p.m.			
3	Dung	1·7	21·7	24·4	+ 12·4
4	NPK	22·0	42·0	41·2	− 1·9
8	Check	2·2	22·2	25·0	+ 12·6
9	Lime	2·4	22·4	24·2	+ 8·0
		Soil solutions, N in the solution, p.p.m.			
3	Dung	0·5	10·5	11·6	+ 10·5
4	NPK	10·5	20·5	19·8	− 3·4
8	Check	0·5	10·5	11·0	+ 4·8
9	Lime	0·3	10·3	11·3	+ 9·7

Owing, however, to the interfering ions present in the extracts an aliquot below 2 ml. is advisable. Soil solutions should also be neutralized before the test.

Analytical procedure and precautions.—The NaCl-treated purified activated charcoal[4] (0·1 g.) is added to 25 ml. of filtered soil extracts or soil solutions. The contents are left for about 10 minutes with occasional shaking and then filtered, using Whatman filter paper No. 40. An aliquot of the decolorized solution (1–2 ml.) is pipetted into a 50-ml. graduated flask and 1 drop of bromothymol blue added. The solution is neutralized with 1% sodium hydroxide solution. The normal procedure as for nesslerization is then followed.

For testing the validity of the technique, Morgan and 2·5% acetic acid extracts (soil : reagent, 1 : 2 ; 1 minute's shaking) and alcohol-displaced soil solutions[5] were taken from fresh soils from a series of long-term manurial-trial plots at the Harlington Field Station. Values for the ammonia-nitrogen in soil extracts and soil solutions and the recovery of added ammonia are given in Table IV.

As was expected, the ammonia contents of these normally cultivated soils, except those from the NPK plots, were rather low. The recovery of added ammonia from the extracts and soil solutions showed errors ranging from − 3·4 to + 12·6%. With the exception of the NPK plot all errors of recovery were on the positive side, with a mean value of + 8·1%. Maximum permissible quantities of aliquots were used since, except for the NPK plot, low concentrations of ammonium ions were present. It is therefore reasonable to suggest that the interfering ions in the test solutions affected the determination, and also that direct nesslerization is not applicable to certain soil types that contain large amounts of easily soluble iron and manganese.

Usually, large amounts of interfering cations present in the test solution cause higher readings in nesslerization, and in serious cases the nesslerized solutions develop turbidity.[1] In such circumstances direct nesslerization is not advisable, and a suitable alternative technique is to distil the ammonia in the extracts and soil solution in the presence of magnesia into 0·1N-hydrochloric acid containing 1 drop of bromothymol blue, the ammonia in the distillate being determined by nesslerization as before.

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FUMIGATION OF AGRICULTURAL PRODUCTS. VIII.*— Penetration and Sorption of Methyl Bromide in Wheat Fumigated at Reduced Pressures†

By A. K. M. EL NAHAL

Penetration of a sack of wheat by methyl bromide occurs readily at atmospheric pressure. Sustained-vacuum fumigation produced little improvement in penetration, but some increase in the residual fumigant was noted in this method. Vacuum fumigation with simultaneous admission of air and fumigant gave results in all material respects the same as were obtained by fumigation for the same period at atmospheric pressure. The ' air-washing ' procedure was found to be less effective than the low sorption of methyl bromide on wheat would suggest. Residual methyl bromide in vacuum-fumigated wheat has been investigated. The enhanced interstitial concentrations found in sacks of wheat fumigated under sustained vacuum with ethylene oxide and methyl bromide may be associated with desorption from wheat drying out under reduced pressures.

Methyl bromide is one of the fumigants most frequently used in current fumigation practice both at atmospheric and at reduced pressures.[1, 2] It is sorbed much less by stored products than is ethylene oxide or hydrogen cyanide, but, of the small residues left after airing the fumigated material, a rather higher proportion is so firmly retained as to appear chemically combined. Recently, Brown & Heuser[3] have discussed the penetration of methyl bromide under atmospheric pressure, sustained vacuum, and vacuum fumigation with simultaneous admission of air and fumigant into boxes of dates, with some consideration of the penetration into wheat and groundnuts. This work overlaps to a limited extent that reported here, but they reported a less comprehensive investigation for wheat than was made by them for boxed dates, and to complete the comparative investigation reported in Parts VI and VII of this series the results obtained with methyl bromide as fumigant are reported here.

Experimental

The design of the experiments and their practical conduct were exactly the same as reported for ethylene oxide in Part VII, except for the determination of the fumigant. Gaseous methyl bromide was determined by the catalytic combustion method described by Russell,[4] and the residues left after fumigation were estimated by Lubatti's technique.[5] The greatest differences between replicated samples were 1 and 3% at the high and low levels of dosage for the gas, and 0·5 to 4% for the corresponding residues of fumigant in the wheat.

Results and discussion

Fig. 1 (*a*, *b* and *c*) gives the concentrations of gas found in the free space and in the wheat during the three types of fumigation. In accordance with the usage of Page *et al.*[6] these methods are termed ' sustained-vacuum fumigation ', ' fumigation at atmospheric pressure ' and ' vacuum fumigation with simultaneous admission of air and fumigant '.

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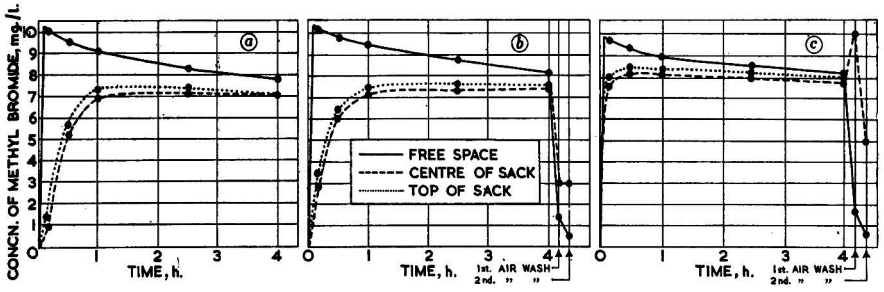

FIG. 1.—*Penetration of methyl bromide into wheat fumigated by three different methods*

(*a*) Fumigation at atmospheric pressure
(*b*) Vacuum fumigation with simultaneous admission of air and fumigant
(*c*) Sustained-vacuum fumigation

Methyl bromide penetrated readily at atmospheric pressure giving a penetration factor[3] of 64% after 4 h. This result is the more striking because it was obtained with wheat of 17% moisture content, this higher level being chosen for representation in Fig. 1 because only for such moist wheat was there any appreciable concentration gradient in the sack. Comparable penetration factors for ethylene oxide and hydrogen cyanide are 14·1 and 0·25%, respectively. Brown & Heuser obtained a penetration factor of 72% in the centre of a 140-lb. sack of wheat fumigated for 3 h. at atmospheric pressure.

When the drier wheat was fumigated under sustained vacuum the concentrations inside the sack built up to greater values than occurred in the free space. When this curious phenomenon was reported in Part VII, where it was encountered in experiments with ethylene oxide, it was associated with wheat of high moisture content. With methyl bromide, only dry wheat provided the high interstitial concentrations. This phenomenon is quite distinct from the enhanced concentrations obtained in wheat[3] when the pressure is restored during the course of a fumigation in which appreciable diffusion into the grain has occurred.

Although the improvement effected by the sustained-vacuum fumigation is shown by the curves in Fig. 1 (*c*), this improvement is, for methyl bromide fumigations of wheat, of little economic importance, a result in agreement with Brown & Heuser's findings.[3] As with the other two fumigants, vacuum fumigation with simultaneous admission of air and fumigant gave negligibly improved penetration compared with fumigations at atmospheric pressure. Tables I and II summarize the concentration–time products and amounts of residual fumigant found when methyl bromide is applied by these three methods.

The investigation of the ' air-washing ' procedure confirms that it is less effective in sweeping away the traces of fumigant from the interstitial spaces than might be expected. After methyl bromide was applied by vacuum fumigation with simultaneous admission of air and fumigant, the air-wash procedure is little more efficient than when ethylene oxide or hydrogen cyanide is the fumigant. The rise in concentration in the grain, when the pressure has been restored after a sustained-vacuum fumigation, is scarcely balanced by the first cycle of air-washing. This rise of concentration may be utilized by delaying the restoration of atmospheric pressure after dosage for a period and then continuing the fumigation under atmospheric pressure.[7] As methyl bromide is less strongly sorbed than hydrogen cyanide or ethylene oxide one might expect desorption to reduce the efficiency of air-washing much less. This expectation is only partly fulfilled, probably because the time of desorption even under ideal conditions is much slower than the period of air-washing.

Residual methyl bromide recovered from the fumigated grain is generally related to the concentration–time product attained at the site sampled. For example, the concentration of residual fumigant in the centre of a sack of dry wheat is higher, by whatever method of fumigation, than in wheat nearer the perimeter.

An analysis of covariance has been made to determine whether any of the factors investigated in this multifactorial experiment induced changes in the residual fumigant not fully accounted for by the associated changes of concentration–time product at the site from which the samples

Table I

Methyl bromide concentration–time products

Moisture content, %	Dose, mg./l.	Method of fumigation	Methyl bromide concentration–time products, mg.h./l.					
			Free space			Centre of sack		
			Fumigation period	Air washes	Total	Fumigation period	Air washes	Total
9·10	6	Atmospheric	22·69	—	22·69	19·74	—	19·74
9·00	10		36·98	—	36·98	32·94	—	32·94
9·05	14		53·38	—	53·38	48·22	—	48·22
8·80	6	Vacuum, with simultaneous admission of air and fumigant	23·34	0·60	23·94	21·01	0·72	21·73
8·90	10		38·18	1·00	39·18	34·76	1·18	35·94
9·20	14		51·81	1·40	53·21	46·43	1·69	48·12
8·85	6	Sustained vacuum	21·54	0·62	22·16	22·06	1·45	23·51
8·80	10		36·80	1·04	37·84	38·23	2·14	40·37
9·00	14		50·35	1·40	51·75	52·21	3·44	55·65
13·00	6	Atmospheric	21·70	—	21·70	16·39	—	16·39
13·00	10		36·21	—	36·21	30·59	—	30·59
13·15	14		48·25	—	48·25	41·18	—	41·18
13·00	6	Vacuum, with simultaneous admission of air and fumigant	21·98	0·57	22·55	18·60	0·75	19·35
12·95	10		37·19	0·97	38·16	31·17	1·22	32·39
12·85	14		50·62	1·33	51·95	43·49	1·66	45·15
12·95	6	Sustained vacuum	21·09	0·57	21·66	21·05	1·47	22·52
13·07	10		35·24	1·02	36·26	37·66	2·83	40·49
13·02	14		50·05	1·41	51·46	51·25	3·86	55·11
17·03	6	Atmospheric	21·60	—	21·60	16·37	—	16·37
17·15	10		35·01	—	35·01	25·75	—	25·75
17·26	14		49·51	—	49·51	38·42	—	38·42
17·10	6	Vacuum, with simultaneous admission of air and fumigant	21·50	0·56	22·06	17·26	0·82	18·08
17·05	10		36·10	0·93	37·03	27·21	1·34	28·55
17·07	14		51·37	1·31	52·68	41·00	1·89	42·89
17·07	6	Sustained vacuum	20·80	0·57	21·37	20·25	1·32	21·57
17·00	10		35·05	0·97	36·02	31·76	2·71	34·47
17·20	14		48·83	1·36	50·19	47·01	3·72	50·73

were taken. Whereas the corresponding analyses relating to the fumigation with hydrogen cyanide (Part VI) and ethylene oxide (Part VII) revealed only simple discrepancies of this nature, the results of the methyl bromide experiments are more complicated. Sorption of methyl bromide under reduced pressure is significantly more marked than at atmospheric pressure, when allowance is made for the differing concentration–time products under those conditions. It should not be thought, however, that because a factor attains significance in these extensively planned experiments, economic importance is on that account alone to be attached to it; the total sorption, rather than the fraction not consistent with the concentration–time product of fumigant attained, is the matter of economic concern. As there is evidence that reducing the total pressure does not, of itself, influence sorption on stored products[7, 8] this problem needs further investigation.

When the influence of moisture-content differences was examined in the covariance analysis a marked increase of sorption with moisture content was found. This increase is considerably in excess of that predicted by the reduction in the concentration–time product around the sample of wheat analysed. Moreover, the difference between sorption in the centre of the sack and that occurring towards its perimeter is significantly greater than the difference between the corresponding concentration–time products. One interpretation of these discrepancies is that, during the early part of the fumigation, sorption proceeds rapidly, particularly if the wheat is moist. At low pressures, however, the moisture content of the grain is progressively reduced by evaporation into the free space. As this loss of water will proceed from the perimeter of the sack inwards, and the sorptive capacity of the wheat decreases as it loses water, desorption is to be expected from the outer layers of wheat in the sack. In the absence of air currents high

Table II

Residual methyl bromide in wheat, p.p.m.

Moisture content, %	Dose, mg./l.	Method of fumigation	Residual methyl bromide, p.p.m. Top of sack Total	Centre of sack Total
9·10	6	Atmospheric	2·90	3·11
9·00	10		3·66	4·00
9·05	14		5·48	5·54
8·80	6	Vacuum, with simultaneous admission of air and fumigant	2·27	2·13
8·80	10		3·43	3·65
9·20	14		6·88	6·48
8·85	6	Sustained vacuum	2·48	2·71
8·80	10		4·00	4·33
9·00	14		6·07	6·97
13·00	6	Atmospheric	5·02	5·35
12·93	10		7·01	8·98
13·15	14		8·12	11·60
13·00	6	Vacuum, with simultaneous admission of air and fumigant	3·26	3·94
12·95	10		6·27	6·95
12·85	14		8·96	10·41
12·95	6	Sustained vacuum	3·23	4·87
13·07	10		5·29	8·55
13·02	14		7·50	10·41
17·03	6	Atmospheric	5·44	5·63
17·15	10		9·99	9·82
17·26	14		13·65	15·18
17·10	6	Vacuum, with simultaneous admission of air and fumigant	5·28	5·80
17·05	10		9·05	9·29
17·07	14		11·97	10·89
17·07	6	Sustained vacuum	5·66	6·92
17·00	10		7·73	11·05
17·20	14		14·01	17·85

concentrations will build up in the interstitial spaces. Jones[9] has shown that in a limited air-space, without replacement of the air, evaporation under reduced pressure is likely to be confined to the outer layers of the individual grains. This conclusion is reinforced by consideration of the limited amount of water (about 0·01% of the total weight of grain present) that could evaporate into the free space of the loaded chamber used in these experiments.

Unless the sorptive capacity of the surface of the wheat is modified during the experiment, a rise in the amount of residual fumigant should be balanced by a corresponding fall in the concentration–time product around the sample analysed. The increased sorption on the wheat of higher moisture content used in these experiments is just balanced by the associated changes in the concentration–time products when hydrogen cyanide is the fumigant. Hydrogen cyanide fumigations do not show the enhanced intergranular concentrations noted above at reduced pressures, but methyl bromide and ethylene oxide fumigations do. When these two fumigants are used, increased sorption on moister wheat is not accompanied by a corresponding fall in the concentration–time products attained near the sample, indicating that the gases have moved from the free space into the bulk of grain down the concentration gradient caused by sorption. This theory fails, however, to explain why the effects depend so markedly on the moisture content of the grain.

If the biological changes that affect the mortality produced by fumigations under reduced pressure are ignored, the results presented in this paper support the contention of Brown & Heuser[3] that vacuum fumigation of wheat with methyl bromide is a scarcely necessary refinement, in view of the excellent results obtained with fumigation at atmospheric pressure. However, judgment should be reserved for the products with more restricted intergranular spaces and for fumigants that penetrate less well because of heavy sorption on the commodity. When the biological effects of reduced pressures are considered, a balanced judgment of vacuum

fumigation becomes more difficult to reach. A review of the different factors that need to be considered in any particular instance will be provided when the results of other investigations, supplementing those reported here, are available.

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FUMIGATION OF AGRICULTURAL PRODUCTS. IX.*— Sorption of Fumigants at Reduced Pressures

By A. B. P. PAGE and R. E. BLACKITH

The adoption of vacuum-fumigation techniques alters the rate of penetration of fumigants into stored products. The sorption of fumigants by wheat and other seeds is known to depend on the concentration–time product attained at the position from which the sample for analysis is taken, and on the shape of the curve representing the rise and fall of fumigant concentration at that position. Reports of laboratory investigations suggested that reduced pressure did not influence sorption. Trials on a pilot scale have suggested that some influence was exerted. This discrepancy can be eliminated by allowing for the influence of reduced pressures on the distribution of fumigants in space and time within the commodity during pilot-scale trials. Reduced pressures, *per se*, do not modify sorption in the experiments analysed.

The sorption of fumigants on stored products during vacuum fumigations has been the subject of much speculation. Claims have been made that at reduced pressures sorption is reduced.[1–3] Other references indicate that it may increase.[4–6] Still other sources suggest that little or no difference is to be found.[7, 8] Some methods of vacuum fumigation give rise to better penetration of stored products by fumigants, so that, unless allowance is made for improved penetration, measurements of residues of the fumigant in the commodity may be misleading.[9, 10] Moreover, it is known that the relative contributions of concentration of fumigant and of exposure period to a given concentration–time product influence sorption.

A concentration that rises rapidly and falls asymptotically to a low value may describe a curve that encloses the same area over the time axis, yet gives a larger value for fumigant sorbed than one that builds up more slowly. Sorption isotherms for hydrogen cyanide published by Lubatti[11] show that wheat sorbs 1580 p.p.m. from 25 mg./l. applied for 46 hours, but only 620 p.p.m. from 7·0 mg./l. applied for 164 hours. In each instance the concentration–time product applied was 1150 mg. h./l. Similar results may be adduced from sorption isotherms of trichloroacetonitrile and of ethylene oxide on wheat,[12] and of methyl bromide on wheat and on onion seed.[13]

Bhambhani has shown that the sorption of hydrogen cyanide by grain weevils depends

* Part VIII: preceding paper

on the shape of the concentration–time curve in the same way as does sorption on wheat.[14] Many, though not all, examples of the alleged protective stupefaction of insects seem likely to be attributable to this phenomenon. For example, El Nahal[15] found that grain weevils in the free space outside a sack of grain were consistently more susceptible to hydrogen cyanide or methyl bromide at a given local concentration–time product than weevils buried in the grain, where the rate of ingress is slower. The form of the concentration–time curve cannot be ignored when assessing the results of vacuum fumigations.

In laboratory experiments Turtle has shown that the sorption of hydrogen cyanide on both wheat and sultanas at a sustained total pressure of 6 mm. for 24 hours is not appreciably different from that resulting from fumigation for a similar period at atmospheric pressure.[8] The partial pressure of the fumigant was the same in both sets of fumigations, but 24 hours is long compared with a representative period of 4 hours for vacuum fumigations. Turtle indicated that sorption should depend on the partial pressure of the fumigant, and not on the total pressure.

Brown & Heuser[7] found that the effect of sorption on the rate of penetration of methyl bromide or hydrogen cyanide into a bulk of material is unaffected by the total pressure at which the experiments are done.

These conclusions suggest that, when sorption is being considered, little importance needs to be attached to the total pressure during a fumigation. On the other hand, experiments by El Nahal, which made full allowance for concentration–time product differences but not for changes in the shape of the concentration–time curve, yielded different conclusions. El Nahal's conclusions agreed that sorption of ethylene oxide on wheat was independent of total pressure,[16] but suggested that, at reduced pressures, sorption of hydrogen cyanide and methyl bromide was greater than at atmospheric pressure.[10, 17] Moreover, Bhambhani[14] has shown that sorption of hydrogen cyanide on *Calandra granaria* is from 1·5 to 3 times as great at 2 cm. total pressure as at atmospheric pressure. The exact value depends on the way in which a given concentration–time product is presented to the grain weevils. The sorption isotherms for the insects were very similar to those found by Lubatti[11] for wheat, and sorption of hydrogen cyanide on wheat and on insects may be expected to follow a similar course at reduced pressures.

The economic importance of being able to distinguish between these apparently clear yet incompatible sets of interpretations is not restricted to effects that are sufficiently marked to be, in themselves, matters of economic concern. Any qualitatively established result, however unimportant quantitatively, is liable to influence decisions as to the adoption of expensive vacuum-fumigation equipment, decisions that have in the past been made partly on the basis of effects whose existence is doubtful.

Re-analysis of El Nahal's data

In the absence of a definite theoretical basis for estimates of the influence on sorption of changes of shape of the concentration–time curve, the only reliable method of assessing the part played by this effect seemed to be by means of a covariance analysis. El Nahal's data afford the only published record of a fully factorial set of experiments amenable to such analysis, and it was by this means that El Nahal was able to show that the changes in the concentration–time products attained in a sack of wheat did not always wholly account for the enhanced sorption at low pressures,[10, 16, 17] or for all the changes in the mortality of test insects.[15]

A complete re-analysis of El Nahal's data was therefore undertaken, so that adjustment could be made for contributions to the enhanced sorption in the form of a double covariance analysis.[18] The experiments using vacuum fumigation with simultaneous admission of air and fumigant were omitted from the analysis because the results were so close to those obtained at atmospheric pressure. Automatic allowance is made in the analysis for the correlation that exists between the concentration–time product at the site of sampling and the rate of penetration of the fumigant. This rate of penetration was used, in default of any clear theoretical indication to the contrary, as a measure of the differences of form of the concentration–time curves.

Results of the re-analysis

For clarity, the fractions of the difference between sorption at atmospheric and at sustained reduced pressure are shown in Fig. 1, in the form of percentages of the total sorption. This

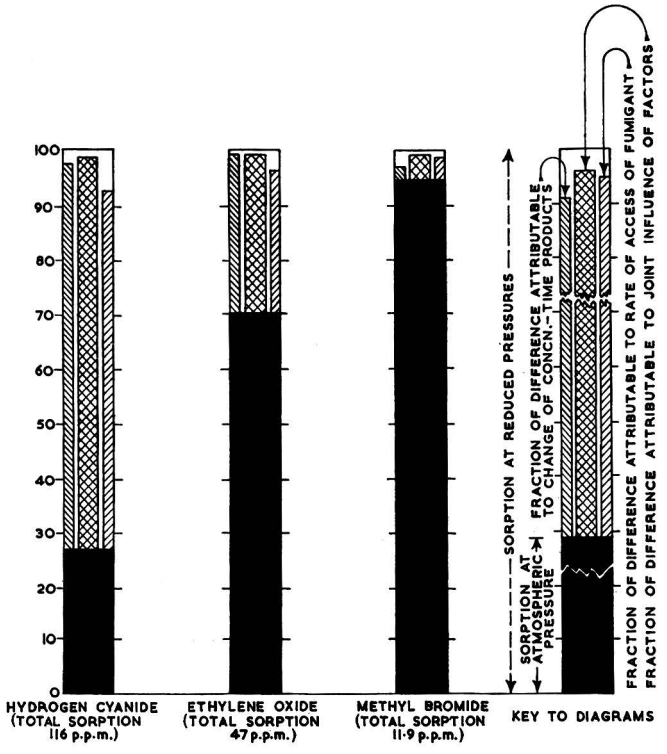

FIG. 1.—*Components of the increased sorption of three fumigants during fumigations at reduced pressures*

form of graphical representation involves some over-simplification of the results of the analysis, but it does focus attention on the most important features. The total sorption of hydrogen cyanide is so much greater than that of ethylene oxide or methyl bromide that if an absolute scale were employed for Fig. 1 the details revealed by the analysis would be obscured. The influence of sustained reduced pressure on either concentration–time products or rates of access to the centre of the sack account severally for most of the observed difference in sorption. Much information about this difference is common to both measures, which is another way of saying that their values are highly correlated. Some degree of independence is shown, however, and both these sources of variation together account for 99·52, 97·80 and 94·62%, respectively, of the observed difference in sorption of hydrogen cyanide, ethylene oxide and methyl bromide. Although the remainder is, as El Nahal has shown, significantly greater than the experimental error for the experiments with hydrogen cyanide and methyl bromide when only concentration–time products are used in the analysis, the joint use of both sources of information predicts the total change of sorption to within the expected sampling limits.

Discussion

The results of this re-analysis emphasize the importance of care in the choice of words to describe vacuum-fumigation experiments. It is true to say that, in the fumigation of a substantial bulk of a commodity such as wheat at sustained reduced pressure, sorption of any of the three fumigants is likely to be substantially greater than at atmospheric pressure. It is also true that sorption on wheat at reduced pressures appears to be no greater than at atmospheric pressure. Sustained-vacuum fumigation alters the temporal and spatial distribution of fumigant in a mass of grain so that sorption is enhanced.

The fact that more hydrogen cyanide can be recovered from grain weevils fumigated at

2 cm. total pressure than under otherwise comparable conditions at atmospheric pressure suggests that the margin of safety between the tolerance of a pest and that of the commodity it infests may be greater in sustained-vacuum fumigations. The changes in fumigant distribution are usually common to both commodity and infestation, but increased sorption at low pressures seems to be confined to insects. Moreover, the action of reduced pressures on the rate of loss of water from the insect can become important in relatively dry environments.[14, 19]

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APPARATUS FOR THE DETERMINATION OF HIGH CONCENTRATIONS OF METHYL BROMIDE IN FUMIGATION

By D. LOVEDAY

An apparatus is described for the determination of the concentration of methyl bromide in air over the range of 0–35 mg./l. The apparatus is based on the method of determination previously described by Call,[1] which uses the length of coloured stain, produced by interaction of bromine, as a combustion product of methyl bromide, with paper impregnated with fluorescein. In the apparatus described here, however, a swept-sample technique is used to obtain a representative sample, and buffered phenol red is employed as indicator.

Introduction

Methyl bromide now used to fumigate dried fruit is employed in concentrations up to 30 mg./l. An apparatus has already been described by Call[1] for the rapid estimation of low concentrations of the order of 0·01–0·1 mg./l. The principle of the method is the interaction of bromine, liberated by combustion, with a solution of a dye on a strip of filter paper covering a grooved block. The bromine vapour flows along this groove at a constant rate. The length of stain formed on the paper is proportional to the concentration of the gas. However, the method of sampling in this technique is not necessarily suitable for the estimation

of high concentrations, since the small volume of sample necessary to give a conveniently small stain would prohibit its use. The large volume of dead space in the apparatus and sampling line would in fact be comparable with the size of sample taken. The apparatus described here eliminates this difficulty by taking a swept sample.

Experimental

High concentrations of methyl bromide of about 35 mg./l. were first prepared in a steel fumigation-chamber of 470-l. capacity, and the concentrations inside the chamber were accurately determined by withdrawing samples in evacuated sampling tubes of 50-ml. capacity. The samples were passed over a hot platinum wire to decompose the methyl bromide,[2] and the bromine liberated was absorbed in caustic soda solution and determined by the argentometric potentiometric method of Russell.[3] Successively lower concentrations were prepared by evacuating the chamber to the desired pressure, followed by the readmittance of air.

In the apparatus to be described, a method of measurement similar to that of Call was used, i.e. after combustion the gas sample is drawn along a groove in a block of metal at a definite rate of flow and allowed to interact with a suitably sensitized paper pressed flat over the groove. The length of visible stain varies with the conditions and concentration but, provided that other factors remain constant, it varies only with the concentration of gas in the original sample.

Sampling by this method consists of two distinct operations: (1) The gas is drawn continuously from the chamber through a sampling tube for a standard length of time by means of a suitable pump. (2) It is then trapped by closing three-way taps at each end of the tube. This sequence is followed by rotation of the taps to an alternative position to permit (*a*) admittance of air at the end of the tube and (*b*) withdrawal, by a small centrifugal fan, of the resulting mixture of air and sample from the tube through the furnace and past the impregnated paper. By this means it is possible to obtain samples of small volume about 10 ml., without trouble due to dead space in the sampling line, which may be of a similar volume. A detailed explanation of the apparatus is given below (see also Fig. 5).

Factors affecting the length of stain

Concentration of gas.—Fig. 1 illustrates the relationship between length of stain and concentration of gas when a buffered phenol-red indicator with a gelatin-coated paper is used. Although a characteristic of the stains is a diffuse area at the end or 'tail', it is possible to

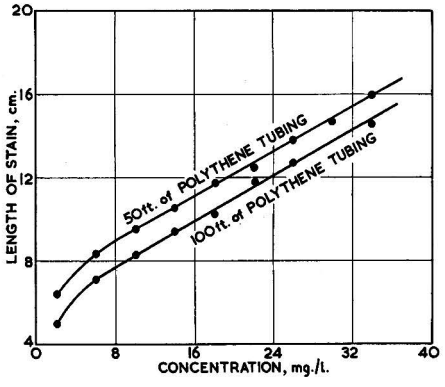

FIG. 1.—*Variation of length of stain with concentration of methyl bromide*

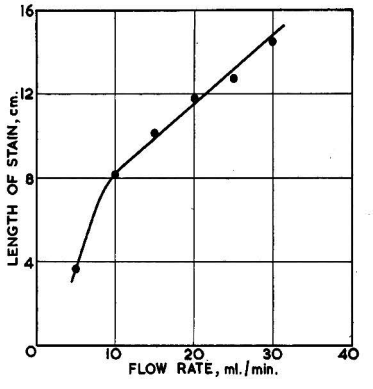

FIG. 2.—*Variation of length of stain with flow rate at a concentration of 10 mg./l.*

estimate their length to within 1 mm. and the points appear to lie close to a straight line. A similar relationship has been observed for concentrations between 0 and 80 mg./l.

Flow rate.—The variation in length of stain with the flow rate of the sample through the groove is shown in Fig. 2. Call has shown that the definition of stains is sharper with lower

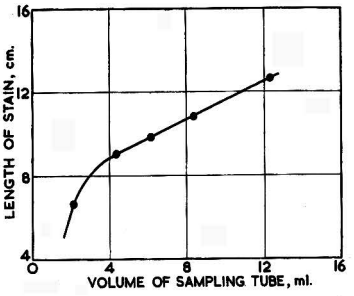

FIG. 3.—*Variation of length of stain with volume of sampling tube at a concentration of 26 mg./l.*

flow rates if fluorescein is used on the paper. This is also true for phenol red. With flow rates higher than 30 ml./min. the increase in length of the diffuse tail gives rise to errors in estimating the length and does not permit accurate measurement. Moreover, the over-all contrast of the stain decreases. The flow rate used in practice for giving a well-defined stain is about 15 ml./min. and it will be seen from the graph that maintenance of a stable flow rate is important for obtaining consistent results.

Volume of sampling tube.—It will be observed from Fig. 3 that the length of stain is not so sensitive to changes in the volume of sampling tube as to changes in flow rate. A cylinder of 12-ml. capacity and 0·45-cm. bore was found to be the most suitable size for giving stains of convenient length for concentrations of 30 mg./l.

Paper and indicator.—Different types of paper and indicator have been tested, including Whatman's No. 544 soaked in fluorescein. However, greatest contrast is secured with photographic bromide paper fixed with sodium thiosulphate, washed with water and soaked in phenol red in the presence of a buffer. Double-weight glossy paper (Ilford Bromide B3 1K) was found to be convenient, since the glossy surface enhanced contrast of colour and provided a good seal to the block when moist, thus preventing air leaks. The interaction between the bromine and phenol red absorbed on the paper is:

$$\underset{\text{Phenol red}}{C_{19}H_{14}O_5S} + 4Br_2 \rightarrow \underset{\text{Bromophenol blue}}{C_{19}H_{10}O_5Br_4S} + 4HBr$$

The pH range of colour change of phenol red is 6·8 (yellow)–8 (red) and that of bromophenol blue is 3·0 (yellow)–4·6 (violet). Since four molecules of hydrobromic acid are formed during the interaction it is necessary to establish a buffer on the paper in order to prevent the loss of the dark-violet colour of the alkaline form of bromophenol blue in the presence of excess of acid.

The most suitable concentration of phenol red in the bath for soaking the papers was found to be 0·1%, as this was sufficient to give a good contrasting violet stain against a yellow background at a buffered pH of 6. Any pH more acid than this tended to permit bleaching of the stain when high concentrations of bromine were present. The buffer used to attain this pH was sodium dihydrogen phosphate and caustic soda. The use of a solution that contained 20% (w/v) of sodium dihydrogen phosphate as buffer necessitated soaking the paper for a period of not more than three hours in order to obtain a sufficiently dense stain. However, such a concentration of the phosphate tended to salt-out the phenol red; hence a lower concentration, 3·5% (w/v), was found to be preferable, although this necessitated a soaking period of 48 hours.

The bath containing dye and buffer could be used for a week without trouble, but for longer periods it was found advisable to add 0·05% of *p*-cresol in order to prevent the growth of organisms capable of liquefying gelatin. This low concentration of *p*-cresol did not affect the stain. On preliminary investigation it was not found possible to store the papers at 100% r.h. after immersion in the bath since, although the papers remained moist, a definite alteration of the length of stain occurred from that previously obtained. Thus all papers had to be used immediately after withdrawal from the bath, followed by subsequent removal of surplus solution by pressing firmly between two sheets of filter paper.

The stains obtained with these papers were found to be permanent, fading being negligible, even in daylight, over a period of a year or more.

Combustion of methyl bromide.—Since the amount of methyl bromide in the sample taken by this apparatus is about 100 times greater than that taken by Call's apparatus, it was decided to use a rather larger furnace. This furnace is shown diagrammatically in Fig. 4 and

consists of quartz tubing A, of internal diameter 0·15 cm. and wall thickness 0·05 cm., wound with 34 turns of 34-gauge nichrome wire, B, with a spacing of 2 mm. between turns. This winding was surrounded by an outer asbestos tube, C, internal diameter 0·9 cm., and the intervening space was packed with asbestos wool. Catalytic decomposition of the methyl bromide was found to be possible by the use of a coil of platinum wire, D, 8 cm. long, inside the quartz tube. This permitted complete decomposition at about 700°, a temperature lower than that necessary in the absence of catalyst. The consumption of current at this temperature was 1·8 A at 20 V.

Water is formed as a reaction product and trouble was experienced from its condensation on cooled parts of the furnace tube leading to the block. This resulted in absorption of bromine from samples decomposed subsequently. The amount of bromine from each sample is small, approximately 0·3 mg.; thus the cumulative effect of water collecting over several determinations may be large. To eliminate this trouble the distance between the heated part of the furnace and the groove in the block was kept to a minimum, that is 3·5 cm.

Sampling line.—It has been found practicable to sample concentrations with this apparatus through quite long distances of polythene tubing instead of lead capillary previously used, since losses due to absorption are not significant by this method. The initial set of calibration stains for the instrument were obtained through 50 ft. of polythene tubing, internal diameter 1·5 mm., wall thickness 0·5 mm.

However, a second set of stains for the same concentration obtained by sampling through 100 ft. of wider-bore tubing (internal diameter 2 mm.) were found to have a slightly reduced length (cf. Fig. 1); thus it seems necessary, when calibrating the instrument, to use a length of tubing appreciably the same as that to be used in subsequent determinations.

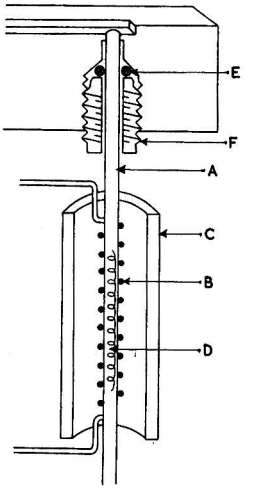

FIG. 4.—*Section through block and furnace*

Periods of sampling and measurement.—With a rotary pump and 50 ft. of polythene tubing, internal diameter 1·5 mm., the rate of flow of gas through the apparatus is approximately 1 litre/min. With this rate, 15 seconds is found to be an adequate sampling period, although for the first sample of a series it may be wise to increase this figure by 10 seconds in order to allow for initial absorption of fumigant. The length of time necessary to complete the second part of the operation, i.e. the actual measurement, was observed to be 60 seconds.

Extension to other gases.—Call has already made a brief survey of suitable impregnated paper for the estimation of other gases, including hydrogen cyanide and hydrogen sulphide. These papers may, on further investigation, prove to be useful for the estimation of high concentrations of such gases with the present method.

Constructional details.—The apparatus is shown diagrammatically in Fig. 5, and in practice all the components, with the exception of the exterior pump, may be assembled into a compact form in a wood or metal box. The apparatus consists of a tube, A, leading to a vacuum pump with a positive action, a motor and centrifugal fan, B, for providing suction for measurement of the sample, a furnace, C, the grooved block, D, with its pressure plate, E, between which plates the sensitized paper is placed, and three-way taps at F, G and H. The furnace has already been described. The block, 19 cm. × 2 cm. × 1·5 cm., was made of brass with the grooved face polished; the groove was 1 mm. wide, 1 mm. deep and 16 cm. long. Holes were bored through the block at either end of the groove, widening from 1 mm. to 1 cm. in diameter, to provide a means of connecting inlet and outlet tubes. A convenient joint (see Fig. 4) between the block and the quartz tube of the furnace is made by inserting the tube and rubber ring, E, into a sleeve under the block, and screwing in a collar, F, which squeezes the rubber ring around the tube thus holding it in position.

In order to prevent corrosion, the grooved block was lacquered with Bakelite lacquer No. L 3128. A substantial layer of this lacquer was found to be necessary and important,

since any metal exposed in the groove to the bromine vapour removed a considerable proportion by interaction and gave completely unreliable results. The pressure plate E may be kept under pressure on the block by means of a suitable spring-loaded lever. In the suction end of the block at I was included a filter packed with soda-lime in order to protect the metal parts of the apparatus from bromine vapour and dust. A needle valve and flow meter were inserted at J and K so as to maintain a suitable rate of flow.

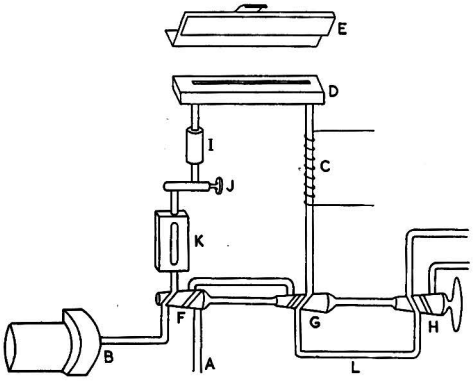

FIG. 5.—*Diagram of complete apparatus*

Although in the initial experiments three glass three-way taps were used, as shown in the diagram, it has been found possible to replace these by a tap with a single barrel constructed in metal (see Fig. 6). Care was taken to exclude as much grease and dirt from the barrel of

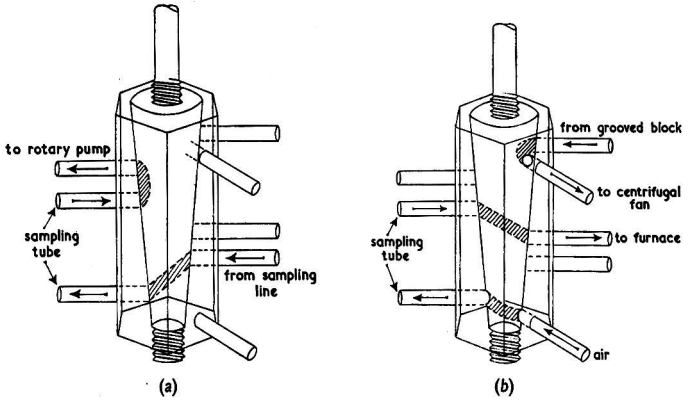

FIG. 6.—*Diagram of composite tap in two positions: (a) sampling, (b) measuring*

the tap as possible, while leaving sufficient lubrication for the tap to remain gas-tight. The two positions of the tap were defined by means of a spring-loaded projection engaging a cleft in the rim of a disc attached to the barrel. Sampling tube L (Fig. 5) was constructed of a length of copper tubing, 80 cm. long and 0·45 cm. in internal diameter, coiled into three compact turns.

In the use of this apparatus at the dockside, for example, an exterior form of suction was necessary that was positive enough to draw samples through lengths of tubing. In practice it was found possible to use the suction side of a tyre inflator that was built into the

lorry. However, over short lengths of polythene tubing, of about 10–20 ft., the small centrifugal blower used for measurement could be utilized for both sampling and measurement. This small blower was actuated by a 24-v a.c./d.c. motor.

Procedure.—The furnace is switched on before use in order to allow time for it to warm. Subsequently the centrifugal fan and rotary pump are started. The impregnated paper is pressed between filter papers and inserted between the pressure plate and the grooved plate. The tap is turned to the position shown in Fig. 6*a* so as to permit gas from the chamber to be drawn along the sampling line and straight through the sampling tube to the motor. After

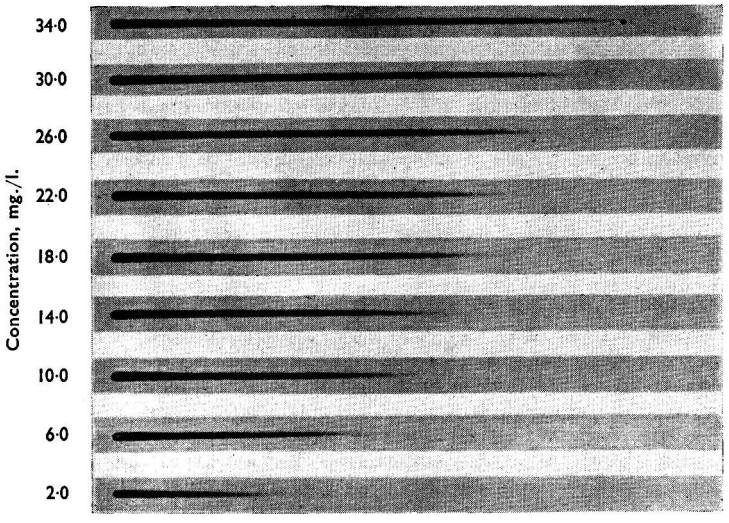

FIG. 7.—*Set of standard stains for high concentration of methyl bromide*

15 seconds, or sufficient time for a representative sample of gas to have passed through the tube, the tap is turned to the position shown in Fig. 6*b*, thus allowing air to displace the sample trapped and to permit its passage through the furnace and along the test paper placed over the groove. Measurement of the length of stains is unnecessary (cf. Call[1]) but they may be compared against a set of standard stains (Fig. 7). By this means it is possible to measure differences of concentrations of at least 2 mg./l. and the apparatus is simple enough for operation by unskilled workers.

Acknowledgments

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VEGETABLE OILS. III.*—*Mallotus philippinensis* Muell. Arg. Seed Oil

By R. C. CALDERWOOD and F. D. GUNSTONE

Kamlolenic acid is shown to be 18-hydroxyelaeostearic acid and the configuration of the α- and β-isomers is almost completely established. The component acids of *Mallotus philippinensis* seed oil are reported and the potential use of this material as a drying oil is discussed.

For some time now one of us has been engaged in a study of naturally occurring hydroxy-acids in the belief that the structure of these compounds might have some bearing on theories concerning the biogenesis of unsaturated acids in seed oils.[1, 2] Our interest was naturally aroused by a report on *Mallotus philippinensis* seed oil, which was said to contain an unsaturated hydroxy-acid designated kamlolenic acid.[3, 4] Subsequently Puntambekar[5] suggested that kamlolenic acid was not a hydroxy- but a keto-acid. In view of this discrepancy we resolved to investigate this problem; some of our results have already been reported in a preliminary communication[6] and in this paper we report the complete proof that kamlolenic acid is 18-hydroxyelaeostearic acid, together with the quantitative investigation of the component acids of this oil. While this work was in progress two additional papers on this subject were published in India,[7, 8] and these will be discussed below.

The composition of *Mallotus philippinensis* seed oil

Mallotus philippinensis Muell. Arg., also known as monkey face tree, or as kamala, and by several synonyms, including *Rottlera tinctoria* Roxb. and *Croton philippensis* Lumk, is a small evergreen tree found throughout tropical India. The fruit affords a powder, used locally as a dye and a drug, which is known to contain rottlerin.

The seeds, 100 of which weighed about 4 g., were brown or black spheres about $\frac{1}{8}$ in. in diameter; removal of the outer shell revealed a white kernel. Attempts to grind the seeds in a mill were not always successful because the crushed seeds readily matted and choked the mill (cf. [5]), and it was advisable, at least for the first extraction, to crush the seeds in a mortar. These were subsequently extracted with light petroleum (b.p. 40–60°) in a Soxhlet extractor. The resulting oil (15–20%) was dark-coloured, semi-solid at room temperature, and completely solid at 0°; it showed evidence of hydrolytic rancidity. Because of the instability of the highly unsaturated kamlolenic acid the usual precautions were observed. Some characteristics of this sample of kamala oil are compared with previously recorded values in Table I, from which it is apparent that the oil used in the present investigation is less unsaturated than that examined by other workers. The iodine value, of course, does not represent the true unsaturation of the oil because of the presence of conjugated unsaturation in one of the constituents.

The component acids have been determined by the method described by Hilditch & Riley[9] and Riley[10] for oils containing conjugated acids. The oil (67·3 g.) was hydrolysed, unsaponifiable

Table I

Characteristics of kamala oil

	Present work	Aggarwal *et al.*[3]	Singh & Saran *	Forest Res. Inst.†	Puntambekar[5]
Yield of oil,‡ %	15–20	about 24	—	—	22–24
Acid value	66·2	6·4	11·3	19·0	5·7
Saponification value	203·4	195·0	207·6	170·3	178·3
Iodine value (Wijs)	124·1	166·8	157·3	183·2 (Hanus)	183·2 (Hanus)
Acetyl value	—	16–44	47	49	49
Carbonyl value	—	nil	—	—	26·0
Unsaponifiable, %	2·6	1·7	1·9	1·8	1·8

* *Curr. Sci.*, 1942, **11**, 360
† *For. Res. India*, 1941–42, Pt. I, p. 92 } Quoted by Aggarwal *et al.*[3]
‡ These values represent the amount extracted with light petroleum and are based on the whole seed

* Part II: *J. Sci. Fd Agric.*, 1953, **4**, 129

Table II

Low-temperature crystallization and spectrophotometric examination

	Mixed acids	A	B	C	D	Total
Solvent						
Ether–light petroleum (− 20°)		insol.	insol.	sol.	sol.	
Methanol (− 20°)		insol.	sol.	—	—	
Methanol (− 45°)		—	—	insol.	sol.	
Weight, g.		16·33	5·62	13·67	13·75	
Percentage of total		33·1	11·4	27·7	27·8	
$E^{1\%}_{cm}$						
Unisomerized at 271 mμ	623	1120	1429	178	155	
234 mμ	63·5	98·0	141	20·2	29·4	
Isomerized at 268 mμ*	523	894	1156	151	142	
234 mμ †	244	140	201	92·3	524	
Component acids, % (wt.)						
Kamlolenic	35·6	64·0	81·7	10·2	8·9	
Linoleic	18·4	0	2·8	7·7	55·7	
Oleic + saturated	46·0	36·0	15·5	82·1	35·4	
Component acids, increments, % (wt.)						
Kamlolenic		21·2	9·3	2·8	2·5	35·8
Linoleic		—	0·3	2·1	15·5	17·9
Oleic + saturated		11·9	1·8	22·8	9·8	46·3

Isomerization carried out at 170°/15 min.* and 180°/60 min.†

material (1·73 g., 2·6%) removed[11] and the mixed acids (64·1 g.) were recovered. The acids (49·5 g.) dissolved in ether (250 ml.) and light petroleum (500 ml., b.p. 40–60°) were crystallized at − 20°, the crystalline fractions and the acids remaining in solution being subsequently crystallized from methanol (10 ml./g.) at − 20° and − 45° respectively. The four fractions so obtained were examined spectrophotometrically, before and after alkali-isomerization. The results are recorded in Table II, and Table III gives values of $E^{1\%}_{1\,cm.}$, some of which are required for the calculation of the results given in Table II. These values have been measured on pure α- and β-kamlolenic acids, prepared as described below.

The composition given in Table II has been confirmed and elaborated in the following experiments.

The quantity of hydroxy-acid may be derived from the acetyl value, which has been determined, not on the original oil that polymerizes when heated with acetic anhydride,[3] but on the mixed hydrogenated esters. Some oil (29·0 g.) was hydrogenated in ethyl acetate solution, with 10% palladium–charcoal as catalyst, until the separation of insoluble glycerides stopped the reaction. The hydrogenation product was hydrolysed, unsaponifiable material removed (2·6%), and the acids were esterified by refluxing with methanol containing a little concentrated sulphuric acid. A portion of the esters was then acetylated and the equivalent of the ester before and after acetylation determined in quadruplicate. These results (299·9 and 237·9 respectively) indicate an acetyl value of 56·6, which corresponds to 33·3% (wt.) of hydroxystearic acid or 33·0% (wt.) of hydroxyoleic acid. Although the acetylated esters had an iodine value

Table III

Absorption values ($E^{1\%}_{1\,cm.}$) for α- and β-kamlolenic acids

	λ, mμ	$E^{1\%}_{1\,cm.}$	λ, mμ	$E^{1\%}_{1\,cm.}$	λ, mμ	$E^{1\%}_{1\,cm.}$	λ, mμ	$E^{1\%}_{1\,cm.}$
Unisomerized acid								
α-Kamlolenic acid	234	165	262	1310	271	1750*	282	1380
β-Kamlolenic acid	—	—	259	1370	269	1880†	280	1460
Isomerized acid								
α-Kamlolenic acid (180°/60 min.)	234	215	—	—	—	—	—	—
α-Kamlolenic acid (170°/15 min.)	—	—	—	—	268	1380	—	—

Aggarwal *et al.*[8] give 1800 (270–270·5 mμ) * and 1990 (268 mμ) †

Table IV

Fractionation of hydrogenated, acetylated, methyl esters of kamala-seed oil

Fraction No.	Wt., g.	I.V.	S.E. (Sap. equiv.)	C_{14}	C_{16}	C_{18}	Hydroxy-C_{18}
1	3·15	0·8	267·9	0·25	2·90	—	—
2	2·97	7·1	278·4	—	2·05	0·92	—
3	2·97	13·1	295·1	—	0·30	2·67	—
4	2·92	11·9	296·2	—	0·19	2·73	—
5	2·99	9·9	296·4	—	0·19	2·80	—
6	2·78	7·8	295·6	—	—	2·74	0·04
7	3·02	6·3	295·2	—	—	2·97	0·05
8	2·67	6·7	229·0	—	—	1·47	1·20
9	2·60	7·5	187·8	—	—	0·33	2·27
10	2·46	20·7	212·0	—	—	—	2·46
Residue	3·46	—	—	—	—	—	3·46
Total	31·99			0·25	5·63	16·63	9·48
			Esters, %	0·78	17·60	51·99	29·63
			Acids, %	0·79	18·15	53·89	27·17
					19%	54%	27%

Note : The correction for the fact that the original oil contains unsaturated C_{18} acids rather than saturated C_{18} acids has not been made, since this will not affect the result expressed to the nearest unit per cent.

of 46·9 the ultra-violet-absorption curve indicated the absence of conjugated polyethenoid material. (The significance of these results is discussed below.)

The content of palmitic acid may be determined by fractional distillation of another sample of the mixed hydrogenated acetylated esters.[10, 12] There was evidence of some decomposition towards the end of the distillation. The results (shown in Table IV) indicate the presence of 19% of palmitic acid (including a trace of myristic acid).

Bertram oxidation[13] shows that the mixed acids contain only 18% of saturated acids.

A bromination experiment confirmed the absence of linolenic acid and the presence of linoleic acid in fraction D, and dihydroxystearic acid (m.p. 129–131°) was obtained by oxidation[14] of fraction C, proving that oleic acid was present.

Taken together, these results lead to the following composition for the mixed acids of *M. philippinensis* seed oil: palmitic acid 18, oleic acid 28, linoleic acid 18 and kamlolenic acid 36%.

Kamlolenic acid

α-Kamlolenic acid.—The mixed acids (6·4 g.) of kamala oil, crystallized from a mixture of ether and light petroleum (b.p. 40–60°) and then repeatedly from ethyl acetate, gave a sample of α-kamlolenic acid, m.p. 72–75° (literature,[8] 78–79°) (Found: C, 73·3; H, 10·1. Calc. for $C_{18}H_{30}O_3$: C, 73·4; H, 10·3%). The ultra-violet-absorption curve, which is very similar to that recorded for α-elaeostearic acid, shows three absorption maxima at 262 mμ, 271 mμ and 282 mμ (see Table III).

β-Kamlolenic acid.—The mixed acids (5·4 g.) were crystallized from a mixture of ether and light petroleum (b.p. 40–60°) and the resulting crystals, dissolved in benzene (20 ml.) containing a little iodine, were exposed to ultra-violet light for three hours. The material that separated from the cold benzene solution (1·25 g., m.p. 83–86°) was repeatedly crystallized from ethyl acetate. The melting point changed very little (85–87°, literature,[8] 90–91°) but the intensity of absorption rose to the maximum values given in Table III and then fell with further crystallization, probably owing to oxidation (Found: C, 73·3; H, 10·1%).

Ozonolysis of α-kamlolenic acid.—Ozone was passed through a solution of methyl α-kamlolenate (3·3 g. prepared from a 90% concentrate of kamlolenic acid) in acetic acid at about 10° for four hours. The contents of the reaction flask were then stirred and surrounded by cold water during addition of ether (50 ml.) followed by zinc dust (10 g.) and water (10 g.), portionwise and alternately. The bath temperature was raised to 50° during one hour and subsequently maintained at 100° for two hours. The solution was then decanted from the zinc and zinc acetate which was washed with water and with ether, and the solutions plus washings were extracted with ether, washed with water, potassium hydroxide solution, and water again, dried

and then distilled. The distillate (0·645 g., b.p. 70–80°/0·4 mm.) was methyl 8-formyloctanoate, which readily formed a 2 : 4-dinitrophenylhydrazone,* m.p. 53–55° (Found : C, 52·6 ; H, 6·0 ; N, 15·5. $C_{16}H_{22}O_6N_4$ requires C, 52·5 ; H, 6·1 ; N, 15·3%), and a semicarbazone, m.p. 107–109° (literature,[14a] 107°) (Found : C, 54·4 ; H, 8·1 ; N, 17·7. Calc. for $C_{11}H_{21}O_3N_3$: C, 54·3 ; H, 8·7 ; N, 17·3%).

Discussion

The structure of kamlolenic acid.—In a series of papers by Aggarwal *et al.*,[3, 4, 7, 8] it was finally concluded that kamlolenic acid is 18-hydroxyoctadeca-9 : 11 : 13-trienoic acid (I). (Other incorrect structures were suggested in the earlier reports and the correct structure was only described after the present investigation had been started.) This conclusion is based on the following facts : reduction gives 18-hydroxystearic acid after absorption of three mol. of hydrogen, oxidation with potassium permanganate affords azelaic acid, and the ultra-violet absorption indicates that a conjugated triethenoid system is present. These observations also permit another solution, in which kamlolenic acid is 18-hydroxyoctadeca-5 : 7 : 9-trienoic acid (II), but structure (I) is preferred because of the ' fact that in naturally occurring unsaturated acids unsaturation is generally found after the seven carbon chain attached to the carboxyl group '.[7]

$$HO\cdot[CH_2]_4\cdot CH{:}CH\cdot CH{:}CH\cdot CH{:}CH\cdot[CH_2]_7\cdot CO_2H \quad \text{(I)}$$

$$HO\cdot[CH_2]_8\cdot CH{:}CH\cdot CH{:}CH\cdot CH{:}CH\cdot[CH_2]_3\cdot CO_2H \quad \text{(II)}$$

$$HO\cdot[CH_2]_{10}\cdot CH{:}CH\cdot CH{:}CH\cdot CH_2\cdot CO\cdot CH_2\cdot CO_2H \quad \text{(III)}$$

Puntambekar[5] has questioned these results and, on the basis of a carbonyl value for both oil and mixed acids, has suggested that a keto-acid is present accompanied by elaeostearic acid or an isomer of elaeostearic acid. Oxidation of the ' solid unsaturated ' acids gave valeric and undecanoic acids, and, of various possible structures, 3-oxo-octadeca-5 : 7-dienoic acid (III) together with octadeca-9 : 11 : 13-trienoic acid (elaeostearic) was preferred.

Our results have confirmed those of Aggarwal *et al.* Like them we have been unable to obtain any evidence of carbonyl-group reactivity, and hydrogenation and permanganate oxidation have given similar results. Our experiments have already been reported briefly[6] and are not recorded further, since Aggarwal *et al.*[7] have now published full details of their experiments. We have now shown, however, that ozonolysis of methyl kamlolenate affords methyl 8-formyloctanoate, which is possible only from structure (I). Kamlolenic acid is accordingly shown to be 18-hydroxyoctadeca-9 : 11 : 13-trienoic acid, and the only point in doubt is the geometrical configuration of the double bonds. Oxidation and hydrogenation experiments show clearly that α- and β-kamlolenic acid differ only in geometrical configuration, and thus correspond to the isomeric forms of other long-chain conjugated acids such as elaeostearic, licanic and parinaric. Recent work[15, 16] has shown that α-elaeostearic acid is probably the *cis*-9 : *trans*-11 : *trans*-13-isomer whereas the β-acid has the *trans*-9 : *trans*-11 : *trans*-13-configuration. The method of preparation of β-kamlolenic acid indicates that one or more *cis*-bonds have been changed to the *trans*-configuration, and the lower values for $\lambda_{max.}$ observed in the ultra-violet-absorption spectrum of the β-acid suggest that this compound is more *trans* than the α-acid. Further evidence is available from the infra-red spectra which have been determined by Mr. N. H. E. Ahlers. Owing to the low solubility of these acids in the usual solvents the absorption in the region of 10 μ only was measured (carbon disulphide solution) but the results show the similarity between α- and β-kamlolenic acid and α- and β-elaeostearic acid respectively. The α-acid showed two peaks at 10·09 μ (extinction coefficient 1·35) and 10·38 mμ (0·383) [α-elaeostearic acid : 10·09 μ (1·425) ; 10·37 μ (0·416)],[17] whereas the β-acid showed only a single peak at 10·07 μ [β-elaeostearic acid : 10·06 μ (1·995)].[17] Commenting on the results, Ahlers reports ' α-kamlolenic acid has a *cis–trans–trans-* or a *trans–trans–cis*-configuration and β-kamlolenic acid has a *trans–trans–trans*-configuration '. Aggarwal *et al.*[7] have reported that α-kamlolenic acid formed a maleic anhydride adduct which, on oxidation, gave azelaic acid. On the basis of the stereospecificity of the Diels–Alder reaction this would indicate that the α-acid is 18-hydroxyoctadeca-

* S. Isikawa & A. Miyati (*Sci. Rep. Tokyo Bunrika Daig.*, 1939, [A]**3**, 257 ; *Chem. Abstr.*, 1940, **34**, 981) report a m.p. of 67–68° for this compound

cis-9 : *trans*-11 : *trans*-13-trienoic acid, but, as there is no proof that the liquid adduct did not contain α-kamlolenic acid, the configuration of the α-acid must remain in doubt.

The composition of Mallotus philippinensis *seed oil.*—The results obtained from the spectroscopic data (Table II) involve certain assumptions. The absorption at 234 mμ before isomerization is almost wholly accounted for by the kamlolenic acid that is present, and as the values obtained after correction for this factor are small, variable and spread over the four fractions, they have been neglected. The same applies to the absorption at 268 mμ after isomerization; the portion not accounted for by kamlolenic acid is small and variable and shows no increase in the fraction (D) in which this acid should concentrate. Linolenic acid is accordingly assumed to be absent and this is confirmed by our inability to isolate any hexabromostearic acid from fraction D. These small discrepancies might easily arise from a small error in the constants determined for α-kamlolenic acid.

The acetylation experiment indicates a value of 33% of hydroxy-acid as compared with 36% of conjugated trienoic acid. In view of the difficulties of working with highly unsaturated conjugated oils, these results are accepted as being in agreement and are taken to signify that kamlolenic acid is the only hydroxy-acid and the only conjugated acid present, though similar results would be obtained if the oil contained equal amounts of a non-conjugated hydroxy-acid and of a non-hydroxy conjugated trienoic acid (e.g. elaeostearic).

The content of palmitic acid (19%) as determined by distillation and hydrogenation is so close to that of the total saturated acids (18%) as to suggest that stearic acid is not present. These values, taken in conjunction with those in Table II, lead to the results already given.

Aggarwal *et al.* have not yet quoted any quantitative results though they do state that their oil contains only 12·3% of saturated acids, which appear to be a mixture of palmitic (66%) and myristic acids (34%).[3] Puntambekar[5] has reported the following: isomeric elaeostearic acid 38·4, polyethenoid keto C_{18} acid 25·7, linoleic acid 2·4, oleic acid 28·6, saturated acids 4·9%.

It has been reported[18] that until recently the Euphorbiaceae family was somewhat exceptional in containing some species that elaborate unusual acids in their seed fats, in particular ricinoleic acid, an unsaturated hydroxy-acid, and α-elaeostearic acid, a conjugated triethenoid acid. Although it is now known that hydroxy-acids and conjugated acids occur in the seed fats of other families, it is interesting to note that kamlolenic acid, the first naturally occurring acid to contain a hydroxyl group *and* a conjugated-triene system, has now been discovered in the seed oil of *Mallotus philippinensis*, which belongs to the Euphorbiaceae family.

Hilditch & Riley,[9] in a study of oils containing α-elaeostearic acid, have drawn attention to the absence of linolenic acid and suggest that these two acids do not occur together in the same vegetable fat; they have further reported that diminution in elaeostearic acid content is generally accompanied by an increase in palmitic acid content. Both these generalizations find support in the present results, for the oil appears to contain no linolenic acid and the relatively small proportion of kamlolenic acid (36%) is accompanied by 18% of palmitic acid, which is higher than usual for a highly unsaturated oil of this type.

Several reports from India[19] have suggested that kamala-seed oil is a drying oil, in some ways superior to tung oil. This is not what one would have predicted from the present results though, as already pointed out, the present sample seems to be less unsaturated than some others that have been studied, and it may be that there is a considerable variation in the composition of kamala oil, some samples of which would be more useful as a drying oil than others. The component glycerides of this oil have not been examined but, on the assumption that the rule of even distribution holds, one can say approximately that each triglyceride molecule will contain one molecule of kamlolenic acid, one of oleic acid and a third that would be linoleic or palmitic acid. This would lead to approximately equal quantities of palmito-oleo-kamlolenin and linoleo-oleo-kamlolenin. The latter compound, but not the former, might be a useful constituent of a drying oil. Hilditch[20] states that for an oil to be of use as a drying oil the total fatty acids should contain at least 65% of polyethenoid acids, and one of the polyethenoid acids should form 50% or more of the total fatty acids. Unless, therefore, the present sample is exceptional in its low unsaturation, it seems unlikely that kamala oil will find much use as a drying oil though it may usefully be blended with other drying oils, or be itself treated to give an improved drying oil.

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CHEMICAL WEED-CONTROL AND PASTURE PRODUCTIVITY*

By W. G. TEMPLEMAN

The principal weeds of grassland are unwanted grass species, and, although some possibilities are indicated, no chemical method for their removal in farming practice is yet available. The hormone weedkillers may be used for the control of a number of broad-leaved weeds. Figures are presented from the available information to show the increased herbage production that has resulted from chemical weed-control in grassland, and the need for good management is stressed.

The principal weeds of grassland both in this country and overseas are other grass species. As yet there are no chemical methods that can be adopted in farming practice for selectively killing unwanted grasses and leaving the desired species unharmed. For this reason, among others, the removal of weeds from both leys and permanent grass must generally be achieved by good management. In the worst cases this involves ploughing and reseeding, but in other cases grazing times and periods, stocking densities, combinations of cutting and grazing, the use of lime and fertilizers, drainage and the appropriate mechanical treatments may be arranged to increase herbage yields. Even so it is interesting to note that at Jealott's Hill in 1946 a mixed grass–clover sward was converted into a pure wild white-clover sward by autumn applications of 20–40 lb./acre of *iso*propyl phenylcarbamate (IPC). During 1952 in Colorado a useless

* Read at a joint meeting of the Agriculture Group and the Crop Protection Panel, 17 November, 1953; for a report of the Discussion at the meeting see *Chem. & Ind.*, 1954, p. 124

annual grass, downy brome (*Bromus tectorum*), was selectively eradicated from the valuable perennial grass, blue grama (*Bouteloua gracilis*), by spring or autumn applications of 12–24 lb./acre of *iso*propyl *m*-chlorophenylcarbamate (chloro-IPC) or 3 lb./acre of *p*-chlorophenyldimethylurea (CMU). These results are pointers to future possibilities.

There are, however, many broad-leaved weeds of grassland, and some of these are susceptible to the hormone weedkillers. With these weeds, too, it is often better to plough and reseed or introduce an appropriate system of management; on the other hand, there are many weedy areas that are unploughable or cannot be correctly managed. On these, MCPA (4-chloro-2-methylphenoxyacetic acid) or 2,4-D (2 : 4-dichlorophenoxyacetic acid) may be used. At the same time it must be remembered that the valuable leguminous components of pastures show some susceptibility to these weedkillers.

Many legumes are much more susceptible to MCPA and 2,4-D in the young seedling stage than when the plants are well established. Yellow charlock (*Brassica sinapis*), wild radish (*Raphanus raphanistrum*) and pennycress (*Thlaspi arvense*) frequently infest cereals undersown with mixtures of grass and clover seeds. These weeds can be readily eradicated with sulphuric acid, copper chloride, DNC (2-methyl-4 : 6-dinitrophenol) or dinoseb [2-(1-methylpropyl)-4 : 6-dinitrophenol] if the weeds can be attacked *before* the grass and clover seedlings have emerged through the soil, and no harm whatsoever comes to them. Frequently, however, the weeds and the grass and clover seeds germinate together. The question then arises whether MCPA and 2,4-D can be used, for they deal very satisfactorily with the weeds mentioned above. Table I shows the effect of MCPA on the clover content of young leys judged several weeks after the cereal harvest.[1]

Table I

Effect of MCPA (sodium salt) on clover content of leys undersown in cereals

Centre No.	Early application (clovers not emerged or just emerging)				Late application (clover seedlings established)			
	Spray		Dust		Spray		Dust	
	1 lb./acre	2 lb./acre	2 lb./acre	4 lb./acre	1 lb./acre	2 lb./acre	2 lb./acre	4 lb./acre
No weeds 1	o	o	o	—	—	—	—	—
,, 2	o	—	—	—	o	o	o	o
,, 3	o	o	o	o	..	..	..	..
,, 4	—	—	—	—	—	—	—	—
Weedy 5	o	o	o	—	+	+	—	—
,, 6	o	o	—	—	o	o	o	o
,, 7	o	—	—	—	o	o	o	o

— Seriously depressed
o Not appreciably affected
\+ Substantially increased
.. No application

It will be noticed that the rates of application were rather high and in some cases there was reduction of clover content, which was greatest with the early applications and with the higher doses. Several years' experience since these trials were undertaken shows that MCPA can often be used with benefit on undersown cereal crops infested by a susceptible species. For these conditions a low concentration applied in either high or low volume of solution may be used, and if spraying is delayed until weather conditions are good and the weed has plenty of foliage, which shields the well-established grass and clover plants, excellent results are usually achieved.

Permanent grassland that is weedy and cannot be ploughed or correctly managed to eradicate the weeds lends itself to the use of chemical herbicides. Before World War II, 'spot' treatment with weedkillers such as sodium chlorate was occasionally used. The discovery of the hormone weedkillers, which are translocated in plants and can kill perennial weeds, opened new possibilities in this field. Information is now available on the effectiveness of MCPA, 2,4-D and 2,4,5-T (2 : 4 : 5-trichlorophenoxyacetic acid) on very many weeds of many types of grassland all over the world. As an example, the results of 54 trials in this country carried out by Plant Protection Ltd. are summarized in Table II. The sodium salt of MCPA was used at approximately 2 lb./acre, applied as a low-volume spray, and it was studied both alone and

Table II

Effect of MCPA (sodium salt) on common grassland weeds

Weed		Number of sites where weed present	Control obtained					
Botanical name	Common name		Excellent	Very good	Good	Fair	Poor	Nil
Ranunculus repens	Creeping buttercup	39	4	2	22	8	3	—
*Cirsium arvense**	Creeping thistle	35	1	3	9	14	8	—
Juncus spp.	Rush	12	1	1	3	4	3	—
Senecio jacobaea	Ragwort	8	—	—	1	3	4	—
Taraxacum officinale	Dandelion	30	—	—	1	13	16	—
Plantago major	Plantain	33	—	1	17	13	2	—
Ranunculus bulbosus	Bulbous buttercup	2	—	—	1	1	—	—
Ranunculus acris	Crowfoot	5	1	1	1	2	—	—
Bellis perennis	Daisy	19	—	—	1	3	13	2
Chrysanthemum leucanthemum	Oxeye daisy	4	—	—	—	1	2	1
Rumex crispus	Dock (curled-leaf)	9	—	—	—	1	5	3
Rumex obtusifolius	Dock (broad-leaf)	2	—	—	—	—	2	—
Rumex acetosa	Sorrel	22	—	—	—	3	13	6
Polygonum persicaria	Redshank	5	—	—	—	—	3	2
Polygonum convolvulus	Black bindweed	1	—	—	—	—	1†	—
Convolvulus arvensis	Lesser bindweed	3	—	—	—	—	2	1
Achillea millefolium	Yarrow	18	—	—	—	3	6	9
Hypochaeris radicata	Cat's-ear	7	—	—	1	3	3	—
Prunella vulgaris	Self-heal	10	—	—	—	1	9	—
Carex spp.	Sedge	1	—	—	—	1	—	—
Equisetum spp.	Horsetail	1	—	—	—	1	—	—
Centaurea nigra	Hardheads	12	—	—	2	7	3	—
Potentilla anserina	Silverweed	8	—	—	—	1	3	4
Potentilla reptans	Cinquefoil	6	—	—	—	1	3	2
Lotus corniculatus	Bird's-foot trefoil	5	—	—	—	3	1	1
Matricaria spp. / *Anthemis* spp.	Mayweed	1	—	—	—	—	1	—
Leontodon spp.	Hawkbit	14	—	—	4	9	1	—

* Aerial growth

† Flowering suppressed

when a fertilizer dressing was also given. The type and rate of fertilizer given were appropriate to the individual sites.

Fertilizer treatment seemed to have little effect on the weed control achieved, but improved the sward in a number of trials. In only 15 out of the 54 sites did clover appear to suffer any setback, and in these it was only slight. It is now evident that well-established white or red clover (*Trifolium repens* or *T. pratense*) is much more resistant to MCPA than when in the young stages of growth.

Further trials were carried out by I.C.I. and Plant Protection Ltd. in 1951 and assessments made of the degree of weed control achieved in both years.[2] Table III summarizes these results, which demonstrate that the effectiveness of MCPA on creeping buttercup and creeping thistle in particular can persist for at least two seasons.

For arable crops such as cereals, flax, peas and similar crops it is a simple matter to devise experiments and determine the effect of weed control on crop yield. When enough accurate and properly conducted trials have been made the average benefit can be assessed. For grassland, however, the situation is different. The ultimate criterion to the farmer is what increase of beef, mutton or milk follows eradication of weeds. To get accurate figures from adequately replicated plots and animals is extremely difficult, if not impossible, and, so far as the writer is aware, no data of this kind are yet available. Trials have so far proceeded part way and a few workers have recorded herbage yields after weedkiller application. In the simplest case this means yields of hay. For example, Jealott's Hill undertook a series of nine trials in 1946–47[1] in which 2 and 4 lb./acre of MCPA (sodium salt) were applied in autumn 1946 and in spring 1947 to control creeping buttercup (*Ranunculus repens*) in grassland, and the hay yields were measured in 1947. A summary of these results is given in Table IV.

Although there are a number of reports of the effect of hormone weedkillers on clean stands of legumes and grasses, perhaps the most comprehensive trial reported so far where weeds were present in the pasture is that by Klingman at Lincoln, Nebraska.[3] The trial has so far run for

Table III

Grassland trials I.C.I./Plant Protection Ltd., 1950 and 1951
Analysis of weed-control results based on assessments of summer, 1951

Weed	Spraying year	Average frequency of occurrence at start	No. of sites sprayed	No. of sites where frequency of occurrence in summer, 1951				
				20% or less	21–30%	31–40%	41–50%	Over 50%
Creeping buttercup	1950	73	9	8	1	Nil	Nil	Nil
(*Ranunculus*	1950 and 1951	60	7	7	Nil	Nil	Nil	Nil
repens)	1951	65	7	6	Nil	1	Nil	Nil
Total			23	21	1	1	Nil	Nil
Creeping thistle	1950	82	5	3	1	1	Nil	Nil
(*Cirsium arvense*)	1950 and 1951	76	8	7	Nil	1	Nil	Nil
	1951	64	4	Nil	1	1	1	1
Total			17	10	2	3	1	1
Rushes (*Juncus* spp.)	1950	87	3	2	Nil	1	Nil	Nil
cut before spraying	1950 and 1951	85	2	2	Nil	Nil	Nil	Nil
Total			5	4	Nil	1	Nil	Nil
Rushes (*Juncus* spp.)	1950	85	2	1	1	Nil	Nil	Nil
uncut	1950 and 1951	88	4	3	Nil	Nil	Nil	1
Total			6	4	1	Nil	Nil	1

Table IV

Buttercup trials, 1946–47: summary of results

	Average yields (dry matter), cwt./acre					
	MCPA application, lb./acre					
	Autumn			Spring		
	0	2	4	0	2	4
Total useful herbage	17·6	22·8	22·4	19·0	18·8	19·4
Buttercups*	8·3	2·3	1·7	7·0	1·9	2·0
Clover*	2·4	1·8	0·5	2·8	0·6	0·3

* Average of centres where yields were recorded

Table V

Average total production from pasture at Lincoln, Nebraska,[3] given differential weed-control treatments, 1951–52; dry matter per acre, lb.

Treatment	Total production	Desirable grasses	Weed grasses	Broad-leaved weeds
Check	4469	1244	859	2366
Mown early June	3258	1165	773	1320
Mown early July	2970	1113	1027	830
2,4-D ester, 1 lb./acre, early June	4580	2168	1494	919
2,4-D ester, 1 lb./acre, early July	4412	1928	1658	826
Ploughed, resown smooth brome grass; sprayed 2,4-D ester	4471	2630	1594	247
Ploughed, resown intermediate wheat; sprayed 2,4-D ester	4808	1756	2717	335
Ploughed, resown warm-season grass mixture; sprayed 2,4-D ester	4356	2607	1309	440

two years and production data were obtained from caged areas. The main weeds were ironweed (*Verbena hastata*), hoary vervain (*Verbena stricta*), false boneset (*Kuhnia eupatorioides*), annual ragweed (*Ambrosia artemisifolia*), hairy chess (*Bromus commutatus*) and prairie triple-awn (*Aristida oligantha*). Results are shown in Table V.

Slaats & Stryckers,[4] in Belgium, have proceeded a stage further and measured the yield

Table VI

Pasture experiment at Melle, Belgium; [4] *percentage survival of* Taraxacum *and* Trifolium *and relative yields (control = 100)*

Sprayed 20 August, 1950
Weeds counted 20 October, 1950
Yield determined 12 June, 1951 (at fifth grazing after spraying)

Type of herbicide	Number of products	Active material, kg./hectare	% Survival		Relative yield, %
			Taraxacum officinale	*Trifolium repens*	
MCPA, Na salt	5	2	19·9	83·5	107·3
2,4-D, Na salt	11	2	27·2	83·0	111·0
2,4-D, amine salt	4	1	43·3	107·3	117·0
2,4-D, ester	4	1	21·8	82·8	114·1
Average			27·4	87·1	110·9

of herbage at the fifth grazing after weedkiller application (see Table VI). An increase of 10·9% resulted, but the statistical significance of this difference is not shown.

Reliable data on the utilization of the increased herbage produced are even scantier. Willis,[5] of the Hertfordshire Institute of Agriculture, has measured the amount of herbage ungrazed at the end of three months on plots infested chiefly with bulbous buttercup (*Ranunculus bulbosus*) and on those where it was controlled by MCPA or 2,4-D. Results are given in Table VII.

Table VII

Effect of closeness of grazing; weight of herbage ungrazed at end of approximately 3 months; Buntingford experiment

	Control	MCPA		2,4-D	
		1 lb./acre	2 lb./acre	1 lb./acre	2 lb./acre
Average weight of ungrazed herbage, cwt. dry matter/acre	15·6	12·8	8·2	11·0	9·2
Difference from control	—	2·8	7·4	4·6	6·4

Mean difference from control = 5·3 cwt./acre

Iorweth Jones[6] has also reported the increased stock-carrying capacity of range-land in Oklahoma after the removal of mesquite and sagebrush by a combination of mechanical treatment and 2,4-D. Results are presented in Table VIII.

Table VIII

Brush control on range-land, Woodward, Oklahoma

Mechanical treatment in June
2,4-D, 1 lb. in 3 gal. of Diesel oil/acre in following May
Results on 6-year average, 1946–51

	Natural-bush range	Cleared range
Acres/beast	9·7	5·7
Live-weight gain/beast, lb.	386	388
Live-weight gain/acre/annum, lb.	40	69
Profit/acre, $	4·50	7·70

The indications from these reports are that increased herbage production results from weed control but that, owing to the initial check to legumes in the sward, this benefit is not evident for a period after weedkiller application. It is also clear from general observations that good pasture management after chemical weed-control is essential to get optimum production and to prevent weed re-infestation.

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THE CONTROL OF WEEDS IN TURF*

By R. B. DAWSON

In Great Britain the turf grass crop accounts for about one million acres and is of considerable value. Much of it requires to be in good order throughout the year but surface quality, as distinct from grass quality, varies with the sport or game for which the turf is to be used.

Of all the problems associated with turf management, weed control is pre-eminent, since certain weeds are universal where grass is cut short. Short mowing determines the weed species that are able to persist, and by reducing ground-floor competition permits their increase. By good management a weed-free sward can be kept in this state for a long period, but the production of a weed-free turf is a problem facing most turf users. Methods of control involve mechanical operations, such as raking, and the use of chemicals, varying from the sulphates of ammonia and iron to the plant poisons and then to the growth-regulating substances or 'hormone' weedkillers. Methods of using all these are described, with data on the effects of repeated applications of the selective weedkillers for resistant weeds. The best conditions for use are also described.

Reference is made to 2,4,5-T (2 : 4 : 5-trichlorophenoxyacetic acid) for weed control in turf and also to growth-stunting chemicals such as maleic hydrazide. There is a possibility of controlling growth of long grass (on verges or in cemeteries, for example), but for fine turf there seems to be little likelihood of future application of such materials.

Grass grown as turf is as much a crop as any pasture or meadow. There are about a million acres in Great Britain growing turf for school games, racing, golf, football, cricket, bowls and so on, as well as private lawns. The value of this huge crop of turf cannot readily be assessed but it must obviously be high, whatever system of valuation is adopted.

Turf for games and sport is generally in use for most of the year. The attainment of the necessary standard presents many problems, and often through lack of care the quality of the crop (as represented by the playing surface) drops below that required.

Of all the problems in the proper management of turf, whether used for sport or merely ornament, perhaps weed control is pre-eminent. The problem is universal, since weeds occur under a wide range of soil and climatic conditions.

The commonest turf weeds are daisy, cat's-ear, rib-wort plantain, broad-leaved plantain, buck's-horn, plantain, hawkbit, buttercup, clover, yarrow, pearlwort and self-heal, and clearly there is a common factor influencing the establishment and spread of these weed species.

Mowing

The mowing factor operates on all forms of managed turf and its intensity and frequency have much to do with the spread of turf weeds. There are naturally other secondary factors that exert an influence, but mowing is outstanding. The habit of growth of turf weeds is such that they are able to survive and multiply, in spite of the cutting action of the mower blades.

* Read at a joint meeting of the Agriculture Group and the Crop Protection Panel, 17 November, 1953; for a report of the Discussion at the meeting see *Chem. & Ind.*, 1954, p. 124

The weeds escape the defoliation of the mowing machine which, at the same time, is removing ground-floor competition by ' selective ' removal of grass leaves and even the upper parts of grass shoots. The weeds under these conditions can increase either vegetatively or by seeding below the level of the sole plate of the mower.

Rosette weeds, such as daisy, cat's-ear, the plantains and dandelion, grow closely pressed to the ground, thus escaping punishment by the mower. Some species produce side shoots, which in turn develop into daughter plants, thus building up a closely packed colony. Such weeds are aggressive and the relatively large size of their leaves means that short mown grass competes on very unequal terms. With the mat weeds, for example clover, self-heal, yarrow, pearlwort, mouse-ear chickweed and creeping buttercup, there is an ability to adjust themselves to turf conditions by growth of shortened stems and leaves ; thus they too escape defoliation. They increase by overground or underground stems, so that the area of colonization gradually extends and gives increased competition against the grasses.

Height of cut, i.e. intensity, has an important bearing, especially with pearlwort and moss. Observation shows that pearlwort is rarely difficult where grass is cut at $\frac{3}{4}$–1 in. in height, but can be serious where the height is $\frac{1}{8}$ in. or $\frac{1}{16}$ in., a level not infrequent on some bowling greens. As to moss, a recent example is worth quoting. Two parallel and identical strips of turf were mown respectively at $\frac{1}{4}$ in. and $\frac{1}{8}$ in. After two seasons moss occupied 60% of the area on the $\frac{1}{8}$-in. cut and only 1% of the area on the $\frac{1}{4}$-in. cut. In practice very close cutting is to be deprecated, and best results are obtained by regular mowing, provided that it is not keen.

Weed-free swards

There are two main approaches to this problem of weed control in turf : first, management of swards already weed-free in such a way as to prevent invasion, or management to discourage and eliminate existing weeds ; secondly, cleaning up turf infested with miscellaneous weeds, a process that must obviously be followed up by methods of management designed to prevent reinvasion.

1. By starting with a weed-free turf it is possible to maintain it in this condition by regular but not too keen mowing, and, by appropriate fertilizer treatment, supplemented by applications of sieved friable compost, to maintain a uniform surface over which the mower will pass smoothly and at an even height of cut. Occasional spiking and wire-raking is necessary and lime or lime-containing materials must be avoided, except under special conditions, and the control of earthworms and other pests are necessary adjuncts. Certain experimental plots at the St. Ives Research Station, hand-weeded and de-wormed in autumn 1929 and spring 1930, have been maintained in weed- and worm-free condition for a period of 24 seasons without hand-weeding or other weed-control treatment.

Appropriate fertilizer treatment, plus reduction in intensity, but not frequency, of mowing, can materially reduce weed, and can, in some cases, lead to complete elimination in time.

2. Various methods are adopted for eradication of weeds from an infested sward : patching, hand-weeding, raking and the use of a variety of chemicals having a corrosive or even poisoning effect. Weed eradication in turf demands a combination of operations and can seldom be left to a single cure.

Mechanical methods.—Spread of surface creeping weeds can be checked by surface operations. For example, wire-raking clover runners before mowing and drag-brushing yarrow-infested turf ensure that the runners of clover and leaves of yarrow are largely removed by the mower, and this process helps also with the annual parsley piert. Wire-raking is also useful on mouse-ear chickweed and heath bedstraw, which is often found in heathy turf. Close scything of clover and yarrow is useful, and shaving with a tool not unlike a Dutch hoe, but having sharpened serrations, can help. There is always room for the weed fork to deal with isolated rosette weeds surviving from other treatments, and patching with plugs of clean turf is often the simplest way with small areas of mat weeds and Yorkshire fog (*Holcus* spp.).

Chemicals.—Where isolated weeds require attention on relatively small areas certain corrosive or poisonous chemicals can be used for ' spot ' treatment, for example common salt, sodium chlorate, copper sulphate, sodium arsenite, sulphate of iron and ' tractor vaporizing oil '. All these, when given in sufficient amount to kill weeds, can seriously affect turf grasses and even

retard recolonization of space formerly occupied by weeds. For large areas obviously some special treatment, not harmful to the grass, is needed. Possible materials include lawn sands (containing sulphate of ammonia and sulphate of iron), chlorates, arsenic acid and of course the selective weedkillers based on the synthetic growth-regulators MCPA (4-chloro-2-methylphenoxyacetic acid) and 2,4-D (2 : 4-dichlorophenoxyacetic acid). Experiments with low concentrations of arsenic acid and sodium chlorate show that, although good weed control can be achieved, temporary disfigurement is such that they cannot be widely recommended. On the other hand, lawn sands and selective weedkillers are suitable weapons if used intelligently, and the remainder of this paper deals mainly with them.

Lawn sands.—These consist of sulphate of ammonia and sand, or sulphate of ammonia, sulphate of iron and sand, and have a selective corrosive action on weeds; at the same time, however, the nitrogen encourages rapid growth of grass. In warm sunny weather many weeds can be controlled, though some, such as broad-leaved plantain, cat's-ear and some creeping weeds, are liable to escape, especially if the weather becomes unfavourable. We have concentrated on repeated light dressings applied under favourable conditions and have found that a wide range of weed species can be eliminated with minimum damage to the turf. Thus a mixture of 3 parts of sulphate of ammonia, 1 part of calcined sulphate of iron and 20 parts of carrier, when applied broadcast at from 4 to 6 oz./sq. yd. (10–16 cwt./acre) four or even five times in a season, will give good control of a number of weeds. For more resistant weeds the carrier should be reduced to 10 parts. In both mixtures the carrier should be bulky, thus making the mixture easy to sow. Combinations of sulphate of ammonia, sulphate of iron and sand have been widely used for pearlwort and mouse-ear chickweed. Mixtures of calcined sulphate of iron, dried blood and sand have also been successfully used. Calcined sulphate of iron, alone or with sand as a carrier, is also useful against parsley piert and moss, though with the latter weed the predisposing cause should first be ascertained.

Growth regulators

Perhaps the most outstanding development in turf management in recent years has been the widespread use of MCPA and 2,4-D for eliminating most common weeds with comparatively little detriment to the turf. These substances have not, however, removed the need for sound management, since re-invasion by weeds can soon take place where there is neglect. Nor have they wholly replaced the sulphates of ammonia and iron, or even the hand weeder, but they are a valuable weapon.

Latterly interest has been shown in 2,4,5-T (2 : 4 : 5-trichlorophenoxyacetic acid), originally suggested for dealing with brushwood.

MCPA and 2,4-D.—Rates of application have been the subject of much discussion, owing probably to comparisons with the rates used for annual weeds in this country and those used in the U.S.A. for perennial weeds. Our first experiments at St. Ives Research Station, made with commercial products, were designed to obtain maximum kill at one application and we used amounts of from 2 to 4 lb. of acid equivalent/acre for 2,4-D and up to 5 or even 6 lb. for MCPA, though with MCPA we considered 2 lb. adequate for susceptible weeds, e.g. broad-leaved plantain, under good conditions. Further plot experiments with compounds prepared from the pure acids were carried out. By using these products doubts that might arise as to the amounts of MCPA and 2,4-D applied as commercial products were dispelled. The plots showed that the original amounts could be reduced below those first envisaged, and it was concluded that 2 lb. of MCPA or 2,4-D/acre would be sufficient as a general-purpose application. Field trials and practical experiments prove that 2 lb. of acid equivalent is enough unless repeated application is ruled out, when a larger amount is desirable for dealing with the more resistant weeds, e.g. from 4 to 5 lb./acre. When ester formulations are used there is some risk in using 4 lb./acre and higher amounts are very liable to cause grass damage. With any of these, repeated applications at intervals of from three to four weeks are desirable for resistant weeds and two or three applications at 2 lb. are commonly better than single applications at 4 or 6 lb. Thus three applications of MCPA at 2 lb. with three weeks between gave 100% control of clover, one application of 6 lb. at the start gave 67% control and 6 lb. at the end of the period gave only 50% control. Full results are given in Table I.

The most successful results are obtained in May, June and July, but in practice it is found that good weed control can be obtained when there is active growth, and some good results have been obtained in September, or even early October. Late treatments leave little time, however, for the grass to grow into the spaces formerly occupied by weed. Best conditions are represented by lack of wind and rain, moist soil, and a warm and not too dry atmosphere. Under conditions of high humidity the effect of the weedkiller on leaves has been noticed in as short a period as one hour. Under conditions of drought damage to weeds may be scarcely more noticeable than damage to the turf. It has been found that a suitable dressing of fertilizer, preferably given some days before the weedkiller, is an advantage. Mixed fertilizer/selective weedkiller products have not been found unsatisfactory, though there was a time when one commercial product did cause concern by the damage created.

Much unnecessary attention has been devoted to the relation of mowing to treatment. In our experience the only requirement is that of refraining from mowing for 24 hours after applying the selective weedkiller. One case is known where a considerable area was mown one hour after spraying, with little apparent effect on the results obtained.

Table I

Treatment/acre	*Percentage control* Clover	Daisy	Dandelion	Plantain	Total, main weeds
3 Applications, 2 lb. MCPA (3 weeks between)	100	98	98	100	Almost 100
3 Applications, 2 lb. MCPA (6 weeks between)	86	100	98	100	97
2 Applications, 3 lb. MCPA (3 weeks between)	94	97	98	100	97
2 Applications, 3 lb. MCPA (6 weeks between)	85	100	96	100	95
1 Application, 6 lb. MCPA (at start)	67	87	69	100	81
1 Application, 6 lb. MCPA (at end)	50	70	72	100	67

Fully established plants of common turf grasses vary somewhat in susceptibility, but are sufficiently resistant for all practical purposes; however, newly established turf, whether from seed or sod, is not so resistant and should not be treated for at least three months after sowing or laying. The period allowed depends on progress made by the new turf. Where renovation with seed is called for after treating with 2,4-D or MCPA no difficulty has been experienced with sowings immediately following 2,4-D, but with MCPA occasional failures have been noted when sowing was followed within three to four weeks of application. Under practical conditions this difference is of little importance in turf management.

Efficiency of chemicals in controlling the various weeds of turf.—Annual weeds do not normally present a problem since they usually disappear with mowing, but knotweed is a noteworthy exception. This is common on grounds where muddy conditions have been created by winter play. The weed can be controlled by MCPA or 2,4-D if it is treated in the very young stages, but repeated applications are usually needed to destroy it completely. Since it is a weed of turf used for winter games, where there is only a short time between playing seasons for turf restoration as well as weedkilling, practical difficulties arise in arranging a spray programme in conjunction with the growing of new seedling grass plants. Another annual that has given difficulty on golf and bowling greens is parsley piert, which appears to be remarkably resistant; reference to it has already been made in this paper.

Of the commonly occurring perennials, most difficulty has arisen with pearlwort, wild white clover, yarrow, the speedwells and the mosses. Mosses are little affected and *Veronica* spp., in particular *V. filiformis*, are very resistant to MCPA and 2,4-D. Yarrow and clover can

be controlled with three or four repeated applications of either chemical as a spray though results are variable. In field trials two applications would give quite good results but in practice such success seems to be rare. The control of yarrow and clover seems to have been consistently less successful on a practical scale than on experimental trials. This can probably be ascribed to a number of factors, the chief of which is that more careful attention to efficient spraying is usually given in experimental work than on a practical scale. Pearlwort is undoubtedly resistant to 2,4-D but less so to MCPA, and can be eliminated with repeated applications of this chemical. For creeping buttercup MCPA is better than 2,4-D, but bulbous buttercup is resistant to both.

Experiments carried out on sea-marsh turf (this is lifted and transferred to bowling greens) are interesting, since certain special weeds are concerned; thus sea pink can be eliminated with either MCPA or 2,4-D, sea milkwort can be eradicated with MCPA as spray, but MCPA as powder and 2,4-D as liquid or powder are not so effective. The sea plantain and buck's-horn plantain are both susceptible to each weedkiller.

In general, applications of selective weedkillers are more efficient when given as sprays than as powders or dusts, but powders or dusts are at times very useful for local spot-treatment, since hand applications can be made directly to the weeds or weedy areas.

Our experiments indicate that within certain limits the amount of water used per unit area does not matter greatly provided that the correct amount of active principle is applied. In practice it would seem likely that 20–30 gal./acre is suitable, but there are indications that a rather higher amount of water may be better against mat weeds. Many of the larger areas of sports turf are now treated under contract and, of course, the cost per acre varies considerably according to the amount of ground at any one site. For areas of about 30 acres the cost would be about £3 10*s*. 0*d*.–£3 15*s*. 0*d*. per acre per application.

2,4,5-*T*.—This chemical has received some attention during the last few years and a number of trials have been carried out at St. Ives Research Station with it as well as with MCPA and 2,4-D admixtures. The purpose was to test the material for the control of some of the weeds resistant to 2,4-D or MCPA.

Table II

Treatment/acre	Per cent. of area covered by Yarrow		Clover	
	28.8.52 After one spray	10.10.52 After two sprays	28.8.52 After one spray	10.10.52 After two sprays
A Control	29·9	19·0	31·3	18·1
B 1 lb. 2,4,5-T (amine)	18·5	7·0	2·4	0·4
C 2 lb. 2,4,5-T (amine)	15·3	3·4	0·5	0·0
D 2 lb. 2,4-D (amine) 1 lb. 2,4,5-T (amine)	10·0	1·1	1·0	Trace
E 1 lb. 2,4-D (amine) 1 lb. 2,4,5-T (amine)	9·1	2·0	1·8	Trace
F 1½ lb. 2,4-D (butyl ester) ¾ lb. 2,4,5-T (butyl ester)	9·1	0·3	0·9	0·0
G 2 lb. 2,4-D (amine)	8·7	0·6	5·2	Trace
H 2 lb. MCPA (amine)	14·1	1·6	2·0	Trace
I 2 lb. MCPA (amine) 1 lb. 2,4,5-T (amine)	12·3	2·2	0·4	0·0
J 1 lb. MCPA (amine) 1 lb. 2,4-D (amine)	11·2	1·6	3·6	Trace

In preliminary trials against clover, results seemed to be slightly better and the control was more prolonged when 2,4,5-T was used, but this early promise has not been fulfilled in the later trials. Table II gives the results. A comparison should be made between the results for 2 lb. of MCPA, or 2,4-D or 2,4,5-T after two sprays.

As has been found in other trials the results with 2,4,5-T were better in experiments than

in practice, but little advantage would appear to be gained by incurring the increased cost of the 2,4,5-T, especially when it is known that reinvasion, regardless of treatment, took place after 12 months. Results against knotweed have not been striking, and against *Veronica* spp. and pearlwort they have been poor. A fair amount of scorch of the grasses is caused by 2,4,5-T, especially with ester formulations.

A number of organizations have tried commercial mixtures of 2,4-D and 2,4,5-T against resistant weeds, but we have heard of no outstandingly successful results.

Precautions.—At this stage a warning about the dangers of drift of selective weedkiller used on sports turf to neighbouring farm or garden crops may be given.

Other chemicals.—Sodium arsenite has been claimed in the U.S.A. to be successful against clover. In our trials, rates of from 1 to 10 lb./acre gave considerable scorch and disappointing clover control. Although the grass recovered, the amount of scorch was so great that it would hardly be tolerated in this country. In this respect the results are similar to those secured with arsenic acid in earlier trials.

Growth stunting.—Trichloroacetic acid has been tested at amounts of from 5 to 25 lb./acre for growth-stunting purposes, but damage to grasses proved excessive. Recovery of grass was poor and recovery of weeds was decidedly better. Work has also been done with maleic hydrazide for stunting growth. Our trials suggest that for sports turf mown regularly there is little likelihood of successful future applications of this chemical. In trials in 1952 at two heights of cut, much damage was done and an increase in weed population and annual meadow grass resulted. There was undoubtedly an initial reduction in the amount of cuttings, but later in the season there was increased growth which was still apparent after 12 months. Trials with further plots treated in the spring of 1953 confirm that the initial suppression of growth was accompanied by disfigurement of the turf, and increased growth later on more or less compensated for the original suppression.

With road-side verges there have been more satisfactory results. Here the grass was longer, being 3 to 4 in., and plots treated with 2–4 lb. of maleic hydrazide did show stunted growth. Initial discoloration was marked, but perhaps this would not be unacceptable for this type of turf. Further trials may indeed show the value for growth control on verges and perhaps in places like cemeteries.

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THE CONTROL OF MOSSES IN LAWNS AND SPORTS TURF*

By R. V. BLANDY

Mosses are one of the commonest weeds in lawns and sports turf. The study of individual species, their methods of multiplication, and the situations in which they are found, is necessary before satisfactory control methods can be devised. Some moss species can be controlled by cultural means but, where this is not practicable, chemical methods have to be employed. The chemicals in the past acted only as a temporary palliative, but with the introduction of lawn sands containing calomel (mercurous chloride) a more permanent control is possible. The results of a trial with one lawn sand containing calomel is recorded.

* Read at a joint meeting of the Agriculture Group and the Crop Protection Panel, 17 November, 1953; for a report of the Discussion at the meeting see *Chem. & Ind.*, 1954, p. 124

Introduction

As a prelude to the study of the control of mosses in turf, it is necessary to have knowledge of the individual species of moss, their methods of propagation and, in particular, the conditions under which they thrive.

When it is realized that over 30 species have already been recorded in turf, the size of the problem will be appreciated. Just as certain flowering plant weeds are associated with particular soils, so the various species of moss are often indicative of soil conditions and type of management.

Mosses are much simpler in structure than either ferns or flowering plants. They never have roots, but many species have hair-like rhizoids by which they attach themselves to the substrate and obtain some water and nutrients. Their stems may be erect or creeping, and they branch in a variety of ways. They bear small, delicate, perfectly formed leaves which usually consist of only one layer of cells.

Mosses multiply either by spores or by vegetative means. Many species produce spores only rarely and the reproduction of these is almost entirely vegetative. Even those species that commonly produce spores when growing in undisturbed habitats do so very rarely, if ever, when growing in closely mown turf. Thus, although initial infection by some species may be by spores, the initial infection by many species and the spread within the lawn of them all is most certainly by some form of vegetative multiplication.

Any living fragment, such as a single leaf or a short length of stem, can, under suitable conditions, grow to produce a new plant and ultimately a new colony. The spread of moss throughout an area of turf, when once it is present, is greatly hastened by the constant mowing that is carried out on lawns and sports turf, an operation that cuts off and distributes small living fragments of moss.

When mosses are present in turf they are present in a vegetative condition for the whole year. Many leafy shoots persist through both the hot dry conditions of summer and the frosts of winter, even if some are killed. The persistence of mosses in turf is in part due to their extraordinary resistance to drought. Many species may remain alive for several years even when quite air-dry.

Strictly, moss species should be treated individually. It is more convenient, however, to consider the mosses in two groups: (*a*) the pleurocarps, which have a loose prostrate habit, and (*b*) the acrocarps, which grow in dense erect tufts. Most of the important lawn-weed mosses are pleurocarps. They tend to produce long branching horizontal shoots which can cover the ground quickly. Their rate of effective spread is increased by their reaction to adverse conditions (such as drought). Instead of dying back to a central point, such as the rootstock, as in some flowering plant perennials, they die forwards; the older parts die and the young shoot apices persist in a dormant state until conditions improve. This is another reason why these species colonize a piece of ground so quickly. These species will tolerate considerable shade but are relatively sensitive to drought. Thus they are found in greatest abundance under moist shaded conditions.

The pleurocarps are most obvious in turf in the early spring and late autumn, when both temperature and moisture conditions are favourable to growth. In summer, when the turf is kept short, growth is often limited by drought and the moss material often dies down, giving the brown patches that may become so noticeable.

The acrocarpous mosses differ in several respects from the pleurocarps. Their slow-growing erect stems are always attached to the substrate by numerous rhizoids, and repeated branching tends to produce a series of brush-like tufts or, with some species, a dense smooth green carpet. These species are typically more drought-resistant and require more light than the pleurocarps. They are to be found in more exposed places, and especially where the growth of grasses is very poor because of soil poverty or too close cutting.

There are two ways by which mosses may be controlled in turf: (*a*) by cultural means and (*b*) by the application of chemicals.

Cultural control

Some moss species are restricted to a narrow range of soil conditions, e.g. *Barbula fallax*

Hedw. to calcareous soils and *Pohlia nutans* (Hedw.) Lind. to extreme acid conditions. Species of this type may be eliminated by appropriate soil treatments. Elimination of one species by soil treatment, however, may produce only a temporary improvement, as there are other species that can invade the turf, whatever the soil pH is.

Some of the pleurocarps persist only where the turf is deep enough to afford them protection. More keen mowing will often eliminate them. Small acrocarps, e.g. *Barbula unguiculata* Hedw. and *Barbula convoluta* Hedw., on the other hand, are abundant only in a very short or thin turf on a soil surface that has been consolidated by rolling or treading. Measures taken to change the associated conditions will reduce or eliminate these mosses.

Raking out moss material is a common practice in turf management and can produce a local and temporary reduction in moss material. The effects, however, are short-lived and, as living moss fragments are effectively spread over a large area, raking may seriously aggravate the trouble. Raking can be usefully employed in conjunction with chemical treatment, since, by removing some of the living moss material, it increases the effectiveness of the chemicals applied in dealing with the remainder.

It has often been argued that moss growth is vigorous only where the competition from the turf grasses is low. It has been suggested that good turf management, aimed at promoting the growth of the grass, is all that is necessary to control moss. The extent to which grass can be encouraged, however, is limited by the fact that it must be kept mown; it cannot be allowed to exert its full competitive effect. Thus, there are many pieces of turf in which mosses still thrive even when cultural operations have gone as far as they can, within the limits set by usage, in encouraging the grass and discouraging the moss. On areas of this type a method of effective chemical control is invaluable. Such a method is available and is equally effective on areas where cultural conditions are not good.

Chemical control

Both potassium permanganate and ferrous sulphate have been used for the control of mosses. Potassium permanganate can best be applied to a turf that is old and matted at a rate of ¼–½ oz./gal. of water/sq. yd. This substance apparently not only checks the moss but, so it is claimed, stimulates the breakdown of organic residues.

Under alkaline or near-alkaline conditions moss can often be more effectively controlled by the use of ferrous sulphate. This can be applied at a rate of ½ oz./sq. yd., either in solution with water or in a dry form bulked with sand. Where soil conditions are poor, the chemical can be included in a general fertilizer or top-dressing.

These chemicals, however, will give only a temporary control of moss. When applied they scorch and kill the existing top growth of moss but, as soon as conditions are favourable, the rhizoids below the soil surface and any vegetative matter that remains alive will send out new growth and cause the reappearance of the moss. Thus, if the treatment is applied in May the control may remain effective only until the autumn, when moss growth again becomes rapid.

Recently, however, a new development has taken place which gives an effective and more permanent control of mosses in turf. Booer,[1] during the course of certain pot experiments, found that with a minute trace of mercury or mercury compounds present in soil heavily infested with moss spores no development took place. A small-scale trial was made on mossy paths to establish whether these mercury compounds would control the development of moss under normal conditions from both spores and rhizoids. The results confirmed Booer's original findings and also showed that the mercury compounds, among which was calomel (mercurous chloride), were equally effective. Calomel was, therefore, selected since it is reasonably cheap and classified as non-poisonous.

A further experiment was carried out to determine whether calomel would prevent the growth of the stems and other vegetative fragments. To do this a moss *Eurhynchium praelongum* (Hedw.) Hobk, one of the commonest species found in turf, was cut into fine pieces and spread evenly over the surface of the soil contained in seed boxes. A dressing of calomel was given at a rate of 1 g./sq. yd. No moss developed on the treated boxes, whereas moss established itself on the untreated boxes. This experiment demonstrated that calomel prevented the

development of the spores, rhizoids and vegetative fragments of this species of moss, and was an effective control of the methods by which the moss is able to establish itself in turf.

Experimental

Calomel was then incorporated with an ordinary lawn sand, containing 20–25% each of ammonium sulphate and ferrous sulphate, to give approximately 1 g. of calomel/sq. yd. when the combined lawn sand was applied at a rate of 4 oz./sq. yd.

A trial, replicated four times, was laid down to test this material for the control of moss, and compared with an untreated control. Each replicate comprised an area of 16 sq. yd. and the dressing was applied on 18 May, 1950, at a rate of 4 oz./sq. yd. After the treatment, the normal scorch produced by a lawn sand containing both ammonium sulphate and ferrous sulphate developed, but by 31 May the scorch effect had disappeared.

On 2 October, 1950, a moss assessment was carried out. Each replicate was divided into 64 identical units. Each unit was assessed separately and placed into one of the following groups: (1) abundant, (2) moderate, (3) slight, (4) trace, (5) nil. The results were:

Mean of units in each group, %

	Abundant	Moderate	Slight	Trace	Nil
Control	17·6	23·5	35·2	8·9	14·9
Treated	0·4	0·8	5·1	17·6	76·1

The plots were kept under continued observation until 1952, and there was no appreciable reinfestation of moss in the treated areas.

Discussion

These results showed that the dressing had effectively controlled the moss and prevented its reappearance for a complete season, thus demonstrating that it is now possible to control moss in lawns by a single annual treatment.

As the fertilizer effect is not always required, further experiments were carried out to determine the amounts of ammonium sulphate and ferrous sulphate just sufficient to kill off the aerial growth of the moss and combining this effect with the long-term control obtained by the introduction of calomel. Such a material could be used at any time of the year and so fit in with the management carried out in the upkeep of the turf for the particular game concerned.

Moss, however, is not only the problem of the greenkeeper. It can also be a serious problem in agricultural grassland, especially on hill-lands in the western districts of Great Britain. These moss-controlling materials are based on the necessity of applying a dressing of 1 g. of calomel/sq. yd.; this entails an expenditure of about £11 an acre, which is obviously uneconomic for the farmer. We have some evidence now to show that certain pleurocarp mosses are controlled with smaller applications of calomel. Further research is proposed on the quantities of calomel necessary to control individual moss species, the results of which may make their control in pastures an economic proposition.

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Conclusions.

Acknowledgments.

References.

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