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RESIDUE ASPECTS OF SOIL FUMIGATION WITH METHYL BROMIDE

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Summary The levels of inorganic bromide formed in soils previously treated with methyl bromide have been determined, and the persistence of the bromide ion in soil and the leaching effect of different degrees of flooding with water have been examined.

Cabbage, carnation, lettuce, radish and tomato plants grown in soils fumigated with methyl bromide were shown to accumulate bromide in the foliage. Studies on the distribution of bromide in carnation, lettuce and tomato plants indicated that more was retained in the older leaves. Relatively small amounts of bromide were found in cucumber and tomato fruit from plants taking up bromide from the soil.

Inorganic bromide at levels expected to be present in soils after treatment with methyl bromide at commercially applied rates was not toxic to cucumber, lettuce or tomato plants, but carnations were particularly sensitive to bromide and were damaged by levels as low as 5 - 15 p.p.m.

INTRODUCTION

With the increasing interest being shown in methyl bromide as a glasshouse soil sterilant, there is a consequent need to know more about the problems associated with the application of this compound. In soil, methyl bromide undergoes degradation with the liberation of appreciable amounts of inorganic bromide. The use of the sterilant may therefore introduce additional effects attributable to this breakdown product.

The present paper is concerned with the formation and persistence of inorganic bromide in methyl bromide-treated soils, its uptake by various plants and its influence on plant growth.

MATERIALS AND METHODS

Carnation and tomato plants were grown in beds of soil previously treated with methyl bromide at dosage rates of 0.5 lb/100 ft^2 (24.4 g/m^2) and 1.5 lb/100 ft^2 (73.2 g/m^2), respectively. Studies were also made of the uptake of bromide by carnation, cucumber, lettuce and tomato plants grown in pots of soil supplemented with a range of concentrations of potassium bromide.

Some of the plant material and soil samples were obtained from Luddington Experimental Horticulture Station, Warwickshire, through the kind cooperation of Miss E.A. Turner of that Station and of Miss M.H. Ebben of this Institute. A number of plant and soil samples were also generously supplied by Mr. D.J. Fuller of the Agricultural Development and Advisory Service, Ministry of Agriculture, Fisheries and Food, Leeds.

Plant material for analysis was dried at 85°C and milled. Soils (5 g), air-dried at 27°C, were eluted in a 13 mm diam column with 30 ml 0.1M KCL and the total extract used for analysis of inorganic bromide by a modification of the procedure of Turner (1964). Tissue samples (0.5 g or 1.0 g) were ashed with alcoholic KOH at 500°C overnight, the residue extracted with 30 ml water and, depending on the bromide content of the material, the whole volume or an aliquot taken for analysis by the above-mentioned method.

RESULTS

Levels of inorganic bromide formed in soil

Fumigation of soil with methyl bromide can lead to the release of substantial amounts of inorganic bromide. In glasshouse beds of loam or loam/peat mixtures (loam:peat, 8:2 and 5:5 v/v) exposed to the fumigant at the relatively low rate of 0.5 lb/100 ft^2 (24.4 g/m^2) under polythene sheeting for 2-5 days, soil bromide levels rose from 0.8 - 1.8 p.p.m. dry weight before treatment to an average of 18.6 p.p.m. over all the plots. Soil beds covered for five days had a slightly higher average content of 20.4 p.p.m., compared with 16.9 p.p.m. in the beds covered for two days only. In further experiments, the amount of bromide formed in a peat-rich compost (loam:peat, 2:8 v/v) was found to be more than double that formed in a compost low in peat (loam:peat, 8:2 v/v) or in loam alone.

Bromide determinations have also been made on soils (0-6 and 6-12 in cores) from a number of commercial holdings in Devon, Lancashire and Yorkshire, which had been fumigated with methyl bromide. The period between fumigation and sampling was 4-6 months, the soils varying from a fine sandy loam to a peaty loam. The individual findings are not strictly comparable because of differences in conditions and methods of application, subsequent cultivation procedures, etc., but they showed that treatment at the commonly used rate of 1.5 lb methyl bromide/100 ft^2 (73.2 g/m^2) led to the appearance in the soil of quantities of inorganic bromide ranging from 10.2 - 60.9 p.p.m.

Persistence of inorganic bromide in soil

Beds of soil in unheated glasshouses at Luddington E.H.S. were given different methyl bromide treatments and were subsequently planted with a tomato crop. Analyses of the soil (0-6 in cores) are summarised in Table 1:

Table 1

Inorganic bromide levels in methyl bromide-treated soil

Methyl bromide applied (lb/100 ft^2)	Period between fumigation and sampling (weeks)	Soil bromide (p.p.m. dry wt.)
0	-	0.8
1)	3.5	(9.5
1.5)		(11.9
0.5)	29	(1.8
1)		(3.0
2		(4.6
0.5	55	1.2
0.5)	80	(1.3
1		(1.2
2)		(1.5

Untreated plots contained less than 1 p.p.m. of bromide. This level was increased following fumigation, about 10 p.p.m. being present 3.5 weeks after the application. There was still a noticeably elevated level 29 weeks after treatment, despite an intervening summer watering regime, and the soil bromide was still slightly above the untreated value more than one year afterwards.

Removal of inorganic bromide from soil in any quantity requires substantial flooding with water. Table 2 shows the effect of different degrees of watering on the bromide content of 0-6 in cores from beds of loam/peat mixture (5:5, v/v), previously treated with 0.5 lb methyl bromide/100 ft^2 (24.4 g/m^2). More than three inches of water (14 gal/yd^2; 76.2 l/m^2), applied as far as possible in a single operation, was required to produce a reduction in bromide content of practical value. Flooding was somewhat less effective when the loam content of the beds was higher. Thus with a loam/peat ratio of 8:2, addition of 3 in and 6 in of water gave reductions of 41.5% and 72.4%, respectively.

Table 2

Effect of flooding on the inorganic bromide content of soil

Extent of flooding (inches of water)	Sheeting time (days)	Bromide content of soil (p.p.m. dry wt.) Before flooding	After flooding	Percentage decrease
0	-	18.0	17.1	5.0
1.5	(2	15.3	12.0	21.6
	(5	25.6	22.0	14.1
3	4	20.7	9.9	52.2
6	4	21.1	3.3	84.4

The movement of bromide in soil flooded with water was examined in more detail. Glasshouse beds of brick earth loam were fumigated with methyl bromide at the rate of 1.5 lb/100 ft^2 (73.2 g/m^2) and subsequently treated with 2.5 in of water, analyses for bromide being made at various depths down to 26 in. Fumigation resulted in an appreciable rise in soil bromide to a depth of at least 14 in (Table 3) and there was evidence of penetration of the sterilant to at least 26 in. The effect of flooding was most marked in the top 2 in of soil but was less so at lower depths due to the downward transfer of the bromide.

Table 3

Movement of inorganic bromide in soil following flooding

Depth of core sample (in)	Bromide content of soil (p.p.m. dry wt.) Before fumigation	Before flooding	After flooding	Percentage decrease
0-2	1.5	12.5	3.9	68.8
6-8	1.3	11.2	7.9	31.3
12-14	0.6	9.7	6.9	28.9
18-20	0.4	6.1	4.8	21.3
24-26	0.7	2.1	-	-

Accumulation of bromide by plants

A number of plants grown on methyl bromide-treated soil under unheated glass at Luddington E.H.S. were examined for their ability to take up inorganic bromide. As shown in Table 4 the accumulation in the foliage which occurred was more marked when the fumigant was applied at the rates of 1-2 lb/100 ft^2 (48.8 - 97.6 g/m^2) and when a shorter time had elapsed between fumigation and planting. Even quite small increases in soil bromide level resulted in noticeable increases in leaf tissue content above those of control plants.

Table 4

Bromide content of the foliage of plants grown in methyl bromide-treated soil

Methyl bromide applied (lb/100 ft^2)	Period between fumigation and planting (weeks)	Soil Bromide (p.p.m.)	Bromide content (p.p.m. dry wt.) Lettuce	Cabbage	Radish
0	-	0.8	39	14	37
	(31	1.8	426	876	841
0.5	(57	-	205	73	151
	(83	1.3	175	46	43
	(6	9.5	2320	2430	3080
1	(31	3.0	766	875	1148
	(83	1.2	43	91	45
1.5	6	11.9	2333	2562	4037
	(31	4.6	874	2037	2330
2	(83	1.5	65	351	180

(Age of plants at sampling: lettuce, 18 weeks; cabbage, 19 weeks; radish, 13.5 weeks)

Similar findings have been obtained with tomato and carnation plants. Tomato plants obtained from Luddington E.H.S. which had been planted one week after soil fumigation at the rate of 1 lb methyl bromide/100 ft^2 (48.8 g/m^2) had accumulated up to 735 p.p.m. bromide in the leaves after 17 weeks growth. In experiments at this Institute, tomato plants in soil fumigated with 1.5 lb methyl bromide/100 ft^2 (73.2 g/m^2) and then flooded with 2.5 in water, accumulated up to 1824 p.p.m. of bromide in the lower leaves after 8 weeks growth. The bromide content of the foliage decreased from the base to the top of the plants, concentrations of 418 - 696 p.p.m. being found in the growing tip.

Carnation plants grown in beds treated with 0.5 lb methyl bromide/100 ft^2 (24.4 g/m^2) accumulated up to 3900 p.p.m. bromide in the leaves 9 weeks after planting. As found for the tomato, the lower leaves contained considerably more bromide than the upper leaves or growing tip.

As illustrated in Table 4, concentrations of bromide in plant tissue generally reflected the level of bromide in the soil. This relationship has been further examined in a range of plants grown in soil supplemented with known amounts of potassium bromide. Relatively low levels of bromide in the soil, up to 5 p.p.m., resulted in foliage concentrations of 500 p.p.m. or less in tomato or carnation plants but over 1000 p.p.m. in lettuce. Higher soil levels of 50 p.p.m. bromide produced foliage values of 4500 p.p.m. in carnation and 10,000 p.p.m. in the case of lettuce.

Bromide accumulation in fruit

In view of the marked accumulation of bromide which can occur in the foliage of some plants, the analyses on tomato and cucumber plants were extended to the fruit.

Tomato fruit from plants grown on untreated soil had bromide contents of less than 1.5 to 28.5 p.p.m. of dried tissue (mean value for 19 fruit, 11.1 p.p.m.). Ripe fruit from plants grown on methyl bromide-treated soil (dosage rate 1.5 lb/100 ft^2; 73.2 g/m^2) ranged from 87.0 to 436.7 p.p.m. (mean of 23 fruit, 190.0 p.p.m.). Corresponding values for green fruit were 85.2 to 382.6 p.p.m. (mean of 40 fruit, 215.4 p.p.m.). There was no obvious relationship between the bromide content of the fruit and the position on the plant of the truss from which the fruit had been taken.

Information on the cucumber is limited to analyses of a series of plants grown in pots to which potassium bromide had been added. Fruit from plants in the absence of supplementary bromide contained 25 p.p.m. of dried tissue. For soils containing 5-15 p.p.m. bromide, fruit contents ranged from 19.4 - 26.7 p.p.m. and for soils containing 30-100 p.p.m. bromide the fruit content rose from 45 p.p.m. to a maximum of 91.6 p.p.m.

Phytotoxicity due to inorganic bromide

Experiments on plants grown in pots of soil supplemented with potassium bromide have shown that lettuce is particularly insensitive to the presence of bromide in the soil, as much as 5000 p.p.m. having no marked effect on growth. Over the range 5-100 p.p.m., within which soil levels resulting from methyl bromide fumigations by commercial applicators would be expected to fall, no detectable phytotoxic effects were observed in cucumber or tomato plants.

Carnation plants, on the other hand, are highly susceptible to the presence of bromide in the soil. Phytotoxic damage was obtained with soil levels of 15 p.p.m. and occasionally at lower levels, most of the plants recovering to make good regrowth after an initial check. However, few plants survived at levels of 30 p.p.m. or above. The symptoms observed were a chlorosis and shrivelling of the 4th, 5th or 6th pair of leaves from the base of the plant, first noticeable near the base of the leaf. These symptoms spread to higher pairs of leaves and in severe cases affected the growing tip.

DISCUSSION

The use of methyl bromide as a soil sterilant results in the formation of inorganic bromide due to the interaction of the compound with organic matter. Factors influencing the level of inorganic bromide produced in soil include (i) those determining the penetration and persistence of methyl bromide itself, namely the rate at which it was applied, the time of containment of the gas in the soil (the sheeting time), the extent of sorption on to soil components (particularly peat), soil compaction, moisture content and temperature; (ii) those affecting the subsequent chemical breakdown, e.g., the organic nature of the soil, its pH, moisture content and temperature.

The present results show that increased soil bromide levels are associated with a higher methyl bromide application rate and a longer sheeting time, as well as with a greater content of organic matter. In addition, treatment of soil with the sterilant at rates used in current commercial practice can result in inorganic bromide levels which result in appreciable accumulation of bromide in the foliage of some plants and which may lead to phytotoxic damage. Bromide can persist for a considerable period in soils and is lost only slowly as a result of normal cultural and watering procedures.

Substantial reduction of soil bromide levels requires extensive flooding of the soil with water (Drosihn et al, 1968). The results obtained here would suggest a minimum application of three inches of water (14 gal/yd^2; 76.2 l/m^2) applied in one operation, and preferably more depending on the amount of bromide initially present. Higher proportions of peat in the soil appear to favour the formation of increased levels of inorganic bromide, but aid in the more efficient leaching of the soil by water.

Uptake of bromide from soil treated with either bromine-containing fumigants or inorganic bromide has been reported for a variety of plants, e.g. beet, cabbage, carrot, lima and snap beans, lemon, orange, tobacco, tomato (see Martin, 1966), and more recently carnation (Drosihn et al, 1968) and wheat (Brown and Jenkinson, 1971). The present work provides similar data for cabbage, carnation, cucumber, lettuce, radish and tomato. Of these plants, cabbage, carnation, lettuce and radish were all found to accumulate bromide to a considerable extent. Furthermore, distribution studies in the tomato and carnation showed that tissue bromide concentrations were greater in the older and lower leaves. Similarly in the lettuce, the bulk of the bromide present was in the outer leaves, with relatively small amounts in the centre portion.

Fruit from cucumber and tomato plants grown on bromide-containing soils had higher bromide contents than fruit from control plants, but the accumulation was small compared with that in leaves. Maximum values for tomato fruit from plants grown in soil treated with 1.5 lb methyl bromide/100 ft^2 (73.2 g/m^2) were not more than 437 p.p.m. of dried tissue, which is equivalent to 28 p.p.m. on a fresh weight basis. Comparable values have also been obtained by O.F. Lubatti and N.G.M. Hague (personal communication). Low bromide contents were also found in cucumbers grown under similar conditions. These products would therefore contribute only small amounts of bromide to the average diet.

Phytotoxic damage due to the presence of inorganic bromide in soil has been observed in a number of plants (Martin, 1966). Although this does not appear to constitute a problem in the cultivation of lettuce, tomato and cucumber, carnations are markedly affected (Drosihn et al, 1968; Scholten, 1968,) and the growth studies described above indicate that damage can occur at soil levels as low as 5 - 15 p.p.m. Consequently, unless the bromide content of soils fumigated with methyl bromide can be appreciably reduced by adequate flooding, there is a risk of incurring crop losses in the subsequent planting of carnations.

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CONTROL OF BLACK ROOT ROT OF CUCUMBER (PHOMOPSIS SCLEROTIOIDES) WITH METHYL BROMIDE

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Summary Soil naturally infested with black root rot of cucumber (Phomopsis sclerotioides) was fumigated with methyl bromide at various concentration time products (C.T.P's). A C.T.P. of 4530 mg h/l. gave a significant increase in number and weight of cucumbers cropped, and a significant decrease in root infection.

INTRODUCTION

Ebben and Last (1968) fumigated Phomopsis infested soil at ca. 2 lb/100 ft^2 (90 g/m^2) for 96 hours and obtained a significant increase in wieght of cucumbers cut after 3 months cropping, but Wiggel and Simpson (1969) reported that methyl bromide applied at 1.5 lb/100 ft^2 (73 g/m^2) for 72 hours neither increased yield nor controlled root infection after 3 months of cropping.

In this paper the effect of methyl bromide on P. sclerotioides is investigated. The infested soil was fumigated in pots in a fumigation chamber.

METHOD AND MATERIALS

Phomopsis infested soil obtained from a glasshouse in the Vale of Evesham was fumigated at field capacity in 10 in clay pots. Fumigations were done in two types of metal chamber. In one of 5500 l. the methyl bromide was introduced by vapourisation via a dosing device from a 40 lb cylinder. In the smaller chamber (345 l.) the methyl bromide was injected by syringe. Methyl bromide in a 150 ml Erlenmeyer flask was cooled to - 18°C by solid CO_2, in a large thermos vessel, and injected through a sealed silicone rubber placenta by means of a cooled, graduated 10 ml hypodermic syringe. Details of the fumigations done are given in Table 1.

Table 1

Fumigation details

Experiment	Chamber Volume l.	Concentration MeBr mg/l.	Time h	C.T.P. mg h/l.
1	5500	63	24	1512
	5500	63	48	3024
	5500	63	96	4536
2	345	47	24	1132
	345	47	48	2265
	345	47	96	4530
3	345	47	96)	
	345	94	48)	4530
	345	189	24)	

Two week old seedlings of cucumber

In Experiment 1 replication was fourfold. In Experiments 2 and 3 replication was threefold and each experiment was done twice. The pots were aired in the glasshouse for one week, when two week old seedlings of cucumber "Pepinex" were planted. The plants were cordon grown and fed with an organic summer fertiliser (Solufeed) diluted $\frac{1}{250}$ at every watering. Upward growth of plants was stopped at 8.2 ft (2.5 m).

In each experiment plants were mulched with Levington compost after six weeks. After 12 weeks of cropping the percentage of root rot was assessed by the Root Rot Index of Wiggell and Simpson (1969).

RESULTS

Results of Experiment 1 are given in Table 2.

The plants were attacked by red spider mite and infected with mildew, neither of which was controlled. Although the yields were low, it was clear that Phomopsis was controlled at 4536 mg h/l., and there was a satisfactory increase in yield.

Table 2

Effect of methyl bromide on yield of cucumbers and incidence of black root rot

C.T.P. mg h/l.	Percentage increase or decrease in yield of cucumbers		Percentage root rot
	Weight	Number	
0	-	-	81.4
1512	- 33.1	- 27.3	92.5
3024	+ 8.9	+ 9.6	74.3
4536	+ 60.2	+ 54.7	48.7

Mean control yield per plant 4.0 cucumbers (1.2 kg)

In Experiment 2 the range of C.T.P's was similar to those in the preliminary experiment. Cucumber yield at 4530 mg h/l was more than double that of the untreated control (Table 3). There was also evidence that larger cucumbers were produced when the root rot was controlled.

Table 3

Effect of methyl bromide on yield of cucumbers and incidence of black root rot

C.T.P. mg h/l	Percentage increase or decrease in yield of cucumbers		Percentage root rot
	Weight	Number	
0	-	-	94.9
1132	- 37.0	- 35.2	95.8
2265	+ 25.3	+ 8.2	84.9
4530	+154.4	+121.6	46.6

Mean control yield per plant 6.2 cucumbers (1.7 kg)

The pattern of cropping is shown in Fig. 1.

In chamber fumigations to control insects and nematodes it has been found that the response of the organism is proportional to the concentration time product (C.T.P.). Since this relationship has never been investigated for fungal control with methyl bromide, Experiment 3 was set up.

Results are given in Table 4.

Table 4

Effect of concentration/time combinations of methyl bromide on yield of cucumbers and incidence of black root rot

Concentration mg/l.	Time h	C.T.P. mg h/l.	Percentage increase in yield of cucumbers		% Root rot
			Weight	Number	
0	0	0	-	-	95.0
189.0	24	4530	+ 68.1	+ 52.6	56.5
894.4	48	4530	+ 78.2	+ 60.3	55.0
47.2	96	4530	+ 92.6	+ 65.8	54.0

Mean control yield per plant 7.0 cucumbers (1.9 kg)

Pattern of cropping is shown in Fig. 2.

DISCUSSION

Methyl bromide applied at a C.T.P. of 4530 mg h/l. gave a significant increase in weight and number of cucumbers cut in all three experiments. Lower C.T.P's resulted in yield reductions as compared with the controls, for which there has been

no satisfactory explanation. There is however evidence (Wilhelm, 1971), on the field scale, of low doses of methyl bromide causing greater incidence of Verticillium wilt in strawberries. In some cases only slight increases in yield occurred with little or no control of the fungus. There was no significant difference in yield of cucumbers after methyl bromide was applied in different combinations of concentration and time to give a C.T.P. of 4530 mg h/l., but from considerations of cost the grower would probably opt for the longer time and lower concentration.

These experiments did not take into account reintroduction of this fungus from soil which might have been inadequately fumigated, i.e. infection at lower depths in soil, nor did they indicate whether fumigation would be effective in a cropping period as long as six months.

If, however, a C.T.P. of 4500 mg h/l was achieved throughout the area penetrated by cucumber roots, then there ought to be adequate control of black root rot for at least one growing season. This dose would be attained with an application of methyl bromide at 1.5 lb/100 ft^2 (73 g/m^2) for 96 hours.

Even in glasshouses where soil is steam sterilised, Phomopsis control is only effective for one year (Ebben personal communication). Root attack by the fungus towards the end of the growing season after sterilisation should have little effect on the yield during that season, but could result in a high inoculum level for the following season, a subject which requires further investigation.

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Fig. 1

Number of cucumbers cropped in Expt. 2.

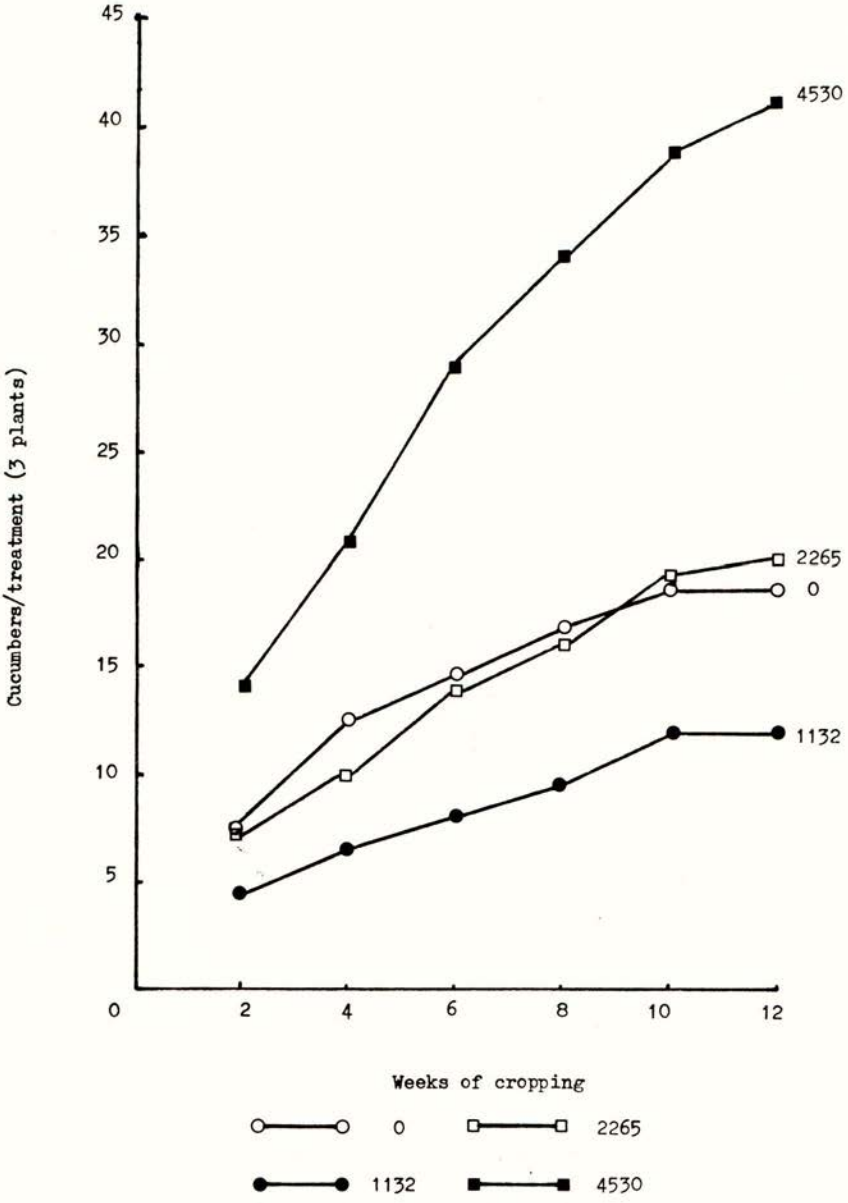
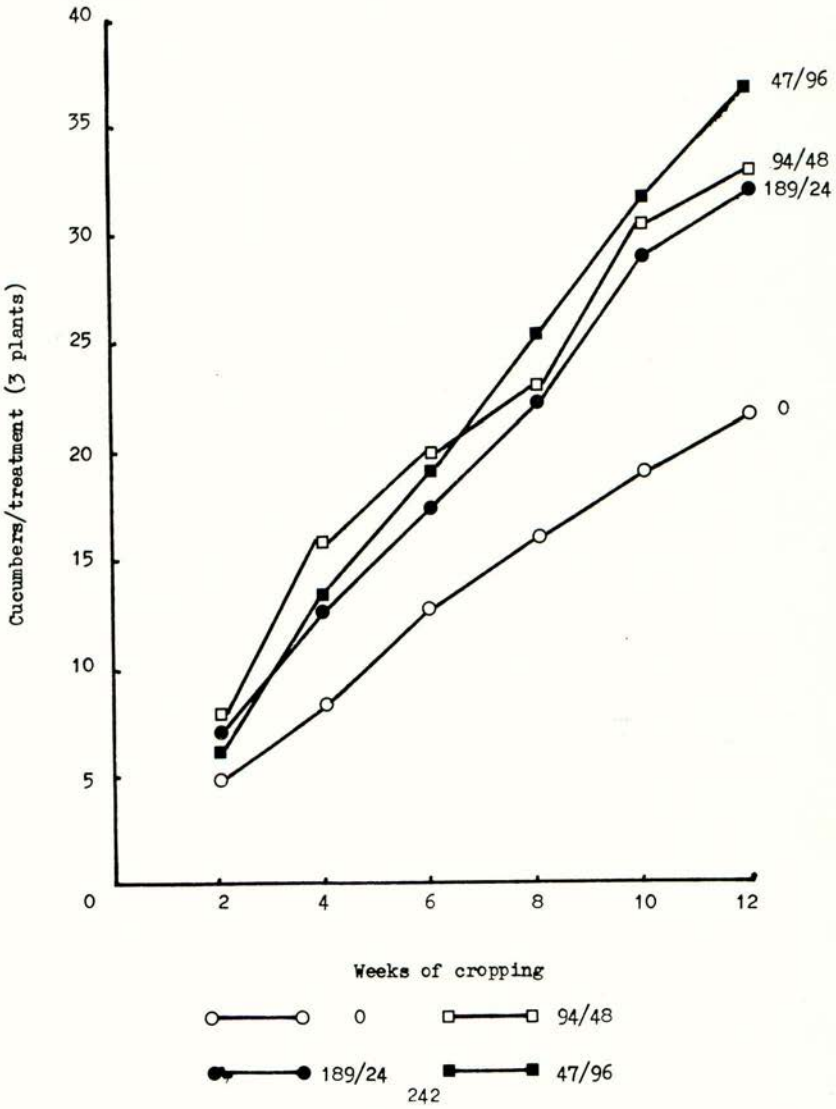


Fig. 2.

Number of cucumbers cropped in Expt. 2.



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TOMATO BROWN ROOT ROT: THE BUILD-UP OF SOIL INOCULUM AND ITS CONTROL BY FUMIGATION

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Summary Soil-borne inoculum of Pyrenochaeta lycopersici the causal pathogen of tomato brown root rot builds up in soils cropped continuously with tomatoes to maximum levels which vary in different soils. Assessments of the % of the root system with brown lesions can be correlated with yield loss and give a measure of the inoculum levels which are damaging. Control is effected when soil sterilisation decreases soil inoculum density below these critical levels for at least one crop.

INTRODUCTION

Brown root rot (BRR) of tomatoes caused by the fungus Pyrenochaeta lycopersici, is a disease which can be classed as an example of soil sickness, in that the causal fungus is soil-borne and at low inoculum levels no aerial shoot symptoms are seen, the host plant acting as an apparently symptomless carrier. As soil inoculum levels and disease severity on roots increase, aerial growth visibly deteriorates and yields are decreased.

Root symptoms, in the form of brown lesions on younger roots and the development of brown corky bark on older roots, increase in severity on roots sampled throughout the life of the crop. Root loss occurs, but is less easily defined although it is this loss of root which is primarily responsible for the starvation and poor growth of the plant. The pathogen is almost certainly ubiquitous in many soils and has been isolated from many non-solanaceous hosts (Termohlen 1962), but only increases to damaging levels when its most susceptible host the edible cultivars of the tomato Lycopersicon esculentum are grown.

The relationship between disease severity, the inoculum potential of the pathogen and the disease potential of the host plant has been defined as:

Disease severity = inoculum potential x disease potential; where inoculum potential is a combination of inoculum density and the capacity of the pathogen to invade (Baker 1968). Soil sterilants exert their main effect on the inoculum density of the pathogen, although conditions in the soil after the use of different sterilants may affect the disease potential of the host. Where different concentrations of the same sterilant can be applied however, as with methyl bromide, the effects on disease severity are probably due to changes in initial inoculum density in the fumigated soil.

DISEASE ASSESSMENT

It is not easy to assess soil inoculum levels directly, especially for a slow growing root-inhabiting pathogen such as P. lycopersici thus to assess

the severity of the infestation the % of the root system with brown lesions (Preece 1964, Last et al 1966) and the loss of yield have been used as indicators.

On replicated plot experiments at Glasshouse Crops Research Institute and Luddington Experimental Horticultural Station, the correlation between % BRR assessments two and four months after planting and final yields was highly significant (P = 0.001). At Luddington E.H.S. on soils treated with three concentrations of methyl bromide either for two years consecutively or only in the first year, yields were depressed by more than 17% when BRR levels were greater than 15% and 33% two and four months after planting respectively. At G.C.R.I. in plots steamed one, two, or three times, or not at all in a three year period, a similar decrease in yield was associated with BRR assessments greater than 18% and 23% two and four months after planting.

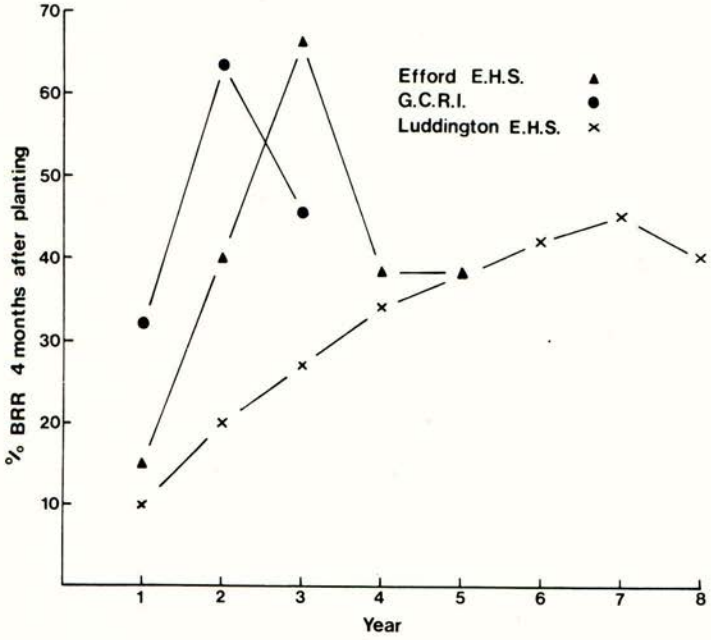
In two out of the three seasons no significant correlation was found between end of season root rot assessments (6 months after planting) and final yields. At least two factors could affect this, firstly visual assessments of root infection between 30-80%, which are levels usually found on roots at this time in the season, are likely to be overestimated (Smith et al 1969) and secondly late assessments are often observed to be lower than would be expected on plots where % BRR levels were high earlier in the season; an effect probably analogous to the relation between initial and final levels of populations of Heterodera rostochiensis reported by Hague & Hesling (1958). Brown root rot assessments at 2 and 4 months after planting have therefore been used in preference to end of season assessments as measures of disease severity.

RELATIONSHIP BETWEEN SEVERITY OF DISEASE AND YIELD

Last et al (1969) showed that on a site at Luddington E.H.S. in a cold glasshouse where tomatoes had never been grown previously, disease severity measured by % BRR on root systems increased regularly and could be defined mathematically over a 5-year period on untreated plots. Data for the 3 years subsequent to this work suggests that disease severity has now reached a maximum, the build-up of root rot in 8 consecutive years of tomato cropping shows a sigmoid curve typical of the increase in disease severity with increasing inoculum density (Baker, 1968). The mean % BRR on root systems 4 months after planting is shown in Fig. 1 and has increased from very low initial levels to 40-50% in the eighth year. Yields from plants in this untreated soil were not significantly less than those from sterilised soil until the 4th year of cropping, when a decrease in yield of 14% occurred when BRR reached levels of 30% 4 months after planting. In the 8th crop, yields were decreased by 25%.

Assessments of BRR on tomato roots from two other sites show a much more rapid build-up to higher levels of BRR. The % BRR 4 months after planting reaching levels of over 60% in the 2nd and 3rd years of cropping without soil sterilisation on sites at G.C.R.I. and Efford E.H.S. respectively (Fig. 1). Prior to these trials tomatoes had been grown annually for 9 years in a heated house at G.C.R.I. and for 4 years out of 8 in a cold house at Efford E.H.S. At both sites assessments of BRR decreased in the years following the peak but yields did not improve.

Fig. 1 Incidence of BRR on tomatoes on unsterilised soil



Relative yields from untreated plots on all three sites show a consistent decrease when plotted against (a) increasing % BRR assessments 4 months after planting (Fig. 2) and (b) the numbers of tomato crops grown irrespective of any soil sterilant treatment between crops (Fig. 3).

Fig. 2

Relation of BRR assessments to yields of tomatoes on unsterilised soils

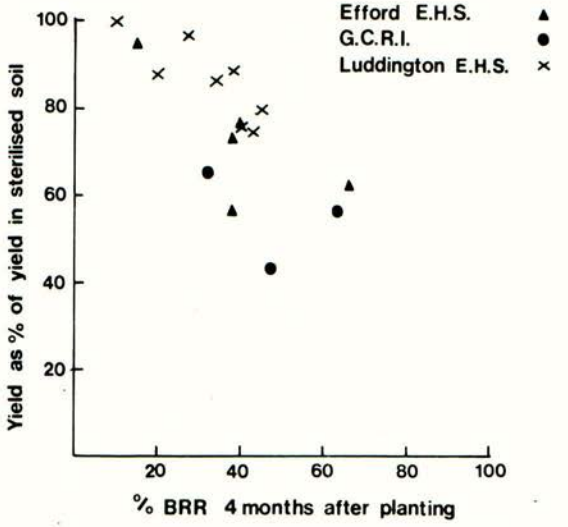
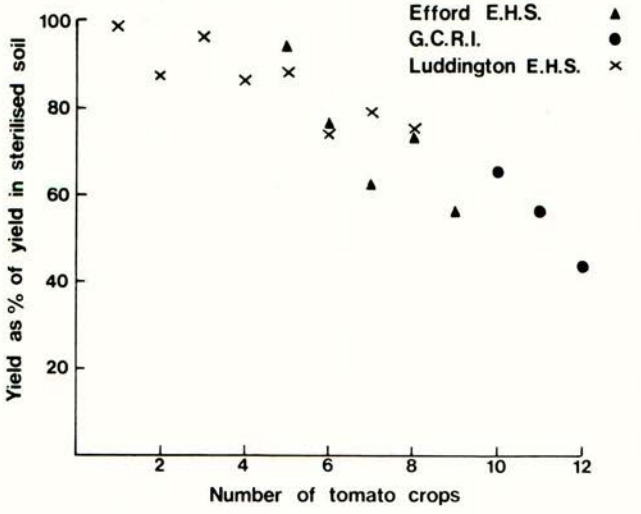


Fig. 3

Decrease in tomato yields on unsterilised soil with increasing numbers of tomato crops

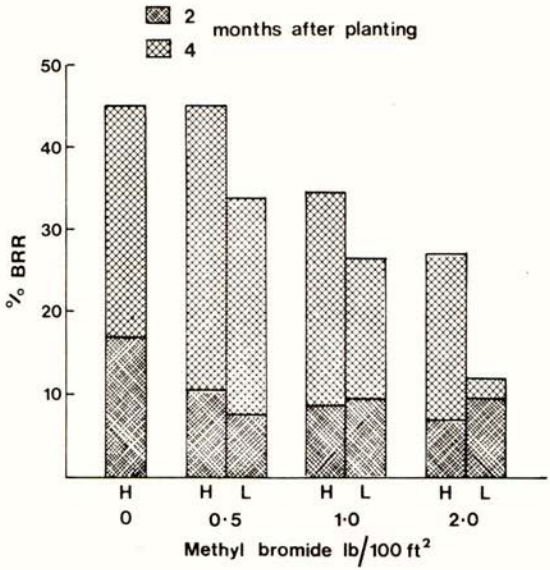


It seems possible therefore that high brown root rot levels developing on untreated plots at G.C.R.I. and Efford result from the longer cropping history and greater build-up of inoculum in the soil over the years. Even in sterilised soil some build-up of BRR would occur if the within-season increase in inoculum were greater than the decrease resulting from sterilisation. The % BRR corresponding with damaging levels of inoculum may however be similar on all three sites, circa 30% BRR 4 months after planting giving approximately 15% decrease in yield.

CONTROL OF BRR WITH METHYL BROMIDE

The effect of soil fumigation with methyl bromide in reducing inoculum of BRR to below critical levels is shown in results obtained from soils of relatively low disease levels at Luddington E.H.S. Plots used in an earlier trial were designated as of high or low inoculum density according to the previous season's BRR assessment. (Mean 4 month BRR 30% and 17% respectively). Three concentrations of methyl bromide 0.5, 1.0 and 2.0 lbs/100 ft^2 (25, 50 and 100 g/m^2) were applied in spring and sheeted for 4 days to two 'high' and two 'low' inoculum plots, each $13\frac{1}{2}$ x 4 ft. The efficacy of the fumigations, assessed as % BRR in the following tomato crop, are shown in Figure 4. The general effect of increasing concentrations of methyl bromide was to decrease the % of BRR in soils of similar inoculum backgrounds, but where initial inoculum levels were high, twice the concentration of fumigant was required for the same control. All fumigant treatments, except 0.5 lbs/100ft^2 on high inoculum plots, gave significant increases in yields.

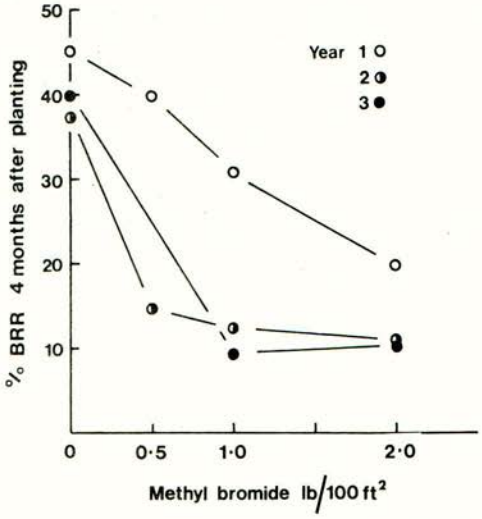
Fig. 4 Effect of 3 concentrations of methyl bromide on the incidence of BRR in soils of high [H] and low [L] initial inoculum levels.



Application of methyl bromide under varying conditions or to different soil types can give different effective dosages (= concentration x time products) and these results show that with different initial levels of soil inoculum, control of BRR would vary. Even where yield loss is prevented, high levels of BRR could build up by the end of the season after the application of minimal effective concentrations to heavily infested soils.

The effect of increasing dosages of methyl bromide on the incidence of BRR in the first year after applications of 0.5, 1.0 and 2.0 lb methyl bromide/ 100ft^2 is shown in Fig. 5 the % BRR 4 months after planting was 40, 30 and 20% respectively, compared with 45% on untreated plots. When fumigation was repeated the following year all 3 dosages decreased BRR to levels below 15%, but after fumigation in the third successive year further decrease of BRR was minimal.

Fig. 5 Effect of 3 concentrations of methyl bromide applied annually for 3 years on the incidence of BRR



The effective dosage of methyl bromide in the soil can be varied by changing the quantity applied and also by varying the period for which the soil is covered before aeration. At Stockbridge House E.H.S. covering sheets were left on the soil for 2, 4 or 12 days after applying 1.0 lb methyl bromide/ 100ft^2. On the following crops the % BRR 2 months after planting was 12, 17 and 16% for 2, 4 and 12 days cover treatments respectively and yields were all similar. When a second crop was grown without further fumigation 2-month

BRR assessments rose to 27, 24 and 19%, but on re-fumigated plots the levels of BRR were only 10, 14 and 9% respectively for the 2, 4 and 12 day cover treatments. Where soil had been covered for 4 or 12 days, one fumigation controlled brown root rot adequately on two crops and no yield losses resulted, but were the soil was covered for only 2 days, control was effective for one crop only and significant losses in yield resulted in the second year.

At Luddington E.H.S. control of brown root rot for two crops was also effective but levels of 37, 29 and 31% BRR developed in the second crop 4 months after planting on soils originally fumigated with 0.5, 1.0 and 2.0 lb methyl bromide/100ft^2 respectively. Fumigation before the third crop decreased BRR to mean 10.9% compared to 10.3% in plots fumigated annually.

DISCUSSION

A consideration of the disease levels of brown root rot occurring on different sites suggests that in untreated soil starting from minimal levels of soil inoculum, disease severity builds up to a maximum under continuous tomato cropping, and thereafter where soil is not sterilised, fluctuates at these relatively high levels. Commercially, these disease levels are unacceptable and can give yield decreases of 30-50%. The aim of soil sterilisation is to reduce inoculum density (assessed above as % BRR) to below those critical levels at which root loss and consequently yield loss occurs. This critical inoculum density is likely to vary with conditions which affect root growth, the rate of root loss and the effect of root loss on the plant. Higher levels of brown root rot can be tolerated by a crop where new root production balances the root loss caused by the pathogen, but greater root growth will also provide larger amounts of host tissue available for infection and therefore, in these circumstances, inoculum levels are likely to build up faster during one season.

Soil fumigation will destroy only a proportion of the inoculum present so the control of disease will depend on the initial levels of pathogenic inoculum present, as well as on the efficiency of the sterilant. (Bald & Jefferson 1956). Where inoculum is decreased below critical levels no further improvement in yields will result, and repeated sterilisation may be unnecessary if the rate of inoculum build up during the crop is low, and optimum conditions for root growth are maintained.

Acknowledgments

I wish to acknowledge the cooperation of Directors and Staff at Efford, Luddington and Stockbridge House Experimental Horticulture Stations.

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ROOT ROT AND WILT OF FIELD BEANS (VICIA FABA)

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Summary Wilted field beans with blackened tap roots and few functional laterals are common and were widespread in 1970 when many crops were stunted, yellow and yielded very little. Possible causes include the pea and bean weevil, Sitona lineatus, which damages leaves and roots and transmits viruses; the stem eelworm, Ditylenchus dipsaci; the fungi Fusarium oxysporum, F. avenaceum, Rhizoctonia solani, Pythium spp. and Phytophthora megasperma. In field experiments, aldicarb, which is insecticidal and nematicidal and the fungicide, dexon, both strikingly decreased wilt symptoms and increased growth and yield; benomyl had less effect, formalin and BHC least of all. Only P. megasperma caused root rot and wilt of plants inoculated in pots, but was not found regularly in rotted roots from the field.

INTRODUCTION

Now that bean aphids can be controlled by insecticides, spring sown beans have attractions as a break crop between cereals susceptible to foot and root rots, but in recent years they have suffered loss from fungi attacking the leaves, virus diseases and unexplained poor growth. The agricultural returns for field bean acreages in England and Wales in 1963 were 57,000 acres (23,100 ha). By 1968 the acreage had increased fourfold, but in 1969 there was a small decrease followed by a further decrease in 1970 to about 189,000 acres (76,500 ha) (Ministry of Agriculture, Fisheries and Food, 1968,1969,1971a,1971b). During this time yields fluctuated widely and at Rothamsted spring beans grown in rotation with wheat, potatoes and barley gave yields ranging from 30.7 to 5.6 cwt/ac (3815 to 703 Kg/ha) (Table 1).

Table 1

Mean yield of beans in the cultivation - weedkiller experiment, Rothamsted

Year	Cwt/ac	Kg/ha	Variety
1961	18.7	(2347)	Tick
62	29.0	(3640)	Tick 30B
63	32.8	(4164)	Winter beans
64	27.3	(3426)	Spring beans
65	25.0	(3138)	Winter beans
66	30.4	(3815)	Pedigree Tick
67	30.7	(3853)	Tarvin
68	21.1	(2648)	Maris Bead
69	24.6	(3087)	Maris Bead
70	5.6	(703)	Maris Bead

Late sowing and a dry May and June were partly responsible for the especially poor growth in 1970, but many crops were stunted and yellow, their tap roots dry and blackened and their lateral roots mostly rotted away. These symptoms occur every year, but are more widespread in some years than others. Several different pests and pathogens seem to be involved. The pea and bean weevil, Sitona lineatus damages leaves and can transmit some viruses and later its larvae feed on roots and nodules. The stem eelworm, Ditylenchus dipsaci damages roots and stems as it migrates upwards to infest the seed pods. The fungi, Fusarium oxysporum, F. avenaceum, Rhizoctonia solani and Pythium spp. are common in diseased roots.

Hornby and Salt (1969) and Hornby, Salt and Phillips (1970) reported that isolates of these fungi from affected roots did not reproduce the root rot and wilt symptoms in plants in pots, nor were these symptoms caused by viruses or herbicide. However, another fungus, Phytophthora megasperma, found in wilted bean roots on two fields, one at Saxmundham, Suffolk and the other at Rothamsted (Barnfield) produced black root rot and wilt when inoculated into steamed soil in which beans were sown (Salt and Hornby, 1971). Although P. megasperma is a virulent pathogen of bean roots, it was not always found in rotted roots; possibly it may have been there earlier, but was replaced by other fungi once the symptoms were advanced.

To evaluate the relative importance of these fungi and pests under field conditions, chemicals having wide biocidal, fungicidal and insecticidal properties were used in experiments on Barnfield in 1970 and 1971.

METHODS

Experiment 1, 1970 Twenty plots 12 ft 3 ins x 5 ft (3.7 m x 1.5 m) were marked out in two blocks of five on Barnfield plot 3 (no manures) and another two blocks of five on plot 4 (30 lbs/ac (34 Kg/ha)P, 200(224)K, 80(90)Na and 20(22)Mg). The following chemicals were applied as dusts to the soil surface on 20 March and the soil immediately rotary cultivated:

0	None
N	10% aldicarb ('Temik') at 10 lbs/ac a.i. (11.2 Kg/ha)
F	50% benomyl at 20 lbs/ac a.i. (22.4 Kg/ha)
I	50% BHC at 2 lbs/ac a.i. (2.2 Kg/ha)
NFI	The three chemicals as above applied together

Beans cv. Maris Bead were drilled at 200 lbs/ac (224 Kg/ha) on 20 April, basal simazine weedkiller was applied on 24 April at 1 lb/ac (1.1 Kg/ha), and demton-s-methyl insecticide at 3.5 oz/ac (245 g/ha) on 17 June. Four of the seven rows were harvested by hand from each plot on 3 September.

Experiment 2, 1971 Twenty four plots 12 ft 3 ins x 7 ft 6 ins (3.7 m x 2.3 m) were marked out as four blocks of six on a different part of Barnfield plot 3 (unmanured). The following chemicals were applied as dusts and rotary cultivated on 26 February, except that formalin was applied as a drench on 25 November 1970.

0	None
N	10% Aldicarb at 10 lbs/ac a.i. (11.2 Kg/ha)
F	70% Dexon at 70 lbs/ac a.i. (78.5 Kg/ha)
I	25% BHC at 4 lbs/ac a.i. (4.5 Kg/ha)
B	Formalin at 266 gal/ac (2988 l/ha) of a 38% solution of formaldehyde in 4840 gal/ac (54368 l/ha) water

NB Aldicarb and formalin at the rates shown above

Maris Bead was drilled on 4 March, simazine applied on 10 March, and insecticide, demton-s-methyl on 1 July. Four rows each 6 ft (1.8 m) long were harvested by hand on 25 August.

Root disease estimations Five to ten plants per plot were lifted at random from the three rows not required for harvest. After washing, four areas of each plant, namely the lowest 10 cm of stem above ground, the hypocotyl from ground level to the seed attachment, the tap root below the seed and the lateral roots were scored on the following scale in 1970:

(a)	Clean or slightly discoloured	0
(b)	Moderately discoloured	1
(c)	All black with rotting cortex	2
(d)	Roots few or lacking, applied to laterals only	3

In 1971 the whole tap root and laterals were assessed on this scale and a percentage disease rating calculated from the sum of all scores, 100% indicating that all roots were black and most laterals missing.

Identification of fungi Plating diseased roots on agar was an unreliable way of determining what fungi were present. Pythium spp. and Rhizoctonia solani were suppressed by surface sterilizing with sodium hypochlorite, and on rich media, such as potato-dextrose agar, the plates were quickly overrun by Fusaria and species of Mucor and Rhizopus. Water agar enabled many different fungi to grow weakly and sporulate. Phytophthora megasperma was never recovered on agar from field samples, even when the roots contained oospores, and special techniques were necessary to isolate it in pure culture. Roots were cleared and decolourized (Phillips and Hayman, 1970) to allow microscopic examination for Pythium and Phytophthora oospores, Rhizoctonia mycelium, mycorrhizal infection by Endogone spp, and sporangia and resting spores of Olpidium brassicae. The main disadvantages were that only small samples could be examined and we could not detect Fusarium spp.

Virus diseases In 1971, pea enation virus (aphid-transmitted), bean leaf-roll virus (aphid transmitted) and broad bean stain virus (weevil-transmitted) were all present in the experimental crop. All three diseases were recorded under the heading of 'virus-infected plants'.

RESULTS

Experiment 1 Aldicarb decreased the percentage of plants wilted (Fig. 1), and increased growth and yield greatly, especially in unmanured plots (Table 2). Benomyl had much less affect and BHC none. Aldicarb greatly decreased the number of nematodes in the soil during May and the percentage of plants infested by stem eelworm during August (Table 3). It also prevented leaf damage by Sitona lineatus, which was severe in other plots, and decreased the number of S. lineatus larvae recovered from the soil. Despite these spectacular effects on pest control and yield, none of the chemicals substantially decreased the disease rating of roots, either on plants in May or August (Table 4).

Fig.1. Incidence of wilt, Barnfield 1970

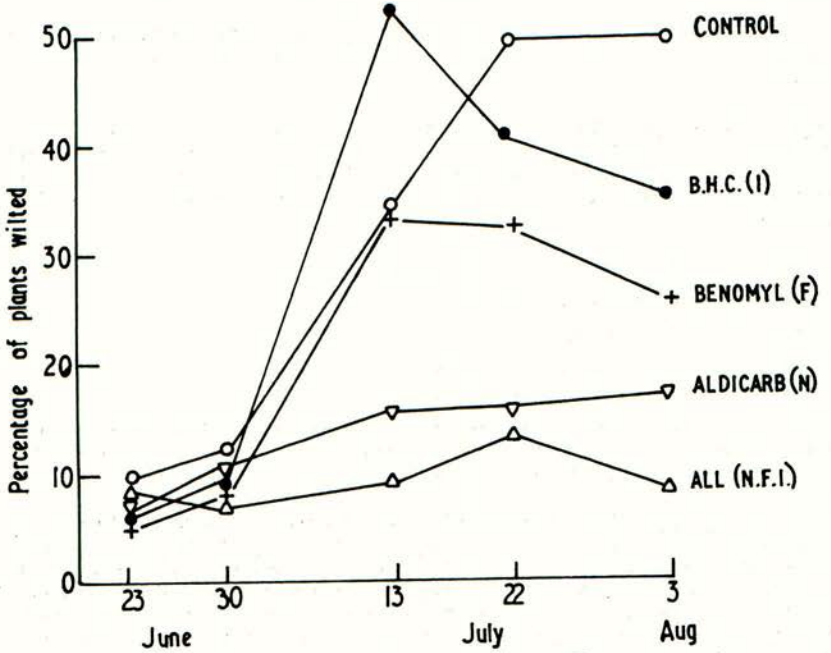

Fig 2 Incidence of wilt, Barnfield 1971

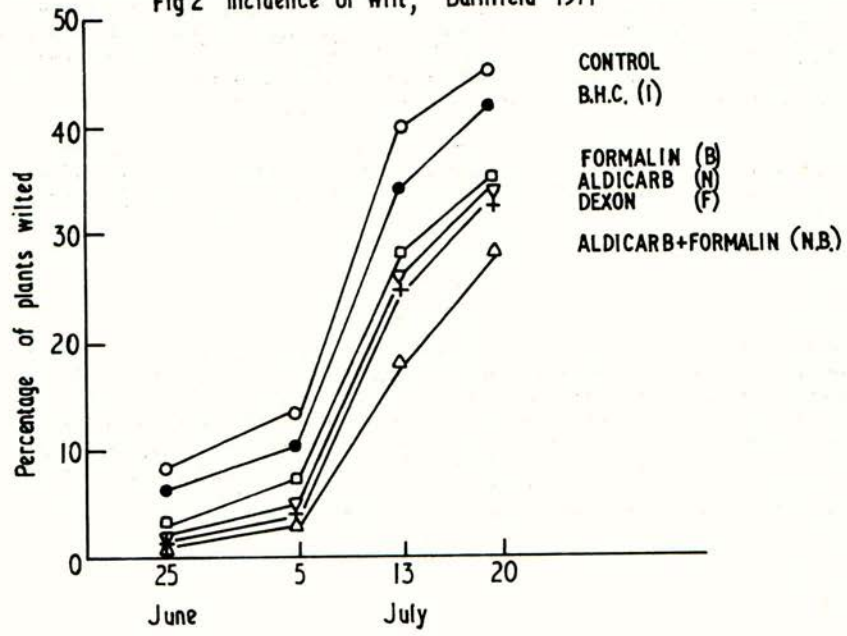

Table 2

Grain yields cwt/ac (t/ha) from Barnfield, 1970

Manure	0	Aldicarb(N)	Benomyl(F)	BHC(I)	(NFI)	Mean
			± 1.72 (215.9)*			
0	9.5 (1192)	19.5 (2447)	15.7 (1970)	13.3 (1669)	16.3 (2046)	14.9 (1870)
PKNaMg	15.3 (1920)	20.2 (2535)	15.5 (1945)	13.1 (1644)	23.0 (2886)	17.4 (2184)
Mean	12.4 (1569)	19.8 (2485)	15.6 (1958)	13.2 (1657)	19.7 (2472)	16.2 (2033)
			± 1.22 (153.1)			

* For use in horizontal and interaction comparisons
Mean dry matter percentage = 79.4

Table 3

Effect of treatments on nematode numbers

Treatment	Number in soil samples, (21.5.70) Tylenchids	Others	Total	% plants with D. dipsaci, (3.8.70)
0	81	373	454	31
Aldicarb(N)	34	66	100	5
Benomyl(F)	88	221	309	20
BHC(I)	33	190	223	36
NFI	20	23	43	3

Table 4

Disease rating of roots (%) in 1970

Treatment	May	August
0	26	74
Aldicarb(N)	39	69
Benomyl(F)	24	72
BHC(I)	30	71
NFI	37	67

Experiment 2. Aldicarb again controlled S. lineatus, decreased the percentage of wilted (Fig. 2) and virus-infected plants and increased growth and yield. (Table 5)

Table 5

Summary of the 'Chemical Control of Soil-borne Pathogens' experiment, Barnfield, 1971

Treatment	Plant height (cm) 25.6.71	Virus-infected plants (%) 25.6.71	Disease rating (%) 5.7.71	Yield (85% dry matter) cwt/ac	Kg/ha
				± 0.87	± 109.2
0	76	20	74	8.6	1079
Dexon(F)	94	12	57	15.6	1958
BHC(I)	82	13	59	12.0	1506
Aldicarb(N)	98	6	47	19.3	2422
Formalin(B)	77	11	66	9.8	1230
NB	92	8	49	15.1	1885

The fungicide dexon decreased wilt and root disease rating almost as much as did aldicarb, but had little or no effect on S. lineatus damage or virus infection. All chemicals decreased disease ratings of roots in July, aldicarb most and formalin least (Table 5). The percentages of plants infested by stem eelworm were fewer in plots treated with aldicarb (25%) or with aldicarb and formalin (23%) than in controls (73%), but were unaffected by other chemical treatments.

Microscopic examination of 30, 1 cm lengths of cleared and stained lateral roots per treatment from random samples taken in July showed that dexon controlled P. megasperma and aldicarb decreased the number of roots infected by Pythium spp. (Table 6)

Table 6

The percentage frequencies of occurrence of fungi in 1 cm lengths of root in July

Treatment	Phytophthora megasperma	Pythium spp.	Olpidium brassicae	Endogone spp.
0	27	20	73	37
Dexon	0	37	93	37
BHC	20	20	77	23
Aldicarb	17	3	73	27
Formalin	20	20	77	23

Infection by Olpidium brassicae was widespread and unaffected by any of the treatments. A later examination of roots from wilted plants only, gave a similar result; P. megasperma was not found in plants from dexon treated soils and occurred on fewer than one in five roots from other treatments.

DISCUSSION

The poor yields in 1970 could be attributed partly to late sowing followed by drought in May and June. Irrigation almost doubled yields in one experiment at Rothamsted, but in our experiments chemicals applied to soil before sowing were equally effective. This suggests that much damage was pathological, but may have been accentuated by the dry, early summer.

P. megasperma can cause root rot and wilt and severely checks the growth of plants that survive, but the fungus has been found on relatively few wilted plants and therefore its role as the principle pathogen remains in doubt. On Barnfield, P. megasperma is present on many young wilted plants during May and June, before root rot symptoms appear on other fields where the fungus has not been found. In July when wilting is first apparent on other fields, P. megasperma is more difficult to find on Barnfield, although wilting increases rapidly (Figs. 1-2). It seems wilting in July has other causes and the large response of plants to aldicarb suggests that insect pests and nematodes may be responsible.

Responses to aldicarb are complex because it controlled not only weevils and nematodes, but decreased Pythium spp. and the spread of viruses, all of which are beneficial effects. Benomyl was ineffective against P. megasperma and Pythium spp., but it seems to have had some effect on other fungi. Although we failed to reproduce symptoms with Fusarium and Rhizoctonia solani in plants in pots, the possibility of an association in the field between insect and nematode damage and fungal infections needs examination.

Acknowledgments

We are grateful to Mr. D. Hooper for nematode counts, Mr. R. Bardner and Mr. K.E. Fletcher for observations on S. lineatus and Dr. A.J. Cockbain for identifying virus diseases.

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BRITISH CROP PROTECTION COUNCIL

Proc. 6th Br. Insectic. Fungic. Conf. (1971)

CHEMICAL CONTROL OF VERTICILLIUM DAHLIAE AND HETERODERA ROSTOCHIENSIS ON POTATOES

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Summary Aldicarb rotavated into the soil decreased the numbers of H. rostochiensis pathotype A and increased the yield of potatoes. Benomyl applied to seed tubers did not affect yield, but rotavating a greater amount into soil decreased Verticillium symptoms and increased potato yield. Methyl bromide, which decreased both nematode numbers and Verticillium symptoms, increased potato yield by more than the sum of the increases from the other two chemicals.

INTRODUCTION

Verticillium wilt of potatoes is widespread in England (Isaac & Harrison, 1968) and so is the potato cyst-nematode (Jones & Parrott, 1968). H. rostochiensis pathotype A and Verticillium dahliae interact: symptoms of wilt in potatoes grown in pots appear about 2 weeks earlier and are more severe when H. rostochiensis populations exceed 9 eggs/g soil than when the fungus is present alone (Fig. 1). The entry of the fungus into the root is made easier where developing females rupture the root surface (Corbett & Hide, 1971).

Conflicting results have been reported on effects of fungicides and nematicides on land where V. dahliae and Pratylenchus penetrans occur together. Thus, Cetas (1970) found that either the fungicide, benomyl, or the nematicide, aldicarb, each increased yield of potatoes, whereas Easton (1970) found no increase with aldicarb nor disulfoton, but chloropicrin increased yields.

Our paper describes experiments on silty clay loam soil of Broadmead Field at Woburn, Beds., which contained up to 20 eggs H. rostochiensis/g soil and was infested with V. dahliae. We used a nematicide (aldicarb), a fungicide (benomyl) and a general biocide (methyl bromide), to assess the effect of each organism on yield and the extent the two interact.

MATERIALS AND METHODS

The treatments were applied to plots in randomised blocks and replicated four times. Aldicarb, as a 10% granular compound was rotavated into the soil at 6 lb. a.i./acre. For the first experiment Benomyl, was dusted on the seed tubers using 3 lb a.i. in 10 lb of kaolin per ton of tubers, but in experiment 2, 20 lb a.i./acre was rotavated into the soil. Methyl bromide was applied at 2 lb./100 sq. ft. under gas tight sheets.

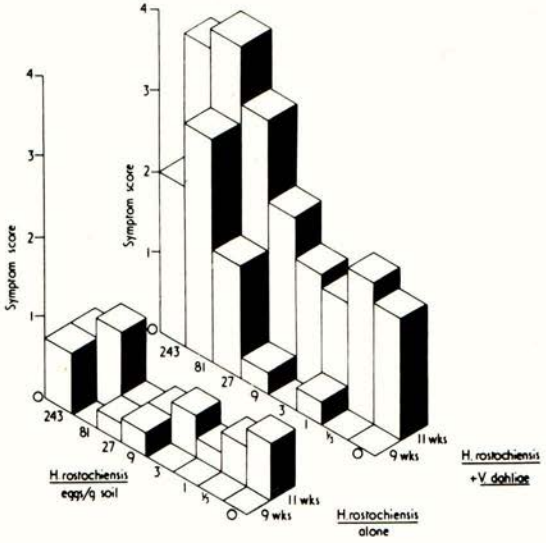

Fig. 1. Symptom development of potato plants in pots 9 and 11 weeks after inoculation with H. rostochiensis and V. dahliae

White females of H. rostochiensis on the roots of carefully lifted plants were estimated by washing and rubbing the roots over a 20 mesh sieve and counting those retained on a 60 mesh sieve held below. Cysts were counted after extracting them from soil in a Fenwick can, and eggs were counted in a water suspension after crushing the cysts. All counts were transformed to log (x + 1) for analysis, but actual counts are given in the tables.

V. dahliae propagules in air dried soil were estimated after planting on ethanol-streptomycin agar, using the method of Harrison & Livingston (1966).

In experiment 1, tractors were run three times during the season along rows of some untreated plots and some plots treated with methyl bromide and the yields from the rows taken separately. Yields from these rows were no less than in other rows with the same treatment. Experiment 1 was started in 1969 when the variety King Edward was planted. In 1970 it was continued without further chemical treatments and with the variety Pentland Crown. Experiment 2, done in 1970 only, was planted with Pentland Dell.

RESULTS

In the first year of experiment 1, foliage symptoms of V. dahliae were most severe in untreated and in benomyl-treated plots, and plants in these plots died

early in August. Plants in plots treated with methyl bromide grew larger and remained vigorous until the crop was lifted in September. Aldicarb also delayed haulm senescence, but almost all dead stems in treated and untreated plots contained the black microsclerotia of V. dahliae at harvest.

Table 1

Total tubers (ton/acre)

Variety & year	Untreated	Aldicarb	Benomyl	Methyl Bromide
Experiment 1				
King Edward, 1969	9.96	14.23**	10.44	17.66***
Pentland Crown, 1970	3.49	7.48*	3.30	8.09**
Experiment 2				
Pentland Dell, 1970	1.96	6.26**	4.84*	14.18***

Significantly different from untreated at 5%, *; 1%, **; and 0.1%, ***

In the second year of the experiment, plants grew less well than in the first, and in June 1970 plants in untreated and benomyl-treated plots were smaller than in those treated with aldicarb or methyl bromide. Plants in untreated and benomyl-treated plots died during July, whereas in aldicarb- and benomyl-treated plots they survived until they were lifted. Tuber yields reflected these growth differences in both years (Table 1).

In experiment 2, plants in untreated plots grew less well than in treated plots and symptoms of Verticillium appeared in them during June. Plants in aldicarb- and benomyl-treated plots grew well, but were less vigorous than in plots treated with methyl bromide.

Both methyl bromide and benomyl prevented microsclerotia of V. dahliae developing in most stems. Yields are in Table 1.

Table 2

Counts of H. rostochiensis & V. dahliae in Experiment 1

	Untreated	Aldicarb	Benomyl	Methyl Bromide
June, 1969				
White females/g root	20	7	61	4*
After harvest, 1969				
Cysts/100g soil	29	5**	30	6**
Eggs/g soil	60	16*	96	9**
After harvest, 1970				
Cysts/100g soil	129	50*	112	47**
Eggs/g soil	398	155	407	187
Propagules/g soil	22.5	25.6	15.6	13.1

Significantly different from untreated at 5%, * and 1%, **.

Aldicarb and methyl bromide decreased numbers of H. rostochiensis on roots of plants while growing in experiment 1 and of cysts and eggs in soil at harvest (Table 2). At the end of the second year, soil treated with methyl bromide or aldicarb contained significantly fewer cysts than in untreated soil, but not significantly fewer eggs of H. rostochiensis and propagules of V. dahliae.

Table 3

H. rostochiensis & V. dahliae in Experiment 2

	Untreated	Aldicarb	Benomyl	Methyl Bromide
After harvest, 1970				
Cysts/100g soil	110	32**	52	46*
Eggs/g soil	417	55**	151	120*
Propagules/g soil	66.3	73.1	25.0**	32.5*

Significantly different from untreated at 5%, * and 1%, **

In experiment 2, cysts and eggs of H. rostochiensis were fewer in soil of treated than untreated plots but not significantly so in benomyl plots. By contrast, plots treated with benomyl or methyl bromide had significantly fewer V. dahliae propagules than those untreated or treated with aldicarb (Table 3).

DISCUSSION

In both experiments, aldicarb killed nematodes and increased yield. Benomyl applied to seed tubers (Experiment 1) did not affect yield, but increased it when applied to soil (Experiment 2) and decreased Verticillium; it did not affect nematode numbers significantly. Methyl bromide always increased yield by significantly more than either aldicarb or benomyl, but had no more effect on nematode numbers after harvest than aldicarb; it lessened the severity of Verticillium wilt and decreased the number of propagules in soil after lifting. The beneficial action of methyl bromide and aldicarb in decreasing nematodes and increasing yields persisted into the second season.

Our results confirm the interaction between the two pathogens observed with plants in pots. Where both occur, yields can be increased by controlling either, but the increase from controlling both greatly exceeds the combined increases from controlling each separately. That the beneficial effects of methyl bromide and aldicarb extend to a second year indicate that methyl bromide diminishes the populations of nematodes and fungus, and aldicarb the nematode, below those able to increase to damaging numbers during one season. Other workers have shown similar benefits lasting more than one year from fumigating soil infested with Verticillium spp. (Miller & Hawkins, 1969, Taylor 'et al', 1970).

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SPECIFIC REPLANT DISEASES OF TREE FRUITS AND THEIR CONTROL BY SOIL FUMIGATION

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Summary Pre-planting fumigation of orchard or fruit-tree nursery sites was an effective means of improving the growth of apples and cherries otherwise retarded by specific replant diseases on land previously occupied by the same crop. Of the fumigants tested, chloropicrin gave the most consistently satisfactory results and a dosage of 25 gal/ac (281 l/ha) proved adequate on loam soils.

Resistant cultivars were not found, but among the apples used, rootstock M.IX benefited particularly from fumigation and there were indications that the scion Cox was more responsive than Laxton's Fortune.

INTRODUCTION

The stimulus for this research was the existence of a long-standing practical problem in the re-establishment of orchards or fruit-tree nurseries. Our objectives were, in descending order of priority, to establish practical control measures, to compare the reactions of commonly grown stock and scion cultivars to the disease and to its control and, where possible, to learn something of the cuases of the problem. The trials were, with one exception, carried out on soils in which apple or cherry had previously been grown and used apple and/or cherry test plants.

In his review, Savory (1966), proposed the name 'specific replant disease' to distinguish the field problem with which we were concerned from other other, more vaguely defined conditions, often known as 'soil sickness' or 'replant problems'. Specific replant diseases of fruit trees have been known in the U.K. for over 200 years and have been dealt with in the past by cultural means, such as crop rotation (exemplified by the growers' maxim 'Don't plant pip after pip or stone after stone') or by refilling the planting hole with fresh soil.

Specific replant diseases are characterised by several properties, of which the following are the most important.

1. Specificity - difficulty is experienced in establishing a plant species in a soil in which it, or a closely related species, has previously been grown, e.g. apples may grow poorly on old apple or pear land and cherries fare badly on old stone-fruit land, whereas apples usually grow normally after stone-fruit, as do cherries after pome fruit.

2. Persistence - intervals of five, or even ten or more years between the grubbing of the first crop and the planting of the second usually have little influence on severity of symptoms in the replanted crop.

3. Symptoms - there are no reliable diagnostic symptoms but the root system is usually reduced and discoloured, while the shoot is stunted.

4. Recovery - affected plants transplanted to fresh soil soon resume normal growth i.e. the causal factor(s) are not systemic.

Many causes have been postulated for specific replant diseases, such as nutrient imbalance, specific toxins and little-known pathogens, but convincing experimental proof is lacking. Recent work on apple replant disease has largely discounted toxins (Savory, 1969) and plant-parasitic nematodes (Pitcher, Way and Savory, 1966; Hoestra, 1968) as causal factors. Hoestra (1965) and Sewell (unpublished) have, however, demonstrated that the common soil fungus Thielaviopsis basicola (Berk. and Br.) Ferr. will stunt cherries but has no effect on the growth of apples. The existence of a comparable apple-specific organism has not yet been demonstrated and further work is necessary to assess the true role of T. basicola in the etiology of cherry replant disease.

The data given below have been selected from a series of trials carried out at East Malling Research Station over the past ten years by the authors and others.

METHOD AND MATERIALS

Land

All the trials were carried out on loam soils overlying the Hythe Beds of the Lower Greensand, and the site selected for each trial had previously supported either a mature or a dense stand of either cherries (Trials 1 and 3-5) or apples or pears (Trials 2 and 6-12). It was assumed that the site had been completely occupied by the roots of the previous fruit crop and that the performance of the test plants in the various experiments was, therefore, not materially affected by the exact location of the original trees. Brief details of the sites are given in Table 1. In trials 1-4 the population of the principal tylenchid nematodes present were assessed before and after treatment.

Table 1

Details of sites used

Trial	Soil series and texture	Previous species	Duration of previous planting (years)	Interval between grubbing and planting (years)
1	Barming: sandy loam	Cherry	9	2
2	" " "	Apple	46	1
3	" " "	Cherry	9	3
4	" " "	Cherry	9	4
5	Millhall: calcareous fine sandy loam	Cherry	14	1
6	Langley: silt loam	Apple	4	2
7	Malling: sandy loam	Apple	50	1
8	Langley: medium loam	Apple	50	1
9	Lowlands: fine sandy loam	Apple	18	1
10	Langley: medium loam	Apple	50	6
11	Millhall: calcareous fine sandy loam	Pear	11	0
12	Langley: silt loam	Apple	4	2

Soil treatment

The following commercial materials were used in one or more of the trials:

1. Chloropicrin (CP), a technical grade of trichloronitromethane.

2. Dichloropropane-dichloropropene mixture (DD), containing 50% w/w 1,3-dichloropropene.

3. Chlorobromopropene (CBP), containing 55% w/w 1,chloro-3,bromopropene and other related hydrocarbons.

4. Methyl isothiocyanate (MIT), containing 40% w/v isothiocyanatomethane.

5. 'Di-Trapex (WN 12)', 20% w/w methyl isothiocyanate in chlorinated C_3 hydrocarbons, including 1,3-dichloropropene.

6. Dazomet (DAZ), containing 85% w/w tetrahydro-3,5-dimethyl-2H-1,3,5-thiadiazine-2-thione, yielding 38% methyl isothiocyanate.

7. Methyl bromide (MB), technical grade bromomethane.

Materials 1-5 were injected into the soil, at 6 in depth and 9 in spacing, in early autumn. Dazomet powder was applied to the soil surface and mechanically incorporated to a depth of about 10 in, methyl bromide gas was released at the soil surface under a polythene sheet.

Before treatment all the sites were deep ploughed and later rotavated, often several times, to obtain a fine tilth 8-12 in deep. After fumigation, all plots, with the exception of those receiving MB, were rolled to give a partial gas seal. Not less than two weeks after fumigation the soil was cultivated to allow any remaining vapour to disperse. All treatments were fully randomised and adequately replicated.

Plants

Rooted layers were used as test plants in Trials 1, 2, 4, 6, 8 and 9, while in the remaining trials maiden trees were used. The test plants were raised on unfumigated nursery sites and all were average grade planting stock, with the exception of those used in Trials 8 and 9, in which the size selected was below that normally used commercially.

Unit plot size varied between experiments but was never less than that considered adequate to contain the root growth made in the season after planting.

RESULTS

Initial experiments

Trials 1 and 2

Two sister experiments were set up in autumn 1961 to study the growth of apple and cherry layers on the sites of former apple and cherry plantings. In each case the effect of a single, high dosage of chloropicrin (60 gal/ac) was compared with normal planting practice, i.e. no fumigation.

Table 2

Results of Trials 1 and 2

Trial	Treatment	Extension growth per plant (cm) Cherry F12/1	Apple M.II
1. (previous fruit crop cherries)	Untreated	29.2	52.1
	CP 60 gal/ac	75.2	70.3
	S.E.	7.0	1.4
2. (previous fruit crop apples)	Untreated	58.8	46.3
	CP 60 gal/ac	68.0	69.3
	S.E.	4.3	2.4

Table 2 shows that cherries following cherries grew very poorly in untreated soil and that growth was more than doubled in fumigated soil. In contrast, apples grew well in untreated cherry soil and benefited much less from fumigation. Apples following apples grew tolerably well, even in untreated soil and fumigation gave only a small, although significant growth increase. Cherries grew well in apple soil and the effects of fumigation were not significant. It was concluded that whereas in Trial 1 cherry replant disease was very evident, apple replant disease was of little importance in Trial 2. Both trials gave clear evidence of the specificity of the respective replant diseases and of the effectiveness of chloropicrin as a control.

Subsequent experiments with cherry

Trials 3 and 4

To evaluate the effectiveness of a range of soil fumigants two further experiments were carried out on cherry land adjacent to that used for Trial 1. The results are given in Table 3. The consistently superior performance of chloropicrin in these trials led to the adoption of this material, applied at a rate of 25 gal/ac (281 l/ha), as a standard fumigation treatment on cherry land (Trial 5).

Trial 5

The varietal susceptibility of worked trees was compared on another site. The trial compared the response to fumigation of eight scion cultivars, all low budded on the rootstock F12/1, the test plant used in the previous cherry trials. Although cultivar x fumigation interactions were detectable these were relatively unimportant. Fumigation greatly improved the growth of all cultivars tested, which, in the absence of fumigation, were extremely stunted (Table 4).

Subsequent experiments with apple

The first fumigant screening trial was carried out before any means of assessing soils for their apple replant disease status was available. These trials were set up on the lines of those carried out on cherry land, but were designed to compare the efficacy of various fumigants in controlling apple replant disease. In

this experiment, as in Trial 2, the growth of control plants was reasonably good, consequently fumigation responses were low and differences between fumigants unimportant. It was concluded that apple replant disease was relatively unimportant on the site used and consequently the data are not included here.

Table 3

Trials 3 and 4, comparisons between soil fumigants on cherry land

Treatment		Extension growth (cm) per plant of cherry F12/1 Trial 3	Trial 4
Untreated		30.0	52.0
DAZ	400 lb/ac (448 kg/ha)	50.0	-
MIT	60 gal/ac(674 l/ha)	67.5	-
DD	25 " " (281 " ")	-	79.1
DD	50 " " (562 " ")	85.0	88.5
MB	250 lb/ac (281 kg/ha)	-	70.1
MB	500 " " (562 " ")	-	82.5
CBP	25 gal/ac(281 l/ha)	-	89.8
CBP	50 " " (562 " ")	-	78.0
CP	10 " " (112 " ")	79.5	99.5
CP	24 " " (268 " ")	92.0	-
CP	60 " " (674 " ")	97.5	118.8
L.S.D. (P = 0.05)			
(a) between fumigation treatments		11.7	21.6
(b) between treatments and control		10.2	18.8

Table 4

Trial 5, the growth of eight cherry cultivars on cherry land

Cultivar	Extension growth per plant (cm) Untreated	CP 25 gal/ac
Schrecken	0.0	230
Roundel	0.0	305
Merton Favourite	1.0	231
Early Rivers	2.1	344
Merton Bigarreau	7.5	499
Bigarreau Gaucher	37.6	347
Noir de Guben	56.1	401
Merton Glory	58.8	540

S.E. for treatment comparison within varieties 43.9

Trials 6 and 7

Further experience on sites more heavily infected with apple replant disease has shown, however, that chloropicrin can be very effective in stimulating growth, and that it can be superior to both WN 12 and DD (Tables 5 and 6).

Table 5

Trial 6, the growth of two apple rootstock cultivars on apple land

Treatment		Extension growth per plant (cm) M.IX	M.II
Untreated		17.6	32.4
WN 12	30 gal/ac	20.0	41.7
WN 12	40 " "	19.6	42.1
WN 12	50 " "	23.6	43.0
CP	25 " "	38.1	53.0
S.E.		0.89	1.54

Table 6

Trial 7, the growth of two apple scion cultivars on apple land

Treatment		Extension growth per plant (cm) Cox/M.II	Fortune/M.II
Untreated		263	168
DD	50 gal/ac	281	184
CP	10 " "	464	269
CP	25 " "	607	294
S.E.		12.6	

The lower of the two rates of chloropicrin used in Trial 7 was clearly only partially controlling apple replant disease. In view of these results and those with cherry replant disease, 25 gal/ac (281 l/ha) was also adopted as the standard dosage on apple land (Trials 8-12).

Cultivar performance

Trials 8 and 9

Among the range of apple rootstock and scion cultivars normally used, none were known to be resistant to replant disease. Trials carried out to compare the response of rootstock layers to soil fumigation indicate, however, that certain cultivars suffer more severely than others from growth retardation on replant sites (Table 7, and Savory, 1967).

Table 7

Trials 8 and 9, the growth of five apple rootstock cultivars on apple land either untreated (U) or fumigated with CP at 25 gal/ac

Cultivar	Extension growth per plant (cm)				Increment in diameter squared (mm^2)			
	Trial 8		Trial 9		Trial 8		Trial 9	
	U	CP	U	CP	U	CP	U	CP
M.IX	21	89	56	88	7	67	23	64
M.26	59	145	134	171	21	111	70	107
MM.106	58	170	121	166	22	170	71	112
MM.111	53	163	109	161	18	106	47	82
M.II	66	133	100	123	22	92	37	44
L.S.D. (P = 0.05)	-		-		24.5		14.5	

The extremely poor growth of the M.IX controls in Trial 8 contrasts with that of other cultivars. This factor shows up particularly sharply in terms of stem thickening and is emphasised by the very high percentage increase in growth resulting from fumigation. On the lighter soil of Trial 9 (see Table 1) replant factors could hardly be considered important since the plants grew reasonably well on untreated soil and the responses to fumigation were relatively modest. These growth increases can therefore be regarded as largely 'non-specific' and it is noteworthy that, even in this context, M.IX as again the cultivar benefiting most from soil fumigation.

Trials 10 and 11

Further evidence of the sensitivity of M.IX layers to replant disease was provided by other experiments - one on the site of a former apple orchard, the other following pears (Table 8). On one site (Trial 10), growth on untreated soil was so poor that a proportion of the control plants died.

Table 8

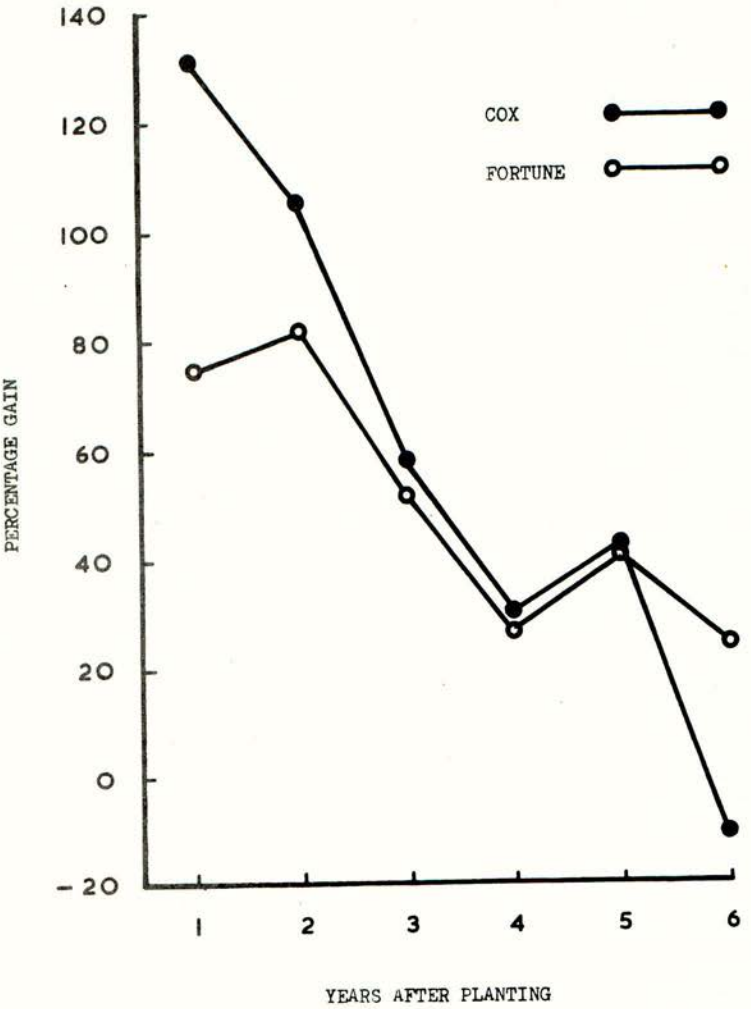
Trials 10 and 11, the growth of apple (M.IX layers) on apple and pear land

Treatment	Extension growth per plant (cm)	
	Trial 10 (apple land)	Trial 11 (pear land)
Untreated	10.8	18.0
CP 25 gal/ac	60.2	52.8

Trials 7 and 12

Sensitivity to replant diseases among scion cultivars appears rather less marked than between rootstocks. Table 9 summarises data from two trials in each of which the proportional response to fumigation of the cultivar Cox has been greater than

Fig. 1. Trial 7, Annual shoot growth: percentage gain from fumigation



that of Fortune. Thus Cox may be rather more sensitive than some other varieties.

Table 9

Trials 7 and 12, the growth of two apple scion cultivars on apple land

	Extension growth per plant (cm)				
	Cox/M.II		Fortune/M.II		
	Untreated	CP 25 gal/ac	Untreated	CP 25 gal/ac	S.E.
Trial 7	263	607	168	294	12.6
Trial 12	339	840	185	370	31.8

Longer term aspects of replant disease control

The results of all the above trials have related to the responses obtained in the first year after planting, and have necessarily been measured in terms of vegetative growth. While this is a major consideration to the nurseryman, yield is more important to the fruit grower. At the present time, data is available from only one long-term experiment with apple.

Trial 7, planted in spring 1964 on old apple land, compares the influence of several soil treatments on the performance of Cox and Fortune on rootstock M.II. Only two treatments need to considered here, trees planted in the centre of a 6 x 6 square of soil fumigated with chloropicrin at 25 gal/ac and trees planted in unfumigated soil.

As noted in Table 9, the initial response of Cox to fumigation was considerable, the amount of shoot growth made by such trees exceeding that of the controls by 131 per cent. With the exception of a small resurgence in the fifth year, the magnitude of this improvement in shoot growth declined annually and in the sixth year after planting no increase was obtained (Fig. 1). At this age the girth of the fumigated trees was 19 per cent greater than that of the controls.

Trees of Fortune made less growth than Cox and initial responses to fumigation were less also; from the second year onwards the pattern of response largely resembled that of Cox.

In 1970, the sixth year after planting, the trees, particularly Cox, bore their first sizeable crop (Table 10). Fumigation appreciably increased the yield of Cox but in the case of Fortune, where the overall yield was much less, the small gains during the first five years were not extended to the sixth.

DISCUSSION

The choice of chloropicrin as the soil fumigant to be used in the initial trials was based on reports from Denmark (Groven, 1958) and the Netherlands (Hoestra, pers. comm.), indicating its potential usefulness in replant situations. The trials reported here have confirmed the value of this material for the control of replant diseases of apple and cherry and for the latter species in particular it is clearly the most effective fumigant of the range tested. Despite the disadvantage of

relatively high cost and the problems associated with handling this material, soil fumigation with chloropicrin remains the most appropriate treatment for these diseases.

Table 10

Apple yields to the sixth year after planting on apple land either untreated (U) or fumigated with CP at 25 gal/ac

Cultivar		Accumulated yield* 1966-1969	Yield* 1970
Cox/M.II	U	16	70
	CP	64	106
Fortune/M.II	U	11	56
	CP	29	59

*Number of fruits per tree

Evidence is scant on the distribution of cherry replant disease, but experience suggests that most cherry sites will retard the growth of a subsequent crop of the same species. Our trials support other evidence that not all sites of former apple plantings are affected by specific apple replant disease. Savory (1967) and Hoestra (1968) have indicated that severe effects are more likely to occur on alkaline soils.

Where localised soil fumigation is carried out it is clear that large short-term vegetative responses are obtainable on sites affected with specific replant diseases. Soil fumigation is therefore specially worth considering as a control measure where short-term vegetative responses are important, for example, in fruit tree nurseries. In the case of the dwarfing rootstock M.IX it may also be worthy of consideration even where specific replant effects are not anticipated.

The decline of the percentage vegetative response shown by localised fumigation in Trial 7 could be regarded as an illustration of the point made by Savory (1966), that the adverse effects of replant diseases in untreated soil became less severe with time. However, an alternative explanation is suggested by the work of Jackson (1971), which indicated that shoot growth is checked as the roots grow out of the fumigated zone.

The use of soil fumigation in commerce for orchard planting assumes an economic gain in yield. Hoestra (1968) has been able to demonstrate considerable yield increases in the third to fifth years after planting the precocious combination James Grieve/M.IX. These trees were planted in chloropicrin-fumigated strips 1.3 m wide on soils which greatly inhibited the growth of the control trees. Soils showing less severe replant effects and planted with trees slower to come into bearing, as in Trial 7, may show a less economic result.

The nematode samples taken in Trials 1-4, (for details see Pitcher *et al.*, 1966) gave no support to the theory that tylenchid nematodes, especially *Pratylenchus* spp., are primarily responsible. Indeed, the most severe growth retardations were found in Trials 1, 3 and 4, where the tylenchid nematode populations were extremely low.

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Dr. B.M. Savory (present address May and Baker Limited, Fyfield Road, Ongar, Essex) was closely involved in several of the trials reported here and his contribution to this work is therefore gratefully acknowledged.

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RESISTANCE OF CUCUMBER POWDERY MILDEW TO DIMETHIRIMOL

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Summary Soil applications of dimethirimol gave excellent control of cucumber powdery mildew (Sphaerotheca fuliginea) in commercial glasshouses in 1968 and 1969. In 1970 there were many reports of failures in control in Holland, and a few in England and W. Germany. Mildew isolates from 41 sites of failure were tested against dimethirimol by a leaf-disc method. Thirty-nine isolates tolerated higher applied concentrations (mostly 10-25 times higher) than seven other isolates from sites of satisfactory control. From this and other evidence resistance to dimethirimol is considered the main cause of the inadequate disease control. Resistant mildew was still widespread in Holland in May 1971, after a year in which very little dimethirimol had been used. Resistance has been detected only in glasshouses in N.W. Europe. It has not been encountered in field-grown crops, or in other parts of the world where dimethirimol is used.

Resistant isolates were at least as vigorous in growth and sporulation as sensitive isolates. They remained sensitive to benomyl, dinocap and quinomethionate. Resistance in vivo was paralleled by resistance in spore germination tests in vitro. Spores and mycelium of resistant isolates treated with labelled dimethirimol took up at least as much fungicide as sensitive isolates. The ways in which resistant isolates originated and spread are unknown.

INTRODUCTION

Dimethirimol is a pyrimidine fungicide which has a potent systemic action against cucurbit powdery mildews (Elias et al., 1968; Geoghegan, 1969). It has a direct fungitoxic effect on certain powdery mildews (Bent, 1970), but little or no action on some other powdery mildews or on a wide range of other pathogenic fungi.

Soil applications of aqueous solutions of dimethirimol hydrochloride ('Milcurb'*, 'PP' 675*) have been used widely since 1968 for mildew control in commercial cucumber houses. In 1968 and 1969 results were generally excellent; the few cases of failure were attributable either to mis-use or to poor uptake when applied to old, moribund plants. However in 1970 many reports of inadequate mildew control were received from growers in Holland. There were also a few complaints from sites in England and W. Germany which could not be

* Trade Marks of Plant Protection Ltd.

attributed reasonably to mis-use or to poor uptake. It soon became clear that most of the trouble was associated with powdery mildew which tolerated unexpectedly high concentrations of dimethirimol.

Available information on the incidence and nature of dimethirimol resistance in cucurbit powdery mildew is reported in this paper.

METHODS

The response of powdery mildew isolates to dimethirimol and other fungicides was assayed mainly by the following procedure. Infected leaves were collected in polythene bags. Within four hours they were removed and sporulating mildew colonies were tapped against the upper surface of healthy young cucumber leaves to give a visible coating of inoculum. Discs (12 mm diam) were cut from the inoculated leaves and floated on fungicide solutions (10 ml) in 5 cm diam Petri dishes (3 discs per dish). Fungicide concentrations were in six 2·5-fold dilutions from 5·0 to 0·052 p.p.m.; two dishes were used for each concentration, and four dishes contained water only. The closed dishes were kept at ambient temperature in a glasshouse or laboratory, away from direct sunlight. To minimise cross-contamination, instruments and working surfaces were wiped with damp tissue before each strain was handled. After 7-10 days, each disc was scored as 4, 3, 2, 1 or 0, corresponding to visible mildew covering 0, trace-5, 6-25, 26-60 or 61-100 percent of the disc area. A simplified method of presenting the results has been adopted in this paper : response is expressed as a 'tolerance level', this being the highest of the tested concentrations which allowed some degree of visible mildew growth on the discs.

Germination in vitro was examined by incubating young spores on a cellulose acetate membrane (4·25 cm diam, 6-10 μm thick), laid within a closed Petri dish partly lined with moist cotton-wool. Each membrane had been impregnated with water or with dimethirimol solution (to an extent of 2·0-2·5 ml per ml membrane material), by flotation for 30 min directly before transfer to the Petri dish and application of spores. After 24 hours at 25°C, 200-300 spores were examined, and those with a germ-tube longer than half the spore diameter were recorded as germinated.

RESISTANCE IN HOLLAND

Although there were trials in 1967 and 1968, dimethirimol was first used widely in Dutch glasshouses in 1969. It was applied as 20 ml of a 12,500 p.p.m. solution to the soil at the base of each established plant (>2 metres high); 400 or 200 p.p.m. solutions were applied in a similar way to smaller plants by a few growers. These treatments inhibited the growth and sporulation of existing mildew lesions within a few days, and gave virtually complete protection for 6-10 weeks. Nearly all growers were very satisfied with the results. Rather poor control was reported from houses where old plants were treated in July-August, 1969, and this was attributed to poor uptake. Mildew attacks in January or early February, 1970, were also well controlled. However in March-April, 1970 many failures were reported. Considerable amounts of sporulating mildew appeared within 2-3 weeks of treatment, and existing colonies were unaffected. Samples of 'Milcurb' were analysed for dimethirimol content, and found to be satisfactory.

In April 1970, mildew samples were taken from six sites of complaint in the Rotterdam area, and tested for response to dimethirimol by the leaf-disc method described above. One isolate had a tolerance level of 5 p.p.m., four gave values of 0·8 - 2 p.p.m. and one was as sensitive as 'control' isolates from Jealott's Hill and from the Laboratory of Phytopathology, Wageningen (tolerance levels 0·32 p.p.m.).

These results increased our suspicion of widespread decrease in the sensitivity of cucumber mildew to dimethirimol. Hence a more comprehensive study was made in May-July 1970, when mildew samples from 38 sites were tested by the leaf disc method. The samples were tested in batches of 3-8 at a time, and the isolate used routinely at Jealott's Hill for fungicide testing (isolate JH) was included as a 'sensitive standard' in most tests.

Thirty-one of the samples came from sites of poor control, and of these 29 were less sensitive than isolate JH and 2 were equally sensitive. The 6 isolates from sites of good control were equally or more sensitive than the isolate JH. Mildew from one grower who had not used dimethirimol was more susceptible than the JH isolate. Results of this survey are presented in a simplified form in Table 1. The sensitivity of isolate JH apparently varied from test to test; this probably resulted from a parallel variation in inoculum potential or in general vigour of mildew development, since response to benomyl which was included in these tests varied in a similar way.

Table 1

Sensitivity to dimethirimol of cucumber powdery mildew from Dutch glasshouses

Tolerance level (p.p.m.)*	Number of isolates	
	From sites of poor control	From sites of good control
5·0 or >5·0	8	-
2·0	9	-
0·80	10	-
0·32	2	1
0·13	-	-
0·05 or < 0·05	2	5

* Isolate JH, tolerance levels : 0·05 in 1 test, 0·13 in 2, 0·32 in 2, 0·80 in 1.

Thus there was a clear correlation between failure in commercial disease control in 1970 and degree of tolerance to the fungicide in leaf-disc tests. It was not practicable to investigate every site where dimethirimol was used in Holland, but resistant mildew was probably present in the great majority of houses where inadequate control was experienced. Tolerance levels recorded for most isolates from sites of poor control varied between 0·8 and 5·0 or >5·0 p.p.m. (Table 1). In general, each isolate was tested once only, and some of this variation probably resulted from experimental error. However, mildew samples from two sites were taken and tested on three separate occasions. In each of these tests mildew from one site gave a tolerance level of 0·8 p.p.m., and that from

the other a level of 5·0 p.p.m. (isolate JH scored 0·13 or 0·32 in these tests). From these results, and also from close observations of the tests and consideration of the full data, we concluded that there were real differences in degree of resistance between certain sites. We do not know whether isolates from these sites were genetically distinct strains; they may have been heterokaryons or mixtures of strains with different proportions of resistant and sensitive genotypes.

A further, more limited survey was conducted in Holland in May 1971. This involved 10 sites, which had been examined for resistance in 1970. The 1971 results (Table 2) indicated that resistance had apparently increased at eight sites and decreased at two sites.

Table 2

Sensitivity to dimethirimol of powdery mildew taken from the same Dutch glasshouses in 1970 and in 1971

Site	Tolerance level (p.p.m.)	
	1970	1971
A	>5·0	>0·05
B	>5·0	2·0
C	2·0	>5·0
D	2·0	2·0*
E	2·0	>5·0
F	0·05	2·0
G	2·0	>5·0
H	0·80	2·0
I	0·80	5·0
J	5·0	>5·0

* The full data showed slightly greater resistance at this site in 1971.

In 1970 we tried to discover how the resistant mildew originated and spread by asking the growers at 44 sites of failure and 7 sites of good control, including all those from which mildew samples had been taken, to complete a questionnaire. This included questions regarding details of dimethirimol applications in 1968, 1969 and 1970, the degree of control obtained; the dates of appearance of mildew, the source of the plants, the conditions of growth, the soil type and method of irrigation, the use of the mite predator Phytoseiulus, etc.

It is impossible to record the answers in full, but the following conclusions were drawn from the completed questionnaires and from visits and interviews. Glasshouse structures, growing temperatures and irrigation systems were similar at all sites. The cucumbers were grown in soil (ca. 15 cm) over new straw bales. The mite predator, where used, was obtained from the same supplier and had been bred on bean plants. The resistant strains appeared widely in Holland at a similar time, mostly in March-April, 1970, but were more prevalent in the largest and most intensive cucumber-growing area, around Rotterdam, than in the two other areas in northern Holland. No initial focus of infection could be discerned. Except for one site where irrigation was inadequate, the plants were growing with normal vigour. Methods of cultivation were similar to those used in 1969.

Failures occurred on both sandy and peaty soils. Twenty-nine of the growers in this survey had obtained good control (at least 6 weeks' protection) in 1969, and five had late-season failures (applications in July-August, 1969). Of the 29 with good results in 1969, 24 achieved little or no control in 1970. Of the five with poor results in 1969, three had resistant strains and no control in 1970, one had a sensitive strain but poor results and one had a sensitive strain and good results. Of two adjacent growers using the same soil and the same rate of dimethirimol at the same time, one obtained satisfactory control and the other did not. Infection of dimethirimol-treated plants (as of untreated plants) tended to be worse near windows and doors.

Samples of leaves of dimethirimol-treated plants were taken from six sites of failure, and dimethirimol was extracted with methanol. Extracted dimethirimol was estimated by bioassay against isolate JH. The six samples were found to contain 1-3 times (average 2·2) the mean tissue concentration of dimethirimol known to give 95-100% protection against isolate JH. This suggested that uptake of dimethirimol was not a limiting factor. This conclusion was supported by observations of slight marginal leaf necrosis, commercially negligible, at some sites; similar symptoms had been induced in earlier glasshouse experiments with dimethirimol at concentrations well above those required for complete protection.

RESISTANCE IN ENGLAND AND WEST GERMANY

England

In general dimethirimol proved very effective in English glasshouses in 1968-1971, but a few instances of failure in disease control coupled with relatively high tolerance in the pathogen have occurred.

The first indication of the existence of a resistance problem arose at the Glasshouse Crops Research Institute, Littlehampton, Sussex, early in 1969. Dimethirimol had been used for about a year, in several ways including routine application as a soil treatment at 100 p.p.m. to protect experimental cucumber plants in pots. Towards the end of this period there was a fall in performance. Tests at the G.C.R.I. indicated that this was a complex phenomenon, probably associated to some extent with absorption by the growing medium and with Phomopsis root infections, but also with increased tolerance to dimethirimol. By the spring of 1970 the decrease in control was more marked and other control measures were adopted. Leaf-disc tests indicated a tolerance level of 5 p.p.m. In November-December 1970 all cucumbers were removed for 6 weeks and weeds (which often carry powdery mildews and may well be the main means of overwintering of cucumber mildew) were eradicated from the site as far as possible. A sensitive isolate was introduced early in 1971, for use in fungicide tests, and this has remained susceptible for at least 6 months.

Resistance in commercial glasshouses in England was first encountered in 1970, when there were six proved occurrences : May - Hook, Hampshire; June - Fernhurst, Sussex; July - Lea Valley, Herts; August - Slough, Bucks; September - Hull, Yorkshire; November - West Drayton, Middlesex. These involved single holdings, the incidence of resistance in 1970 being far more localised in England than in Holland. In addition there were four suspected cases of resistance (in Yorkshire, Kent, Middlesex and Isle of Wight) which could not be investigated because the plants were destroyed. Tolerance levels for the six English isolates were 5·0 p.p.m. or above (3 isolates), 2·0 p.p.m. (2 isolates) and 0·8 p.p.m. (1 isolate); isolates from sites where dimethirimol performed well

gave tolerance levels of 0·13-0·32 p.p.m. Background enquiries were made systematically, as in Holland, but again they cast no light on the origin of resistance.

The general level of mildew in 1971 was very low, and consequently usage of dimethirimol was small; two sites where control was inadequate (in the Lea Valley and in the Isle of Wight) were found to have dimethirimol-resistant mildew.

Growers with resistant mildew in 1970 were specially advised to burn their plants at the end of the season, to clean out the glasshouses thoroughly and to destroy weeds in and around the houses. It is difficult to assess the efficiency of these measures in eliminating resistant mildew or reducing its spread. Little or no mildew developed in these houses in 1971, but this could have resulted from generally adverse conditions. At two of the houses, where sufficient mildew developed for assessment of resistance in 1971, the pathogen was found to be sensitive to dimethirimol. It is not known whether these houses contained new strains, or the formerly resistant strains which had persisted from the previous year and had lost their resistance.

West Germany

During August-September, 1970, nine reports of failure with dimethirimol were received. Only four could be investigated, and three of these were associated with dimethirimol resistance. It is notable that Dutch-grown plants are commonly used in Germany and that there is close liaison between Dutch and German growers.

ABSENCE OF RESISTANCE IN OTHER COUNTRIES

Israel

Dimethirimol has been used extensively since 1969 as soil treatments and as sprays for control of cucurbit mildews in the open-air and under cover. The level of performance has remained high, and benomyl-tolerant powdery mildew has been well controlled by dimethirimol.

Spain

Dimethirimol granules ('Melozel'*, 'Zelcurb'*) applied in the irrigation furrows have been used widely in the Alicante and Murcia areas in 1970 and 1971, for systemic control of powdery mildew on melons. There has been no evidence of field resistance to the treatment, and several mildew isolates proved sensitive in leaf-disc tests (tolerance level 0·32 p.p.m.).

Canary Islands

Dimethirimol was introduced as a soil treatment for cucumbers under cover in October, 1970. An initial survey of mildew response was made in September, 1970, and all five isolates tested proved sensitive (tolerance level 0·32 p.p.m.). Subsequent dimethirimol treatments in October 1970 - January 1971 proved very effective, and there were no cases of suspected resistance.

* Trade marks of Zeltia Agraria S.A.

NATURE OF RESISTANCE

Two powdery mildews have been recorded on cucurbits : Sphaerotheca fuliginea (which seems more common) and Erysiphe cichoracearum. In Holland and England, both sensitive and resistant isolates were identified as S.fuliginea by microscopic examination of conidial characteristics. Thus the occurrence of resistance does not appear to stem from any change in genus. Resistant isolates grew and sporulated on untreated leaves and leaf-discs at least as well as and often more vigorously than sensitive isolates.

The most resistant Dutch isolate (VK, tolerance level 5-10 p.p.m.) was compared with isolate JH for response to dimethirimol applied as soil drenches and protective sprays, to see whether the differences in behaviour revealed by the leaf-disc method were also shown by these more practical methods of application. The isolates were maintained on stock plants and tested in separate glasshouses. Marked differences in response to sprays and soil drenches were obtained between the two isolates (Table 3).

Table 3

Response of two isolates of cucumber powdery mildew to spray and soil treatments with dimethirimol

Test	Concentration of dimethirimol solution (p.p.m.)	Degree of disease control* (10 days after inoculation)	
		Isolate JH	Isolate VK
Soil drench (10 ml) to seedlings in 4 cm pots; cotyledons inoculated 2 days later.	200	4	3
	100	3	2
	25	3	0
Soil drench (25 ml) to 50 cm high plants in 20 cm pots; leaves inoculated 2 days later.	5000	4	4
	1250	4	1
	500	3	0
	250	2	0
High-volume spray to seedlings in 4 cm pots; cotyledons inoculated 2 days later	300	4	1
	100	4	0
	50	4	0

* Grades 4, 3, 2, 1, 0 correspond to visible mildew covering 0, trace-5, 6-25, 26-60, 61-100 per cent of leaf or cotyledon.

In leaf-disc tests and in tests involving soil treatments to seedlings., isolate VK proved as sensitive as isolate JH to benomyl. To judge from growers' experience, dimethirimol-resistant mildew remained sensitive to dinocap and quinomethionate.

Conidia of one highly resistant and two sensitive isolates originating in England were treated in vitro with dimethirimol. Fungicide-impregnated cellulose-acetate membranes were used as substrate (see Methods section). It was found

that the spores of the spores of the resistant strain germinated normally when exposed to dimethirimol concentrations at which germination of sensitive strains was severely inhibited (Table 4). Thus the resistant reaction can be attributed entirely to a change in the pathogen itself, and it is unnecessary to invoke some alteration in the host-parasite interaction.

Table 4

Effect of dimethirimol on spore germination of sensitive and resistant isolates of S. fuliginea

Concentration of dimethirimol solution (p.p.m.)	Spore germination (%)		
	Sensitive strains		Resistant strain
	(1)*	(2)*	(3)*
10·0	0	2	50
1·0	0	3	50
0·1	21	31	49
0	40	47	51

* Tolerance levels in leaf-disc tests were (1) 0·32, (2) 0·80, (3)>10 p.p.m.

Uptake of ^{14}C-dimethirimol by sensitive and resistant isolates of S.fuliginea has been compared. The results will be presented fully elsewhere; briefly, uptake of dimethirimol by spores incubated on impregnated membranes and by mycelium on treated leaf-discs was at least as great in resistant as in sensitive isolates. Possible differences in the cellular distribution or in the metabolism of acquired dimethirimol in sensitive and resistant isolates are under study.

Resistant isolate VK was maintained for experimental purposes on untreated stock plants in Holland for 3 months without detectable change in its response to dimethirimol. Of two resistant isolates similarly maintained at Jealott's Hill, one retained a tolerance level above 5 p.p.m. for 6 months but the second became increasingly sensitive, the tolerance level gradually declining from 5 p.p.m. to 0·8 p.p.m. over a 6-week period. Thus it appears that the resistance of some but not all isolates is stable in the absence of fungicide; evidence for the maintenance of resistance in the commercial glasshouse areas in Holland was mentioned earlier.

By transferring isolates through successive leaf-disc cultures in increasing dimethirimol concentrations it is possible to induce gradual increases in tolerance level from 0·32 to 12·0 p.p.m. This has been achieved by successive 1·5-fold increases in applied dimethirimol concentration. It has also proved possible to 'train' isolates to 2·5-fold increases, but attempts with 5-fold increases in dose have been unsuccessful so far.

DISCUSSION

Several earlier occurrences of fungicide resistance, involving a wide range of compounds such as biphenyl, hexachlorbenzene and organo-mercurials, are well documented. However, the appearance of dimethirimol-tolerant infections of S. fuliginea in N.W. Europe in 1970 was surprising in its suddenness and in the

in the short period of prior usage of the fungicide. Some problems of resistance to benomyl, particularly resistance of cucurbit powdery mildew in Israel, seem to bear a closer resemblance to the phenomenon described here.

There are many important questions which we cannot answer. Did dimethirimol resistance originate from one point, or separately at several sites? Did it arise by selection of existing strains, or de novo either fortuitously or by induction? What is the biochemical mechanism of resistance, and could it be overcome? Is the risk of rapid resistance a special feature of systemic fungicides in general? Is it a particular risk in cucurbit powdery mildew, or in glasshouse crops or in other systems of intensive and continuous growing? What are the best ways of minimising the risk of resistance, or of restricting its spread? Will it be possible to predict resistance? It is important (but impracticable here) to speculate upon these questions, in the light of our own findings and of other investigations into pathogen resistance to fungicides, insecticides and bactericides.

It seems likely from the results of the laboratory 'training' experiments that the presence of sub-lethal doses of dimethirimol within cucumber plants could increase the risk of emergence of resistant mildew. Consequently, the recommendation for late-season application on older plants, which tend to take up the fungicide relatively poorly, has been withdrawn. Recommendations for the use of dimethirimol on young plants (< 2 metres high) have also been withdrawn, in order both to restrict its use to established plants where alternative treatments are inconvenient and relatively ineffective, and to avoid the risk of resistant mildew arising on nursery plants which are sent out to many growers.

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BENOMYL-INDUCED INSTABILITY OF ASPERGILLUS DIPLOIDS

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Summary Attempts to induce mutations detectable as sectoring in Aspergillus nidulans colonies are reported. No sectoring was found in colonies grown on benomyl containing medium from haploid conidia, but abundant sectors were induced in colonies grown from heterozygous diploid conidia under the same conditions. Most of these induced segregants were haploid. It is suggested that benomyl either interferes with the normal process of chromosome segregation or it induces chromosome breaks which lead directly to genetic instability.

INTRODUCTION

Aspergillus nidulans has been a popular organism for genetical research for many years (Pontecorvo et al, 1953). Its popularity stems principally from its versatility. It offers several systems for formal genetic analysis (Pontecorvo, 1959), and several levels of organisation for investigating gene action and mutagenesis. In its haploid form it is a convenient organism for investigating gene mutation (Siddiqi 1962) and the diploid form is easily adapted for the genetic detection of chromosomal mutations (Kafer 1963).

Benomyl (methyl 1-(butyl carbamoyl) -2-benzimidazole carbamate) has molecular similarities to the purine bases of nucleic acid, and it is conceivable that either it or compounds derived from it in the cell may be mutagenic. The investigation reported here was undertaken to decide whether benomyl is mutagenic in A. nidulans. Some of the results reported here were published previously (Hastie 1970).

METHODS AND MATERIALS

The three haploid strains of A. nidulans used originate from the Genetics Department, University of Glasgow. These had the genotypes bi-1; bi-1 w (Y): ad-9 meth A-17 y (bi = biotinless, ad = adenineless, meth = methionineless, w = white conidia and y = yellow conidia). A heterozygous diploid strain was selected by the technique of Roper (1952). The haploid parents were bi-1 w (Y) and ad-9 meth A-17 y. All these mutant alleles are recessive so that the heterozygous diploid had no specialised nutritional requirements and wild-type conidial colour (green).

Uninucleate conidia were exposed to benomyl by inoculating and growing them on Aspergillus complete agar medium containing benomyl. The benomyl was added to the medium immediately before pouring the plates. The general culture methods were those given by Pontecorvo et al (1953), and cultures were incubated at 37°C.

RESULTS

Effect of benomyl on haploid Conidia of the haploid strain bi-1 were spread on complete medium containing 0, 0.25 and 0.5 ppm (parts per million) benomyl. The survival of these haploid conidia was rather lower than that quoted for diploids in

Table 1. All survivors formed colonies with green conidia and no differently coloured sectors were seen in about 2000 survivors of each treatment.

Effect of benomyl on diploid Conidia of the selected heterozygous diploid were inoculated in the same conditions as the haploid referred to above. The results of the treatments are given in Table 1. The survival is the number of conidia which formed colonies, and segregation is scored as the number of colonies containing either white or yellow sectors.

Table 1

Effect of benomyl on survival and segregation of a diploid A. nidulans

Treatment	Survival		Colonies showing Segregation	
	Number	Per cent	Number	Per cent
Control (no benomyl)	87	100	4.7	5.4
0.25 ppm benomyl	75.3	86.6	31	41.2
0.5 ppm benomyl	42.7	49.1	30.6	71.6

Numbers are means of four replicates

Benomyl reduced both the size and the number of colonies as with haploid inoculum. The segregants were not only more numerous on the benomyl containing medium but also occupied a much larger area of the colony, indicating that in the presence of benomyl segregational events (sectoring) occurred earlier in colony growth. Most spontaneous sectors consisted of only a few conidiophores.

Spontaneous segregants from heterozygous diploid A. nidulans are either haploid or diploid (Pontecorvo 1959), and the two classes are readily distinguished by measuring the conidia borne on them. Conidia containing a diploid nucleus have twice the volume of those containing a haploid nucleus. The proportions of haploid and diploid segregants from colonies growing in the presence and absence of benomyl are compared in Table 2. The segregants recorded there included those recorded in Table 1, and also others from later experiments. The results show that benomyl significantly increased the proportion of haploid segregants. The proportion of haploids among all spontaneous segregants (8/62 = 11.1%) agrees with the limits of 10% to 20% haploids quoted by Pontecorvo (1959) for spontaneous segregations.

Table 2

Comparison of the relative numbers of haploid and diploid segregants recovered with and without benomyl

Treatment	Diploid Segregants	Haploid Segregants	Total
Control (no benomyl)	54	8	62
0.5 ppm benomyl	42	81	123
Total	96	89	185

Contingency chi^2 = 44.4 (P 0.01)

DISCUSSION

Numerous genes are known in A. nidulans which can mutate to alleles causing unusual conidial colours (Dorn 1967; Clutterbuck 1969). If a mutation occurs during the growth of a colony from a haploid conidium a sector with mutant conidial colour is formed. Such sectors occur following treatments with nitrous acid (Siddiqi 1962). The absence of such sectors when the haploid strain was grown on benomyl containing medium seems to imply that benomyl does not cause gene mutations. It therefore seems necessary to seek other reasons for the sectoring induced in the heterozygous diploid.

Diploid strains of A. nidulans heterozygous for genes affecting conidial colour sector spontaneously at low frequency. These spontaneous segregants are formed principally by mitotic recombination, which gives homozygous diploid segregants, and haploidisation which yields haploid segregants (Pontecorvo 1959). The genetic effect of the benomyl on the heterozygous diploid was two-fold. There was both an overall increased frequency of segregation and also a larger proportion of the benomyl induced segregants were haploid.

Spontaneous haploidisation occurs through a series of non-equational nuclear divisions at which there is mitotic non-disjunction (Kafer, 1961). Benomyl may cause increased segregation by increasing the frequency of mitotic non-disjunction. This implies that benomyl acts by increasing the rate of the spontaneous process. Alternatively benomyl may induce segregation by a scheme like that proposed by Kafer (1963) to explain the segregation of A. nidulans caused by mutagens which break chromosomes. Chromosome breakage leads to the formation of acentric fragments. These are excluded from daughter nuclei at subsequent nuclear divisions. The daughter nuclei are therefore hemizygous for the corresponding chromosome regions, and these nuclei tend to be unstable. Both these alternative schemes for inducing segregation would be undetectable in haploids because they have no heterozygosity which can segregate.

To distinguish between the two mechanisms suggested it is necessary to decide whether benomyl causes chromosome breaks. The chromosomes of A. nidulans are very small, and chromosome breaks would be very difficult to detect cytologically. However chromosome breakage occasionally leads to the formation of translocations. These can quite easily be detected genetically in diploid A. nidulans (Kafer 1963), and a search for these would enable a distinction to be made between the proposed mechanisms.

Benomyl is mutagenic to A. nidulans, but perhaps only in the limited sense that its effects lead to changes in chromosome number. It is obviously important to investigate whether it has similar effects at sub-lethal doses in other organisms.

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INTERACTIONS BETWEEN SYSTEMIC FUNGICIDES AND BARLEY GENOTYPES: THEIR IMPLICATIONS IN THE CONTROL OF MILDEW

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Summary A study was made of the effectiveness of benomyl and ethirimol in the control of mildew (Erysiphe graminis hordei) on barley varieties believed to differ in their inherent levels of partial resistance and of the differential response of the varieties to the specific chemicals as measured by disease control. Regression analysis on data involving mildew infection at different dose rates of ethirimol and benomyl showed that the variety with the highest inherent resistance (Vada) was relatively less responsive to increased fungicide application over the concentrations tested than were the other varieties, but it required the least amount of chemical for absolute control of mildew. These results are discussed in relation to the genetic systems controlling response and inherent resistance, and their significance in the integrated control of disease.

INTRODUCTION

During the last 20 years cereal breeders have largely been concerned with the incorporation of race-specific resistance into cultivated varieties as a means of mildew control. When effective, such resistance confers near immunity to the crop, but experience has shown that due to the emergence of new physiologic races its effectiveness in practice is short lived. More recently, increasing attention has been paid to the development of varieties with high levels of adult plant resistance. This type of resistance appears to be more stable with reference to physiologic races, but its expression is incomplete and influenced by the stage of growth of the plant and by fluctuations in the environment (Jones and Hayes, 1971). The introduction of translocatable fungicides with activity against barley mildew (Erysiphe graminis hordei), such as ethirimol (Bebbington et al, 1969) and benomyl (Doodson and Saunders, 1969), presents the possibility of integrating their use with adult plant resistance to give stable disease control.

The experiments reported here were designed to investigate the response to translocatable fungicides of barley genotypes differing in levels of adult plant resistance to mildew, and thus to study the feasibility of integrating these control measures.

METHOD AND MATERIALS

The experiments have been designed for precise assessments of mildew infection on single plants subjected to chemical treatments. For all the mildew experiments, the four spring barley varieties Vada, Archer, Plumage Archer and Haisa were selected on the basis of their seedling susceptibility to all races tested and of differences in their expression of adult plant resistance.

Experiment 1: Benomyl soil drench : Glasshouse experiment; single inoculation.

Single plants of the four varieties were grown together in 18 cm pots in John Innes No. 2 potting compost. Five different dose rates of the chemical together with a water control were applied as a soil drench at a rate of 20 ml per pot giving 0, 1, 2, 4, 8 and 16 mg of benomyl a.i. per pot with 5 replicates. The plants were grown in a mildew-free glasshouse, chemically treated at the first leaf stage, and inoculated 24 hours later by shaking mildew-infected plants over them. The percentage leaf area covered with mildew was assessed 10 days after inoculation when the plants were in the 4th leaf stage. Ethirimol treatments were also included, but the results were variable. This was attributed to the method of application of the chemical, consequently the following experiment was designed to overcome this problem.

Experiment 2: Ethirimol soil mix : Glasshouse experiment; single inoculation.

Pots were filled to within 5 cm from the top with John Innes No. 2 potting compost. Finely sieved soil was thoroughly mixed with ethirimol in a drum to give final concentrations of 0, 0.8, 4, 16, 40 and 80 mg a.i. per 20 g of soil. One 20 g aliquot was shaken evenly over the surface of the soil in each pot to give 5 replicates of each treatment. A germinated seed of each of the four varieties was placed in each pot and covered with 3 cm of vermiculite. The plants were grown in a mildew-free glasshouse and were inoculated as described above at the 3rd leaf stage. The percentage leaf area covered with mildew was assessed 10 days after inoculation. This technique which gave more precise results was used subsequently to study the chemical response of adult plants under conditions of continuous inoculum in the field.

Experiment 3: Ethirimol soil mix : Field experiment; continuous inoculation.

Different quantities of ethirimol were mixed in a revolving drum with John Innes No. 3 compost in which the peat had been replaced by vermiculite. Whalehide pots 9 cm diameter were filled with treated soil giving ten chemical treatments at final concentrations of 0, 25, 37.5, 50, 75, 100, 150, 200, 300 and 400 mg a.i. per pot. Single germinated seeds were planted in each pot in a glasshouse where the plants were grown to the 2nd leaf stage prior to transplanting to the field. Single drills of Plumage Archer, which was to act as the mildew spreader, had been previously sown to provide a 10 x 10 lattice of 100 squares each measuring 60 x 60 cm. Within each square one pot of each of the four varieties was planted. Ten replicates of each of the ten treatments were arranged in a randomized split block design with the varieties providing the last split. The spreader drills were dusted with spores from heavily infected potted plants to initiate mildew infection. The percentage leaf area covered with mildew was assessed on a whole plant basis at the flag leaf stage of growth and again after ear emergence.

Experiment 4: Five chemicals : Field experiment.

The two varieties Vada and Plumage Archer were used and similar methods to those described for experiment 3 were employed. Benomyl, dodemorph, tridemorph, tetrachloroquinoxaline and dimethirimol were incorporated into soil mixes to give concentrations of 0, 25, 37.5, 50, 75, 100, 150, 200, 300 and 400 mg a.i. per pot.

RESULTS

The mean levels of mildew infection, expressed as percentage and angular transformation for the first three experiments, are given in Table 1a-d. The analysis of variance of the transformed data (Table 2) shows that variety differences were significant in all experiments and that there were significant variety x dosage interactions in Experiments 2 and 3.

Table 1

Mean percent leaf cover of mildew on four spring barley varieties (Treatment = mg a.i. per pot)

Treatment	Vada		Archer		Plumage Archer		Haisa	
	%	Angle	%	Angle	%	Angle	%	Angle
	a) Experiment 1. Benomyl soil drench glasshouse							
0	23.3	28.9	46.7	43.0	68.3	55.8	43.3	41.1
1	18.3	24.2	46.7	42.4	70.0	57.6	30.0	32.3
2	11.6	19.5	48.3	44.0	65.0	53.8	28.3	31.8
4	7.0	14.9	58.3	49.9	66.7	55.4	31.6	34.0
8	7.7	15.2	41.7	40.0	61.7	51.9	22.0	26.0
16	4.0	8.9	36.7	31.9	38.7	34.9	25.0	29.2
S.E. for the difference between two treatment means for the same or different varieties = ± 10.79								
	b) Experiment 2. Ethirimol soil mixture, glasshouse							
0	5.6	13.1	25.0	29.8	36.0	36.7	20.0	25.5
0.8	7.0	15.1	21.4	26.9	28.0	31.7	21.0	27.2
4	2.8	9.3	14.6	21.1	13.6	20.5	10.4	18.0
8	2.0	7.0	11.0	18.6	9.4	17.2	8.6	16.3
16	1.4	4.0	6.4	13.2	4.0	11.3	3.0	8.6
40	0.0	0.0	0.2	1.1	1.0	4.4	1.0	4.3
80	0.0	0.0	0.2	1.1	0.4	2.3	0.2	1.2
S.E. for the differences between the two treatment means for the same or different varieties = ± 3.29								
	c) Experiment 3(a). Ethirimol soil mixture. Field, 6/7/71							
0	14.0	21.5	66.0	54.5	59.0	50.1	58.5	49.9
25	3.1	6.7	32.5	34.1	31.5	31.7	23.1	27.0
37.5	0.8	3.0	23.5	25.0	23.5	25.9	25.0	28.6
50	0.4	2.3	19.5	23.4	17.6	22.2	15.0	20.7
75	0.2	1.1	22.5	26.3	21.0	25.6	6.9	10.4
100	0.5	1.3	4.9	10.8	5.4	11.3	2.0	6.0
150	0.1	1.0	4.8	9.4	3.8	6.6	3.8	7.6
200	0.0	0.0	3.5	8.7	1.5	4.7	0.9	3.6
300	0.1	0.6	1.0	4.2	1.4	4.1	1.7	4.3
400	0.1	0.6	1.0	4.2	0.5	2.9	0.4	2.3
S.E. for the difference between two treatment means for the same or different varieties = ± 4.02								
	d) Experiment 3(b). Ethirimol soil mixture. Field, 27/7/71							
0	10.5	18.5	35.0	36.1	41.0	39.6	38.0	37.8
25	5.5	12.2	15.8	22.9	24.7	29.3	18.8	23.7
37.5	6.1	12.6	9.2	15.0	15.8	21.4	23.3	26.8
50	3.9	10.6	13.1	19.1	17.0	24.2	18.6	23.8
75	3.2	9.5	15.1	20.9	22.5	27.4	12.4	17.1
100	3.6	8.6	7.4	13.2	14.0	19.9	9.6	19.4
150	2.9	8.6	6.1	11.9	12.8	18.6	13.9	18.3
200	1.7	5.8	5.7	9.0	8.5	17.1	4.9	10.9
300	1.4	5.0	2.2	6.0	3.3	6.4	7.5	12.3
400	1.8	3.3	5.3	12.0	6.2	11.3	4.4	9.4
S.E. for the difference between two treatment means for the same or different varieties = ± 3.76								

Table 2

Summary of analysis of variance of angular transformed data for % leaf covered with mildew in Experiments 1, 2 & 3

Source	Degrees of freedom and mean squares for data from: Experiment 1		Experiment 2		Experiment 3(a)		Experiment 3(b)	
	d.f.	M.S.	d.f.	M.S.	d.f.	M.S.	d.f.	M.S.
Blocks	2	97.92	4	153.8*	9	214.3	9	279.0**
Dose rates(D)	25	436.1	6	2,027.7***	9	6,769.4***	9	2,223.7***
Error (a)	10	515.3	24	41.24	81	138.1	81	93.30
Varieties (V)	3	3,598.5***	3	791.06***	3	5,452.3***	3	2,858.7***
D x V	15	47.45	18	58.14***	27	315.0***	27	110.5*
Error (b)	36	61.16	84	22.26	270	62.00	270	63.2

Experiment 1. The highest dosage of benomyl reduced mildew infection on all four varieties (Table 1a). Vada had a significantly smaller area of the leaf infected with mildew than the other three varieties. The absolute and relative reduction in mildew after chemical treatment was greatest in Vada in which, for example, 16 mg a.i. benomyl gave over a five-fold reduction compared with less than a two-fold decrease for the other varieties.

Experiment 2. The mean level of mildew infection in this experiment was lower than in Experiment 1, but the varieties showed the same relative degrees of infection in the control pots. Significant reduction of mildew was obtained with ethirimol for all varieties, infection being virtually eliminated at the 40 mg a.i. dose.

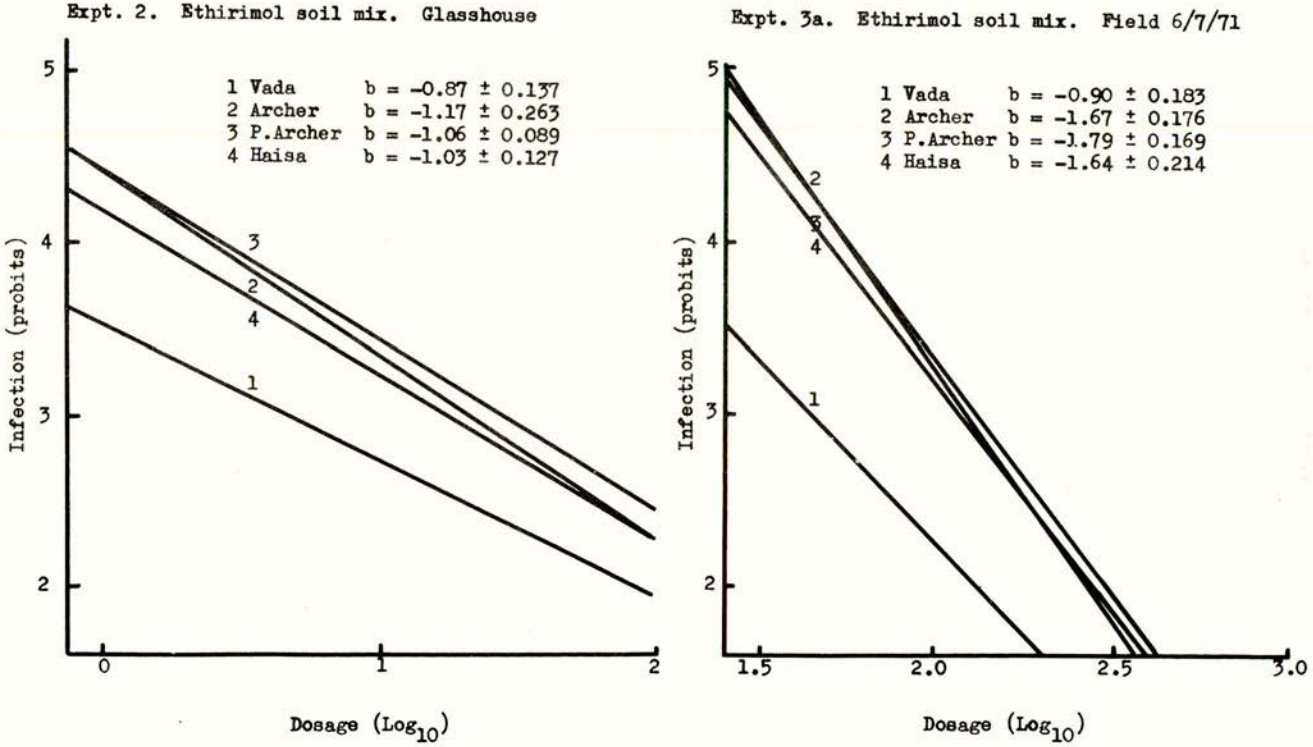
Experiment 3. The responses of the four varieties to ethirimol under conditions of natural infection in the field when assessed at two stages of adult growth, are given in Table 1c and d. Significant reductions in mildew at both stages of growth were obtained on all varieties after treatment. The relative reductions in mildew at the flag leaf stage at the four lower dose levels were greater for Vada than for the other three varieties. Under these treatments Plumage Archer and Archer were less responsive than Haisa. The virtual complete control obtained with the highest level (400 mg a.i.) just prior to ear emergence was not so marked three weeks later.

Table 3

The lowest dose rate (mg a.i.) at which five fungicides significantly reduced the plant height of barley

Variety	Dimethirimol	Dodemorph	Tetra-chloro-quinoxaline	Benomyl	Tridemorph
Vada	200	50	200	150	25 (100*)
Plumage Archer	50	25	200	400	25 (37.5*)

Fig. 1. Response of barley genotypes to ethirimol



Experiment 4. Dimethirimol, dodemorph and benomyl at the concentrations tested reduced mildew on both Vada and Plumage Archer, but the pattern of response was variable especially where growth was retarded due to chemical treatment. Significant reduction in plant height was observed at different dose levels (Table 3). Plumage Archer was more sensitive than Vada to dodemorph, dimethirimol and tridemorph. With benomyl the order was reversed, but the results in this case were more variable.

DISCUSSION

Several points of interest emerge from an interpretation of the significant interactions observed between varieties and fungicides in these experiments.

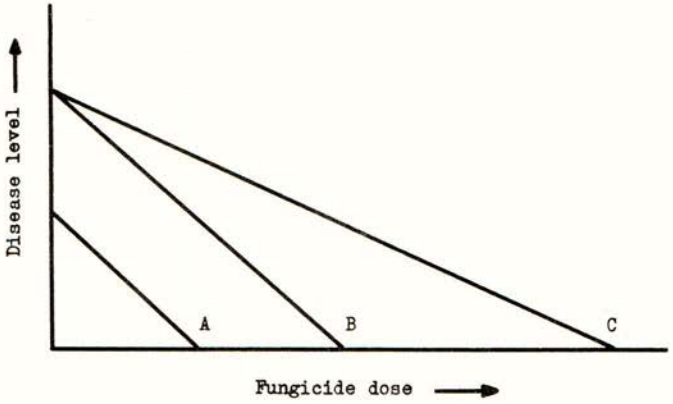
Both benomyl and ethirimol proved effective in reducing mildew on the four varieties tested. Vada had the least amount of mildew in the absence of the chemical treatments, reflecting its relative resistance even at the juvenile stage. It also required considerably less chemical to give virtually complete mildew control. This complementation of the partial resistance of Vada and, to a lesser extent, Haisa is of great practical significance since it provides the opportunity for stable control of mildew with the application of a minimum amount of fungicide.

The assessment of the relative response of the different varieties to the fungicide treatment presents some difficulty since the activity of the chemical in the plant is expressed only through the growth of the mildew. Also the expression of race non-specific resistance as measured by % leaf area infected can vary according to the level of inoculum and the stage of growth of the plant (Jones and Hayes, 1971). The relatively high reduction in mildew at the lower concentrations of benomyl in Experiment 1 and the 79% reduction in mildew in the field plots at flag emergence following the application of 25 mg a.i. ethirimol, suggests that Vada may be highly responsive to low concentrations of the fungicide. However, assessments of the relative responses of the varieties to ethirimol by regression of the log dose on probit infection show that, over the range of concentrations tested, Vada is less responsive to increased levels of the fungicide than the other three varieties (Fig. 1a and b). These differences in response are probably due to genetic differences in physiological processes reflected in differential uptake, translocation, persistence etc.

It is not possible from the results of these experiments with a limited number of genotypes to establish conclusively whether varieties with the same level of inherent mildew resistance respond similarly to varying doses of the fungicide. To do this it will be necessary to test many varieties, with varying levels of inherent resistance, against a wide range of chemical concentrations. It is probable that varieties differing in genetic resistance to mildew have different rates of response to fungicides as shown diagrammatically in Fig. 2. Variety A would be the most desirable model to aim for, since it combines a high level of inherent resistance to mildew with a high rate of response to applied fungicide. However, if such a variety is not available, it would be preferable from the standpoint of disease control to grow variety B, rather than variety C. Both have a lower level of inherent resistance but the former is more responsive to the fungicide.

If the negative association between the inherent level of mildew resistance (y) and the rate of response (regression value "b") observed in these experiments proves to be general, then it will be possible to select genotypes giving the best response to the fungicide on the basis of their mean level of resistance. A lack of association would indicate that the inherent resistance and response to the chemical are under separate genetic control and hence capable of improvement by breeding.

Fig. 2. Theoretical responses of genotypes A, B and C to a systemic fungicide



The results of Experiment 4 reveal that, compared with Plumage Archer, Vada is relatively more resistant to the adverse effects of increasing concentrations of three different chemicals when applied as soil treatments. We also have evidence that varietal differences in damage occur following foliar applications of tridemorph. The existence of such varietal differences is perhaps not surprising in the light of previous results showing that genetic differences exist for resistance to damage from chemicals applied as herbicides and insecticides (Hayes et al 1965). This provides the opportunity for breeders in collaboration with chemists to produce varieties and chemicals which complement each other not only in providing maximum expression of resistance to a pathogen, but also in exhibiting minimal adverse effect of the chemical applied.

Acknowledgements

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THE STRUCTURE OF MYCOPLASMA AND THE PROBLEM OF CONTROL METHODS

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SUMMARY. The ultrastructural features of plant and animal mycoplasmas are discussed together with their possible mode of replication as intracellular organisms. Control may be similar to virus infection of plants.

The existence of mycoplasmas as pathogenic organisms was established during the early part of this century and they are now considered to be the smallest free-living micro-organisms. They have been estimated to fall within a size range of about 120 nm to 600 nm across (1 nanometre = 1 millimicron). The mycoplasma cell lacks any form of wall structure and is bounded by a single unit-membrane. From morphological and hydrodynamic studies it has been demonstrated that mycoplasmas possess ribosomes of the bacterial type. Slender strands which have been interpreted from electron micrographs as being DNA were observed to be loosely distributed in the mycoplasma cell cytoplasm. Recent studies on the carbohydrate composition have indicated that the percentage of dry weight varies according to the strains analysed. It has also been established that mycoplasmas have a number of enzyme systems associated with the respiratory pathways.

Most of the early work on the isolation and identification of mycoplasmas resulted from investigations concerned with diseases of cattle, fowls and man. From these studies it was possible to establish a number of biological, morphological and chemical characteristics which were considered by Sabin (1941) as forming a basis for their classification as pleuropneumonia-like organisms (PPLO). It should be mentioned that similar bodies were also found to be associated with a number of bacteria and these were described by Kleineberger-Nobel (1947) as being L-forms of bacteria. The observations on bacteria have raised a number of controversial points concerning the

relationship between bacterial L-forms and mycoplasmas.

The study of mycoplasmas and their association with a variety of diseases in animals and plants is of considerable economical importance. Moreover, it is becoming clear that a number of diseases originally attributed to virus infection now appear to be caused by mycoplasmas or mycoplasma-like bodies. The fact that these organisms have been isolated from plants, together with other reasons, has resulted in the name mycoplasma being considered as being most suitable, rather than the early PPLO classification (cf. Nowak 1929).

Although mycoplasmas can be detected with the aid of light microscopy, ultrastructural studies with the aid of electron microscopy have revealed a number of basic morphological features. Fig. 1. These features are of particular interest when they are linked to biological techniques. Specimens of mycoplasmas are usually prepared from colonies grown on artificial media. There are few publications dealing with the morphological aspects of mycoplasma and host cell interaction.

In the case of plants it is becoming clear that there is more information derived from electron micrographs showing the presence of mycoplasma or mycoplasma-like organisms within the plant cell cytoplasm compared with animal cell systems. These bodies are identical in their morphology to the mycoplasmas isolated from animal infections and grown on artificial media. Some of the diseases in plants together with their insect vectors in which mycoplasma has been detected are list in Table 1. There are several reports which have appeared in the literature and serve as examples for demonstrating the presence of mycoplasma-like structures or mycoplasma in diseased plants. Shikata et al. (1968) found large concentrations of the agent in phloem tissues removed from sugar-cane plants with white leaf disease. Similar findings were reported by Maramorosch et al. (1968) in a study of the Philippine rice yellow dwarf disease. Spike disease of sandal trees in Southern India is an interesting example, showing large numbers of phloem cells containing mycoplasma-like bodies. This disease was for many years considered to be caused by virus infection (Hull et al. 1969).

Electron micrographs of Siberian wallflower tissues prepared by thin sectioning methods show extensive replication of mycoplasma bodies in the

cytoplasm of cells. Fig. 2. The presence of mycoplasma as intracellular structures raises a number of important questions in basic cell biology and also problems in the approach for possible treatment of infected plants. From the evidence derived from electron micrographs there is a strong indication that mycoplasma are being assembled within the cells in a similar manner to some of the more complex virus particles.

Table 1.

Plant diseases which have mycoplasma-like bodies associated with them.

Disease	Transmission leafhopper	Phyllody* and witches broom symptoms
Aster yellows	+	+
Clover dwarf	+	+
Clover phyllody	+	+
Corn stunt	+	
Cotton phyllody		+
Crimean yellows		+
Legume little leaf		+
Mulberry dwarf	+	+
Oat sterile dwarf	+	
Parastolbur	+	+
Paulownia witches broom		+
Potato witches broom	+	+
Rice grassy stunt	+	
Rice yellow dwarf	+	
Stolbur	+	+
Sugar cane white leaf	+	
'Pea mycoplasm'	Aphid	

*Phyllody: Floral parts developing more or less as normal foliage leaves.

Recent investigations have revealed that diseases in plants caused by mycoplasmas are widespread and aster yellows is a typical example. Treatment of the various plants to control the insect vectors is of obvious economical importance. Some success has already been achieved by the application of

insecticides against the insect vectors, which is essentially similar to the current methods used to control the spread of virus infection.

It has been established that mycoplasmas do not react to the majority of antibiotics, but experiments have shown that growth can be inhibited by tetracycline hydrochloric. From the current experiments on both animal and plant mycoplasmas it is evident that treatment of mycoplasma diseases presents some unusual problems. On the one hand it is possible to isolate the organisms from the host and culture them on a suitable artificial media, but on the other hand the observations on plant diseases show mycoplasma replicating in the cell cytoplasm and in some instances within the associated insect vector cells (cf. Chen and Granados, 1970).

For the future, it is clear that progress in terms of complete treatment will only be made possible following further studies on the basic cell biology, mechanism of replication and chemical composition of mycoplasmas (cf. Horne, 1970). One of the immediate problems facing plant pathologists is that of developing techniques to isolate mycoplasmas from plants and grow them on artificial media along similar lines now well established for studying mycoplasma diseases in animals and man.

The reader is referred to the following publications dealing with mycoplasmas generally.

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The electron micrographs shown in Figs. 1 and 2 are from recent unpublished work by Miss A. Plaskitt of the John Innes Institute.

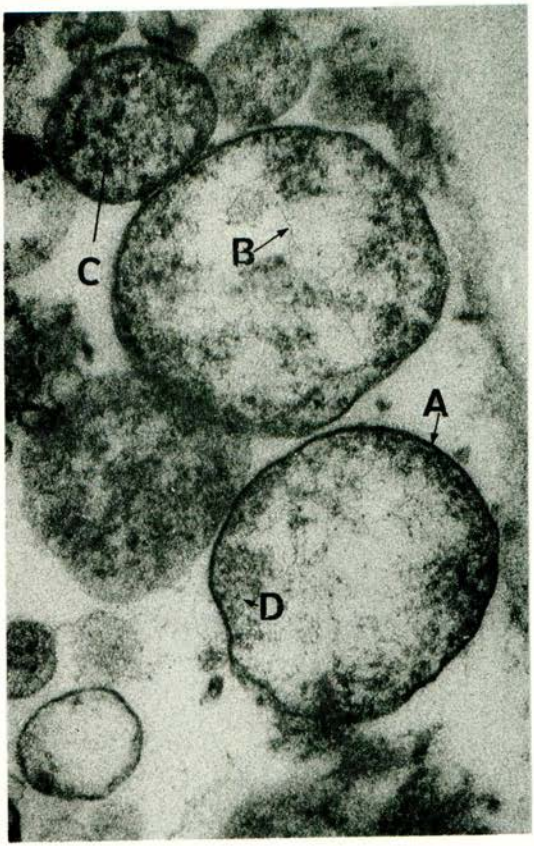

FIG. 1. Electron micrograph of a thin section from phloem tissue showing typical mycoplasma bodies in cells with clover phyllody. Magnification 96,000X. The ultrastructural features which are characteristic of most mycoplasmas prepared by thin section methods are indicated below:

(A) Unit membrane (B) Strands of DNA (C) Ribosomes

(D) Fine cytoplasmic contents

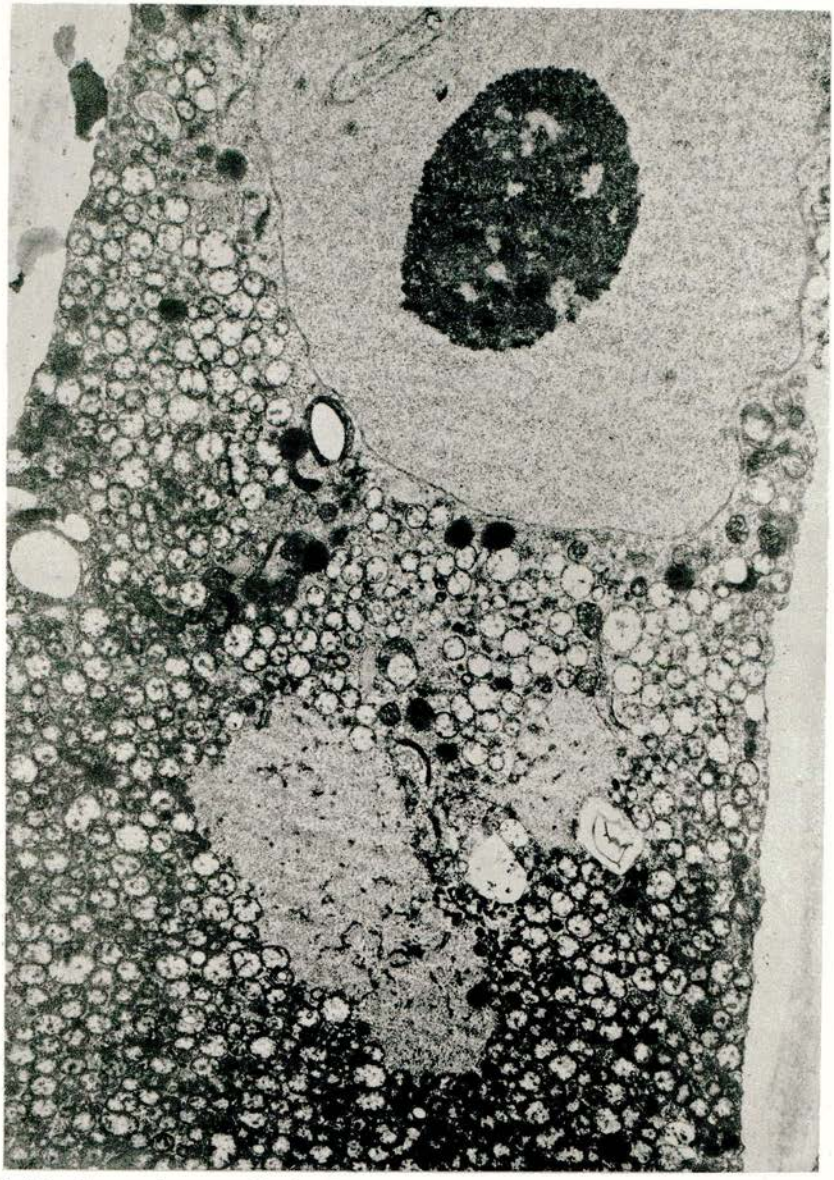

FIG. 2. Electron micrograph of a thin section from Siberian wallflower shows the presence of large numbers of mycoplasma-like bodies within the cell cytoplasm. The size range of the particles vary from about 150 nm diameter corresponding to the dense elementary bodies, to larger structures of about 500 nm across. These structures are morphologically identical to animal mycoplasmas. Mag. 11,000X.

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EPIDEMIOLOGY AND CONTROL OF WEEVIL-TRANSMITTED VIRUSES IN FIELD BEANS

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Summary Broad bean stain virus and Echtes Ackerbohnemosaik-Virus sometimes cause indistinguishable mottle and mosaic leaf symptoms in field beans. Combined incidence of the two viruses ranged from 2 to 92% in nine crops of field beans examined in 1970 and 1971. Early infection of field beans by either virus can decrease yield by c. 70%. Both viruses are transmitted through seed and by adult weevils of the genera Apion and Sitona. Of treatments tested, aldicarb applied to the soil at drilling time best controlled adult weevils and checked spread of both viruses; gamma BHC applied to the foliage was much less effective.

INTRODUCTION

Between 1957 and 1962 only three viruses, all aphid-transmitted, were common in crops of field beans (Vicia faba L.) in the U.K.; bean (pea) leaf roll, pea enation mosaic and pea mosaic (Heathcote & Gibbs, 1962; Aldrich et al., 1965). However, broad bean stain virus (BBSV) (Lloyd et al., 1965; Gibbs et al., 1968) was found in field beans at Rothamsted in 1969 (Cockbain, 1970) and Echtes Ackerbohnemosaik-Virus (EAMV) (Quantz, 1953), syn., broad bean true mosaic virus, was isolated from seed harvested at Rothamsted in 1970. Before 1971, studies of the incidence of BBSV in field beans probably confused infection with BBSV and EAMV (Cockbain, 1971).

BBSV and EAMV have similar-sized isometric particles, they are seed-borne and cause indistinguishable symptoms in broad beans (Gibbs et al., 1968) and sometimes indistinguishable symptoms in field beans. Gibbs et al.(1968) failed to transmit the two viruses by aphids, bees, nematodes or weevils but recently BBSV was transmitted by adult weevils of the genera Apion and Sitona (Cockbain, 1971) and this paper reports that these weevils also transmit EAMV.

RESULTS

Symptoms

Field beans naturally-infected with EAMV often had more severe leaf symptoms

than those infected with BBSV, but sometimes the symptoms were indistinguishable. As in broad beans (Gibbs et al., 1968), the severity of symptoms differed on successive leaves. Indeed, some leaves appeared normal whereas others showed symptoms ranging from a faint green mosaic or mottle to a severe yellow mottle and distortion.

Incidence

Table 1 gives the proportion of plants with symptoms of BBSV, EAMV and other viruses in six crops of the variety Maris Bead at different sites in July 1970 and 1971. BBSV and EAMV are combined because it is sometimes impossible to distinguish their symptoms. Usually they were more common than bean leaf roll virus (BLRV), pea enation mosaic virus (PEMV) and pea mosaic virus (PMV). In other crops not listed in Table 1 the proportion of plants with symptoms of BBSV/EAMV ranged from 5 to 54%.

Table 1

Incidence of viruses in field beans

Year	Site	% plants infected with: BBSV/EAMV	BLRV	PEMV, PMV and other viruses
1970	Broom's Barn, Suffolk	19	5	0
	Rothamsted, Herts.	60	16	1
	Woburn Farm, Beds.	22	6	2
1971	Bridget's Farm, Hants.	92	17	15
	Broom's Barn, Suffolk	2	29	3
	Rothamsted, Herts.	30	30	3

Serological tests on sixty plants with obvious symptoms of BBSV/EAMV from crops in 1971 showed that 37% were infected with BBSV and 63% with EAMV.

Effects on yield

One experiment in 1970 assessed the effects of natural infection with BBSV (and possibly EAMV) on the yield of field beans (Cockbain, 1971). Plants that showed symptoms respectively before, during or after flowering yielded 76%, 62% and 31% less weight of seed than plants without symptoms. In 1971, plants that were naturally infected before flowering and which showed very mild mottle or mosaic symptoms (mainly BBSV) yielded 70% less, and plants with severe mottle or mosaic symptoms (mainly EAMV) 76% less, than uninfected plants.

Some plants infected with BBSV or EAMV through the seed flowered but none set seed in crops grown in 1971.

Transmission through seed

Of a sample of 400 seeds harvested at Rothamsted in 1970, nine produced seedlings infected with BBSV, and four with EAMV. Eight out of fourteen other stocks

of field-bean seed from different sources also contained some infected seeds; maximum incidence of BBSV was 0.3%, and of EAMV, 1.7%.

Transmission by weevils

The only known vectors of BBSV are species of Apion and Sitona (Cockbain, 1971); these weevils also transmit EAMV.

Weevils were collected during June 1971 from a crop of field beans in which 12% of plants showed symptoms of BBSV/EAMV; they were then caged for three days on healthy bean seedlings. EAMV was transmitted by 10% of A. vorax and 1% of S. lineatus; BBSV was transmitted by 8% of A. vorax but not by S. lineatus.

EAMV was transmitted by 53% of A. vorax, but not by A. aestivum, A. aethiops, A. apricans, A. assimile and A. pisi, when these were caged first for five days on infected plants and then for five days on healthy seedlings in the glasshouse.

Control by insecticides

Insecticides were used in two field experiments intended primarily to identify the vectors of these viruses and to estimate how weevil- and aphid-infestations and virus infection affected yield. Only results relevant to the spread and control of BBSV and EAMV are considered here.

In the 1970 experiment, five treatments were replicated five times on plots measuring 6.4 x 4.0 m:

1. Untreated,
2. gamma BHC watered on the soil surface and then rotavated-in before drilling in early April, 1 lb a.i./acre (1.12 kg/ha),
3. gamma BHC sprayed on the foliage five times between mid-May and late July, 0.5 lb a.i./acre (0.56 kg/ha) each time,
4. Menazon sprayed on the foliage five times between early June and early August, 0.25 lb a.i./acre (0.28 kg/ha) each time,
5. Aldicarb ('Temik') dusted on the soil surface and then rotavated-in before drilling, 10 lb a.i./acre (11.2 kg/ha).

The second experiment, in 1971, had larger plots (12.2 x 10.7 m) and four replicates of six treatments; gamma BHC was not applied to the soil, and aldicarb was applied at two rates (1 and 10 lb a.i./acre : 1.12 and 11.2 kg/ha) before drilling at the beginning of March; malathion (1lb a.i./acre : 1.12 kg/ha), gamma BHC and menazon were sprayed on the foliage three times between late April and late June.

Fewer than 0.5% of seedlings emerged infected with BBSV/EAMV in the 1970 experiment, whereas 1% did in 1971, but the viruses spread more in 1970 (Table 2). In both years the viruses seemed to spread most in June; adults of Apion (mainly A. vorax) were more abundant, and adults of Sitona (mainly S. lineatus) less abundant, in June 1970 than in 1971. This is a further indication that A. vorax, although much less abundant than S. lineatus on field beans, is a more efficient vector of BBSV and EAMV.

The larger amount of aldicarb decreased adult weevils and spread of BBSV and EAMV most in both years (Table 2); the smaller amount had a similar effect to malathion and lessened the numbers of Sitona but did not significantly decrease the incidence of BBSV and EAMV. Spraying with gamma BHC seemed not to affect numbers of adult weevils but lessened the incidence of BBSV and EAMV by a third in 1970 and, like menazon, by a half in 1971.

Table 2

Effects of insecticides on number of adult weevils and incidence of BBSV and EAMV

Year	Treatment	No. of adults in June		% plants infected with BBSV/EAMV in July
		Sitona*	Apion*	
1970	Untreated	6.0	2.0	48
	γBHC (soil)	6.8	3.0	50
	γBHC (foliage)	9.4	1.6	31
	Menazon (foliage)	4.6	2.2	40
	Aldicarb (soil)	0	0.8	19
	S.E.	±1.28	±0.47	±4.4
1971	Untreated	12.8	0.8	17
	γBHC (foliage)	10.3	0.3	9
	Malathion (foliage)	5.0	0.3	16
	Menazon (foliage)	10.0	0.8	9
	Aldicarb (soil ; 1 lb)	7.3	0.3	13
	Aldicarb (soil ; 10 lb)	0	0	5
	S.E.	±1.19	±0.21	±2.2

* No. per 50 shoots; mean of 5 (1970) or 4 (1971) replicates

Possibly some of these treatments, especially sprays of gamma BHC, would have been more effective had they been applied to whole crops instead of to small plots, because their effectiveness may have been diminished by weevils immigrating from nearby untreated plots.

DISCUSSION

Present results indicate that BBSV and EAMV spread rapidly within a crop when some seedlings emerge infected and when weevils, especially A. vorax, are abundant. Although insecticides that are effective against weevils may be useful in slowing the spread of BBSV and EAMV within a crop, it may eventually be cheaper and safer to ensure that seed stocks are free from infection. The possibility of heat-treating infected seed stocks to prevent emergence of infected seedlings is being investigated.

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THE CONTROL OF INSECT-TRANSMITTED VIRUSES OF CEREALS

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Summary Barley yellow dwarf virus is the most widespread and damaging of insect-transmitted viruses of cereal crops in Britain but the losses it causes differ greatly in different parts of the country and from year to year. Isolates of the virus differ in virulence and not all are transmitted equally well by the several species of aphid vector. Crops in south and south western counties are worst affected because the mild winters enable vectors to overwinter on hosts of the virus infected with virulent isolates.

Field studies of the occurrence of infective aphids in eastern counties have helped to show when insecticides should be applied, but the virus spread too little in the experiments for spraying to have large effects on yield. It is hoped that virus incidence can eventually be predicted from knowledge of overwintering vectors and when and how many carry virus into the crops.

INTRODUCTION

In Britain only two diseases of cereals spread by insects are known to be economically important. Barley yellow dwarf is caused by a virus (BYDV) which is transmitted by several species of aphids and occurs in forms differing in virulence. European wheat striate mosaic (EWSM) (Plumb, 1971), which is spread by planthoppers has similarities to virus diseases, but its causal agent is not known. Although EWSM is extremely damaging it is seldom prevalent and this paper is confined to BYDV.

Doodson and Saunders (1970a,b) infected many commercial varieties of cereals with BYDV and recorded losses up to 90% of yield in the spring cvs. 'Blenda' (oats) and 'Deba Abed' (barley). Natural infection is seldom so damaging as experiments at Rothamsted showed (Table 1), and Doodson and Saunders (1969) estimated losses of 5-10% although larger losses occur (Watson, 1959).

The epidemiology of BYDV is complicated by the different conditions its several vectors require to complete their life-cycles, by differences in virulence and vector specificity between virus isolates and in their prevalence in different parts of the country (Table 2).

This paper describes methods used during the past three years to measure the population of aphids, their infectivity and effectiveness as vectors of different BYDV isolates, and the possible correlations between the results and field incidence and effect of infection on yield.

Table 1

Effects of aphids, BYDV and insecticide on yield of Barley (cv. 'Zephyr'), Rothamsted 1969

Grain yield [cwt/ac (Tonnes/ha)]

Introduced: Aphid	None	R. padi	S. avenae	R. padi	S. avenae	
Virus	-	-	-	+	+	
No insecticide	48.8 (6.15)	42.8 (5.4)	49.1 (6.17)	42.1 (5.28)	24.6 (3.08)	S.E. ±1.72 (0.22)
Insecticide	48.2 (6.05)	45.1 (5.7)	45.0 (5.65)	39.6 (4.97)	27.1 (3.4)	

Table 2

Origin, virulence and vectors of BYDV isolates tested at Rothamsted, 1961-68

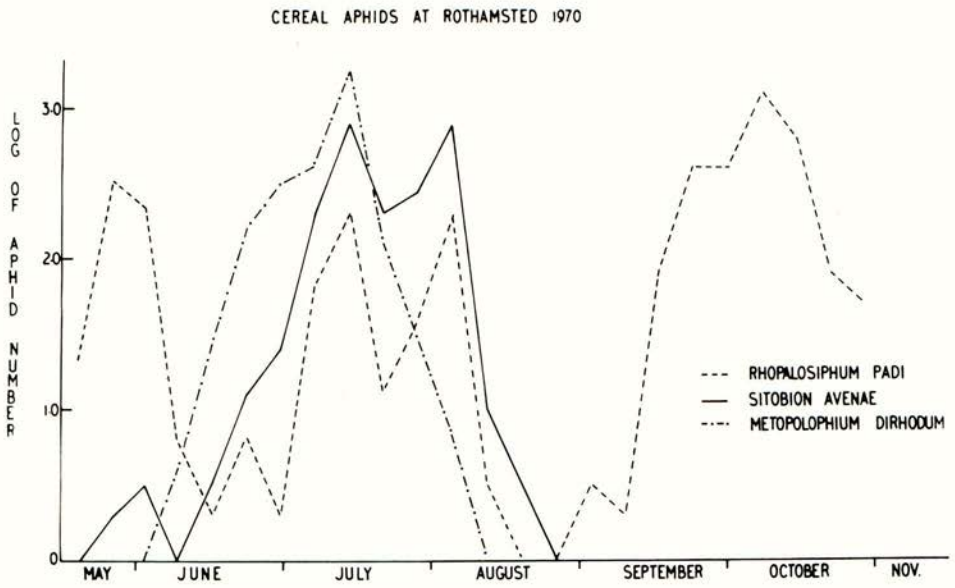
Origin:	South & West Britain		North & East Britain	
Isolate:	Avirulent	Virulent	Avirulent	Virulent
Vectors:				
R. padi	5	8	14	7
S. avenae	4	4	17	5
S. fragariae	1	2	8	4
M. dirhodum	0	0	2	0
Total isolates	6	9	36	14

EXPERIMENTS AND RESULTS

Aphid numbers and percentage infectivity

Catches in suction traps operated by the Rothamsted Entomology department (Taylor, et al., 1969) indicated the country-wide incidence of aphids 40 ft (12.2 m) above ground; Fig. 1 gives the numbers of the three most important vectors of BYDV, the bird-cherry aphid (Rhopalosiphum padi), grain aphid (Sitobion avenae) and the rose-grain aphid (Metopolophium dirhodum) caught during 1970.

Fig. 1



Their occurrence followed similar patterns in 1969 and 1971, with slight differences in date and number. All three aphids became numerous during the major growth period of their cereal hosts. In addition, R. padi was temporarily prevalent during May, and also during September-October, when more are flying than at any other time.

To test them for virus, aphids were caught alive in smaller suction traps sampling at 4 ft (1.22 m) above ground. These traps were run continuously and flying aphids were collected between 9 am and 4 pm and, when most prevalent, also overnight. Each catch was sorted in a transparent plastic box and aphids thought to be R. padi, S. avenae, or M. dirhodum were removed to glass tubes and identified microscopically. Cereal aphids were paired, according to species, on the coleoptiles of oat seedlings cv. 'Blenda'. The aphids were confined to the test plants for two days, when if still alive, they were removed and their identification checked. Collections were made from the beginning of May until cereal aphids stopped flying, usually in the first or second week in November. The test plants were kept until they showed symptoms of infection with BYDV, or up to a maximum of 6 weeks. Symptoms were compared with those caused by isolates of known severity and the percentage of infective aphids calculated according to Gibbs and Gower (1960). Usually all potential BYDV vectors caught were tested, but occasionally only a sample. Table 3 summarises the proportion of aphids that proved infective.

Each year the proportion of infective aphids was small at anytime, although the number entering crops could be considerable. The first infective aphid was always R. padi; it occurred earliest in 1971, when scattered infected plants were visible before the end of May. In 1970 the first record did not coincide with infection in the field and no further infective R. padi were trapped until July. In 1971 all aphid species became infective 10-14 days earlier than in the previous two years, but transmission tests showed that the virus that was producing symptoms in the field

in May was transmitted so much more readily by R. padi that it was improbable it would be spread much by other aphids, and R. padi was infrequent during June.

Table 3

Infectivity of trapped aphids

Aphid	1st infective	% infective	Total tested
	1969		
R. padi	13 June	9.5	328
S. avenae	23 June	5.9	269
M. dirhodum	21 July	2.7	37
	% of all aphids carrying virus	7.7	634
	1970		
R. padi	27 May	2.3	259
S. avenae	22 June	7.3	210
M. dirhodum	13 July	4.1	320
	% of all aphids carrying virus	4.4	789
	1971		
R. padi	19 May	-	
S. avenae	9 June	-	
M. dirhodum	30 June	-	

In 1969 and 1970, respectively, the proportion of aphids carrying virus increased slowly to a maximum of 15% and 10% at mid-July. This may be a more reliable estimate of the prevalence of BYDV in cereal crops than testing bulk leaf samples, which records only the proportion of crops in which the disease occurs. Most infection was after mid-June so little yield was lost. Results for 1971 are not yet analysed; the early appearance of virulent isolates this year threatened much loss, but the disease did not spread in Hertfordshire, perhaps because aphids able to transmit these isolates were rare later.

In autumn 1970 only 1% of R. padi carried virus. This suggested that infection of autumn-sown cereals would be unimportant in the east. Observations in 1971 showed that crops emerging during late October or early November had about 5% of plants infected, and crops emerging 2-3 weeks earlier had 20%.

In 1969, R. padi had the greatest proportion of infective aphids, but as many infective S. avenae were caught because more were flying. In 1970, R. padi had the fewest infective individuals and S. avenae and M. dirhodum had equal numbers.

Field experiments

In 1969, 1970 and 1971 BYDV was introduced artificially by infesting 2 ft (0.61 m) x 4 ft (1.21 m) areas in the centre of 21 ft (6.4 m) x 40 ft (12.2 m) plots of spring barley, 'Zephyr', with infective S. avenae and R. padi during the last two weeks in May; half the plots were sprayed with insecticide 10 days later. Aphids multiplied in 1969 and 1970, and although the artificially introduced S. avenae-transmitted virus halved the yield (Table 1) of the infested areas, it did not spread and there was no significant yield loss from naturally introduced virus.

Some plots were also infested with non-infective S. avenae, which multiplied rapidly in 1970 and infested plots that were sprayed with menazon 10 days later yielded 11.5% more than the unsprayed plots.

In 1971 parts of a spring barley crop cv. 'Julia' were treated with insecticide on different dates and the incidence of aphids and virus recorded. Three treatments were used.

(i) Granules containing 10% phorate applied at 40 lb/ac (44.8 kg/ha), 4 lb a.i./ac (4.48 kg a.i./ha) by 'Lospred' applicator immediately before drilling.

(ii) Spray with menazon 0.5 pt/a.i./acre (0.7 l.a.i./ha) in 30 gal of water on 24 May.

(iii) As (ii) on 23 June.

The treatments were replicated 4 times on plots 21 ft (6.4 m) x 80 ft (24.4 m). Yields are not yet known but the recorded incidence of infected plants (Table 4) suggests that any differences between plots will be small.

Table 4

Number of plants with BYDV symptoms on different sampling dates and after different insecticide treatments.

Treatment	Plants* with BYDV symptoms in 21 ft (6.4 m) x 80 ft (24.4 m) area 4/6/71	24/6/71	3/7/71
GE	7.5✓	55.7	77.0
-	12.3	78.2	89.2
SM	10.7	66.7	87.0
-	9.0	64.7	79.2
SL	10.0	76.2	70.5
-	10.0	55.2	95.7
S.E.	±0.66	±7.8	±12.2

*mean of 4 replicates

✓significantly different from controls at 0.05 level.

GE - granular insecticide (phorate 4.48 kg a.i./ha) to seed bed

SM - menazon spray 24 May

SL - menazon spray 23 June

\- - controls for each treatment

Incidence of BYDV was significantly decreased on only one occasion and only by the granular insecticide added to the seed bed. These results agree with conclusions from trapping live aphids, that virus was introduced early but spread little.

DISCUSSION

During the last three years BYDV has not been spread enough to effect cereal yields in eastern counties, although it has entered the crop at different times (Table 3). Elsewhere, especially in 1971, BYDV was more prevalent and may have

significantly decreased yield, but unfortunately we do not have detailed knowledge about its incidence or infectivity of aphids in these areas. Hence, much of our discussion must be hypothesis based on information gathered from areas of little virus incidence. Trapping and testing live aphids helps to explain disease development, but will need doing in several areas for several years to know whether there are overwintering conditions that can be used to predict severity of outbreak in spring-sown crops, as has been done with sugar beet yellows (Watson, 1966).

Insecticide, applied as granules to the seedbed, or by spraying early in May, possibly together with herbicide, might have been profitable in the south and west in 1971, but certainly not in the east. In some districts such control may be worth while most years, but elsewhere it is necessary to predict when BYDV may be damaging. Evidently, the disease is most important in the south-west of England, where the viruses seem more virulent than elsewhere (Table 2) and more of the aphid vectors, especially R. padi, overwinter. Also, the mild, damp springs that often delay sowing favour multiplication of aphids, so crops become infected when younger than is usual in eastern counties.

Infection of autumn-sown crops depends very much on numbers of infective aphids when the crops emerge. Where BYDV is expected insecticide added to the seed-bed or a spray immediately after the seedlings emerge might be profitable; or drilling might be delayed until aphids are few (Fig. 1). An unanswered question affecting autumn infection is what happens to R. padi between the large summer and autumn populations (Fig. 1) ? The many trapped during autumn suggest that they had multiplied; if this happened on a rosaceous host the new generationswould be virus-free, but if on grasses, which are susceptible to BYDV (Watson and Mulligan, 1960; Doodson, 1967), many could be infective. The greater incidence of infection of autumn-sown cereals in the south west in 1970-71 than in the east, could be explained were rosaceous species the late-summer hosts in the east and grasses in the west. Similar arguments can be used to try and explain early spring infection by R. padi. Prunus padus is the primary host of R. padi but occurs only sparsely as an ornamental tree in the southern half of Britain, so probably only a few R. padi lay eggs on it. In the south and west, R. padi might also overwinter viviparously on grasses and therefore migrate carrying virus to the spring crops. In the colder east and north few can survive on grasses, so most spring migrants would be virus-free. The few plants that were infected early in eastern counties in 1971 may reflect the comparatively mild winter, which may have allowed R. padi to survive on grasses in the east.

A common practice, especially in the west, is to plough grass leys and sow with winter wheat. The shorter the time between ploughing and sowing, the greater is the danger of infection with BYDV because of aphids present on the grass, and early infection from this source would greatly decrease yield.

Watson and Mulligan (1960) showed that yield losses depend on the virulence of the isolate and the age of the plant when infected. Present information suggests that, for infection to decrease yield significantly, at least 20% of the crop must be infected by the middle of June, i.e. showing symptoms by early July. Therefore the period during which infection must be prevented is before mid-June. Aphids, especially S. avenae on ears, often become abundant late, and affect yield directly as they did on artificially infested plots in 1970. We do not know what effect virus has at this stage and cannot estimate it directly as ripening obscures leaf symptoms. Aphid populations are also much affected by fungal and insect parasites and predators. During 1971 in eastern counties many aphids were parasitised and by mid-July there were more live aphids on plots sprayed with insecticide early in June than on unsprayed plots. This emphasises the need for specific aphicides rather than general insecticides and shows that insecticidal treatments might not be giving the expected protection.

In conclusion, the epidemiological effects of the biology of the three main BYDV vectors can be summarised.

R. padi introduces virus into spring-sown cereals; infection of spring-sown crops depends on the numbers that overwinter on hosts of the virus. It also spreads virus within crops, but probably only locally as it infests the stem base. It is usually less numerous than other cereal aphids during summer, but very large numbers fly during the autumn and may infect autumn-sown crops. It is the most important aphid in the west (Table 2).

S. avenae brings virus into spring-sown crops, and during summer spreads virus in both autumn and spring-sown crops. It usually introduces virus later than R. padi and is most important in the east. It can cause direct damage as a pest.

M. dirhodum does not introduce virus and spreads it inefficiently, but because its populations are sometimes so great, it may be important both in transmitting virus and damaging plants directly.

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THE SPREAD AND CONTROL OF NETTLEHEAD AND OTHER DISEASES OF HOP ASSOCIATED WITH ARABIS MOSAIC VIRUS

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Summary Nettlehead, severe split leaf blotch and bare bine are serious and prevalent diseases of English hops that are associated with the presence of an unusual strain of arabis mosaic virus. This is disseminated in many of the stocks being raised within the main hop-growing areas and is transmitted by the dagger nematode (Xiphinema diversicaudatum).

The hop is not a good host of the nematode and many areas are not infested or support only low scattered populations. This suggests that the present high level of virus infection is due more to the use of infected stock than to spread by nematodes.

Present losses can be decreased by selecting sites free from dagger nematode for all new plantings and by the increased use of virus-tested planting material. Fumigation and fallowing are being tried for use at the relatively few sites with a consistently high population of infective nematodes.

INTRODUCTION

There are approximately 17,000 acres (6,900 ha) of hops in England producing an annual crop of 10,000 tons (10,160 tonnes) worth £7-8 million to the 550 or so growers involved. Yields and profitability are seriously affected by pests and fungus diseases. However, there has been no estimate of the losses caused by virus diseases, although some of these have long been known to be widespread, severe and prevalent. Nettlehead disease was first described in 1574 and hop mosaic and split leaf blotch have been studied for almost 50 years.

Progress in the control of the principal virus diseases has been impeded by inadequate information on their etiology and on the identity of their vectors. This situation was transformed by the discovery of an unusual strain of arabis mosaic virus (AMV) in hop (Bock, 1966) that is transmitted by the dagger nematode (Xiphinema diversicaudatum) and associated with nettlehead and split leaf blotch diseases and also with the 'bare bine' condition. This paper summarises present information on these diseases and on their control.

Nettlehead and other diseases associated with arabis mosaic virus

The strain of AMV occurring in hop differs from those isolated from other crop plants in that it seldom causes symptoms in herbaceous indicator plants. Any

symptoms that do develop are slight, slow to appear and often preceded or obscured by those of prunus necrotic ringspot virus, which is present in all commercial clones of English hops. A serological technique provides an alternative method of detecting AMV in the sap squeezed from young hop leaves or shoots. This method is quick, gives reliable results and has been used extensively to assess the incidence of AMV in commercial plantings.

The first association of AMV with nettlehead disease (Bock, 1966) has been confirmed by all subsequent tests on material of different varieties from widely separated areas in the West Midlands and in the south east. Nettlehead is common in both regions and must cause substantial losses as affected plants are severely stunted and produce little crop (Legg, 1959). Symptoms are most obvious in early summer, when affected leaves develop a mottle or vein clearing, with enations growing from the undersides of the mid-rib and main veins. Leaves are rolled upwards and the shoots fail to climb so that they fall away from the supporting strings (Keyworth and Davies, 1946).

AMV is present in all hops with nettlehead disease, but the virus also occurs in many other hops and sites where nettlehead has never been reported. In the widely grown variety 'Fuggle', infection is always associated with severe split leaf blotch disease (Keyworth, 1951), which seems to predispose plants to nettlehead and often precedes it (Legg and Ormerod, 1964). Severe blotch alone decreases growth and crop; in aggregate it causes greater losses than nettlehead, which although more damaging, is much less prevalent (Legg, 1959).

When growth commences in the spring, 'Fuggle' plants that will later develop severe blotch have a weak unthrifty appearance. Unusually few shoots develop and these have a characteristic curvature, dark pigmentation and leaves that are few, small and retarded (Keyworth, 1951). This transient 'bare bine' condition is just as widespread in other varieties as severe blotch in 'Fuggles' and there is a similar association with AMV, but no blotch. Bare bine in these varieties may be followed by nettlehead but most plants recover and become symptomless until the following spring. Nevertheless their yield is diminished (Neve and Thompson, unpublished) and the aggregate effect on crop is likely to be substantial.

The transmission of nettlehead and the hop strain of AMV by dagger nematodes

Nettlehead disease often recurs and spreads slowly at the same site in successive crops; it tends to be particularly prevalent alongside hedgerows, where hedges have been grubbed and when hops follow permanent pasture or orchards (Keyworth and Hitchcock, 1948). These features suggest that infection is soil-borne, but early attempts to confirm this were unsuccessful. Moreover, inoculations to herbaceous hosts failed to detect AMV or any of the other nematode-transmitted viruses (Legg, 1964a) and the first hop soils examined contained few nematodes of known vector species.

The situation was transformed by the recovery of substantial numbers of dagger nematodes from soils where nettlehead was spreading and by the ability to detect the hop strain of AMV (Bock, 1966). This prompted transmission experiments with hand-picked dagger nematodes, which were done concurrently with those of R.B. Valdez, in our laboratory. High rates of transmission of AMV have been obtained between hops. Nettlehead was also transmitted to hop seedlings by nematodes obtained from the soil around infected plants in the field or in the glasshouse. These results emphasize the importance of dagger nematodes in hop soils and are consistent with traditional observations because the nematode can persist in fallow soil and spreads slowly

amongst its host plants. Moreover, the species is numerous in hedgerow soils and often occurs in smaller numbers in permanent pasture or orchard land, but seldom where arable crops are grown.

Control

The total losses caused by nettlehead and the other diseases associated with AMV cannot be assessed on present evidence, although they are likely to be considerable as the productivity of many plantings is seriously diminished. Some become totally uneconomic and have to be replaced prematurely, while other sites have to be abandoned and new ones found, with the additional expense of erecting costly wirework and supporting poles. The information now available allows a more rational approach to control measures than hitherto and is of great significance to growers.

Planting material

Growers have long been urged to plant 'A plus' Ministry-certified sets raised from specially selected stocks in isolated areas (Legg, 1964b). The original justification for this advice was the lower incidence of nettlehead in plantings made with 'A plus' material compared with those of unselected 'A' and uncertified stocks propagated within the hop-growing areas (Legg, 1964a and unpublished). Despite this there has been only a limited demand for 'A plus' sets and two-thirds of all recent plantings comprised unselected material (Thresh and Ormerod, 1971).

The superior performance of 'A plus' plantings is explained by their initial freedom from AMV, which is now known to be common in many of the unselected commercial stocks. This emphasizes the importance of the original advice. The use of carefully selected material for all new plantings will avoid introducing virus to hitherto non-infective populations of dagger nematodes and will decrease the present widespread occurrence of infection at sites free from the nematode.

Site

Growers can now be more discriminating than previously in selecting sites least likely to be affected by nettlehead and related diseases. Increasing use is already being made of the facilities of the Agricultural Development and Advisory Service for sampling soils to assay nematode populations. Unfortunately there is no convenient way of testing their infectivity and there are limits to the number of routine samples that can be collected and processed. There are also problems in developing a reliable sampling procedure for an organism so patchily distributed in the huge volumes of top-soil involved.

Despite these limitations it is usually possible to locate the relatively few sites where dagger nematodes are widespread and numerous. Hop growing should cease on such sites with a previous history of virus disease, unless an effective fumigation or other preplanting technique can be developed. Sites with dagger nematodes but no previous history of hop cultivation should also be avoided wherever possible, pending further information on the precise risks involved to a subsequent crop of hops. On no account should they be planted with inferior material.

Present indications are that hop is not a good host of dagger nematodes, that consistently high populations of the nematode are restricted to relatively few sites and that many areas are infested or support only low, scattered populations. Growers do not seem to have been seriously inconvenienced by the need to find nematode-free land for new plantings and usually they have been able to offer an alternative area should one be rejected. Indeed, the present high level of infec-

tion seems to be due more to the continued use of inferior planting material than to spread by infective nematodes.

Fallowing and crop rotation

Fallowing and crop rotation offer only limited prospects of eliminating dagger nematodes from hop soils because the nematode can feed and reproduce on the roots of many crop and weed hosts and can survive long periods in bare soil. Nevertheless, virus does not persist through the larval moults and the long-lived adults may not retain virus indefinitely. A replicated trial at Headcorn, Kent, has indicated that their infectivity declines sharply in the second year of bare fallowing of nettlehead land. Similar trends have been apparent from observations elsewhere and it is the standard practice of some growers to fallow for two seasons before replanting. This is not always possible when hops are in great demand and the immediate replanting of many infected sites in 1968-1969 and 1969-1970 gave the maximum opportunity for infective nematodes to survive (Thresh and Ormerod, 1971).

Fumigation

Growers may be particularly anxious to retain the wirework and continue hop production in certain areas despite the likelihood of nettlehead recurring. In these circumstances fumigation to control nematodes offers a possible solution, even though it may be expensive and require special soil preparation.

Satisfactory control will be especially difficult to achieve in the hop, which is a deep-rooted perennial, often grown in very heavy clays. Dagger nematodes may occur to a depth of 3 ft (0.9 m) and a mean population of only one individual per 200 ml soil sample is equivalent to about 12,000 around a single hop root system.

Despite these difficulties, some success has been achieved in a series of field trials. Methyl bromide applied at 480 lb/ac (540 kg/ha) at Headcorn, Kent, under suboptimal soil conditions in the late spring of 1967, failed to control virus or vector and replanted 'Fuggle' hops were severely affected by blotch and nettlehead within three years. By contrast, an autumn application of a mixture of dichloropropene and dichloropropane (DD) by hand injection, at the rate of 25 gal/ac (282 l/ha), effectively controlled nematodes in some plots and halved the overall incidence of nettlehead and AMV in the second growing season compared with untreated controls. A similar application at the rate of 50 gal/ac (563 l/ha) in 1968 was even more effective and the population occurring down to 24in(0.6 m) was only 2% of that in untreated plots.

The original trials and the first results of later ones have justified further experiments involving the injection of much larger areas by tractor-drawn machines. Adaptations of the Auchincruive and Leaper-Ramsay machines developed for injecting potato and sugar beet land, respectively, and also a Danish machine designed for use in tree nurseries have been used in trials. All three machines have also been used recently by growers in attempts at commercial control.

Future prospects

Present measures are intended primarily to avoid disease. There is no practical method of curing infected plants in the field and no evidence of immunity or of a level of resistance or tolerance to virus or vector that could be exploited by plant breeding. The advice that can be offered to growers to avoid the recurrence of infection at sites with an extensive infestation of infective nematodes is limited at present but current studies on fallowing and fumigation offer some prospect of success. However, as the present chemicals are phytotoxic and can be

used only before planting, there is an urgent need for materials that can be used prophylactically and that will penetrate heavy soils to great depths. Further work is also required on the crop and weed hosts of the hop strain of AMV to be able to predict the incidence of infection in hop plantings at fresh sites.

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SUCCESSFUL RESTRICTION OF THE SPREAD OF NONPERSISTENT VIRUSES BY NEW METHODS OF VIRUS VECTOR CONTROL

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Summary Following the successful use of systemic insecticides to decrease the spread of potato virus Y in seed potato crops other methods of control were studied. Field trials showed that a combination of dimethoate and mineral oil decreased the spread of potato virus Y in potato crops and of pea mosaic virus in field bean crops more than either preparation alone. This combined treatment will be complemented in the future using substances with a deterrent action.

INTRODUCTION

Insecticides can effectively decrease the spread of aphid-transmitted persistent viruses, e.g. decrease the spread of potato leaf roll virus. However, attempts to control spread of nonpersistent viruses, especially nonpersistent viruses of potato, have often failed. Nevertheless, chemical control of aphids acting as vectors of nonpersistent viruses can be successful under certain conditions. Joint experiments made in the GDR by VEB Chemiekombinat Bitterfeld and VVB Saat- und Pflanzgut showed that vector control to decrease spread of such viruses was most effective in large, enclosed planting areas.

METHODS AND MATERIALS

In the following experiments virus vector control was carried out according to instructions from a specially organised warning service, based on the biology of the potato aphids. Spray treatments with 900 ml/ha of formulated dimethoate ('Bi 58 EC'), 600 ml/ha of formulated demephion ('Tinox 50'), or 10 l./ha of an aerial spray based on dimethoate ('FIP') were applied twice during the growing period, which is economically acceptable as it has been shown that effective virus vector control and a decrease in virus incidence can be obtained with a few sprays of a systemic insecticide (Dubnik, 1969a & b, 1970, 1971; Sass et al., 1970).

RESULTS

The effect of vector control on virus incidence in seed potato crops is shown in Tables 1 - 4, using the 'tuber index' test.

Table 1

Control of virus spread with dimethoate at Dorst-Zobbenitz
Results of the 1969, 1970 and 1971 progeny tests

(Two treatments each of 900 ml/ha in all potato fields)

Year	Untreated		Treated	
	% virus-infected plants	% non-persistent viruses	% virus-infected plants	% non-persistent viruses
1969	3.8	1.9	0.8	0.6
1970	6.8	2.3	1.8	0.2
1971	10.0	4.0	4.8	1.7
Mean	6.9	2.7	2.5	0.8

Table 2

Control of virus spread with demephion at Kerkau
Results of the 1970 progeny tests (i.e. 1969 crop)

(Two treatments each of 600 ml/ha in a seed potato crop)

Variety	Untreated		Treated	
	% virus-infected plants	% non-persistent viruses	% virus-infected plants	% non-persistent viruses
Pirat	10.0	3.1	6.6	3.6
Spartaan	9.4	2.8	5.0	0.0

Table 3

Control of virus spread with demephion and dimethoate at Kerkau
Results of the 1971 progeny tests (i.e. 1970 crop)

(First treatment with demephion then an aerial spray of dimethoate)

Variety	Untreated		Treated	
	% virus-infected plants	% non-persistent viruses	% virus-infected plants	% non-persistent viruses
Pirat	15.0	10.5	6.3	4.8
(Initial infection)	6.6	3.6	6.6	3.6
Ora	7.0	1.5	4.0	1.5
(Initial infection)	3.0	1.8	3.0	1.8

Table 4

Control of virus spread in 1969 with two aerial sprays of demephion
Results of the 1970 progeny tests

Variety	Untreated		Treated	
	% virus-infected plants	% non-persistent viruses	% virus-infected plants	% non-persistent viruses
Auriga	2.1	1.7	0.0	0.0
Pirat	2.8	2.3	0.6	0.6
Ora	6.5	2.6	2.1	1.9

The results obtained from all experiments (Tables 1 - 4) showed that a decrease in virus incidence, including that of nonpersistent viruses can be achieved by this control method, but we considered it only partially successful and studied other methods of controlling the spread of virus in seed potato crops in the GDR, especially that of nonpersistent viruses.

It has been known for a number of years that the spread of nonpersistent viruses can be decreased by means of oil sprays. These do not decrease the spread of persistent viruses, and semipersistent viruses are not considered here. Such oil sprays do not control the virus vectors as such, but protect plants from aphid-transmitted viruses, i.e. prevent acquisition of virus. Several mineral and vegetable oils and films of milk have proved effective. We studied a series of mineral oils for their protective action against transmission of pea mosaic virus (PMV) by Myzus persicae (Sulz.), with Vicia faba L. as the infection source and the test plant. Of the samples tested, none was without effect or gave widely differing results, so we selected the mineral oil 'W 6735' for our next experiments.

This mineral oil gave 69% protection against PMV. However, the protective effect of mineral oil against the transmission of viruses by aphids does not last for long and for an effective decrease in the spread of nonpersistent viruses under field conditions sprays would have to be applied at weekly intervals. These findings agree with those of other authors who carried out treatments at weekly intervals using similar experimental layouts. Such weekly treatments would be too expensive and would damage crops. Although the oil spray method is at present not fully developed for practical use, it may be economically important in the future if an increase in the persistence of the action of mineral oils can be achieved.

Theoretical considerations led us to assume that a combined application of mineral oil and insecticide would enable us to reduce the number of sprays. This would combine the protective action of mineral oil, inhibiting aphid transmission of nonpersistent viruses, with the aphicidal action of an insecticide, which ideally should be persistent and systemic in action. If systemic insecticides are applied alone, viruliferous aphids feeding on a plant may infect it with a nonpersistent (stylet-borne) virus before they take effect. If, however, a combination of a systemic insecticide and a mineral oil is applied, aphids will feed on the plant tissue but infection with stylet-borne viruses will be prevented by the oil and the insecticide will kill aphids a long time before the action of the mineral oil subsides. Transmission of persistent viruses will also be prevented by the systemic insecticide.

The use of an insecticide-oil combination has been suggested by other workers on theoretical grounds (Hein, 1965; Vanderveken, 1968; Vanderveken et al., 1968;

Cousins & Grison, 1969) but until recently experimental studies were only made by Bradley (1968), who commented at the First International Congress of Plant Pathology in London that: 'Oil sprays plus insecticides give no better results'.

In our own laboratory trials on transmission of pea mosaic virus by M. persicae, we obtained, as was to be expected, no better results from treating a V. faba plant with a mineral oil-insecticide combination than with a mineral oil emulsion alone, at least not within the period of time where the mineral oil treatment was still effective. In field experiments, however, the combination of mineral oil and insecticide proved to be superior to a treatment by mineral oil alone (Table 5). In a field experiment in 1969, using as a model PMV and V. faba var. 'Fribo', we studied several treatments for their suitability to restrict spread of nonpersistent viruses. The treatments were applied once a week.

Table 5

Control of spread of PMV to Vicia faba with dimethoate and mineral oil at Aschersleben in 1970

(Plots of 10 m^2, treated weekly, 4 replicates of each treatment)

Treatment	% PMV-infected plants	Relative difference	Absolute difference
Untreated control	44.06	260.30	27.14
Dimethoate (0.075% Bi 58 EC)	16.93	100	0
Mineral oil (2% W 6735)	14.90	88	2.03
Dimethoate plus mineral oil	8.19	48.40	8.73

Standard error of difference: 2.15

LSD 5 abs. 4.69	LSD 1 abs. 6.58	LSD 0.1 abs. 9.30
LSD 5 rel. 27.71	LSD 1 rel. 38.90	LSD 0.1 rel. 54.92

The differences in Table 5 are significant as shown by an analysis of variance. To obtain these differences it proved useful to produce a very high infection 'pressure', by including plots of infected plants. Weekly sprays with dimethoate almost completely prevented aphid infestation.

In 1970 we also studied the effect of a combined mineral oil-insecticide spray on virus spread in a seed potato crop. The crop received three sprays only. The results are shown in Table 6. This experiment, too, illustrated the greater effect obtained from a combination of insecticide and mineral oil. Further experiments are planned to confirm these results. A combined insecticide-mineral oil spray can be more effective and more economical than the use of an insecticide alone, and where insecticides are expected to be effective only when used over large crop-growing regions, when combined with mineral oil may control virus spread in individual fields.

Table 6

Control of virus spread in seed potatoes with mineral oil and dimethoate
Results of the 1970 progeny tests (i.e. 1970 crop)

(3 applications of each treatment)

Treatment	% virus-infected plants	% non-persistent viruses
Untreated control	9.4	6.3
Mineral oil (2% W 6735)	4.4	4.4
Dimethoate (900 ml/ha Bi 58 EC)	4.9	4.9
2% oil plus 600 ml/ha dimethoate	2.4	2.4
Initial virus infection	0.6	0.4

Finally, there is hope that virus spread can be decreased by using chemicals that deter aphids from probing or feeding on plant tissue. In screening tests to find substances with a repellant effect on aphids (Zschiegner et al., in the press) preparations were found which had an inhibiting effect on the transmission of non-persistent viruses by aphid vectors.

We hope to achieve further success in restricting virus spread in seed potato crops by close co-operation between the chemical industry, scientific institutes and agriculture in this country.

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