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CHEMICAL CONTROL OF STEM NEMATODE (DITYLENCHUS DIPSACI) OF BULB ONIONS

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Summary During 1968–71 seventeen replicated field experiments, one pot experiment, and ten observation studies were done in eastern England on the chemical control of stem nematode (Ditylenchus dipsaci), attacking bulb onions grown from seed and from setts. The soil fumigants D-D mixture dichloropropene (Telone) and dazomet gave inconsistent results when applied the autumn before onions were grown. Dazomet applied in spring was phytotoxic. Seed dressings of methomyl and Ciba C14421 with or without dieldrin as pellets, and Plant Protection Ltd JF2535, JF2641, Boots RD18502 and Geigy GS13006 were not effective and usually were phytotoxic. Methomyl and Du Pont 1410 applied as soil sprays were promising. Granular formulations of aldicarb, Geigy GS13006, Plant Protection Ltd R21341, R17210, PP211 (pirimiphos-ethyl), disulfoton, phorate, thionazin, 'Terracour P', 'Neosar', 'Nemacur P', prophos (Mocap, Ciba C14421, methomyl, parathion, chlorfenvinphos, mecarphon (MC2420) and Du Pont 1410 were tested at various rates both bow-wave and over the drills after germination. Experiments were made on three soil types; sandy loam, very fine sandy loam and peaty loam. Of the chemicals tested, thionazin was satisfactory only on peaty loam soil and 'Nemacur P' and Du Pont 1410 on sandy loam and very fine sandy loam. 'Nemacur P' was not satisfactory on peaty loam.

INTRODUCTION

Stem nematode (Ditylenchus dipsaci) is a serious pest of bulb onions grown from seed. The severity of the problem varies from year to year but it is a continual threat, especially in areas where flower bulbs, strawberries, peas, beans, oats and cloves are grown in the same rotation as onions.

Seed is known to be a source of infestation (Wallace and Wood, 1943), but with the advent of methyl bromide fumigation (Lubatti and Blackith, 1956) and the growing of seed in areas comparatively free from eelworm, the risk of infestation from this source is now slight. The greatest hazard is from the resident population of nematodes in the soil. A very wide range of crop and weed hosts grow in all the major onion producing areas, so that once a field is infested it is almost impossible to eradicate the pest. Furthermore, because of the unpredictable nature of stem nematode attacks, all onion fields should be considered to be potentially infested. Usually the infestation is patchy (Kaai, 1967) and often below the level which soil sampling can detect. Onion fields are often large so that the system of forecasting in use in the Netherlands has only limited application (Seinhorst, 1956; Kleijburg, 1967).

In 1968 therefore, we began a series of experiments to find an inexpensive and non-phytotoxic chemical treatment. As there were about 28 experiments and observation studies, only selected results are given in this paper; wherever possible yields of onions/plot have been adopted as the standard but full analyses and conversion of all the data have not yet been made; it is intended to report these experiments more fully elsewhere.

METHODS

The field trials were done at sites where onions had previously failed or partly failed due to stem nematode. Plot sizes varied but all were designed to yield at least 300 onions if treatment was successful. The soil fumigants DD mixture and dichloropropene (Telone) were applied with time injectors followed by rolling; dazomet was hand-broadcast and plots were rotavated twice afterwards. Granules were applied by the 'pepper-pot' method to a two-inch band before seed were drilled either by hand, with a precision drill or with a hand-drill. Granules applied over the top of plants covered a slightly wider band than bow-wave treatment. In one experiment granules were broadcast by hand and raked into the soil surface. Seed dressings were either pelleted (by Germains' of Kings Lynn) or applied in powder form with methyl cellulose as a sticker.

The number of experiments at each site varied from year to year but the main sites are listed in Table 1 and experimental details in Table 2. Altogether there were 17 replicated field experiments and 10 unreplicated observation studies and single row screening trials.

Table 1

Sites of experiments, 1968-1971

Site	Place	Soil texture	Year of experiments
A	Langtoft	Sandy loam	1969, 1970, 1971
B	West Deeping	Sandy loam	1969, 1970
C	Kirton	Very fine sandy loam	1968, 1969, 1970, 1971
D	Prickwillow	Peaty loam	1969
E	Martham	Sandy loam	1969
F	Barway	Peaty loam	1970, 1971
G	Holbeach	Very fine sandy loam	1970

The variety in all experiments was Elsom's Early Mainorop, although at Barway in 1971 a few rows of cv. Bola were drilled. Both were Rijnsburger type. The onion setts were Stuttgart Giant. Nematode counts were made both from soil and plants, and detailed germination counts, the proportions of plants infested at various times during the season, numbers, weight and keeping quality of onions harvested were recorded. Only the yields and brief comments about the other measurements are given in this paper.

Table 2

Numbers and Titles of Experiments

Experiment No.	Site	Title
1	A	Soil fumigants compared with methomyl spray 1969
2	B	" " " " " " "
3	A	Time and rates of application of granular nematicides 1970
4	B	" " " " " " "
5	A	Timing, rates and topping-up of granules 1971
6	C	Granular nematicides to control eelworm on oats, onions and tulips 1968
7	D	Phytotoxicity experiment with broadcast granules
8	C	" " " bow-wave "
9	C	Granular nematicides 1969
10	E	Seed-dressings compared with granules, 1969
11	C	Comparison of onion setts and transplants 1970
12	C	Granular nematicides 1971
13	F	" " 1970
14	F	Seed-dressings 1970
15	G	Granular nematicides 1970
16	G	Seed-dressings 1970
17	F	Granular nematicides 1971

RESULTS

(a) Comparison of fumigants and granular nematicides on onions grown from seed and from setts

Onions failed at Sites A and B in 1968 and experiments 1-4 were designed to compare soil fumigants applied in autumn and spring, a methomyl soil treatment and various rates and methods of applying two granular nematicides aldicarb and prophos (Mocap). In the two later experiments (Table 4) the onions were grown both from setts and from seed.

Table 3

Yield of onions from experiments 1 and 2, 1969

Treatment	Yield (cwt/acre) Site A	Site B
D-D mixture 400 lb/ac.	69	17
Dichloropropene 320 lb/ac.	52	19
Dichloropropene 240 lb/ac.	63	14
Dazonmet 200 lb/ac.	16	115
Dichloropropene 200 lb/ac.	32	24
Methomyl w.p. 6 lb a.i.	9	14
Control - no treatment	0	0
S.E. treatment means (15 d.f. with controls excluded)	± 8.6	± 7.8

All plants died on the control plots at both sites, and none of the treatments prevented the plants from becoming infested; symptomless plants at harvest time were found to contain live nematodes. The results were contradictory in that D-D mixture and dichloropropene worked well at Site A but not at Site B. Dazomet worked well at Site B but not at Site A where it was phytotoxic. Methomyl was phytotoxic at both sites.

Both growers and advisers had contended that onion setts were less liable to attack by stem nematode than crops grown from seed. There was one replicated field trial and three observation studies to test this in 1970. Tests before planting at Site C revealed a small resident population of stem nematioles already in the setts. As in Experiments 1 and 2 the general yield of Experiments 3 and 4 was poor, and the observation studies merely confirmed that aldicarb greatly reduced infestation of setts and slightly improved yield of sound onions.

Table 4

Yields of onions from Experiments 3 and 4, 1970

Treatment	Yield (tons/acre) Site A Setts	Site A Seed	Site B Seed
Control, no treatment	6.7	2.7	4.4
Prophos (2 lb a.i at drilling	6.8	4.8	5.3
Prophos (1 lb a.i at drilling + 1 lb a.i at emergence	6.7	3.2	4.6
Prophos (2 lb a.i at emergence	6.3	3.6	3.5
Prophos (6 lb a.i broadcast before drilling	7.1	4.5	4.6
Dazomet 160 lb a.i before drilling		3.1	6.1
Aldicarb (2 lb a.i at drilling	-	-	5.4
Aldicarb (1 lb a.i at drilling + 1 lb a.i ay emergence	-	-	5.6
Aldicarb (2 lb a.i at emergence	-	-	4.6
Aldicarb (6 lb a.i broadcast before drilling	-	-	5.5
S.E. treatment means	± 0.36	± 0.54	0.59
Degrees of freedom	12	15	27

After drilling there was a drought which reduced both initial germination and nematode attack. Later in the season many seeds germinated and plants became infested with nematodes but yields were small. Dazomet was phytotoxic at both sites. The plants were yellow and weak in spring but made rapid growth following rain in July; dazamet greatly increased the proportion of thick-necked onions.

(b) Seed dressings

Several experiments were made with seed dressings at all sites except D. There was a promising pot experiment in 1969 at Cambridge and a field experiment the same year at Site E, when Geigy GS 13006 emerged as a possible candidate for further study. All the other chemicals, rates and methods of application (inoluding pelleting) were either phytotoxic, failed to control stem nematode, or both. Chemicals tested were: methomyl and Ciba C14421 pelletted with and without dieldrin; Plant Protection Ltd JF2535; JF2641; Boots RD18502; and Geigy GS13006 all as dry powder dressings at various rates.

(c) Further experiments with granular nematicides

A promising initial experiment was done at Site C in 1968 when thionazin and aldicarb granules applied with the seed allowed onions to survive to harvest compared with untreated controls in which all the plants died. Concurrently with our work, experiments in Poland had established that for mineral soils 4 kg a.i/ha thionazin granules gave good results (Brseski, 1970). We tested a wide range of chemicals, rates and timings of application, including aldicarb wherever possible as a standard against which to measure the performance of other chemicals.

(i) Phytotoxicity in the absence of stem nematode

Only one experiment (7 at Site D in 1969) was made with broadcast granules on peaty loam soil. The crop was not harvested.

Table 5

Phytotoxicity test at Site D, 1969; Experiment 7

Granule	lb a.i/acre	Plants/row ft 4 weeks after treatment
Geigy GS13006	1.25	13.2
Aldicarb	1.25	9.6
Plant Protection Ltd R21341	0.63	10.8
Disulfoton	2.34	10.4
Plant Protection Ltd R17210	2.50	10.7
Pirimiphos-ethyl (PP211)	2.50	7.4
Thionazin	4.60	7.1
Phorate	5.00	13.6
Control (a) No granules	-	13.6
Control (b) No granules	-	11.8
L.S.D (p = 0.05)		5.14

At the rates applied pirimiphos-ethyl and thionazin were phytotoxic but the other chemicals did not affect plant establishment. In the second experiment at Site C phorate at 1 lb a.i/acre and 'Terracur P' at 6 lb a.i/acre retarded growth of onions, but aldicarb at 2 lb, thionazin at 2 lb, parathion at 1 lb and C14421 at 20 lb were not phytotoxic.

(ii) Experiments on soil infested with stem nematode

The yields of uncleaned onions from Experiment 5 at Site A are given in Table 6. Each treatment plot had an adjacent untreated control.

Table 6

Yields of onions from Experiment 5, 1971

Treatment lb a.i/acre and method	Kg/plot of uncleaned onions Yield of treated plots	Yield of untreated plots
Aldicarb 2 bow wave	20.1	6.5
Phorate 2 " "	12.7	6.8
Phorate 2 " " + 2 lb top	22.5	9.0
Phorate 2 " " + 2 x 2 lb top	20.0	6.8
'Nemacur' 2 " "	16.2	6.3
'Nemacur' 2 " " + 2 lb top	21.6	6.4
'Nemacur' 2 " " + 2 x 2 lb top	23.2	8.6
Thionazin 2 " "	5.0	4.5
Thionazin 2 " " + 2 lb top	18.5	6.8
Thionazin 2 " " + 2 x 2 lb top	20.1	10.0

The bow wave application alone of phorate and 'Nemacur' was not sufficient to control the infestation and ensure survival of all the seedlings, and thionazin at 2 lb a.i. was very disappointing. Yields were good when one or two additional applications of chemical were made over the top of the seedlings.

In 1969 and 1971 granular formulations of nine nematicides were compared at Site C. Two of the nematicides were applied at very high rates, but the other seven at normal rates per acre. The yields are given in Table 7 and 8.

Table 7

Yields of onions from Experiment 8, 1969

Treatment and lb a.i/acre	Kg/plot cleaned onions
Aldicarb 2	16.3
Methomyl 1	11.4
Phorate 1	4.9
Parathion 1	5.5
Prophos 2	8.3
Thionazin 2	9.3
'Terracur P' 2	8.5
Neosar 55	0
C14421 13	0
Control - no granules	0.3

Table 8

Yield of onions from Experiment 12, 1971
(Kg/plot)

Chemical	lb a.i/acre 0.75	1.5	3.0
Aldicarb	18.0	17.3	18.8
'Nemacur'	12.8	16.1	18.5
Thionazin	5.1	6.2	9.9
Du Pont 1410	15.3	18.2	18.6
Control		1.7	

All the treatments greatly improved yield but thionazin was not as good as aldicarb, 'Nemacur' or Du Pont 1410.

At Site C, crops grown from seed in plots treated with aldicarb always yielded more than 10 tons of onions/acre (25 metric tonnes per 4 otare). Thionazin, even at the highest rate used (3 lb a.i./acre) did not perform well enough for a general recommendation to be made for its use on silt soils but 'Nemacur' and Du Pont 1410 were promising. Methomyl was promising too whereas all other nematicides did not give sufficiently good results to warrant further tests.

The eelworm attack was very patchy at Site F in 1970, and because the onion seed was not drilled until 4 May there was only a very light attack at Site G. Aldicarb at 2 and 4 lb a.i/acre was again used as the standard against which phorate, thionazin, disulfoton and prophos at 3 and 6 lb a.i/acre were compared. Yields at both sites were moderate (about 7 tons/acre) but almost any of the chemicals prevented the nematodes entering seedlings when they were used at a sufficiently high rate. In 1971 the experiment at Site F was similar in design to the one at Site A, so that each treated plot had an adjacent untreated control. The yields of uncleaned onions are given in Table 9. Granules were applied either bow-wave or over the plants (top) some weeks after germination when the plants were known to be infested.

Table 9

Yields of onions, from experiment 17, 1971

Treatment lb a.i/acre and method	Kg/plot uncleaned onions* Yield	Difference from control
Phorate 4 bow-wave	6.3	+ 4.9
Phorate 4 " " + 4 top	5.8	+ 4.4
Phorate 0 " " 4 or 8 top	2.0	+ 0.9
Control (untreated)	1.4	-
'Nemacur' 4 bow-wave	9.3	+ 3.3
'Nemacur' 4 " " + 4 top	8.6	+ 2.9
'Nemacur' 0 " " 4 or 8 top	6.6	- 0.5
Control (untreated)	7.2	-
Thionazin 4 bow-wave	13.6	+ 12.0
Thionazin 4 " " + 4 top	14.2	+ 12.1
Thionazin 0 " " 4 or 8 top	4.1	+ 1.4
Control (untreated)	0.4	-
Aldicarb 2 bow-wave	16.4	+ 10.7
Aldicarb 2 " " + 2 top	19.5	+ 13.7
Aldicarb 0 " " 2 or 4 top	4.2	+ 1.8
Control (untreated)	4.5	-

* In this experiment 1 kg/plot is approx. equivalent to 1 ton/acre

A crop of 12-14 tons/acre is regarded as satisfactory by most growers, and from such a heavily infested site the results achieved by thionazin and aldicarb were most encouraging. Phorate and 'Nemacur' were disappointing in this experiment, possibly because of the peaty nature of the soil.

DISCUSSION

It became clear that onion seed drilled late was less liable to attack than seed sown early. It also seemed that if protection of the young seedlings could be achieved for a relatively short period it might be possible to prevent early invasion and loss of plant and thus produce a harvestable yield of bulb onions. For this reason the experiments in 1970 and 1971 were intended to find a treatment suitable for the three main soil types on which onions are grown in England. Unfortunately no single rate, timings or chemical can yet be recommended. However, these results strongly suggest that chemical control of stem nematode is not only feasible but highly practicable, and depending upon the price of the newer materials economic for growers to use as a routine practice. Aldicarb, thionazin, 'Nemacur P' and Du Pont 1410 merit further consideration when applied as granules at or soon after drilling.

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CONTROL OF STEM NEMATODE (DITYLENCHUS DIPSACI) IN ONIONS

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Summary The experiments described relate to the control of stem nematode in onion crops, grown from sets and from seed, in infested soil. It was found that the application of Dazomet reduced the nematode population by as much as 96% and that this reduction, though enabling a crop to be produced, was not sufficient to prevent a high incidence of diseased bulbs at harvest. In the case of crops grown from onion sets, best results were obtained by treating the soil with Dazomet and by steeping the sets in a thionazin (nemafos) immediately prior to planting. With seed-sown crops, best results were obtained where Dazomet was used as a soil treatment and was later followed by a thionazin treatment applied either as a drench or as a granular top-dressing.

INTRODUCTION

The experiments now described were designed to investigate the possibility of controlling stem nematode (Ditylenchus dipsaci) in onions grown in infested soil - (a) when onion sets are sown and (b) when onion seed is sown. The object was to produce a crop of onions in heavily infested soil by either killing the nematodes with a soil nematicide prior to planting or by protecting the growing plants with a systemic nematicide. It was also decided to test different combinations of soil and plant treatments. The experiments were done in fields in which onion crops had been severely affected by the pest the previous year, the actual area covered by the experiments having been the sites of complete crop failures. The soil was a loamy sand developed on sands of marine origin.

METHODS AND MATERIALS

The experiment in which onion sets were sown was done in 1968 and repeated in 1969. It was of a random 2x3x3 factorial design with three replicates. Plots were 24 feet long and five drills wide. Dazomet (at rates 0, 2 and 3 oz/yd^2) and D.D. (at rates 0, 400 and 600 lb/ac) were used as soil sterilants, and these treatments were combined with pre-plant steeps and post-plant drenches. The Dazomet and D.D. treatments were applied in December. The steep consisted of immersing the onion sets in thionazin (4 pints 46% e.c. in 100 gal water) for 1 hour immediately prior to planting. Thionazin was also used as a drench (6 pints 46% e.c. in 100 gal water) and was applied 30 days after planting.

The experiment in which onion seed was sown was done in 1970 and was repeated in 1971 as the previous years results were inconclusive. It was of a random factorial design with five replicates. Plots were 20 feet long and 4 drills wide. The treatments were as follows:-

1. Control
2. Dazomet @ 3 oz/yd^2 + top dressing of thionazin granules @ 50 lb/ac

3. Dazomet @ 3 oz/yd^2 + thionazin drench (1 gal 46% e.c/100 gals water)
4. Thionazin granules at sowing (@ 50 lb/acre)
5. Thionazin granules at sowing (@ 40 lb/acre) + top dressing of thionazin granules (@ 50 lb/acre)
6. Thionazin granules at sowing (@ 40 lb/acre) + thionazin drench (1 gal 46% e.c/100 gals water)

The Dazomet was applied on February 23 and sowing was done on March 24. The thionazin granules applied at sowing time were put on in a 4" band along the drills (bow-wave technique) and the top-dressing and drench were applied along the row of young plants on June 11.

The effects of the various treatments in both experiments were assessed by making observations on the crop during the growing season and by determining the number and yield of diseased and healthy plants for each treatment.

RESULTS

The results from the "onion set" trial are presented in Table 1 and those from the seed trial in Table 2.

Table 1

Effect of different treatments on mean yield of onions (tons/ac)

Soil Treatment	Bulb Treatment: None			Steep			Steep + Drench		
	Sound	Diseased	Total	Sound	Diseased	Total	Sound	Diseased	Total
Dazomet - None	0.0	0.0	0.0*	5.6	.9	6.5	12.5	1.2	13.7
Dazomet - 2 oz/yd^2	7.9	7.8	15.7	22.1	.9	23.0	17.3	.9	18.2
Dazomet - 3 oz/yd^2	14.4	8.1	22.5	21.2	1.0	22.2	23.0	.5	23.5
D.D. - None	0.0	0.0	0.0*	6.4	1.7	8.1	7.6	2.3	9.9
D.D. - 400 lb/ac	1.0	5.4	6.4	14.1	1.2	15.3	16.6	.5	17.1
D.D. - 600 lb/ac	1.6	4.5	6.1	18.2	1.2	19.4	15.6	.6	16.2

F. test: Soil and bulb treatments highly significant
Differences between "Steep" and "Steep + Drench" treatments not significant
Interactions not significant

* Because of complete crop failure for the "No treatment" levels, analysis of these data could not be obtained.

Table 2

Effect of different treatments on the number and yield of onion bulbs

Treatment	Mean No. of Bulbs* Sound	Diseased	Mean Wt. of Bulbs (lb)* Sound	Diseased
Control	0.0	8.4	0.0	2.2
Dazomet[1] + thionazin top dressing[2]	116.8	19.2	33.6	4.4
Dazomet + thionazin drench[3]	119.0	16.4	32.3	4.6
Thionazin at sowing (@ 50 lb/ac)	10.4	16.0	4.35	5.0
Thionazin at sowing (@ 40 lb/ac) + thionazin top dressing	123.6	25.8	25.7	6.3
Thionazin at sowing (@ 40 lb/ac) + thionazin drench	146.4	24.2	30.1	4.8
S.E.	± 15.9	± 5.6	± 2.4	± 1.4
F. test	***	NS	***	NS

* per 20 ft row harvested

1 Dazomet applied @ 3 oz/yd^2

2 Thionazin granules applied as a top dressing @ 50 lb/ac

3 Thionazin drench (1 gal 46% e.c. in 100 gals water) applied @ 2 gals/plot

DISCUSSION

In both experiments the crop failed completely where no treatment was applied. All plants in these plots had rotted away by mid-July because of the high level of nematode infestation. At sowing time there were averages of 50 and 100 stem nematodes per 200 ml soil in the onion set and seed-sown trials respectively.

In the onion set experiment, plots which received the dazomet treatments only, produced crops which grew well and scored highly in observations made during the growing season. However, it was found at harvest that a high percentage of these bulbs was heavily infested and was not marketable. Pre and post treatment counts showed that the dazomet treatments had reduced the nematode population from an average of 50 to 2 per 200 ml soil. While this reduction was sufficient to allow a crop to become established and grow, the remaining nematodes were obviously capable of causing considerable damage.

Much the same pattern occurred with D.D. but the dazomet treatments were significantly better. D.D. applications reduced the nematode population from an average of 50 to 8 nematodes per 200 ml soil and, as shown in Table 1, this was not sufficient to prevent severe damage to the crop.

Best results, in terms of highest yield and lowest number of infested bulbs, were obtained when dazomet treatment was combined with a pre-plant steep. Differences between "steep" and "steep and drench" bulb treatments were not significant.

In the onion seed experiment the thionazin granules (10%) applied at sowing did not control the pest (Table 2). Despite the high rate of application, yields were very much reduced and over fifty per cent of the produce was heavily infested and unmarketable. Kaai (1968) found that application of thionazin 3 weeks after onions had been sown, gave a better control of attack by the stem nematode than treatments 10 days before or 6 weeks after sowing. The results of the experiment

now described confirm Kaai's findings. Best results were obtained by using either dazomet as a soil sterilant prior to planting or thionazin at sowing time combined with thionazin applied as a drench or as a top dressing.

There was no evidence of phytotoxicity from the thionazin treatments even where the granules were applied at the rate of 50 lb/acre at sowing. This confirmed observations previously made by the author in glasshouse trials (Duggan 1970).

These results are based on the condition of bulbs at harvest and take no account of possible further losses which might occur during storage. This is a factor which needs further investigation.

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EFFECT OF GRANULAR APPLIED 'SYSTEMIC' PESTICIDES ON THE POTATO CYST NEMATODE, HETERODERA ROSTOCHIENSIS WOLL

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Summary Thionazin, phorate, aldicarb and methomyl were applied as broadcast treatments against Heterodera rostochiensis in field plots. Aldicarb and phorate were the most effective nematicides in reducing eelworm multiplication rate and in increasing potato yields.

INTRODUCTION

In the last decade several organophosphorus and carbamoyl oxime compounds applied as granules to the soil have been tested for their efficacy against the potato cyst nematode. In 1961, Frazer and Lindley obtained increased potato yields when thionazin was applied as a band application and the effectiveness of aldicarb has been demonstrated by Dash et al (1969). Phorate granules effectively controlled Pratylenchus penetrans on lilies (Jensen 1966), but satisfactory results were not obtained against Heterodera species, i.e. H. schachtii (Jorgenson 1969) and H. goettingiana (Proctor 1960). Den Ouden (1968) reported that methomyl was not as effective as aldicarb when tested in pot experiments.

This paper reports on the comparative effectiveness of the above mentioned pesticides when applied as broadcast treatments to plots infested with Heterodera rostochiensis.

MATERIAL AND METHODS

A field at the Imperial College Field Station was divided into four blocks corresponding to four levels of natural H. rostochiensis infestation. (Table I). Nine plots each 12 yd (10.9m) by 1 yd (0.91 m) separated by 1 yard wide paths were laid out in each block and planted with a single row of seed potatoes (cultivar Majestic).

In each block, treatments were randomised. Broadcast treatments of commercial granules of thionazin, phorate or aldicarb were applied at rates equivalent to 5 lb. a.i./acre (5.5 kg/ha) or 10 lb. a.i./acre (11.0 kg/ha); methomyl was applied at 5 lb. a.i./acre (5.5 kg/ha) or at 2.5 lb. a.i./acre (2.8 kg/ha). After application the granules were raked in.

To estimate the H. rostochiensis populations, nine random samples were taken from each plot before planting in the Spring (initial population) and in the Autumn (final population). H. rostochiensis cysts were extracted from 200 g. air-dried soil and populations expressed as eggs per gramme of soil (e/g).

The weight of tubers was recorded when potato haulms had died back.

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RESULTS

The eelworm multiplication rate in terms of eggs per g. soil is shown in Table I

Table I

The effect of four granular applied nematicides on the multiplication rate of potato cyst nematode expressed in terms of eggs/g. soil (final : initial population).

	Block 1	2	3	4
Mean initial infestation (eggs/g.)	0.3	1.3	6.6	22.4
S.E.	±0.04	±0.19	±0.55	±2.17
Treatment				
Aldicarb				
5 lb a.i./ac	32.7	8.8	11.1	7.5
10 lb a.i./ac	5.6	0.4	4.7	0.7
Phorate				
5 lb a.i./ac	42.5	35.0	17.0	2.4
10 lb a.i./ac	19.0	14.2	3.7	1.1
Thionazin				
5 lb a.i./ac	52.5	24.1	29.8	8.4
10 lb a.i./ac	24.2	24.2	9.9	8.5
Methomyl				
2.5 lb a.i./ac	32.9	24.8	16.6	17.5
5 lb a.i./ac	30.7	20.8	16.6	2.4
Untreated	82.5	30.2	10.8	4.8

Aldicarb, at 10 lb a.i./acre (11.0 kg/ha) was the only nematicide which kept the nematode multiplication rate at a low level at all initial eelworm densities. Phorate, at 10 lb a.i./acre (11.0 kg/ha) reduced the multiplication rate at the two higher levels of eelworm density.

Largest increases in potato yields (Table 2) were obtained from plots treated with aldicarb, phorate or thionazin at 10 lb a.i./acre (11.0 kg/ha).

Table 2

The effect of four granular applied nematicides on potato yield expressed as percentage increase in yield over untreated.

	2.5 lb a.i./ac	5 lb a.i./ac	10 lb a.i./ac
Aldicarb	-	10	48
Phorate	-	12	44
Thionazin	-	2	34
Methomyl	0	9	-

Mean yield from control plots 5.0 t/a S.E.±1.50 (12,544 kg/ha)

DISCUSSION

Previous workers have shown that the multiplication rate of H. rostochiensis on potatoes may be reduced by organophosphorus nematicides (den Ouden & Seinhorst 1964) or by carbamoyl oxime nematicides (den Ouden 1968). It has been suggested that reduction in eelworm multiplication rate is due to the 'systemic' activity of these nematicides in making the potato plant a poorer nematode host. Pain & Hague (1971) demonstrated that nematicidally, aldicarb at least acts as a 'contact' prison in affecting the mobility of second stage larvae in soil and delaying their entry into potato roots. Pain & Hague also indicated that aldicarb affected the male : female sex ratio in some way, thus causing a reduction in the multiplication rate.

In the present trial, phorate was almost as effective as aldicarb in increasing potato yields and in reducing the multiplication rate at the higher eelworm infestations used in the experiment. Thionazin gave comparable yield increases when applied at 10 lb a.i./acre (11.0 kg/ha) but did not reduce eelworm multiplication rate.

Acknowledgments

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NEMATICIDES AND THE SOIL FAUNA

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Summary The effects of aldicarb (6.8 and 11.2 kg/ha) methomyl (11.2 kg/ha) and dazomet (364 kg/ha) on soil-inhabiting invertebrate populations were studied in field experiments. Overall decreases in populations caused by the nematicides were 38.0%, 54.0% and 41.0% respectively. Numbers of Collembola and Acarina were decreased by all the chemicals but earthworm populations only by dazomet. Predatory mite populations recovered from the effects of the chemicals sooner than those of other mites. None of the nematicides affected insect populations other than those of Collembola.

INTRODUCTION

There is need to know whether pesticides applied to soil affect organisms other than those that they are designed to kill. Some of the effects of insecticides on the soil fauna have been summarized (Edwards, 1965; Edwards & Thompson, 1972) as have those of herbicides (Edwards, 1971). The only report of the effects of nematicides was by Buahin & Edwards (1964) who found that the fumigants D-D, methyl bromide and metham drastically affected the soil fauna, and they later established that these effects could persist for two years after the chemical had dissipated. Most nematicides are general biocides, and can be expected to affect organisms, other than nematodes, including some that are beneficial to soil fertility, aeration and turnover. The results we report were obtained with three of the newer nematicides, aldicarb, methomyl and dazomet, of which the first two are also insecticides.

Methods and Materials

1970

Four plots 13' 4" x 21' 0" (4.08 x 6.4 m) were treated with 10 lb a.i. per acre (11.2 kg/ha) of aldicarb in March 1970, and four other plots left untreated. All plots were sown with field beans (Maris Bead). Four soil cores 2" diam x 6" deep (5 cm x 15 cm) were taken from each plot five months after treatment. The invertebrates were extracted from the soil in high gradient Tullgren funnels (based on those described by Macfadyen, 1961) and they were sorted into broad taxonomic categories (Table 2).

1971

The experiment was on newly-ploughed old grassland that had been thoroughly cultivated. Plots were 3 x 3 m square with 1 m paths between, with four replicates of each treatment and four control plots. Aldicarb and dazomet were applied as granules (10% and 98% respectively) sprinkled evenly over the soil, and methomyl as a solution of 90% emulsifiable powder sprayed over the surface of the soil (see Table 1). Immediately after treatment, all plots were thoroughly rotavated to a depth of 6" (15 cm).

Before treatment, and at approximately monthly intervals thereafter, four soil cores were taken from each plot, and the animals extracted from the soil and sorted as in 1970. Earthworm populations were assessed only once, five months after treatment, by means of 2 ft (0.61 m) square quadrats (5 per plot) on which was poured 2 gal (9.1 l) of dilute formalin (25 cc of 40% formaldehyde in water) (Raw, 1959).

Table 1

Chemicals used in experiments

Common name	Trade name	Chemical name	Mode of action	Dose
aldicarb	Temik	2-methyl-2-(methylthio) propionaldehyde O-(methylcarbamoyl) oxime	contact and systemic	1970 10 lb a.i./acre (11.2 kg a.i./ha)
				1971 6 lb a.i./acre (6.8 kg a.i./ha)
methomyl	Lannate	S-methyl N-(methyl-carbamoyl)oxy thioacetimidate	contact and systemic	10 lb a.i./acre (11.2 kg a.i./ha)
dazomet	Basamid	3,5-dimethyltetrahydro-1,3,5-2H-thiadiazine-2-thione	fumigant	325 a.i. lb/acre (364 kg a.i./ha)

Results and Discussion

Tables 2, 3, 4 and 5 and Figs. 1 to 5 summarize the more important results. Many other groups of animals than those reported were examined, but the effects of the chemicals on them were not usually significant. Although animals were sorted into more detailed taxonomic categories than those shown in the graphs, the general picture for families or species did not differ sufficiently from that for orders to justify including the detailed results.

Table 2

Effects of aldicarb (10 lb a.i. per acre, 11.2 kg per ha) on soil invertebrates (1970)

(Average number per 5 cm core 5 months after treatment)

	Control	aldicarb
Acarina (mites)	62.4	25.1 *
Collembola (springtails)	22.3	5.7 *
Insects	3.8	3.5

* significantly different from control at 5% probability level.

Table 3

Effects of nematicides on some larger invertebrates (1971)

(Total numbers in samples during 5 months after treatment)

	Control	methomyl	aldicarb	dazomet
Symphyla	15	18	7	13
Millipedes	7	8	5	3
Centipedes	16	6 +	4+	6+
Enchytraeid worms	345	328	141*	78*
Insects	146	216	126	155
Total invertebrates	6,980	3,860*	2,667*	2,885*

* Significantly different from control at 5% probability level

\+ Significantly different from control at 5% probability level on one sampling date

Table 4

Effects of nematicides on earthworms

(Number in 20 quadrats)

	Control	Methomyl	Aldicarb	Dazomet
Lumbricus terrestris	22	36	20	11*
Other species	47	42	45	13*

* Significantly different from control at 5% probability level

The preliminary 1970 experiment showed that aldicarb had a quite drastic effect on the smaller soil invertebrates including mites and springtails, even five months after it was applied, and that it was not very toxic to the soil insects. However, the dose applied in this experiment exceeded that usually recommended, so in 1971 a smaller dose was applied and compared with dazomet and methomyl, also at recommended rates.

All three nematicides greatly lessened the total numbers of soil invertebrates over the whole period of the experiment; aldicarb and dazomet to 38.2% and 41.3% of the numbers in untreated soil respectively, but methomyl had less effect and decreased populations only to 54.3%. The chemicals affected the smaller invertebrates much more than the larger ones (Table 3); all three chemicals were toxic to most species of mites and springtails (Figs. 2, 3, 4 and 5 and Table 5). Aldicarb and dazomet, but not methomyl, greatly decreased numbers of enchytraeid worms (Table 3). The effects on earthworms were not conclusively demonstrated, but dazomet had most influence on their numbers, aldicarb very little, and methomyl no obvious effect (Table 4). None of the chemicals seemed to affect populations of soil insects (other than Collembola); this was unexpected, because both aldicarb and methomyl are used as insecticides.

None of these nematicides persist long in soil; dazomet dissipates within 8 weeks, aldicarb within 10 weeks and it is improbable that methomyl persists any longer, although there is no precise information on its persistence. The greatest effects on soil invertebrates were during the first 8 weeks after treatment, but the mite and springtail populations had not fully recovered even after five months. Populations of predatory mites recovered sooner than those of other mites. It seems unlikely that the effects of these chemicals would persist into a second season, but this is still being investigated. Most invertebrate populations recovered sooner from treatments with methomyl than with aldicarb and dazomet.

This experiment may underestimate the effects of dazomet, which is most effective when the soil is warm, but to allow a crop to be grown, we applied it in March, when the soil was only just warm enough to enable it to be effective;

FIG. 1.

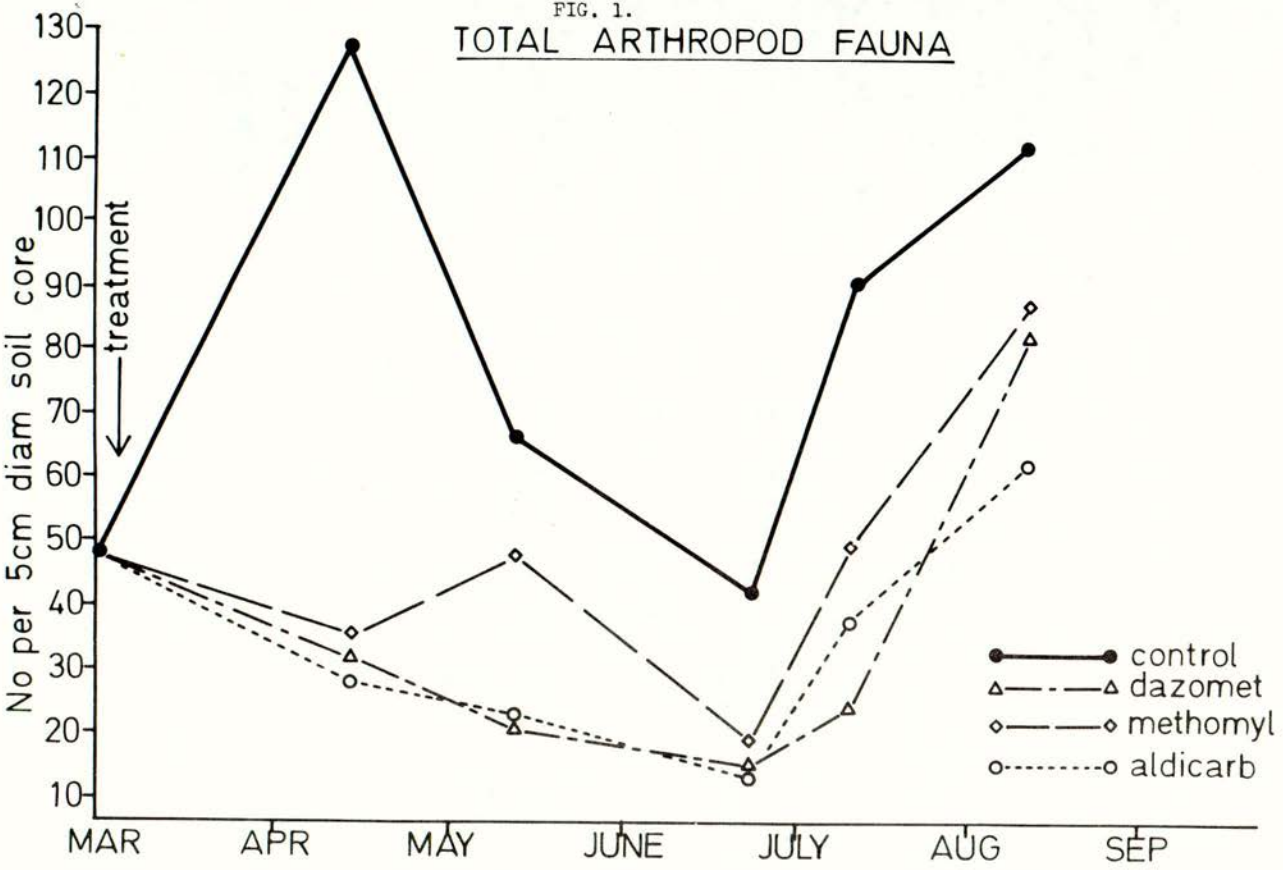
TOTAL ARTHROPOD FAUNA
No per 5cm diam soil core
treatment
130
120
110
100
90
80
70
60
50
40
30
20
10
MAR
APR
MAY
JUNE
JULY
AUG
SEP
control
dazomet
methomyl
aldicarb

FIG. 2.

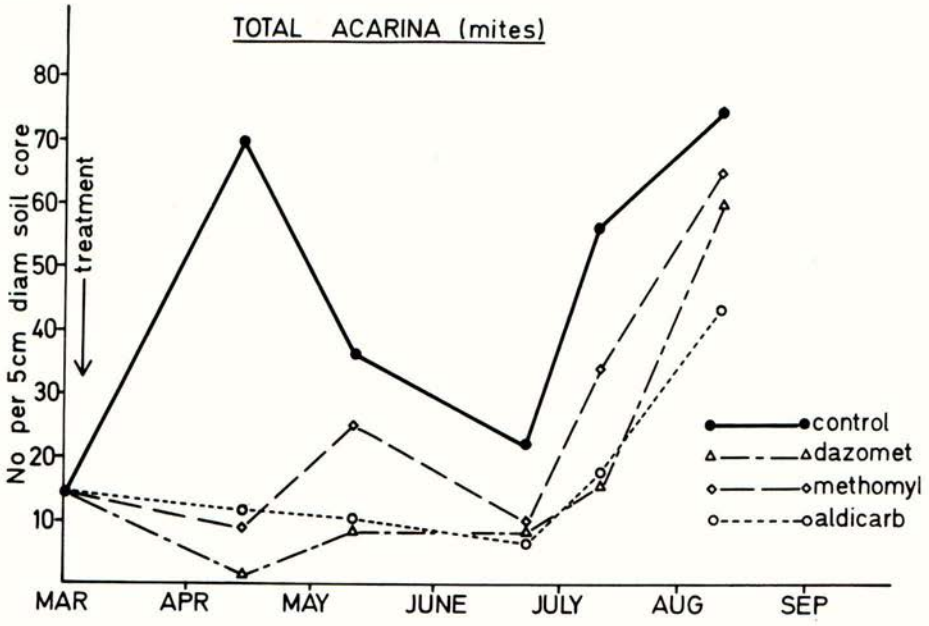
TOTAL ACARINA (mites)
No per 5cm diam soil core
treatment
80
70
60
50
40
30
20
10
MAR
APR
MAY
JUNE
JULY
AUG
SEP
control
dazomet
methomyl
aldicarb

FIG. 3.

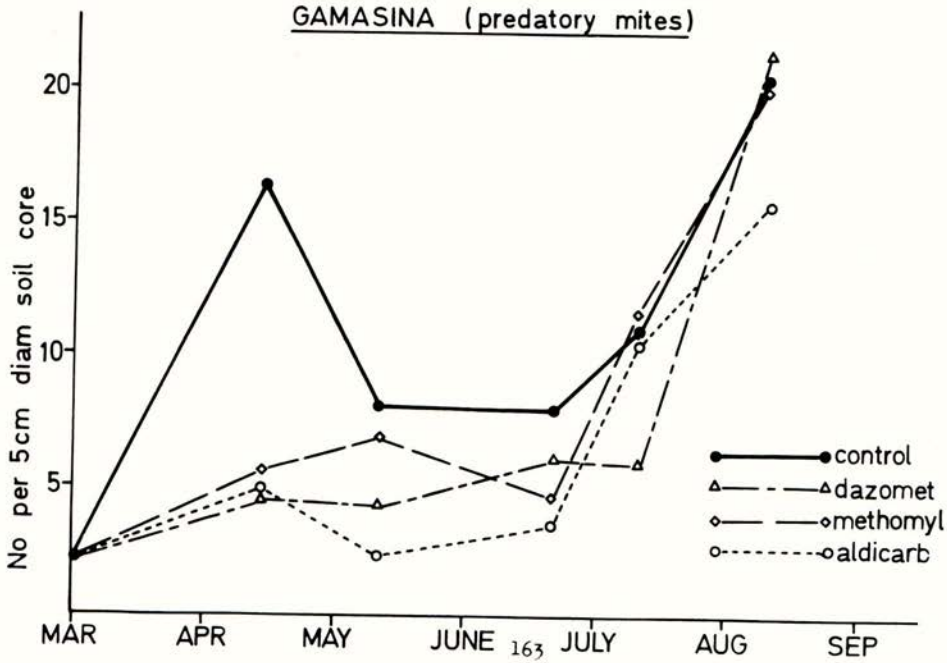
GAMASINA (predatory mites)
No per 5cm diam soil core
20
15
10
5
MAR
APR
MAY
JUNE
JULY
AUG
SEP
control
dazomet
methomyl
aldicarb

FIG. 4.

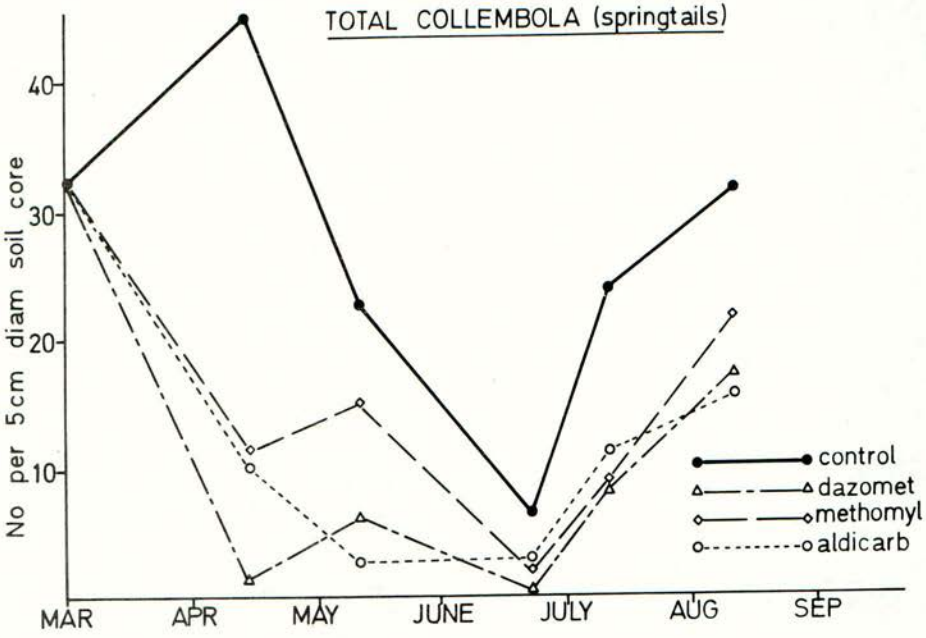
TOTAL COLLEMBOLA (springtails)
No per 5cm diam soil core
40
30
20
10
MAR
APR
MAY
JUNE
JULY
AUG
SEP
control
dazomet
methomyl
aldicarb

FIG. 5.

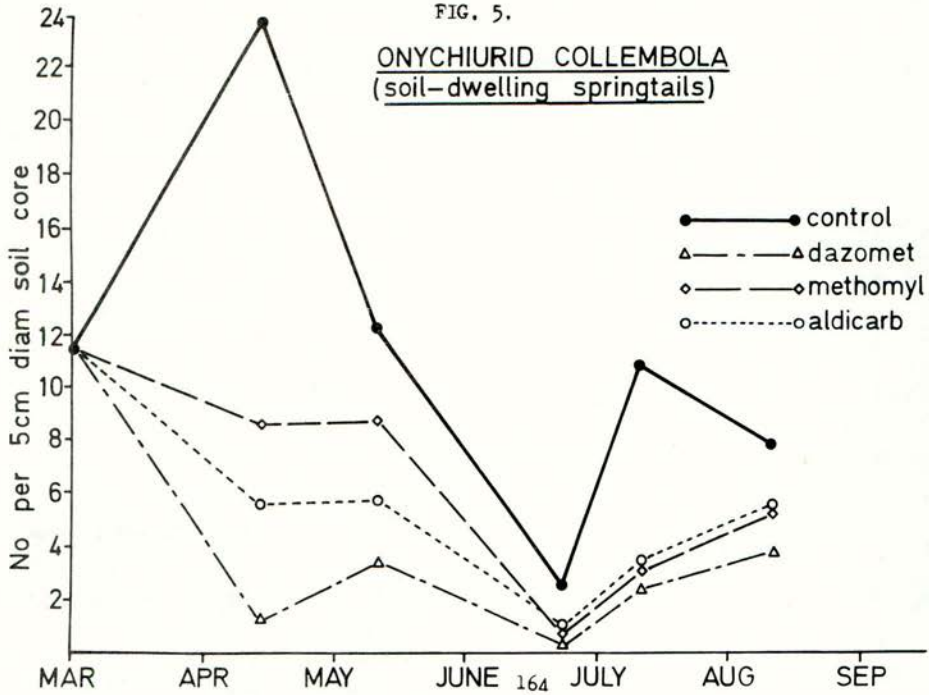
ONYCHIURID COLLEMBOLA
(soil-dwelling springtails)
No per 5cm diam soil core
24
22
20
18
16
14
12
10
8
6
4
2
MAR
APR
MAY
JUNE
JULY
AUG
SEP
control
dazomet
methomyl
aldicarb

Table 5

Statistical analysis of data

* Significant difference at 5% probability level.
\- No significant difference.

	Order or Class	ALDICARB					DAZOMET					METHOMYL				
	Date	14 Apr	12 May	22 Jun	9 Jul	9 Aug	14 Apr	12 May	22 Jun	9 Jul	9 Aug	14 Apr	12 May	22 Jun	9 Jul	9 Aug
ACARINA	Prostigmata (Trombidiformes)	-	-	-	-	-	*	-	-	-	-	-	-	-	-	-
	Mesostigmata (Gamasina)	*	*	*	*	-	*	-	-	-	-	*	-	-	-	-
	Astigmata (Tyroglyphidae)	*	-	-	-	-	*	-	-	-	-	*	-	-	-	-
	Cryptostigmata (Oribatidae)	*	*	*	*	*	*	*	*	*	-	*	-	*	*	*
	Total Acarina	*	*	*	*	-	*	*	-	*	-	*	-	-	-	-
COLLEMBOLA	Onychiuridae	*	-	-	*	-	*	*	-	*	-	*	-	-	*	-
	Isotomidae	*	-	-	-	-	*	-	*	*	-	*	-	-	*	-
	Entomobryidae	*	-	*	-	-	*	-	*	-	-	*	*	*	-	-
	Sminthuridae	-	-	-	-	-	*	-	-	-	-	-	-	-	-	-
	Total Collembola	*	-	-	*	-	*	*	*	*	-	*	-	-	*	-
	Symphyla	-	-	-	-	-	*	-	-	-	-	*	-	-	-	-
	Thysanoptera	-	-	*	-	-	-	-	*	-	-	-	-	*	-	-
	Total Insecta	-	-	-	-	-	*	-	*	-	-	-	-	-	-	-

applied later in spring it might have had even greater effects on the soil fauna.

It is important to assess the effects of these chemicals on predators of nematodes such as centipedes, predatory mites (Gamasina) and springtails, because if populations of these animals are lessened, the nematicide might be much less effective than it would otherwise be. All three of these nematicides did lessen the numbers of all these predators.

It is difficult to assess the importance of the changes in populations of soil invertebrates reported here, because it is unknown whether they contribute to soil fertility. One of them kills the earthworms that may improve soil fertility and they all kill invertebrates that are predators of pest species of nematodes, but there is no evidence here that these effects are prolonged, greatly affect the efficiency of the nematicides or that they influence soil fertility.

Acknowledgements

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VEGETABLE STORAGE DISEASES IN EAST ANGLIA

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Summary A fungicide dip of benomyl at harvest has been used successfully to control the development of Centrospora acerina in celery stored in cool store for 12 weeks, and in hand lifted carrots stored in cool store for 7 months. Other fungicides were not successful.

INTRODUCTION

Since the early 1960's problems of deterioration of vegetables in long term cool storage in East Anglia have been brought to our attention by growers with on-farm storage, processors, and commercial organisations offering cool store facilities. Cool storage is becoming an extension of vegetable growing, and very much the concern of the grower until the produce finally reaches the fresh market outlet or, more commonly, the processing plant. In both cases the success of the enterprise depends on the assurance of continuity of supply of a high quality product over a period of time much beyond the normal harvest season of the vegetable.

Our work has covered three aspects. We first identified the specific organisms which caused the major types of deterioration in celery and carrots in long term store, and then surveyed the distribution of these rots. The third aspect, which is covered in this report, was the evaluation of certain fungicide treatments for the control of the major rots found in stored celery and carrots. It must be stressed that this report refers to relatively long term storage, that is, over 10 weeks for celery and over 5 months for carrots.

At present certain diseases can cause such heavy commercial loss after long term storage that it would be difficult to justify the use of the technique in the absence of control measures. For example, in the mid 1960's, celery losses from Liocrice Rot (Centrospora acerina) reached at least 30% after 8 weeks storage, and losses of 100% were recorded. Lowings (1955) also reported serious losses from this disease from the same area of the country. In addition to direct loss, repacking and sorting of produce adds considerably to the costs of the enterprise, and in the later stages Botrytis may develop where leaf and soil debris are present.

Chanteney carrots from peat and mineral soils in Norfolk, stored from mid October onwards, may start to rot in March from various causes. Three main organisms are involved, namely Centrospora acerina, Botrytis cinerea and, to a lesser extent, Solerotinia solerotiorum (Derbyshire 1971). According to season and other factors there can be either total collapse of produce in bulk bins after 4-6½ months or, under favourable conditions, roots can be stored for 7 months or more with very little deterioration. This variability of keeping potential and quality is not understood at present, but from a growers point of view it is highly unsatisfactory. This uncertainty must be resolved before a prediction of the amount and quality of produce which can be marketed after a certain period, can be made.

Recently certain cultural and fungicide treatments have been considered for the treatment of these vegetables. There are limitations on the type of fungicide which can be used for this purpose, for example:-

1. It must effectively control the fungal pathogen.
2. It must not damage the produce in any way.
3. It must not accelerate senescence.
4. It must be entirely free from operator and consumer hazard.
5. It must not leave an unattractive deposit.
6. It must not taint the produce nor upset the processing method.

These stringent conditions must be adhered to at all times as fungicides without these qualities will never succeed commercially in this field.

Many factors bearing on the success of long term vegetable storage could be discussed, but the purpose of this paper is to present data on the use of fungicidal dips, on an experimental basis, for the control of certain storage diseases. While disease problems hamper the potential development of commercial storage techniques we cannot proceed, but there is evidence that when the disease problems are resolved, there are still important subjects such as the quality of produce for storage which need to be examined in greater depth.

EXPERIMENTAL TECHNIQUE AND THE ORGANISMS INVOLVED

CELERY

Identification of Rots

The chief disease under test in the celery dipping trials was Centrospora acerina. The infected head develops blackish lesions of increasing size at the junction between the petiole base and the celery butt or stem. The position of the rot is most characteristic of this disease, and the colour of the lesions with the torulose hyphae distinguishes them from secondary bacterial infections which are a lighter brown colour. Eventually in the advanced stage of the disease the whole butt and petiole bases may collapse in a black mass with the remains of the petioles loosely attached. This disease rarely spreads in store, but is introduced on the basal area at the start of storage.

Fungicide dips were applied by dipping the base of the petioles and trimmed butt in a 6in. depth of liquid for a few seconds. Alternatively one treatment received a 15 minute soak, and another was applied as a soil drench 11 days before harvest.

Treatments

1. Dicloran dip at ½lb/100 gal.
2. Iodophor as 'Wescodyne' dip at 25 fl. oz/100 gal.
3. Iodophor as 'Wescodyne' dip at 75 fl. oz/100 gal.
4. Benomyl dip at 1lb/100 gal.
5. Benomyl soak for 15 minutes at 1lb/100 gal.
6. Benomyl drench 11 days before lifting at 3 gm/2 gal/sq. yd.
7. Water dip as control.

The design was a randomised block with 4 replications of 2 cubes (boxes) per treatment with samples examined on three occasions.

The celery, var New Dwarf White, was grown on a peat field in Norfolk known to be heavily contaminated with Centrospora disease. The crop was harvested from November 5-9, 1970, dipped directly after trimming of the roots from the butt, and packed at approximately 10-12 heads per cube. The tops were then cut level with the top of the cube removing the majority of the leafy tissue. All the cubes were

packed into 9 unlined bulk bins and stored at 34°F for up to 12 weeks with 95% RH. The temperature in the produce was lowered as rapidly as possible at the beginning of storage. Celery from 3 bulk bins was examined after 6, 9 and 12 weeks. Over 2,000 heads in all were examined for disease incidence.

CARROTS

Identification of Rots

(a) Centrospora acerina

Large blackish sunken lesions containing torulose mycelium occur at the shoulder of the carrot, on the main body of the root, and as a rot at the tip of the tap root. The latter symptom is very characteristic of the disease especially when the tap root is broken in mechanical harvesting. If lesions are incubated in a damp chamber aerial mycelium develops which after a while has a delicate pink tinge in contrast with Alternaria or Stemphylium spp which produce dark mycelium and spores. This disease rarely spreads in store.

(b) Botrytis cinerea

The development of extensive areas covered with grey mould, and later sclerotia, is typical of this disease which can spread rapidly in store. The rot may frequently be soft and the tissue lightish brown in colour. Arsvoll (1966) reported that Botrytis only attacked shrivelled roots but never attacked roots in good physiological condition. Water loss of the produce may certainly be associated with Botrytis spread, and infection also seems more common on mechanically damaged roots.

(c) Selerotinia sclerotiorum

The American name Cottony Soft Rot (Rader, 1952) aptly describes the appearance of this organism on the carrot. From a single infected root, which acts as a disease focus, the mycelium spreads to neighbouring carrots causing a gradual soft rot and watery collapse of the root. Extensive development of this disease in a bulk bin can be important, although in East Anglia serious collapse from the disease has not been found very frequently. For example, out of 5 fields sampled in 1967-68, Selerotinia was only found in one (Derbyshire 1971). The disease was not important in other seasons, nor during the course of the fungicide trial.

Fungicides were applied by completely immersing nylon nets of carrots (about 100 roots) in the solution for a few seconds.

Treatments

1. Water dip as control.
2. Benomyl dip at 1lb/100 gal.
3. Benomyl soak for 15 minutes at 1lb/100 gal.
4. Dicloran dip at ½lb/100 gal.
5. Iodophor dip as 'Wescodyne' at 25 fl. oz/100 gal.
6. Iodophor dip as 'Wescodyne' at 75 fl. oz/100 gal.
7. Sodium O-phenyl phenate dip at 1lb/100 gal.
8. 'Decosan' (10% available chlorine) dip at 4ml/litre.
9. Untreated (no dip at all).

The design was a randomised block with 4 replications of 2 nets per treatment with samples taken on 3 occasions.

The carrots, var Chanteney, mainly over 1¾" diameter suitable for dicing or souping, were harvested by hand on December 15-17 from a peat field in Norfolk known to be heavily contaminated with Centrospora disease. After dipping, the nylon nets were packed into bulk bins and stored at 34°F with 95% RH. Samples of carrots were taken after 3, 5 and 7 months storage. In all, approximately 19,200 carrots were examined individually for disease incidence.

RESULTS

The results of the celery dipping trial are given in Table I. The benomyl dip and soak treatments proved highly successful in controlling Centrospora infection for up to 12 weeks storage. Infection in the benlate drench treatment was delayed in comparison with other treatments but by the twelfth week the disease had reached a high level. A visual examination of the plants suggested that the higher concentration of iodophor may have accelerated senescence and consequently disease incidence.

TABLE I

CELERY Percentage total infection with Centrospora
(data transformed into angles)

Treatment	Weeks in store		
	6	9	12
1. Dicloran	22.4	39.9	45.7
2. Iodophor (low rate)	19.5	46.8	40.6
3. Iodophor (high rate)	20.8	32.7	58.6
4. Benomyl dip	6.5	7.3	0.0
5. Benomyl soak	0.0	5.0	10.8
6. Benomyl drench	4.6	21.5	37.1
7. Water dip	29.2	31.8	44.0
S.E.	4.04	6.30	3.61

The storage conditions were good and the produce was trimmed and handled carefully so that there was very little leaf debris in the cubes. In consequence Botrytis infection was at a low level in this trial.

The results of the carrot dipping trial are given in Tables 2 and 3. There was a gradual decline in percentage marketable roots during the 7 months of storage. Benomyl dips gave the largest number of marketable roots over the whole period, while large numbers of roots treated with the high rate of iodophor, sodium O-phenylphenate and non dipped roots had decayed through bacterial collapse (Pseudomonas marginalis (Group IVA)) by the end of the 7 months. In contract the benomyl treated roots were almost free from bacterial rots. Botrytis and Sclerotinia were at a low level in this trial.

TABLE 2

CARROTS Percentage marketable roots
(data transformed into angles)

Treatment	Months in store		
	3	5	7
1. Water	67.5	62.9	53.2
2. Benomyl dip	81.0	77.4	76.1
3. Benomyl soak	80.2	78.1	77.7
4. Dicloran	61.5	56.0	44.4
5. Iodophor (low rate)	62.8	58.4	50.1
6. Iodophor (high rate)	62.0	60.8	45.2
7. Sodium O-phenylphenate	53.8	55.8	45.4
8. 'Deosan'	66.2	61.5	51.2
9. Untreated	55.8	50.7	34.8
S.E.	2.34	2.84	3.43

TABLE 3

CARROTS Percentage roots infected with Centrospora
(data transformed into angles)

Treatment	Months in store		
	3	5	6
1. Water	19.6	25.3	36.2
2. Benomyl dip	7.0	11.9	13.9
3. Benomyl soak	6.6	8.9	9.5
4. Dicloran	27.0	31.9	37.5
5. Iodophor (low rate)	26.2	26.0	31.5
6. Iodophor (high rate)	26.0	24.9	30.1
7. Sodium O-phenylplenate	29.2	31.1	37.0
8. 'Deosan'	21.2	25.3	32.3
9. Untreated	32.7	36.5	35.0
S.E.	2.66	2.27	2.23

DISCUSSION

In vegetable storage we are dealing with a living product - one which is still metabolising (even if it is at a lower rate than at normal temperature) and therefore physical changes such as dehydration and, in celery, structural changes in the petioles may take place. Chemical changes, defined by Hugh Smith (1964) as down-grade metabolism (or senescence), and microbiological changes, are also going on. It is only the development of disease following senescence which has been studied in this trial and this is the record of one years work carried out in East Anglia. Fungicide dips have not yet been tried using mechanically lifted carrots, which without treatment have been found to store less well than hand lifted roots (Derbyshire 1971). In addition, large carrots were used for the trial, not those suitable for prepacking or canning. It must also be remembered that these results were obtained from roots in which the major rot was Centrospora acerina.

Botrytis and Sclerotinia were only present at low levels. It is not known whether the same fungi cause problems elsewhere in Britain; but the limited world literature on the subject tends to support the opinion that the organisms mentioned are among the main factors in deterioration of celery and carrots.

In earlier surveys, Derbyshire (1971), it was found that there were very strong seasonal influences on incidence and intensity of rots in store and these must be examined in future. Work also needs to be done on the control of water loss in stored produce to reduce the development of Botrytis in both celery and carrots. Fungicide treatment could also influence the development of this disease.

Truscott (1944) working in America, and Lewis and Day (1971) working in Norwich, have found that Centrospora acerina, does not cause serious disease in early storage. It is only when the host plant begins to show signs of senescence, and in consequence, becomes more susceptible to invasion, that the symptoms may develop rapidly. Fungicides such as benomyl used in this context, to delay the later development of the disease in long term storage, will act as one feature in a control programme, but it must be stressed that fungicides alone on badly handled, poor quality produce may only lead to disaster. The success of long term storage will depend to a great extent on the quality of the produce at harvest, but when this is of a high standard, fungicides can give further assurance of a satisfactory marketable sample at the end of the storage period.

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THE CONTROL OF BOTRYTIS POD ROT IN DWARF BEANS

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Summary Replicated experiments carried out over three years and commercial applications assessed in two years, have shown that a treatment of benomyl at 0.5 lb ai/ac (0.56 kg/ha), applied at full-bloom, in medium to high volume will give effective control of pod-rot caused by Botrytis cinerea. Increasing the rate to 1.0 lb a.i./ac (1.12 kg/ha), applied as a single or split treatment, improved the degree of control, but is unlikely to be economic. Routine treatment of dwarf bean crops is likely to prove very beneficial to growers and processors in reducing produce spoilage caused by Botrytis and other fungal diseases. Thiophanate-methyl and BASF 3201 have also proved to be promising materials for this purpose.

INTRODUCTION

Mechanically-harvested dwarf beans (Phaseolus vulgaris) are an increasingly important crop for processing, and the most common and troublesome disease affecting them is pod-rot caused by Botrytis cinerea. The implementation of research findings has resulted in the use of high rates of nitrogen fertilizer, narrower row widths, increased plant density and the more widespread use of irrigation, factors contributing towards creation of an ideal microclimate for the development and spread of fungal diseases. Pods affected by Botrytis cannot be used for processing and those reaching the factory have to be picked out by hand on sorting belts. If more than approximately 10% of the pods are affected it becomes extremely difficult to eliminate them all and the processor may be forced to reject the crop. Produce which is transported long distances to the factory is particularly vulnerable since the infection can quickly develop and spread to previously healthy pods during transit.

It was therefore desirable that investigations should be started in an attempt to control the disease chemically and P.G.R.O. commenced preliminary tests in 1968. Prior to this date little work had been carried out in this country, but in America studies were already in progress on the control of the closely related disease Sclerotinia sclerotiorum (white mould), a more common problem in that country, but only occasionally encountered in our crops. Good control of white mould was obtained by Pegg (1965) using weekly applications of dicloran starting at blossom fall, and control of post-harvest rotting was also achieved with dicloran dips prior to packing. Niedbalski and Rickard (1969) also achieved satisfactory control of the disease with multiple applications of dicloran, provided the initial spray was applied at first blossom. In Natti's (1967) work applications of dicloran, thiabendazole and benomyl were applied as single sprays at full-bloom and post-bloom (4 days later); while one treatment received both sprays. Benomyl proved to be far more effective than either

dicloran or thiabendazole, the two spray treatment providing complete control at the rate of 1.5 lb a.i./ac and almost complete control at 0.75 lb a.i./ac. A single application of 0.75 lb a.i./ac at full-bloom was rather inconsistent and the post-bloom was relatively ineffective.

In work carried out by P.G.R.O. (1968) dichlofluanid as a single or as double spray treatment applied at full-bloom and ten days after full-bloom, proved ineffective against Botrytis cinerea, although some control of Colletotrichum lindemuthianum was achieved. From 1969-71 further experimental work was conducted testing benomyl and other fungicides, which will be discussed in this paper.

METHOD AND MATERIALS

Some replicated experiments (1969-71) were carried out in commercial crops, while others were conducted on the Thornhaugh trial ground. Plots were 108 ft^2 (10 m^2) in a randomised block layout, with three replications. They were harvested by hand at the normal harvesting stage for either quick-freezing or canning, outside rows being discarded. The produce was sorted to establish the weight of Botrytis-affected pods and both total weight (figures not presented) and weight of healthy pods were recorded.

Botrytis can affect pod development in two ways, either it destroys the developing pod, which either drops off the plant or remains very small and shrivelled, or it can affect parts of the otherwise normally developed pod. Because the disease can cause pod-loss in the early stages of development, total yield was recorded from the experiments to see if by preventing this loss yields would be increased. There was no suggestion from the data that this occurred and it is presumed that the plant compensates for the loss of a proportion of pods by producing larger pods or pods which would not have developed otherwise. For this reason the yield of un-blemished pods is presented, as this is of course the only produce of value to the producer and processor.

Data was analysed and where differences occurred at the 5% level of significance between the treated and the untreated they have been indicated by an asterisk. Applications were made with a modified 'OPS' in 1969-70, at a volume of 50 gal/ac (5621/ha); in 1971 a van der Weij sprayer was used, applying a similar volume. In 1969 and 1970 the first application was made at full-bloom (A), when the plant had only a few young pods set, the longest of which would be approx. ¾ in.(19 mm) in length, the later application (B) was made seven to ten days afterwards, when the crop was past the flowering stage and the longest pods measured approx. 4 in. (101 mm). In the 1971 experiments, only one stage of application was tested, the sprays being applied at full-bloom, except at sites 1 and 3 where poor spraying conditions caused delay and the crops were past the full-bloom stage. The rates of chemicals are presented in lb a.i./ac with the equivalent kg/ha following in brackets.

The variety used in this work was Tendercrop, with the exception of sites 2-4 in 1970, at which the varieties were respectively Cascade, Bush Blue Lake 274 and Dynamit respectively and site 3 in 1971 where it was again Bush Blue Lake 274.

In the 1970 commercial trials, eight growers each treated one acre of crop with benomyl at 0.5 lb/ac (0.56 kg/ha) applied at approximately the full-bloom stage of maturity. The rest of the field was left untreated. Applications were made with conventional land sprayers using volumes between 30 and 60 gal/ac (334-668 l/ha), with the exception of three sites, at which a helicopter applied benomyl at a volume of 5 gal/ac (56l/ha) using D625 jets at a pressure of 40 p.s.i. A random sampling method was used at harvest, twenty random 1 yd lengths of row being hand-picked from both treated and untreated areas and the same recordings carried out on the produce as in the replicated experiments. The variety was Tendercrop, except at the Sparham site where it was Cascade.

In the 1971 commercial trials unsprayed areas of approx. 1 acre were left in treated fields to allow assessments to be carried out. A similar sampling method was used to that of the previous year, except that ten random 1 yd lengths of row were used instead of twenty. All these applications were made with land sprayers using 0.5 lb (0.56 kg/ha) of benomyl applied at approx. full-bloom. The 1971 results are presented as the mean of the number of assessments made in each field. On average at least two separate drillings in each field were sampled.

RESULTS

Replicated experiments 1969-71

The percentage reduction (by weight) in the amount of Botrytis affected pods and the yield of healthy pods are presented in Tables 1 & 2. The two-application treatment with benomyl at 0.5 lb (0.56 kg) effectively controlled pod infection caused by Botrytis cinerea and the single application of this material made at the full-bloom stage was also very effective. The later application was generally far less effective. The results for thiophanate-methyl followed a similar pattern, although reflecting a slightly lower level of control than the equivalent rate of benomyl in 1970, at the higher rates tested in 1971 it was equal to benomyl. Thiabendazole was generally less effective and consistent than the two materials already discussed and the limited results with dichlofluanid, mancozeb and thiram-in-oil suggested that they are not capable of giving satisfactory control when used in this way. In the 1971 work BASF 3201 (a benzimidazole derivative) gave good Botrytis control, generally slightly better than that given by benomyl. No significant increases in the yield of healthy pods were obtained in any of the replicated experiments, although generally the yields from the most successful treatments were higher.

Commercial benomyl sprayings 1970-71

The results for the eight field samplings carried out in 1970 and for four fields, involving a total of eleven separate comparisons, sampled in 1971 are presented in table 3. The results of further sampling carried out in 1971 are not yet available.

Table 1

The percentage reduction (by weight) of Botrytis affected pods

Material/Application		Rate lb a.i./ac (kg/ha)	1969	1970 Site 1	1970 Site 2	1970 Site 3	1970 Site 4	1971 Site 1	1971 Site 2	1971 Site 3
benomyl	A	0.5 (0.56)	95	83	77	60	96	86	52	53
"	A	1.0 (1.12)	-	-	-	-	-	100	80	57
"	B	0.5 (0.56)	28	69	76	49	-	-	-	-
"	A&B	0.5 + 0.5	88	100	88	76	100	-	-	-
thiophanate-methyl	A	0.5 (0.56)	-	66	68	39	93	-	-	-
"	A	0.75(0.84)	-	-	-	-	-	90	72	23
"	A	1.50(1.68)	-	-	-	-	-	88	79	63
"	B	0.5 (0.56)	-	100	58	36	-	-	-	-
"	A&B	0.5 + 0.5	-	84	70	66	100	-	-	-
thiabendazole	A	0.5 (0.56)	-	66	29	27	90	50	18	52
"	A	1.0 (1.12)	-	-	-	-	-	63	27	33
"	B	0.5 (0.56)	-	53	44	9	-	-	-	-
"	A&B	0.5 + 0.5	-	49	55	31	63	-	-	-
BASF 3201	A	0.5 (0.56)	-	-	-	-	-	96	61	61
"	A	1.0 (1.12)	-	-	-	-	-	88	84	67
dichlofluanid	A	1.5 (1.68)	-	-	-	-	45	-	-	-
"	A&B	1.5 + 1.5	-	-	-	-	50	-	-	-
mancozeb	A	0.8 (0.90)	20	-	-	-	34	-	-	-
"	B	0.8 (0.90)	0	-	-	-	-	-	-	-
"	A&B	0.8 + 0.8	22	-	-	-	30	-	-	-
thiram-in-oil	A	2.0 (2.24)	13	-	-	-	-	-	-	-
"	B	2.0 (2.24)	0	-	-	-	-	-	-	-
	A&B	2.0 + 2.0	25	-	-	-	-	-	-	-
Untreated - per cent botrytis affected pods			6.0%	0.7%	3.6%	1.5%	3.8%	2.1%	5.3%	1.9%

A Full-bloom
B Seven-ten days after full-bloom

Table 2

The yield of unblemished pods expressed as a percentage of the untreated control

Material/Application		Rate lb a.i./ac (kg/ha)	1969	1970 Site 1	1970 Site 2	1970 Site 3	1970 Site 4	1971 Site 1	1971 Site 2	1971 Site 3
benomyl	A	0.5 (0.56)	101	99	106	107	93	114	109	102
"	A	1.0 (1.12)	-	-	-	-	-	110	106	108
"	B	0.5 (0.56)	116	100	104	95	-	-	-	-
"	A&B	0.5 + 0.5	115	99	100	98	118	-	-	-
thiophanate-methyl	A	0.5 (0.56)	-	98	107	91	80	-	-	-
"	A	0.75 (0.84)	-	-	-	-	-	102	119	102
"	A	1.50 (1.68)	-	-	-	-	-	113	113	103
"	B	0.5 (0.56)	-	97	98	106	-	-	-	-
"	A&B	0.5 + 0.5	-	102	97	107	118	-	-	-
thiabendazole	A	0.5 (0.56)	-	97	97	93	131	108	89	99
"	A	1.0 (1.12)	-	-	-	-	-	110	103	109
"	B	0.5 (0.56)	-	104	104	88	-	-	-	-
"	A&B	0.5 + 0.5	-	98	100	95	108	-	-	-
BASF 3201	A	0.5 (0.56)	-	-	-	-	-	121	118	103
"	A	1.0 (1.12)	-	-	-	-	-	102	117	104
dichlofluanid	A	1.5 (1.68)	-	-	-	-	104	-	-	-
"	A&B	1.5 + 1.5	-	-	-	-	109	-	-	-
mancozeb	A	0.8 (0.90)	111	-	-	-	94	-	-	-
"	B	0.8 (0.90)	94	-	-	-	-	-	-	-
"	A&B	0.8 + 0.8	87	-	-	-	79	-	-	-
thiram-in-oil	A	2.0 (2.24)	110	-	-	-	-	-	-	-
"	B	2.0 (2.24)	96	-	-	-	-	-	-	-
"	A&B	2.0 + 2.0	93	-	-	-	-	-	-	-
Untreated - yield in cwt/ac			31.6	74.5	132.2	137.5	59.3	121.6	113.8	165.4
S.E. as per cent of gen. mean			17.0	7.8	5.8	11.1	18.4	12.1	11.1	10.4

A Full-bloom
B Seven-ten days after full-bloom

In 1970 the benomyl application reduced the amount of Botrytis affected pods by 57%, from 6.9% to 2.7%, while the mean yield of healthy pods was 3% higher than that on the untreated areas. At the three Holbeach sites the percentage of Botrytis affected pods was significantly lower on the treated areas, while at Spalding and the first Holbeach site there was also a significantly higher yield of healthy pods on the treated areas. The level of control achieved in 1971 was lower than in 1970, the mean percentage reduction in the amount of Botrytis affected pods being 27%. The mean yield of healthy pods was 9% higher on the treated areas than on the untreated areas. During most of the period when the 1971 commercial sites were sprayed, weather conditions were not very suitable, with frequent rain and high winds. This may have reduced the efficiency of the treatment.

Table 3

Commercial benomyl spraying results 1970-71

Site or field	% Botrytis affected pods Untreated	Treated	% reduction	Yield of healthy pods as % of untreated
1970				
Boston	5.4	1.8	66	92
Sparham	1.1	0.5	52	93
Spalding	5.5	3.7	34	111*
Riseley	6.5	0.7	89	91
Heydon	1.0	0.7	32	110
/Holbeach	11.6	2.0*	82	151*
/Holbeach	12.7	4.5*	65	74
/Holbeach	11.7	7.7*	34	102
Mean	6.9	2.7	57	103
1971				
Field 1	10.9	5.6*	49	133
Field 2	3.1	2.4	23	100
Field 3	4.2	3.1	26	99
Field 5	7.5	6.9	8	102
Mean	6.4	4.5	27	109

/ sites sprayed by helicopter at low volume.

Taints

Throughout this work samples of produce were taken from treated and untreated plots and after processing were submitted to the Fruit & Vegetable Preservation Research Association, Chipping Campden for the determination of possible taints. The work on benomyl is now completed and it showed that no taints are likely to result from treatment with this chemical. Work is still in progress with the other promising materials tested and to date no taints have been discovered in treated produce.

DISCUSSION

The most successful material tested for the control of Botrytis pod-rot was benomyl, and for optimum results the evidence suggests that it should be applied at full-bloom. In the replicated work, 0.5 lb (0.56 kg) gave adequate but not complete control, although the commercial sprayings at this rate have generally been slightly less consistent and effective. The two application treatment used in 1970 and the double rate treatment in 1971 have shown that higher rates will give more complete control than the 0.5 lb rate, but it is doubtful if they would be economic. The approximate cost of applying 1 lb of 50% wettable powder benomyl is £5, and costings applied to the results of the 1970 commercial spraying assessments showed that the return on the weight of produce saved from spoilage by Botrytis cinerea more than compensated for this expenditure, if the yield was at least 60 cwt/ac and the return per ton of produce was at least £45. Where yields of 70-80 cwt/ac were obtained the treatment was still economic when the produce was costed between £35-40/ton. The lower level of control obtained in 1971 suggests that efficiency may vary from year to year according to conditions, but using a higher rate in order to improve control would probably be uneconomic in years where 0.5 lb gives adequate control.

The most important feature of this work was the reduction of high levels of Botrytis affected produce to more manageable levels. Levels of more than 10% of affected produce were generally reduced by the treatment to approximately 5%, at which level it would be possible for the produce to be sorted in the factory and it would be unlikely that such a crop would be rejected.

There is some evidence that treatment with benomyl at flowering will prevent the spread of anthracnose Colletotrichum lindemuthianum, a disease which causes deep and unsightly lesions on the pods and invariably results in crop rejection. It would therefore appear that a routine spray with benomyl at full-bloom is a worthwhile insurance to reduce the amount of pod-rot caused by Botrytis and to prevent the spread and development of anthracnose too, should this disease be present.

Dwarf beans are still grown in row widths of 16 in. or more and in most crops little difficulty has been experienced in using normal land machines for the application. The three commercial spraying assessments made in 1970 where a helicopter was used, spraying at 5 gal/ac, suggested that this method was equally as effective as land machine spraying and if crops are particularly dense this would be a convenient and safe way to apply benomyl. No phytotoxicity was noted as a result of the low volume application and none was recorded from any treatment in either the replicated experiments or the commercial benomyl sprayings.

Resistance to attack from Botrytis is one of the characteristics looked for in the selection of new varieties and the results from P.G.R.O. dwarf bean variety assessment trials have demonstrated marked varietal differences in this respect. Tendercrop, still the most widely grown variety, is, however, relatively susceptible and until new resistant varieties are introduced there is a need for the chemical control measures already discussed.

Of the other materials tested the most promising are thiophanate-methyl at 0.75 lb (0.84 kg) and BASF 3201 at 0.5 lb (0.56 kg). Both appear to be as effective as benomyl. Thiabendazole, tested as a wettable powder in 1970 and as a liquid formulation in 1971, dichlofluanid, mancozeb and thiram formulated in oil, have not given satisfactory control in these experiments.

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POST-HARVEST DISEASE CONTROL OF POME FRUITS WITH THIABENDAZOLE

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Summary Post-harvest diseases of apples and pears are primarily caused by bull's-eye rot (Gloeosporium perennans), blue mould rot (Penicillium expansum), and grey mould rot (Botrytis sp.). For their control Thiabendazole has been tested in all major fruit growing areas of Europe and the United States. In these experiments the fungicide demonstrated good disease control when applied as a spray before harvest or as a dip or drench after harvest. Treatment with thiabendazole reduced fruit losses during storage, shipping and marketing.

INTRODUCTION

The purpose of storing fruit is to provide a regulated supply to the market over an extended period of time. From the moment apples and pears are placed in storage until they reach the consumer, many tons of stored fruits are damaged or destroyed by pathogenic fungi. During storage alone, losses range from 1-5% in controlled atmosphere and 5-18% in cold storage. Similar losses are incurred when fruit is exported to other countries, due to the shipping time involved and handling before and after harvest.

Since Robinson, et al. (6 & 7) and Staron, et al. (8 & 9) discovered the broad spectrum activity of thiabendazole in 1964, one of the first practical applications was to prevent infection and suppress fungi affecting the fruit after harvest. Crivelli (3) in Italy reported first in 1966 of thiabendazole as a post-harvest fungicide for the control of penicillium decay of oranges. Burden (2) published his findings on the control of crown rot of bananas in 1967, and Pierson (4) mentioned in 1966, the effectiveness of thiabendazole for the control of blue mould of apples. Over the years the prevention and control of post-harvest diseases has received more recognition. With the availability of thiabendazole, losses in pome fruits can now be effectively reduced or virtually eliminated.

Occurance of Pathogenic Fungi Causing Rot

In the United Kingdom and Continental Europe, Gloeosporium sp. is the major pathogen causing rot during storage. Botrytis and Penicillium are of lesser importance.

In the United States this proportion is reversed to the point that Gloeosporium is of little significance. In the apple growing areas of Canada, all three of the fungi are of serious concern to the packing-house operators.

METHODS OF TREATMENT

Commercial post-harvest treatment of pome fruit with thiabendazole involves either a dip tank immersion of loose fruit or spray drenching, with recirculation, of bin contained fruit. These same methods have been used to apply diphenylamine and ethoxyquin in the treatment of the physiological disease, scald. By combining thiabendazole in the same treatment with either diphenylamine or ethoxyquin, it has been found feasible to treat fruit simultaneously for fungal pathogens and scald. Neither DPA or ethoxyquin affect the efficacy of the fungicide. Satisfactory application of the combined treatment requires no special handling other than adequate agitation.

POST-HARVEST TESTING PROCEDURE AND RESULTS

A commercial trial was conducted in New York. The fruit was treated in a 4,000 l. capacity dip-tank, equipped with a sump pump. 9.9 lb of thiabendazole (Formulated as 60% w.p.) was slurried in two 20 l. pails and added to the water filled tank. 4.450 g DPA was then added to the treatment bath. The pump was turned on prior to pouring the chemicals into the tank to provide mixing and agitation of the treatment bath.

A total of 82 bins or 1,476 boxes of McIntosh apples were treated. Two of the bins were dipped simultaneously, by means of a specially equipped forklift truck, for approximately 15-20 seconds.

After treatment, 5 bins including checks were stored at a relative humidity of higher than 80% for detailed evaluation. After 85 days in storage the apples were sorted and the healthy ones in each container were separated from diseased or rotten apples. The criteria for a diseased fruit was the presence of a decay greater than 5 mm in diameter.

Grey mould was the major disease found in the apples examined in this study. Some infection of blue mould was observed in the untreated bins, while none was noticed in those treated with thiabendazole.

The data in Table 1 demonstrates a high degree of rot control with thiabendazole. The data further shows that the water check showed a lower disease incidence than the dry check.

A second commercial trial was conducted in Ontario, Canada. Thiabendazole was applied by means of a drencher. The tank had a capacity of 2,000 l. The unit was equipped with a single pump and a recirculating system. The containers passed on a conveyer belt and each bin, containing 19 boxes, was treated individually by an overhead drencher.

3.6 lb of thiabendazole was slurried in a 20 l. pail and added to approximately 1,800 l. of water, followed by 2.700 g ethoxyquin. 100 bins of McIntosh apples were treated on 11th November, 1970. After treatment, 3 of the treated bins plus an untreated check were placed in storage at 32°F. 111 days after treatment, the 4 bins were removed from storage and the apples in each bin were counted and evaluated for decay.

Blue mould and grey mould were the main diseases found on the apples. Some brown rot and bull's-eye rot were also observed. Thiabendazole reduced storage rot from 6.17% to an average of 1.8% (Table 2).

Table 1

Control of storage rot of apples with thiabendazole, New York experiment

Treatment	Bin No. ***	Number of Rotten Apples per bin*	% Rot
Water check	0	288	13
Dry check	-	353	16
Thiabendazole + DPA**	6	22	1
Thiabendazole + DPA**	14	57	3
Thiabendazole + DPA**	27	42	2

* Approximately 2,160 apples/18 box-bin
** 0.9 lb thiabendazole + 110.2 g DPA/100 l.
*** Sequential number of the 82 bins dip-treated

Table 2

Control of storage rot of apples with thiabendazole, Ontario, Canada experiment

Treatment	Bin No.**	Total No. Fruits	Total No. Rotted Fruits	% Rot
Check	0	2,669	165	6.17
Thiabendazole + Ethoxyquin*	40	2,609	46	1.76
Thiabendazole + Ethoxyquin*	60	2,092	39	1.86
Thiabendazole + Ethoxyquin*	100	2,495	46	1.84

* 0.9 lb thiabendazole + 151.7 g ethoxyquin/100 l.
** Sequential number of the 100 bins dip-treated

In the following, the results of trials conducted at various locations in Germany are reported. As demonstrated in Table 3, in each of the tests, 100 kg of apples were dip-treated in 1,000 ppm thiabendazole suspension. Following the treatment, the fruit was stored at temperatures ranging from 1-6°C at 75-80% relative humidity, for up to 179 days.

In all of these trials, Gloeosporium sp. was determined to be the major pathogen while Botrytis and Penicillium were of lesser importance. Infection ranged from 1.9-21.5%. Even under severe disease conditions, 1,000 ppm of thiabendazole provided excellent control of fungi causing rot during storage.

Table 3

Efficacy of thiabendazole as a dip treatment for apples

Trial Number	Treatment	Concentration in ppm	% Rot	Apple Variety
I	Thiabendazole	1,000	0.05	Jonathan
	Check		3.1	"
II	Thiabendazole	1,000	5.1	Finkenwerder
	Check		23.0	"
III	Thiabendazole	1,000	3.2	Glockenapfel
	Check		9.4	"
IV	Thiabendazole	1,000	0	Golden Delicious
	Check		5.3	" "
V	Thiabendazole		0	Golden Delicious
	Check		20.9	" "

Pre-harvest spray application

In places where a dip treatment for the prevention of storage diseases caused by fungi is not possible, a spray application prior to harvest is an alternative. Trials have been conducted in the United Kingdom to compare the pre-harvest spray, applied before the fruit is picked, with the post-harvest dipping technique.

Thiabendazole was applied as a spray at concentrations of 0.1% and 0.05% nine days prior to harvest. This trial was arranged using 12 individual replicates per treatment and the untreated check. 100 apples were picked randomly from each treated tree and divided into 4 lots, each lot to be examined at the appropriate time. The boxes containing the apples were placed in barn storage for the duration of the trial.

The fungi responsible for the rot included Gloeosporium spp., Penicillium sp., and Sclerotinia fructigena.

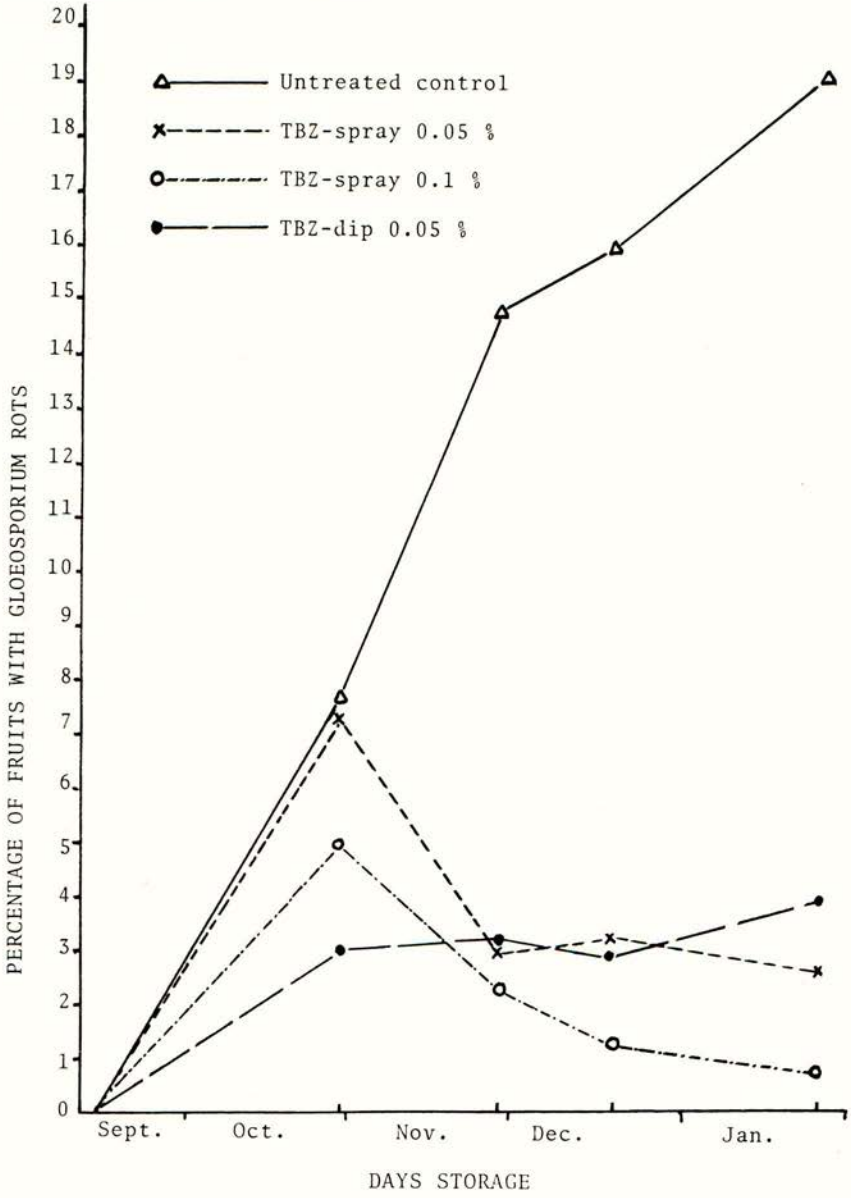
The summarized results for the percentage of Gloeosporium infected apples are given in Figure 1. Thiabendazole at 0.1% applied as a spray provided a 96% control of storage rot. At this concentration one treatment before harvest was as effective as the post-harvest dip treatment at 0.05%.

CONCLUSION

From the commercial or experimental trials, it was concluded that thiabendazole at a concentration of 1,000 ppm effectively controlled fungal diseases during storage. Post-harvest dipping or drenching provided equal control. In areas where farmers are presently not equipped for this type of treatment, trials were carried out to compare pre-harvest spraying with post-harvest dipping. A single pre-harvest spray of thiabendazole was equal to a post-harvest application. For pre-harvest applications, the fungicide must be applied two weeks before harvest at 1,000 ppm concentration.

Figure 1

EFFECT OF THIABENDAZOLE SPRAY TREATMENT ON GLOEOSPORIUM SPP. INFECTION DURING STORAGE



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

DEVELOPMENT OF IN VITRO AND IN VIVO SCREENING PROCEDURES FOR BACTERICIDES

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Summary Bacterial plant diseases differ from fungal plant diseases in several fundamental respects. They are for example caused by a more closely related group of organisms and are frequently more deeply seated in plant tissue. The design of a screen to find suitable chemicals for their control should not therefore be a direct modification of one designed for the control of fungal pathogens. There is a considerable background of technology established for the evaluation of chemicals for the control of animal bacterial diseases which is more relevant. Often anti-bacterial chemicals from these screens which are systemic in mammals are also systemic in plants. Frequently such chemicals are unsuitable for pharmaceutical development because of factors irrelevant to their agricultural use; these, and not compounds primarily developed as fungicides, should be given the highest priority for screening against plant bacteria.

INTRODUCTION

Annual losses of over $59 million are caused by bacterial diseases in twenty five States of USA (Table 1) surveyed by the American Phytopathological Society (Alcorn et al., 1971), and over $14 million is spent annually there on their control. The extent of the world wide losses especially in tropical regions is undoubtedly greater. Thus the potential market for an effective chemical is considerable. Only five genera of bacteria (Erwinia, Pseudomonas, Xanthomonas, Corynebacterium and Agrobacterium) are responsible for most of these losses, (Agrios, 1969) but mycoplasma-like organisms (Hull, 1971) and Streptomyces are also important (Table 2).

Table 1

Aggregate estimated losses from major bacterial diseases in twenty five States of the USA and the ten species causing the most loss*

	Millions of dollars 1967	1968	1969	Reporting States
Aggregate losses - 43 species	53	57	59	24
Pseudomonas alboprecipitans	10	10	10	1
P. syringae	8	10	10	6
P. glycinea	7	7	7	2
Corynebacterium insidiosum	5	7	6	4
Erwinia carotovora	3	4	5	10
Xanthomonas phaseoli	3	3	3	5
Agrobacterium tumefaciens	3	2	2	8
P. andropogonis	2	2	2	1
E. amylovora	2	2	2	13
X. vesicatoria	1	1	1	4

* Adapted from '1970 status of US Phytobacteriology', Phytopathology News 5 (5), May 1971.

Table 2

Families and genera of bacteria that cause disease in plants

Order	Family	Genus	Plant pathogenic species
Pseudomondales	Pseudomonadaceae	Pseudomonas	90
		Xanthomonas	60
Eubacteriales	Rhizobiaceae	Agrobacterium	7
	Enterbacteriaceae	Erwinia	17
	Corynebacteriaceae	Corynebacterium	11
Actinomycetales	Streptomycetaceae	Streptomyces	2
Mycoplasmatales?	Mycoplasmataceae?	Mycoplasma?	up to 43

The relationship between the genera of bacteria are probably closer than those between pathogenic fungi, and it should therefore be possible to select a small range of bacteria for screening which represents a wide range of bacterial plant diseases. On the other hand, the host range of bacteria ranges from potatoes to pears in habit and woodiness, with all the attendant variations in translocation patterns and infection patterns: thus, although it might be expected that chemicals will have a broad spectrum of inherent activity against the bacteria causing plant diseases, it might not be possible to exploit this activity on a wide range of crop plants. Since most bacteria are deep seated in infected tissue, the most effective chemicals are likely to be those which penetrate into and are translocated within host tissues.

Since no known plant bacteria are obligate pathogens, it is possible to carry out a primary screen in vitro, but for further selection of active compounds, in vivo tests need to be made in conditions as close as possible to those occurring in the field.

Development of Screening Procedures In Vitro. In vitro screening for bactericides has several advantages over in vivo screening: particularly it is more economical in time, space and skilled personnel; but it is also capable of selecting inherently bactericidal compounds that could be missed in vivo because of deactivation by the host or phytotoxicity and for which active analogues that do not suffer from these defects might be synthesised. A major objection to in vitro screening is that compounds active only in vivo will be overlooked.

We have adopted the policy of first screening novel untested compounds in vitro as potential bactericides. Compounds active in these tests, together with all compounds active in vivo in other biological screens (e.g. foliage fungicides, growth regulators etc.) are then tested in vivo as bactericides.

There are many in vitro tests established by pharmaceutical bacteriologists and others that are designed to find disinfectants, and bactericides and bacteriostats active against animal diseases. For example, measurements are made of changes of turbidity in liquid cultures following the addition of candidate chemicals. These tests are not entirely suitable for selecting chemicals for application to plants where protection for an extended length of time is more important than quick eradication. Quaternary ammonium surfactants for example are very useful disinfectants (for the surfaces of operating tables and surgeon's hands) but are not in general useful for the control of plant bacteria. Most antibacterial preservatives are also too phytotoxic.

The method we use is a 'poison plate' method in which a suitable amount of test chemical is dissolved in acetone and dispersed in nutrient agar. Provided standardised inoculum is used, the 'poison plate' method gives easily obtained reproducible results using a multipoint inoculator. The choice of organisms used to inoculate the 'poison plate' is rather more difficult. The ten organisms chosen were - Pseudomonas syringae, P. mors-prunorum, P. phaseolicola, Xanthomonas oryzae, X. malvacearum and Corynebacterium michiganense (because of the importance of the genera they represent), Agrobacterium tumefaciens, Erwinia amylovora and Erwinia carotovora (because of their importance as species) and Streptomyces scabies because it is an Actinomycete. No Mycoplasma-like organisms pathogenic in plants are yet included because their in vitro requirements have not been determined. Also the mammalian mycoplasma-like organisms are known to be inhibited by standard antibacterials used against the other bacterial diseases in vivo. The Pseudomonas spp. are similar and one or two may be considered superfluous, but strain differences in susceptibility to toxophores makes their presence in the screen desirable: the difference in susceptibility between strains is sometimes as great as that between different genera. The amount of chemical incorporated in the nutrient agar of the 'poison plate' is a difficult decision to make; too much gives too many false leads, and too little increases the chances of missing a suitable compound. The amount chosen, 50 ppm, is one at which all the commercially established bactericides would have been discovered in vitro. The plates can be read only five days after inoculation if incubated at 25°C. By screening in vitro at first, the need for a separate in vivo phytotoxicity test is reduced. Active compounds masked by phytotoxicity are revealed when the in vitro results are borne in mind.

At the same time as the multi-point inoculations are made for bacterial tests, an in vitro screen is set up using a range of fungi responsible for storage rots etc. Because the team searching for foliage fungicides tests in vivo the same

compounds as we test in vitro, it has been possible to make some check on the efficiency of the in vitro screen in selecting active groups. Of several thousand chemicals tested by the two teams, only two groups of chemicals would have been missed by the in vitro fungicide screen - one specifically active against powdery mildews (obligate parasites) and one active in vivo but not in vitro against Phytophthora infestans on tomato. Many groups where activity was masked by phytotoxicity in initial in vivo screens were discovered in the in vitro screen. It has not yet been possible to make a similar check on the efficiency of the anti-bacterial in vitro screen.

Both types of in vitro screening can be carried out with standard laboratory equipment and are not time consuming to perform. Approximately 10 per cent. of the compounds tested by them are either anti-bacterial or anti-fungal.

Development of Screening Procedures In Vivo. The arguments put forward to support in vivo glasshouse testing are that in some ways it is close to field conditions. In many ways, relevant to bacterial disease development, it cannot be. Lashing rain, wind damage, effect of insect vectors or pests, variable soil type, leaf fall, seasonal changes, and wetness, and other factors cannot easily be routinely simulated in the glasshouse.

The design of the in vivo screen can be biased towards finding systemic or non-systemic bactericides. As most bacterial diseases are either seed borne, deep seated and water spread, or spread by vegetative propagation, our tests are designed to find penetrant, eradicant seed treatments or systemic protectant and eradicant chemicals.

Among the criteria which are generally important in conducting tests are selection of a suitable host/bacterium combination, the standardisation of host symptom expression by standardisation of inoculum environment, chemical application and plant material.

In general the choice of host/parasite combination is dictated by the results of economic surveys. Great care is however needed to select a suitable isolate or race of the pathogen and a suitable cultivar of the host plant. Sometimes this information can be obtained from published data, but often time consuming experiments have to be carried out using large numbers of isolates and plants. If a plant breeder can be befriended, the cultivars he has blacklisted for breeding programmes against the chosen disease organisms can be the basis of a virulence test, if published data is not useful or non existant. We now have combination of Pseudomonas phaseolicola/french bean, Xanthomonas malvacearum/cotton, Xanthomonas oryzae/rice, Corynebacterium michiganense/tomato, Erwinia amylovora/pear and Erwinia carotovora/potato which give excellent, easily assessable symptoms two weeks or less after inoculation.

Pure, virulent isolates must be used and a good stock kept freeze-dried in case of an accidental loss. During our work we lost a virulent X. oryzae isolate because we kept the culture under mineral oil rather than freeze-dried. Some species, for example X. malvacearum, can be kept in a virulent state for years in dead leaf tissue (Bradbury, 1971) others cannot. Overall it is safer to have a reliable freeze-dried isolate.

Since most of the world important bacterial diseases are not endemic in England (the temperature is too low) cultures have to be imported and maintained under Ministry of Agriculture, Fisheries and Food licences. All our in vitro plates and in vivo potted plants and soil are sterilised by autoclaving after assessment. It would be very useful to have a small autoclave actually in the glasshouse for this work.

Many plant pathogenic bacterial elicit a hypersensitive response when injected into plants other than their hosts at high levels of inoculum. This reaction bears little relation to natural infection and should be avoided. In our tests inoculation is by seed soaking, stem pricking or by low pressure spraying foliage with species originally isolated from the host plant used. The age of the isolate is critical, especially in the case of Xanthomonas oryzae where virulence rapidly decreases after 72 hours in vitro. We found that isolates in shake culture are at their most virulent during their log phase of growth. The cell number and density is important as, even in a suitable host, a hypersensitive response can be the result of grossly high levels of inoculum. In our tests, nutrient broth cultures of the density 10^9 cells/ml from a rapidly dividing isolate are used in the seed soak, stem prick and spray. Local lesion assays from high pressure sprays and infiltration by injection by hypodermic needle are not used as they are considered unreliable in this context.

With most tests, four replicate pots are used per chemical treatment. The exceptions are root drenches for the control of Erwinia amylovora (fireblight) on pear and Erwinia carotovora (soft rot /blackleg) on potato, where two replicates are adequate, provided conditions are carefully controlled.

Bean and cotton seed is artificially inoculated by soaking at atmospheric pressure for a few hours (Goth, 1966). The length of soaking is critical: too long and no seedlings emerge, too short and the subsequent leaf infection is too slight. Artificially inoculated seed does not store well: bean seed becomes slowly less heavily infected and cotton seed becomes contam nated with fungi. Freshly infected seed should therefore be prepared as necessary. Any healthy bean and cotton seedlings resulting from chemical treatment of the seed are then subsequently inoculated by spraying with the respective pathogen. Leaf symptoms are assessed later in order to determine whether an eradicant seed dressing also protects against secondary spread of inoculum. Both stages of these tests are best made at 100% relative humidity under a polythene covered frame, and at a temperature between 20-30°C.

Tomato seedlings (Strider, 1970) pear seedlings and potato cuttings are inoculated through stems after soil in which they are growing has been drenched with test chemical. Rice seedlings are inoculated through leaves (Mizukami & Wakimoto, 1969), after drenching the soil and spraying the foliage. All are inoculated by using a pair of vein lifting forceps, the one-pronged tip being wrapped with a cotton wool 'reservoir' dipped periodically into inoculum containing 10^9 cells/ml. Tests are assessed after two weeks in a heated glasshouse (temperature range 20-30°C; the rice at 100% R.H., the tomatoes, potatoes, and pears at ambient R.H. A 0-3 scale is convenient and has been used for assessing individual symptoms; 0 representing full symptom development and 3 no symptom development. Scores are then totalled and a mean grade given on a 0-4 scale by comparison with the standard treatments. 0 is no better control than untreated checks, and 4 much better control than the standards. Consistent results are usually found, except where compounds are intrinsically unstable. Temperature varies in our glasshouse but this appears to have much less effect than the other factors. A stable controlled environment is desirable, but satisfactory results can be obtained without this extra expense, providing other factors are kept to a standard.

DISCUSSION

The screening procedures described have been designed to find anti-bacterial compounds that eradicate seed borne organisms, or have eradicant or systemic protectant properties against secondary infection. Highly effective chemicals have recently been devised for the systemic control of fungi: considering the variety

of anti-bacterial antibiotics used in medicine, the discovery of an effective systemic anti-bacterial compound for use in agriculture is long overdue - the market certainly justifies it. Possibly the reason for this, as explained in the survey made by the American Phytopathological Society, is that in general in American industry, anti-bacterial activity is screened for only when fungicidal activity has already been found. The results of our one year's testing *in vitro* and *in vivo* has shown this approach to be shortsighted.

Plant pathogenic bacteria and some important human pathogens are closely related and although medical anti-bacterials should not be used widely in agriculture because of the danger of the development of resistance, anti-bacterials that are not developed for pharmaceutical reasons might be successfully used in agriculture. Closer links should, therefore, be established between the phytobacteriologist and the medical bacteriologist, and the synthetic chemists working with them.

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PROBLEMS ASSOCIATED WITH THE TESTING OF BACTERICIDES AGAINST PSEUDOMONAS MORSPRUNORUM

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Summary Reasons for discrepancies between field and laboratory results were investigated using a leaf disc technique. Bactericides which were highly effective against a pure culture of Pseudomonas morsprunorum were found to be less effective against a natural field population of the pathogen. Mixing the pure culture with other saprophytic bacteria present on leaf surfaces made the mixed suspension more sensitive to bactericide action but it still showed more resistance than the pure culture alone. The state of the deposit following spraying can effect its activity, although periods of wetness were no more effective than periods of dryness. No factor could be found to account for the inactivity of Bordeaux mixture under laboratory conditions, although it was more effective against a field population on leaf discs than against the pure pathogen.

INTRODUCTION

Bacterial canker of cherry caused by Pseudomonas morsprunorum can be controlled by spraying trees with freshly made Bordeaux mixture but this material damages spray machinery. To facilitate the screening of alternative bactericides, a laboratory technique was devised to test their sterilizing ability on leaf surfaces, using cherry leaf discs dipped in suspensions of pure cultures of P. morsprunorum. Several bactericides were found to have greater activity than Bordeaux mixture (Frick, 1967), but they proved markedly inferior to Bordeaux mixture when tested against natural populations of P. morsprunorum on field trees. The experiments described below examined the following reasons for these discrepancies:- (1) field populations of P. morsprunorum may differ in sensitivity from those in pure culture; (2) the activity of bactericides may be influenced by other bacteria in the leaf surface populations on field trees; (3) artefacts in the laboratory technique may under-estimate the activity of Bordeaux mixture.

METHOD AND MATERIALS

The bactericides were tested against bacterial suspensions dried on the surface of cherry leaf discs by the technique already described (Frick, 1967). Natural field populations of P. morsprunorum were tested in the laboratory by cutting leaf discs from trees and placing these directly into bactericide solutions after washing briefly in sterile water.

For testing bactericides against mixed populations the most common saprophytes were mixed in suspensions in approximately the same proportions that they occurred on leaf surfaces and the suspensions, with or without the inclusion of P. morsprunorum, dried on to the surface of leaf discs before immersion in bactericides.

To examine the activity of bactericides under conditions more representative of field applications, where leaves are not immersed for long periods in spray fluids, leaf discs with a surface film of a pure culture of P. morsprunorum were dipped in bactericides for 15 seconds. The excess bactericide was drained off and the discs were transferred to nutrient broth either immediately, or after storage under dry or moist conditions for two days at 18°C.

The experiments investigating the relative inactivity of Bordeaux mixture in the laboratory screen (Frick, 1967) compared the effects of the following:- method of preparation, concentration, time of immersion, drying of deposits, different wetting agents and agitation of leaf discs during immersion.

RESULTS

Effect of bactericides on natural field populations of P. morsprunorum Compounds which were highly effective against the pure culture of P. morsprunorum, especially the nitrohalogeno-alcohols R.D. 7307, R.D. 7429 and R.D. 7706 and the cinnamylidine derivative NC 1129, were less effective against a natural field population of P. morsprunorum with the exception of phenylmercuric oxinate and silver nitrate (Table 1). Bordeaux mixture, however, was more effective against a natural population. These results agree with those obtained from small-scale field trials. The growth observed in broth was in all cases typical of P. morsprunorum, i.e. slow, regular, non-flocculate, and without the formation of a pellicle.

Effect of bactericides against mixed suspensions of bacteria The addition of P. morsprunorum increased the sensitivity of the mixed suspension of saprophytic bacteria to four of the seven bactericides tested. The response to NC 1129 and Bordeaux mixture was unaffected (Table 2). The bactericidal activity against P. morsprunorum could be reduced by the presence of the saprophytic bacteria (Table 3).

Conditions affecting the activity of the bactericide deposit When a short period of immersion (15 sec) in bactericide solutions was used to represent a field spray application (Table 4) they had little activity compared with that after 30 or 60 min immersion (Table 3). If the bactericide deposit was left on the discs for two days, either in a dry or a moist state, the activity of the nitrohalogeno-alcohols was increased, while that of other bactericides was unaffected. Neither storage under dry or moist conditions improved the activity of Bordeaux mixture. Bactericidal growth from untreated control discs stored under similar conditions (dry or moist) for two days was always characteristic of P. morsprunorum.

Factors affecting the activity of Bordeaux mixture Alternative methods for preparing Bordeaux mixture, e.g. by adding a concentrated solution of lime to a dilute solution of copper sulphate had no effect on its activity, but the copper moiety in the form of sulphate showed greater activity than the calcium hydroxide moiety or the Bordeaux mixture itself. This is due to the greater availability of copper in solution as the sulphate than as the complex form of Bordeaux mixture. Times of immersion from 30-120 minutes, had more effect on the activity of Bordeaux mixture than increases in concentration, as only the strongest Bordeaux (10/15), containing 0.25% copper, had any effect after 30 minutes immersion. Longer contact of the Bordeaux deposit in the dry state with the surface of the leaf disc did not appear to affect its activity up to 90 minutes drying time or even after six days storage (see also Table 4). The inclusion of different wetting agents in the Bordeaux suspension, and the gentle agitation of the discs during immersion, were equally ineffective.

DISCUSSION

Although very active against P. morsprunorum in the field, Bordeaux mixture was less effective in a laboratory screen (Frick, 1967). None of the modifications to the test procedure however increased its activity 'in vitro', or provided a clue to its failure to suppress the growth of P. morsprunorum except in the field. This suggests that leaf exudates or certain atmospheric conditions, or both, are needed to break down this complex mixture into one or more toxic products which can then act as sterilants on the leaf surface. Wiebel et al., (1965) found that a number of compounds which failed to suppress growth of Xanthomonas vesicatoria in vitro, reduced infection of pepper fruits by this bacterium in field trials. They suggested that physiological changes are induced in the host which are unfavourable to the establishment and growth of the pathogen. Jones (1964) has proposed a similar theory to account for the ineffectiveness of Bordeaux mixture under laboratory conditions.

Some bactericides show great promise in the laboratory but are then found to have little or no activity when sprayed in the field. This failure could be due to one of several factors. First, the density of the spray deposit; in other words, the effective concentration in the field may be much lower than that achieved in the laboratory. The difference between the results of 15 seconds immersion and 30 minutes immersion would support this. Second, the compound may not remain in a state readily available to the bacteria for a long enough period, particularly if drying is fairly rapid, but the activity of the bactericides was not greatly improved under moist storage conditions. Third, field populations of bacteria could be more resistant or could be protected on the leaf surface by gums, mucilages, or other bacteria. Compounds with a high activity against the pure culture of P. morsprunorum were found to be far less effective against mixed field populations. In contrast Bordeaux mixture was more effective.

Mixed populations of bacteria and pure cultures react differently when tested against bactericides in the laboratory (Bennett and Baverle, 1960; Truby and Bennett 1964). A bacterium in a mixed population therefore could be more or less sensitive to bactericides than a single strain. P. morsprunorum, although the dominant bacterial pathogen on cherry leaves exists in the field in such a mixed population. The interaction of a suspension of isolates of the main bacterial population of cherry leaves with P. morsprunorum rendered the total population more sensitive to bactericides than the mixed suspension alone, but more resistant to bactericidal action than a pure culture of P. morsprunorum.

These present observations help to understand the results obtained in the field, but there are many complex factors and combination of factors still to be assessed before a true picture of bactericidal action can be obtained. It is also clear that it is very difficult to design a testing technique which will cover every aspect of bactericide activity, and will not flatter one class of bactericides to the detriment of another.

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Table 1

The degree of bactericidal or bacteriostatic activity shown against a natural field population of *Pseudomonas morsprunorum* on leaf discs

Compound	Concn. a.i. (%)	Treatment time (mins.)	No. of culture tubes showing growth* after incubation for 2-3 days	5-7 days	2-3 days in fresh broth
Trichloroisocyanuric acid	0.1	60	1	2	2
1,3-Dichloro-5,5-dimethyl hydantoin	0.2	60	0	2	2
Chlorhexidine diacetate	0.3	60	0	0	4
R.D. 7307	0.05	60	5	5	5
R.D. 7429	0.05	60	5	5	5
R.D. 7706	0.05	60	3	5	5
ω-Nitrostyrene	0.05	30	3	4	5
2,4-Dinitro-1-fluorobenzene	0.05	60	2	2	3
2-(2-Bromo-3-phenylallideneamino) acetohydroxamic acid (NC 1129)	0.075	60	4	5	5
Phenylmercuric oxinate	0.05	60	0	0	0
Silver nitrate	0.1 Ag	30	0	0	0
Dithianion	0.05	60	4	5	5
Bordeaux mixture 2/3	0.05 Cu	30	1	1	1
Bordeaux mixture 4/6	0.1 Cu	30	2	3	3

*No. of tubes out of five; the fewer tubes with growth the greater the compound's activity

Table 2

The degree of bacterial activity after 60 minutes immersion shown against a mixed suspension of leaf surface bacteria with and without the addition of *Pseudomonas morsprunorum*

Compound	Concn. a.i. (%)	No. of tubes showing growth* after incubation of: mixed suspension only for 2 days	mixed suspension only for 5 days	mixed suspension + *P. morsprunorum* for 2 days	mixed suspension + *P. morsprunorum* for 5 days
1,3-Dichloro-5,5-dimethyl hydantoin	0.2	2	3	0	0
Chlorhexidine diacetate	0.3	1	2	0	0
R.D. 7307	0.05	3	5	±	5
ω-Nitrostyrene	0.05	5+++	5	5+	5
2-(2-Bromo-3-phenylallideneamino) acetohydroxamic acid (NC 1129)	0.075	5+++	5	5+++	5
Silver nitrate	0.1 Ag	1	2	0	0
Bordeaux mixture 4/6	0.1 Cu	5+++	5	5+++	5

*out of five. Degree of growth after two days: ± very slight; + slight; +++ heavy

Table 3

The degree of bacterial activity shown against a mixed suspension of leaf surface bacteria with and without the addition of Pseudomonas morsprunorum, compared with the activity shown against the pathogen alone

Compound	Concn. a.i. (%)	Treatment time (mins.)	No. of tubes showing growth* after incubation for 6 days: mixed suspension	mixed suspension +P. morsprunorum	P. morsprunorum only
Trichloroisocyanuric acid	0.1	30	1	0	0
1,3-Dichloro-5,5-dimethyl hydantoin	0.2	30	3	0	0
Chlorhexidine diacetate	0.3	30	0	3	0
R.D. 7307	0.05	60	5++	5++	0
ω-Nitrostyrene	0.05	60	5+++	5+++	0
2-(2-Bromo-3-phenylallideneamino) acetohydroxamic acid (NC 1129)	0.075	60	5	5	0
Silver nitrate	0.1 Ag	30	0	0	0
Bordeaux mixture	0.1 Cu	60	5+++	5+++	5

*out of five. Degree of growth after three days: ++ moderate; +++ heavy

Table 4

The degree of bactericidal or bacteriostatic activity after 15 secs. immersion shown against Pseudomonas morsprunorum when discs are treated in the normal way compared with storing them dry or moist for two days at 18°C before placing in broth

Compound	Concn. a.i. (%)	No. of tubes showing growth* from unstored discs after incubation for		No. of tubes showing growth* from discs stored dry and incubated for		moist and incubated for	
		2 days	5 days	3 days	2 days in fresh broth	3 days	2 days in fresh broth
Chlorhexidine diacetate	0.05	5	5	4		5	5
1,3-Dichloro-5,5-dimethyl hydantoin	0.05	5	5	3		5	5
R.D. 7307	0.05	1	5	0	1	1	2
ω-Nitrostyrene	0.05	4	5	4		5	5
R.D. 7307	0.05	4	5	1	1	0	0
R.D. 7429	0.05	2	5	1	1	2	4
R.D. 7706	0.05	1	5	2	3	0	0
Silver nitrate	0.25 Ag	5	5	5	5	4	
R.D. 7307	0.05	0	4	0		0	
Didecyldimethylammonium bromide	0.2	5	5	5	5	5	5
2:4-Dinitro-1-fluorobenzine	0.05	5	5	5	5	5	5
Bordeaux mixture 4/6	0.1 Cu	5	5	5	5	5	5

*out of five

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

THE VALUE OF BRONOPOL FOR THE CONTROL OF BLACKARM DISEASE OF COTTON

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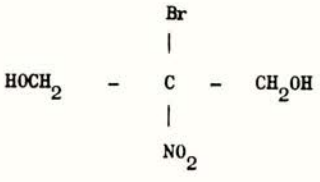
Summary Bronopol is a bacteriostatic substance of low mammalian toxicity discovered by The Boots Company Ltd., which has been tested widely against plant pathogenic bacteria in the laboratory and field. The compound is highly effective against the seed-borne phase of blackarm disease of cotton and is now in use commercially as a dry seed dressing for the control of that disease. It gives yield increases (10-12%) comparable with those for mercurial dressings and is compatible with thiram, captan and dieldrin. Although the compound inhibits the growth of several other plant pathogenic bacteria *in vitro* at low concentrations, it is not so active *in vivo*.

INTRODUCTION

Bronopol, 2-bromo-2-nitropropan-1,3-diol, was one of a series of bromonitroalkanes investigated for antibacterial activity (Clark *et al.*, to be published). The compound exhibits a broad spectrum of antimicrobial activity but low mammalian toxicity (Croshaw *et al.*, 1964) and it has found application as a preservative in cosmetics and pharmaceuticals (Bryce and Smart, 1965; Sykes and Smart, 1969). Unlike many antibacterial compounds, bronopol is as inhibitory to *Pseudomonas aeruginosa* as it is to other Gram-positive and Gram-negative bacteria. This paper describes an evaluation of the activity of the compound against some plant pathogenic bacteria in the laboratory and field.

PHYSICAL AND CHEMICAL PROPERTIES

Structural formula:



Pure bronopol is a colourless to pale brownish-yellow, odourless, crystalline solid which is slightly hygroscopic but stable under normal conditions when formulated as a dispersible powder or in solution. The melting point is about 130°C. Contact with aluminium causes loss of antibacterial activity but the compound appears to be stable in contact with tin.

The solubility of bronopol in water is 25% w/v at 22°C and the vapour pressure is 1.26×10^{-2} μ Hg at 20°C.

TOXICOLOGY

Table 1

Acute toxicity of technical bronopol

Species	Route	LD50 mg/kg
Mouse	oral	270–400
	intraperitoneal	10–25
Rat	oral	180–400
	sub-cutaneous	100–200
	intravenous	50–100
	dermal	64–160 (in acetone)
		>1,600 (in water)
Dog	oral	250
Hen	oral	300–1,000

In subacute tests female rats tolerated up to 100 mg/kg bronopol in aqueous solution for two weeks when administered daily by oral gavage; 300 mg/kg being toxic. In female rabbits, the compound was tolerated at 10 mg/kg daily for 9 days but 30 mg/kg was lethal.

Chronic feeding studies have shown that growth was normal and no haematological or histo-pathological changes occurred in male or female rats fed for 90 days on a diet containing 1,000 ppm of bronopol, equivalent to a daily intake of approximately 100 mg/kg body weight.

No irritation was caused by bronopol to rabbits when applied five times as a 0.5% cream to shaved, intact skin. Single applications of an aqueous solution of 0.5% bronopol or the 12% bronopol seed dressing were non-irritant to the skin of rabbits but repeated applications were moderately irritant. Technical bronopol was slightly irritant to the eyes of rabbits at 1% in normal saline but four times daily instillations of 0.5% evoked no reactions. Bronopol seed dressing (12%) was mildly irritant following a single application of 10 mg.

Inhalation studies with technical bronopol showed no irritation in rats exposed to 0.05 mg of dust per litre of air. Higher concentrations caused eye irritation and respiratory difficulties. Bronopol has shown no teratogenic or antifertility activity in laboratory animals.

Bronopol has been handled over a number of years by people engaged in laboratory work, manufacture and use of agricultural and pharmaceutical formulations of the compound. During this time there have been no reports of irritancy or other untoward reactions attributable to exposure to bronopol.

ACTIVITY AGAINST PLANT PATHOGENIC BACTERIA

In vitro studies

The source of the strains of organisms used are shown in Table 2. They were maintained on peptone-yeast-extract-agar (Glucose 0.1%, Difco Yeast Extract 0.3%. sodium chloride 0.5%. Difco Neopeptone 1.5%, Oxoid Agar 2.0%) and subcultured weekly.

Bacteriostatic activity was determined by preparing plates containing two-fold serial dilutions of bronopol in peptone-yeast-extract agar and surface inoculating them with 0.01 ml of overnight peptone-yeast-extract broth cultures of the test organisms with a multi-point inoculator (Hale and Inkley, 1965). After 24 hours incubation at 26°C, the growth on bronopol-containing plates was compared with that on control plates and the concentration of bronopol that completely inhibited the growth of each organism (MIC) was noted.

The results are given in Table 2 and indicate that all the organisms tested, including the species of Pseudomonas, are susceptible to bronopol.

Table 2

Bacteriostatic activity of bronopol against a range of plant pathogenic bacteria

Species	Source	MIC (µg/ml)
Pseudomonas angulata	NCPPB[1] 1237	12.5
Pseudomonas morsprunorum	NCPPB 560	25.0
Pseudomonas syringae	BSC[2] A495	12.5
Pseudomonas lachrymans	NCPPB 467	25.0
Pseudomonas phaseolicola	NCPPB 52	12.5
Xanthomonas malvacearum	NCPPB 633	12.5
Xanthomonas campestris	NCPPB 528	12.5
Xanthomonas oryzae	NCPPB 793	25.0
Xanthomonas hyacinthi	NCPPB 599	25.0
Agrobacterium tumefaciens	BSC A493	12.5
Erwinina amylovora	NCPPB 595	25.0
Erwinina carotovora	NCPPB 312	25.0
Corynebacterium betae	LRS[3] 144	12.5
Corynebacterium xerosis	NCTC[4] 9755	6.25
Corynebacterium fascians	NCPPB 1488	50.0

[1] NCPPB = National Collection of Plant Pathogenic Bacteria, Hatching Green, Harpenden, Herts.

[2] BSC = Botany School, Downing Street, Cambridge.

[3] LRS = Lenton Research Station, Nottingham, NG7 2QD.

[4] NCTC = National Collection of Type Cultures, Colindale, London, N.W.9.

In vivo studies

The in vitro results were sufficiently encouraging for in vivo studies to be undertaken.

Blackarm disease of cotton (Xanthomonas malvacearum) Naturally infected cotton seed, cultivar S47, was treated with the seed dressing and planted into 1 x 1 yd^2 plots of 24 planting holes; 2 - 3 seeds per hole. Each treatment was replicated four times. Treatments were applied to the seeds twelve days before planting. The plants were assessed for disease symptoms on leaves and stems after eighty-four days. Results are given in Table 3.

Table 3

Activity of 12.5% bronopol seed dressing against blackarm disease of cotton

Treatment	Dose (w/w of seed)	% Diseased Plants	% Control
Bronopol 12.5% a.i.	1:50	2	98.9
	1:100	0	100
	1:150	0	100
Mercurial 8.5% Hg.	1:150	0	100
Untreated	-	50.8	-

Citrus canker of grapefruit (Xanthomonas citri) Young grapefruit plants in pots were sprayed with test chemical and inoculated with a spore suspension (5.6×10^7/ml) 24 hours later. The plants were confined to an inoculation chamber at 28°C for 48 hours and then transferred to a glasshouse bench. Three replicates each of three pots were used for each treatment. Assessment was carried out by selection of the three most infected branches per plant, and six leaves from each branch graded on a scale 0-5 according to the degree of infection. Results are given in Table 4.

Table 4

Activity of foliar applications of bronopol against citrus canker of grapefruit

Treatment	Dose ppm	Average score per treatment	% Control
Bronopol 25% s.p.	1000	20.9	57.9
Bordeaux mixture	1000	27.8	59.5
Untreated	-	68.6	-

Bronopol was moderately active against this disease and was safe as a dormant season spray but tended to cause some phytotoxicity when used as a foliar spray.

Bacterial blast of rice (Xanthomonas oryzae) Each leaf of rice plants growing in pots in a glasshouse was inoculated with X. oryzae and 24 hours later sprayed with test chemical. Two pots were used for each treatment. Eighteen days later, the length of each lesion was measured. Results are indicated in Table 5.

Table 5

Activity of foliar sprays of bronopol against bacterial blast of rice

Treatment	Dose ppm	Length lesions per leaf (mm)	% Control
Bronopol 25% w.p.	500	95.8	2.5
	1000	72.0	26.7
Celomate	1000	29.4	71.1
Untreated	-	98.2	-

Bronopol was phytotoxic to the rice, causing small brown spots on the leaves.

Halo-blight of beans (Pseudomonas phaseolicola) In preliminary laboratory and glasshouse tests at the National Vegetable Research Station, Wellesbourne, Warwick, (J. D. Taylor, Personal communication) bronopol had insufficient activity as a seed treatment for the control of halo-blight. Artificially infected French bean seeds were treated with bronopol dust (12% a.i.) with or without a sticker, and infection of the cotyledons and primary leaves were recorded after germination in compost or on cellulose wadding. Untreated seeds gave approximately 40% infected plants and the treatments gave no significant reduction in this figure.

Soaking seeds for 24 hours in solutions of bronopol at concentrations up to 250 ppm also failed to control infection.

Bacterial diseases of bulbs The following summarises results obtained by use of bronopol for control of bacterial rots in bulbs under glasshouse conditions in Holland (J. Bergman, Personal communication).

Xanthomonas hyacinthi: Neither bulb-dip treatments nor sprays to the growing crop of 4% a.i. bronopol were fully effective in controlling X. hyacinthi.

Erwinia spp. on hyacinths: Bulb-dips and sprays at 2% a.i. were ineffective against Erwinia on hyacinths.

Erwinia carotovora on iris: Dip treatments in 2% a.i. solution produced good control and further field tests are in progress on this species.

Field Trials

Blackarm disease of cotton The excellent in vivo results of bronopol as a seed treatment for the control of blackarm prompted field trials in the major cotton growing areas of the world. During 1966-67, a series of 15 trials in East Africa confirmed the good performance of bronopol which consistently showed equal control to standard mercurial treatments (Table 6). In this and all subsequent trials the experimental seed dressing contained 12% bronopol.

Table 6

Comparison of bronopol and a standard mercurial seed treatment against blackarm disease of cotton in East African trials

Cultivars	Interval (days) between treatment and planting	Treatments*	% Infection
IL 58	6	Bronopol	0
		Mercurial	1.1
		Untreated	36.0
IL 47/10	10	Bronopol	0
		Mercurial	0
		Untreated	50.0
	21	Bronopol	0
		Mercurial	0
		Untreated	58.2
S 47	15	Bronopol	0
		Mercurial	0
		Untreated	50.8
IL 58	192	Bronopol	0.57
		Mercurial	2.05
		Untreated	50.7

*Treatments Bronopol 12% at 1:150 w/w of seed
Mercurial 8.5% Hg. at 1:150 w/w of seed

In 1967, trials were carried out in Nigeria (Dransfield 1968) with similar results to those obtained from East Africa, but lower doses of 1:200 and 1:250 w/w of seed were not so effective (Table 7)

Table 7

Comparison of rates of bronopol with a standard mercurial seed treatment against blackarm disease of cotton in Nigeria

Treatment	Dose w/w seed	% Stands germinated at 10 days	% Seeds germinated at 12 days	% Diseased seedlings at 21 days
Early Trial				
Bronopol	1:150	95.2	60.3	2.73
	1:200	95.5	59.1	4.24
	1:250	92.3	52.6	11.23
Mercurial 5% Hg.	1:200	98.1	57.3	2.72
Copper 45% Cu	1:150	96.3	46.8	3.70
Untreated	-	96.5	60.9	96.04
Late Trial				
Bronopol 12% a.i.	1:150	89.1	65.3	5.01
	1:200	87.0	61.0	11.17
	1:250	97.6	69.6	37.20
Mercurial 5% Hg.	1:200	92.6	68.6	4.83
Copper 45% Cu	1:150	93.1	61.3	13.88
Untreated	-	95.5	69.5	99.93

Cultivar Samaru 26J Sowing dates Early Trial 15/6
Late Trial 14/7

Where blackarm forms part of a bacterial, fungal, and insect pest complex attacking young cotton, e.g. in the Sudan, a 12% bronopol/25% thiram/40% dieldrin seed dressing has produced equal control of blackarm disease to the standard mercurial/dieldrin treatment.

Bacterial diseases of other crops Initial activity against fire-blight of apples (Erwinia amylovora) as a spray application looks promising and further trial work is currently in progress in Denmark.

DISCUSSION

Although bronopol exhibits a high level of activity in vitro against a wide range of plant pathogenic bacteria, the spectrum of effectiveness in vivo appears rather narrow; to date its commercial application is limited to the albeit important disease of blackarm of cotton. The field test results show clearly that bronopol controls the seed borne phase of blackarm of cotton well when used as a seed dressing. In subsequent trials to those reported here, Dransfield (1971) has demonstrated the consistent performance of bronopol as a 12% seed treatment at the rate of 1 lb to 150 lb of seed under practical field conditions.

In Nigeria where blackarm is the only major disease of seedling cotton, yields with bronopol treatment have been improved by 10% - 12% which is similar to increases produced by mercurial treatments.

Bronopol is, however, relatively specific for bacteria and has low antifungal activity. In areas other than Nigeria, blackarm forms part of a complex of cotton seedling problems involving fungi and insect pests and bronopol alone is therefore not sufficient. Where broader spectrum control of seedling diseases and pests is required, mixtures with fungicidal and insecticidal materials are needed to obtain good plant stands. Mixtures of bronopol with thiram, captan and dieldrin as double or triple mixtures are compatible and trials in the Sudan and East Africa have confirmed their efficacy and safety to the crop.

Acknowledgements

We are grateful to our many colleagues in Research Department of The Boots Company Ltd., for information on the chemical, physical and toxicological properties of bronopol, and to Fisons Cambridge Division for East African data on blackarm disease of cotton.

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THE CONTROL OF HALO-BLIGHT OF FRENCH BEANS BY FOLIAR SPRAYS

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(Communicated by R.B. Maude)

Summary In the wet season of 1968, field plots of French beans with 0.4% primary infection of halo-blight (Pseudomonas phaseolicola) had over 33% infected pods at harvest. In the dry season of 1969 a similar level of primary infection resulted in only 3% of infected pods. In both years copper oxychloride or streptomycin sulphate sprays (0.1% a.i., 60 gal/ac (675 l/ha)) applied at ten-day intervals from emergence to flowering time reduced plant and pod infection by about 90%.

INTRODUCTION

Halo-blight of bean occurs in all or most of the bean growing areas of the world (Hayward & Waterson, 1965). It is seed-borne and under suitable conditions a very few infected seeds may initiate severe outbreaks of the disease. Although the use of field sprays against the disease has been studied elsewhere, few such trials have been made in Britain. Two spray trials carried out by the writer at Wellesbourne during the wet season of 1968 and dry season of 1969 are described herein.

METHOD AND MATERIALS

Seed that had been artificially inoculated with Pseudomonas phaseolicola by vacuum impregnation was mixed with clean seed in the proportion of 1:100 and sown in plots. About one third of the plants from the inoculated seed developed typical symptoms of primary infection.

Several spray compounds were tested. In 1968 six applications of each were made at ten-day intervals from plant emergence to flowering. In 1969 only four applications were possible within this period. In neither experiment were the chemicals applied to the pods. In 1968 the sprays were each applied to a single plot approximately 15 x 5 m and in 1969 to quadruplicate randomised plots each 6 m square. In both years plots were sown at the beginning of June. Spread of disease and the percentage of affected pods were recorded.

RESULTS

High rainfall (243 mm June to August) in 1968 produced conditions ideal for the spread of halo-blight. On the control plots 0.4% primary infectors on 13 June resulted in over 40% diseased plants by 1 August and 33% infected pods on

18 September. The only treatments to give significant disease control were sprays of copper oxychloride and streptomycin sulphate at 0.1% a.i. applied at the rate of 60 gal/ac. On plots treated with either chemical only 4.5% of the pods were infected.

In 1969, dry weather (rainfall 112 mm June to August) greatly restricted spread of the disease initially but this was later partially offset by the use of sprinkler irrigation. On the control plots 0.3% primary infection gave 40% infected plants on 19 August but infection was delayed and only 3% of pods were diseased at harvest (26 August). Streptomycin sulphate and copper oxychloride sprays gave significant reduction of the disease on the foliage and pods.

DISCUSSION

The efficiency of both streptomycin sulphate and copper oxychloride in controlling spread of halo-blight was clearly demonstrated in both years. Their economic value was greatest, however, in the wet summer of 1968 when their use converted a commercially unacceptable crop to one in which the incidence of disease on the pods was well below the tolerance level (5% blemished pods) for canning or freezing. It was found that at least four applications of either chemical were necessary to control spread of the disease. It should be noted that sprays were applied from very early in the growth of the crop and this is believed to have contributed to their efficiency.

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CONTROL OF BACTERIAL DISEASES OF TOMATOES IN JERSEY

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Summary The symptoms, incidence and control of bacterial speck (Pseudomonas tomato) and bacterial canker (Corynebacterium michiganense) on glasshouse and outdoor tomatoes in Jersey are described. Cultural and chemical measures reduce the spread of both diseases but considerable crop damage may still occur.

On glasshouse tomatoes cessation of overhead watering and frequent sprays of cupro-ammonium carbonate effectively control bacterial speck but hardly control bacterial canker. On outdoor tomatoes bacterial canker has been eradicated in recent years but bacterial speck is widespread. Copper sprays control leaf infection with bacterial speck but extensive fruit infection may occur if weather conditions are favourable. Further information on the epidemiology of both diseases is required.

INTRODUCTION

In recent years two bacterial diseases have occurred on glasshouse and outdoor tomatoes in Jersey. Bacterial speck (Pseudomonas tomato) was first recognised in 1960 (Phillips, 1961) and has become more prevalent since growers have changed from copper fungicides to maneb for routine spraying of tomatoes against blight (Phytophthora infestans) and stem rot (Didymella lycopersici). Bacterial canker (Corynebacterium michiganense) was confirmed in 1962 (Collingwood, 1964) and has occurred sporadically since that date. Both diseases can overwinter in infected debris and since outdoor tomatoes are grown in the same fields annually, there can be a large source of inoculum available. Nearly 1200 acres (486 ha) of outdoor tomatoes were grown in 1970 and this area can act as a source of disease for both outdoor and glasshouse tomatoes. Other factors encouraging the spread of bacterial diseases in Jersey are the close proximity of outdoor and glasshouse crops, the presence of susceptible tomato plants throughout the year, the practise of frequent overhead watering in glasshouses, the routine application of high volume fungicide sprays and the frequent visiting of other holdings by growers and workers

This paper describes observations made on the symptoms, incidence and control of bacterial diseases of tomatoes in Jersey.

BACTERIAL SPECK

Symptoms and Incidence

Bacterial speck, known locally as "scorch" or "target spot" occurs extensively on outdoor tomatoes and occasionally under glass. Small dark spots (1-2 mm) surrounded by a narrow yellow margin occur on leaves and may coalesce to kill large areas of the leaf. Similar spots also occur on stems and raised black spots (1 mm across) occur on fruit. Two phases of attack occur on outdoor tomatoes: infection soon after planting that results in extensive leaf damage particularly to lower leaves and may result in weak growth of the plants, often accompanied by infection of the flower stalks and consequent dropping of flowers; late infection that results in extensive fruit spotting but is not necessarily accompanied by leaf damage. Wet weather conditions favour the development of the disease in the crop. In glasshouses the disease attacks leaves but fruit infection is rarely seen.

The disease is generally regarded as being seed-borne but this aspect does not seem important in Jersey. Seed saved from severely infected fruit in 1969 produced healthy seedlings even when grown under hot wet conditions in a glass cabinet. The source of disease is assumed to be infected plant debris.

Control

In glasshouses the disease is effectively controlled by the removal of infected leaves, cessation of overhead watering and maintenance of low humidity by ventilation. In addition high volume applications of cupro-ammonium carbonate ("Fungex" at 2 pints/100 gal) may be necessary.

Effective control on outdoor crops is not always achieved. Leaf and stem infection can be reduced by applications of copper but not by dichlorophen. Table 1 shows the results of an unreplicated trial carried out in 1969. Treatments were applied on 17 July and 1 August and the plots assessed on 18 August.

Table 1

Control of bacterial speck on outdoor tomatoes

Chemical	Rate of application /100 gal/acre	Percentage plants infected
50% copper w.p.	3 lb	9.7
50% copper w.p.	1.5 lb	8.4
Dichlorophen*	5 pints	89.0
Dichlorophen*	2.5 pints	89.0
Unsprayed		37.0

* as "Turbicide"

A high incidence of fruit infection can occur with little visible leaf infection and the inclusion of occasional copper sprays in the spray programme seems insufficient to control fruit infection if weather conditions are favourable for its development.

BACTERIAL CANKER

Symptoms and Incidence

Bacterial canker has become more prevalent in glasshouse crops in the last two seasons but has not been seen on outdoor tomatoes since 1965. A number of different symptoms occur in Jersey and the conditions determining which symptoms develop are not fully known. In glasshouse tomatoes infection is first seen when the plants are six feet high and reach the support wires. On leaves the infection occurs as small irregular light areas (1-2 mm across) on the upper surface. These areas later become brown and may merge to form larger infected areas. This stage of the disease greatly resembles damage caused by chemical sprays and has only recently been described on glasshouse tomatoes (Upstone and Lelliott, 1971). Infected fruit show characteristic "birds-eye" spotting; small raised white spots (2-3 mm across) occur, with a dark centre which later erupts giving a rough appearance. Leaf-scorching and fruit infection is usually associated with rapid spread of the disease. Infection on the stem occurs as dry, raised yellowish-white "Mealy" areas, although dark slimy cankers may occur later. Systemic infection results in a slow wilting and eventual death of plants over a period of 6-8 weeks. The epidermis of infected plants is easily separated from the underlying vascular tissue which appears yellowish and granular, later becoming dark brown.

Control

The outbreaks of disease on outdoor tomatoes were effectively eradicated by the removal of infected plants and treatment of the land with metham-sodium. Seed was not saved from the infected crops and no tomatoes were grown in the land the following year.

The disease has proved difficult to control in glasshouse crops. As soon as the disease is recognised, growers are advised to remove all infected leaves and plants, cease overhead watering and apply high volume sprays of cupro-ammonium carbonate ("Fungex" at 2 pints/100 gal) every three days. These measures reduce the spread of disease and cupro-ammonium carbonate sprays are then applied every 7-10 days. Workers are encouraged to exercise thorough hygiene after handling infected plants but this is not always practicable. At the end of the growing season, in addition to the normal steam sterilisation of the soil, glasshouse structures are washed with 2% formaldehyde solution and support wires thoroughly wiped with a cloth soaked in formaldehyde.

DISCUSSION

Using the control measures outlined above, growers achieve satisfactory control of bacterial speck but hardly adequate control of bacterial canker. The latter disease is more persistant and damaging, and requires control measures throughout the season (May to September). Growers find the high degree of hygiene necessary impracticable and usually the disease slowly spreads throughout the holding. Cessation of overhead watering in glasshouse crops is of prime importance in reducing the spread of bacterial diseases but results in poor fruit setting during hot weather and subsequent crop loss. Copper is an effective protectant bactericide but has to be applied frequently and has a hardening effect on tomato plants especially under glass. To achieve better control of these bacterial diseases more information on their epidemiology and an eradicant, systemic bactericide are required.

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CONTROL OF COMMON SCAB OF POTATO

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Summary Incidence of potato common scab, caused by soil-borne Streptomyces scabies, can be decreased by applying chemicals to soil. One such chemical is quintozene, which is now suspected of being carcinogenic. About 120 other chemicals were tested, using potted plants grown in the glasshouse. Most of them, at 50 ppm in soil, either failed to affect scab, or seriously decreased yield of tubers, or both. Those which decreased scab without greatly affecting yield were, in order of increasing effectiveness: 2,5-dimethyl-3-furanilide (BAS 3191F; proposed common name: furcarbanil); (2,4,5-trichlorophenyl) sulphonylmethyl thiocyanate (PH 50-82); 2-pyridine-thiol-1-oxide, Na salt; dinocap phenols (MC 2810); quintozene; and captafol.

In a field trial at Woburn, Beds., quintozene and captafol, each at 70 lb/ac (78 kg/ha), were equally effective. Thus captafol may be a suitable substitute for quintozene.

INTRODUCTION

The incidence of potato common scab, caused by soil-borne Streptomyces scabies, can be decreased by irrigation during the growing season (e.g. Lapwood et al., 1970), or by applying sulphur or quintozene to soil at planting time (e.g. McCreary, 1967; Menzies, 1957; Nøddegaard et al., 1968). However, quintozene may be carcinogenic (Searle, 1966), and its future in plant protection in this country is uncertain.

This paper reports glasshouse tests and one field trial of other possible scab-control chemicals, by applying them to soil.

MATERIALS AND METHODS

The chemicals were used as technically pure samples (glasshouse) or 10-20% dusts (field).

The glasshouse method, which uses potted plants (var. Majestic) has been described (McIntosh, 1970). It gives satisfactory estimates of scab incidence, yield and damage to the plants. All chemicals were used at 50 ppm in soil; quintozene was included in most tests as an internal standard. Scab indices were calculated by standard methods (Large & Honey, 1955; Lapwood & Dyson, 1966). However, the amount of scab in the "nil"-treatment varied from test to test so, to make comparisons simpler, the scab incidence for each chemical treatment was calculated as the percentage of that in the corresponding "nil"-treatment. The figures in Table 1 are the mean percentages from the numbers of tests shown.

In the field trial at Woburn, Beds., the dusts were applied at 70 lb a.i./ac (78

kg/ha) or 35 lb/ac (39 kg/ha) to small plots (4 rows x 20 ft; 5 plots per treatment). The plots were rotavated immediately, and planted the same day (var. Maris Piper). At harvest, yields were estimated from the middle two rows of each plot, and scab indices were calculated from samples of fifty ware tubers from the same rows. The scab figures for this one experiment (Table 2) are, in contrast to those in Table 1, simple scab indices, i.e. they indicate directly the proportions of tuber surfaces disfigured by scabs.

RESULTS AND DISCUSSION

Glasshouse tests

About 120 chemicals were tested. Most of the established fungicides, including the currently-used systemic fungicides, either failed to affect scab, or decreased yield of tubers, or both. Table 1 lists the chemicals which decreased scab at $P < 0.001$ in two or more glasshouse tests, and gives an indication of the effects on yield.

Table 1

Effect of soil treatment (50 ppm) on yield and common scab in glasshouse grown Majestic plants

Treatment	No. of tests	Significance of decrease in yield	Scab incidence
nil	24	-	100
2,3,4,5-tetrachloronitrobenzene	2	not significant	58
2,5-dimethyl-3-furanilide(1)	2	not significant	53
(2,4,5-trichlorophenyl) sulphonylmethyl thiocyanate(2)	2	not significant	50
fluorene	2	$P < 0.001$	47
pentachloropyridine	4	$P < 0.001$	46
2-pyridinethiol-1-oxide, Na salt	3	not significant	46
dinocap phenols(3)	4	$P = 0.02$	37
quintozene	22	$P < 0.001$	32
tecnazene	7	$P < 0.001$	28
binapacryl	2	$P < 0.001$	26
2,3,5,6-tetrachloro-4-nitrophenol	2	not significant	23
captafol	5	not significant	21

(1) Coded by BASF United Kingdom as BAS 3191F; proposed common name: furcarbanil

(2) Coded by Philips-Duphar as PH 50-82

(3) Coded by Murphy Chemical as MC 2810; sometimes known as DNOP

Fluorene, pentachloropyridine, tecnazene and binapacryl decreased the incidence of scab, but also seriously decreased yield. Dinocap phenols (MC 2810) gave fair control of scab but had a slight effect on yield; however, the decrease in yield was significant in only one of the four tests. (The decreased yield from quintozene,

usually about 93% of that in the corresponding "nil"-treatment, was significant in only four of the twenty-two single tests).

The other chemicals did not significantly affect yield. However, 2,3,4,5-tetrachloronitrobenzene and 2,3,5,6-tetrachloro-4-nitrophenol are chemically related to quintozene, and may also be carcinogenic. Although the systemic properties of 2-pyridinethiol-1-oxide are well-known (Rombouts & Kaars Sijpesteijn, 1958), it is not much used on foliage, presumably because of its instability to light; however, the sodium salt does seem to be stable enough in soil to have a significant effect on scab (cf. Allison & Barnes, 1956). Of the remaining chemicals, captafol was clearly outstanding; it did not decrease yield, and gave better control of scab than any other chemical tested. It thus seems to be the most acceptable substitute for quintozene found so far. Further tests with these and other chemicals are in hand.

Finally, and in parenthesis, we noticed in these tests that closely-related chemicals sometimes differed very widely in their effects on scab. Examples of this are:

Effective	Ineffective
dinocap phenols	dinocap
2,3,5,6-tetrachloro-4-nitrophenol	2,3,5,6-tetrachloro-4-nitroanisole
captafol	captan, folpet

Field trial

Table 2 shows some results from a field trial in 1970. The hot dry summer favoured a severe scab attack, and the scab index of 59 from the "nil" plots in 1970 contrasts with the corresponding figures of 31 and 26 in 1969 and 1968.

Table 2

Effect of soil treatment at planting on yield and common scab in a field trial (1970 : var. Maris Piper)

Treatment	Rate lb/ac	(kg/ha)	Total tubers tons/ac	(tonnes/ha)	Scab index
nil			15.1	(37.8)	59
captafol	70	(78)	13.7	(34.5)	37
quintozene	70	(78)	13.5	(34.0)	38
tecnazene	70	(78)	10.3	(25.8)	28
tecnazene	35	(39)	12.1	(30.4)	43
LSD, $P = 0.05$			2.2	(5.5)	9
$P = 0.02$			2.7	(6.7)	11
$P = 0.01$			3.0	(7.5)	12
$P = 0.001$			4.0	(10.1)	17

Tecnazene was the most effective chemical, but it seriously decreased yield even at 35 lb/ac (39 kg/ha). Captafol was as effective as quintozene, although no better, as in the glasshouse tests; it did not significantly decrease yield. Thus the field trial, like the glasshouse tests, indicated that captafol was the best available substitute for quintozene. A further trial is in progress.

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PROSPECTS FOR CONTROL OF POTATO BLACKLEG DISEASE BY THE USE OF STEM CUTTINGS

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Summary Potato blackleg disease, caused by Erwinia carotovora var. atroseptica is widespread and important in Scotland. Experience has shown it cannot be satisfactorily controlled by chemical treatments, agronomic methods or ordinary certification procedures.

Research has demonstrated that the blackleg organism is essentially tuber-borne and on this is based a method for producing seed free of blackleg and certain other diseases by propagating from tested stem cuttings. These uninfected stocks, called VTSC (Virus Tested Stem Cutting) seed, are now the highest grade in the Scottish Seed Potato Certification Scheme.

In 1970 the first VTSC stocks were released into commerce and in 1971, 210 acres of VTSC crops were certified, no blackleg having been found in them. As expected, reinfections have occurred fairly, widely, but the levels of infection were very low. Nevertheless, as the old infected stocks are continually replaced by material derived from stem cuttings, and farm hygiene improved, the general level of blackleg infection should be greatly reduced.

INTRODUCTION

Unlike most potato diseases, potato blackleg, caused by the soft rot coliform bacterium Erwinia carotovora var. atroseptica cannot be satisfactorily controlled by chemical treatments, ordinary certification procedures or agronomic means. Graham and Harper (1967) briefly summarised the epidemiology of the disease and control measures, but they emphasised that these measures were only palliative because tubers in all stocks were carrying infection to a greater or lesser extent and that the ultimate solution would probably be the production of blackleg-free stocks.

The key to freedom from blackleg is the fact, still unappreciated by many, that the causal organism is not part of the natural bacterial flora of the soil, but is tuber-borne. The following evidence that the blackleg organism is not a soil inhabitant in Scotland is based on research begun 20 years ago at East Craigs.

1. Blackleg bacteria could not be isolated from soils in spring just before potatoes were planted.

2. Populations of blackleg bacteria in both artificially inoculated and naturally contaminated soils declined to undetectable levels in a matter of months.

3. Soil bacteria, though not normally inhabitants of water can nevertheless be water-borne, and it was considered that if the blackleg organism were soil-living, it would occur from time to time in the water of ponds and streams. However, the organism could not be isolated from ponds or streams in arable areas.

4. Soft rot coliform infections of hosts other than potato are very rare in Scotland. If the organisms were soil inhabitants, infections should be comparatively common.

On the other hand there is good evidence that blackleg is tuber-borne; for example the organism can easily be isolated from tubers taken from the great majority of potato stocks. The infection cycle begins with the breakdown of the mother tuber, partly by blackleg bacteria. Thus enormous numbers of organisms invade the soil, and come into contact with the daughter tubers, infecting them through lenticels and growth cracks. They can also spread far enough to infect tubers on adjacent plants. Some daughter tubers can be infected via the stolons, but this mode of infection plays only a very minor role in the perpetuation of the disease. By whichever way they are infected, some tubers rot, but the great majority show no symptoms, and the incubation period is prolonged, often spanning the whole of the storage period. When tubers are planted and begin to grow, the bacteria become active, rotting the tuber, which again releases bacteria into the soil, thus completing the annual cycle of tuber infection.

THE PRINCIPLE OF DISEASE CONTROL THROUGH USE OF STEM CUTTINGS

Recent research on a number of potato tuber diseases has emphasised the importance of the mother tuber as the major source of over-wintering disease organisms, and that the infection cycles initially involve underground parts of the potato plant. It follows that if stem cuttings, detached before organisms spread to them, are used to propagate a new generation of tubers, these tubers should be free from infection. However, since blackleg bacteria might spread quickly from the mother tuber into the stem, cuttings have to be tested bacteriologically to ensure they are uninfected.

Use of tested cuttings for production of healthy plants is not new. For instance, it has been developed on some very large nurseries growing carnation cuttings free from certain fungal and bacterial diseases. The technique was first applied to potatoes at Rothamsted Experimental Station, where it was shown that plants could be freed from the fungus disease skin spot (caused by Oospora pustulans) by this method (Hide, Hirst and Griffith, 1969). There is now evidence that nuclear stocks of tubers free from gangrene (Phoma exigua var. foveata) and silver scurf (Helminthosporium atrovirens) can be produced in the same way. The technique for control of blackleg by using stem cuttings was developed at East Craigs.

STEM CUTTINGS IN PRACTICE

In 1967, the Department of Agriculture and Fisheries for Scotland (DAFS), aware that the problem of tuber diseases was acute, decided that available evidence justified the initiation of a project to produce seed free from blackleg and other diseases using stem cuttings from their existing virus-tested stocks. This pilot scheme proved successful and in subsequent years output was stepped up, the eventual aim being to replace existing commercial stocks with material derived from stem cuttings. No one knew exactly what practical difficulties might arise, or how long stocks might remain healthy, but all evidence indicated that the technique had great potential value in countering tuber diseases. To avoid reinfection of the stocks, the DAFS raises and multiplies the material on an upland farm where no commercial crops are grown, and under strict hygienic conditions. Stocks propagated clonally for up to five years from stem cuttings now constitute the highest grade in the Scottish Seed Certification Scheme and are designated "VTSC" (Virus-Tested Stem Cutting) seed. VTSC clones from the DAFS farm were first released to commercial

VTSC producers in 1970. Crops entered for this grade are very rigorously checked for both purity and health and, under the terms of the Scheme, there is no tolerance for blackleg in the growing crop, or for soft rot caused by the blackleg organism in stored tubers.

Production of VTSC Seed on the DAFS farm. Tubers derived from stem cutting material are potted in a sterile peat compost in 6 in. pots under glass in early spring and when stems are 12 in. high the growing point is pinched to cause the buds in the leaf axils to grow into side shoots. When the shoots are about 3 in. long they are removed aseptically. A piece of stem about ½ in. long is cut from the base of lowest cutting on the main stem, and sent to the laboratory for bacteriological testing. It is assumed that if the lowest cutting is free from blackleg bacteria then those higher up the stem will also be free, since the bacteria spread upwards in the stem. The cuttings from each parent plant are wrapped separately in moist absorbent paper and kept separately in small plastic cups until the results of the tests are known, normally within two days.

Cuttings proved to be blackleg-free are rooted in mist propagators, transplanted in 3 in. pots, hardened off in frames and hand planted in the field in June and July. Tubers of commercial size are produced from cuttings of most varieties by the end of the season. Spent haulm is destroyed chemically and tubers lifted carefully for storage. These tubers are used for clonal multiplication in subsequent years.

The Blackleg Test. The pieces of stem for testing are crushed with sterile pliers and the sap spread over plates of MacConkey pectate double layer medium (Stewart, 1962) which are incubated at 25° for 48 hours. On this medium, blackleg organisms form discrete colonies in depressions in the pectate layer, and, if necessary their identity can be confirmed by slide agglutination tests using antiserum prepared against the organism or by biochemical tests.

Over the five-year period 1967-71, 9,300 cuttings have been tested and none have been found infected, probably because the bacteria had not been able to multiply in the mother tuber and spread into the stem by the time the cuttings were taken. However, cuttings from ordinary commercial stocks examined in July in connection with research work have quite often been found to be infected, though the plants from which they were taken showed no symptoms, and for this reason no cuttings are used for routine propagation after mid-June and never from field plots. Some cuttings are contaminated with large numbers of saprophytic bacteria including pectolytic pseudomonads, and these cuttings are discarded as a matter of routine. The average discard rate over the five-year period was 3.7%.

Blackleg and Stem Cutting Material, 1967-70. Over the years 1967-70 approximately 600,000 plants were grown on the DAFS farm and only one plant, of cv Redskin, was found infected. This was detected after lifting as a heel end tuber infection in material grown two years from the cutting stage, but the source of infection could not be discovered. Another 84 samples of suspect tuber material were examined bacteriologically but proved to be uninfected.

In 1970 sufficient VTSC seed was released to commercial growers to plant 44 acres. There were only three instances where this commercial material was found infected and these were investigated to try to discover how infection had been introduced. In the first case one infected plant was found in a crop which appeared to have been attacked by rooks. In the second case seven plants were found of which five had typical blackleg infection arising from the mother tubers; the other two showed stem infections at soil level but the mother tubers were sound and blackleg bacteria could not be isolated from their interiors. The source of infection was not found; it was noteworthy that the infected plants were in the same strip of plots and in an area which had been kept very wet by overspill from a stream above the field where the plots were growing. The third case was particularly

interesting as it demonstrated that blackleg could be introduced easily with contractors' contaminated spraying equipment. The symptoms were soft rot on stems at places where they had been damaged. Nine plants were infected, all adjacent to the wheel tracks, and investigation showed the machinery had been used previously in crops affected with blackleg to the extent of 3-5%. It was claimed that the equipment had been pressure-hosed before use in the crop, but this had apparently not been sufficient to remove infection.

The tubers from all VTSC crops were inspected during storage and only eight stocks contained soft rotted tubers or tubers with heel end necrosis. Two tubers from one of these stocks and 15 from another were found to be infected but the sources of infection could not be discovered.

Blackleg on the DAFS farm in 1971. In 1971 blackleg was more extensive in commercial seed crops in Scotland than in any year since the epidemic of 1966, presumably because weather conditions were favourable for manifestation of infection.

For the first time the disease was discovered spasmodically in tested cuttings planted in the field. Altogether 21 infected stems were found in early September distributed throughout an area which contained 4,000 plants. From the symptoms it was clear that two plants with stem lesions had been infected several weeks previously, and some tubers on these plants showed extensive internal rots caused by the blackleg organism. Both plants were next to wheel tracks suggesting that infection might have been introduced on the wheels. The remaining infections were either on stems at or above soil level or on exposed leaf scars and apparently had been established more recently because lesions had not spread extensively in the stems. The foliage had suffered severe wind damage and due to the exposed situation and the prevailing wet weather, the haulms were senescent and rotting. The rotting material attracted large numbers of insects, mainly fruit flies (Drosophila sp), which were very active in the crop. A number of flies were collected and tested for contamination with blackleg organisms by plating material from crushed bodies; two collections were found to be contaminated and it seems possible that the insects had been responsible for spreading infection.

A further 15 plants were found infected amongst material grown from tubers derived from stem cuttings in previous years. An examination to try to determine the source of infection is in progress, but not all infections originated from the mother tubers. All blackleg infected stocks have been destroyed.

Blackleg in commercial VTSC crops in 1971. Data on blackleg found at inspection in stocks grown by commercial VTSC growers is as follows:

Total number of stocks	Number of stocks rejected for any blackleg	Total acreage entered for VTSC grade	Acreage rejected for any blackleg	Acreage rejected for other reasons
241	52	270	64½	5½

Twenty-four stocks had levels of infection between 0.001% and 0.01% of the plants, 17 stocks had levels between 0.011% and 0.1% and 11 stocks had levels between 0.11% and 0.57%. Of the 49 growers, 27 had all their stocks free from blackleg, despite the fact that the majority were still growing other crops not derived from stem cuttings.

Investigations are still continuing into sources of infection and means of recontamination, but it is already clear that contaminated machinery is important. There is also circumstantial evidence that some blackleg plants developed from tubers attacked by rooks earlier in the season. Rook damage is quite common in certain areas in mid-summer when food is scarce; and it may be that rooks transmit infection by feeding first in ordinary commercial crops and then in VTSC crops.

Problems of preventing reinfection. The data on the occurrence of blackleg in VTSC stocks illustrates that reinfection takes place fairly commonly at present, but the levels of infection are generally very low - much lower than in crops not of stem cutting origin. Nevertheless once the organism has been introduced infection is likely to build up rapidly; hence there is no tolerance for blackleg in VTSC material.

No one should be surprised at the extent of reinfection, considering the amount of infection in all other commercial stocks and the many ways in which organisms could be transferred to blackleg-free stocks. Some of these ways seem obvious; for example the use of contaminated planters, sprayers, tractors, lifting machines and storage equipment, but there is very little experimental evidence of the extent to which these practices spread disease. Experiments have been done at East Craigs on reinfection of VTSC tubers by using a contaminated riddle and spool grader. The graders were contaminated by passing over them tubers with soft rot caused by the blackleg organism. Two treatments were used: VTSC tubers graded immediately after contamination, and after some contamination had been removed by passing 5 cwt healthy tubers over the grader before the VTSC tubers. Grading was done in early March and tubers were stored in trays until planting in April. The results are shown in Table 1.

Table 1

Reinfection of blackleg-free stocks of cv. Arran Pilot by grading

Type of grader	% blackleg appearing in subsequent crop following	
	1. Dressing over contaminated grader	2. As 1. after 5 cwt. healthy tubers had passed over the grader
Spool	16%	2%
Riddle	19%	11%

These results demonstrate the high infectivity of the organism and the necessity for very good hygiene on the farm. Disinfection of all equipment including clothing is essential, but a major practical problem has proved to be the cleaning and disinfection of machinery, which, of course, is not designed with disinfection in mind. Tests have shown that infective material remains lodged in such places as junctions between boards and nut and bolt heads, even after pressure washing. To seal up these sites as well as to provide an easily cleaned surface growers are recommended to paint machinery with a chemically resistant thick paint.

Tests have also shown that many commercial disinfectants such as quaternary ammonium compounds, hypochlorite and some chlorinated phenols are ineffective because they are too readily inactivated by contact with soil and plant debris. The most effective disinfectant found so far is 5% formaldehyde solution containing a wetting agent, but in view of the unpleasant properties of formaldehyde, experiments are continuing at East Craigs to find an odourless, non-toxic, non-corrosive alternative.

Another obvious source of contamination is groundkeepers, but there is no experimental evidence to show how important they are in this respect. However an eight-year rotation is laid down for VTSC crops, and this should greatly minimise the danger from groundkeepers. There is no satisfactory way of destroying them quickly and the development of a herbicide which translocates from foliage to tubers in situ would be a valuable aid in the control of a number of tuber-borne diseases.

Experience with blackleg-free material is bringing to light other possible means of re-infection which were completely obscured by tuber-borne infection. Transmission by rooks is an example, and experiments to test this hypothesis will begin in 1972. If rooks do spread blackleg it is difficult to see how it can be stopped; but it may go some way to explain how crops can become infected despite every reasonable hygienic precaution having been taken.

PROSPECTS FOR THE FUTURE

It would be naive to imagine that merely to take cuttings and propagate from these will, even in the long term, suffice to eliminate blackleg. Stem cutting production is only a first step, but at this stage of development, where islands of disease-free material are being grown in a sea of infection, re-infections are bound to occur. Nevertheless, as old infected stocks are continuously replaced with material derived from stem cuttings, and hygiene improved, the level of blackleg infection should be greatly reduced. Eventually, the disease should no longer cause so much loss and trouble even if it is not entirely eliminated. Dilution of infection should be accelerated by new requirements under the Scottish Certification Scheme, which limit the life of VTSC stocks and the next highest grade "F3" (Foundation Seed) to five and four years respectively. Thus there will be a continuous downward flow of high grade material through the grades. Coupled with these conditions the Department are carrying out a vigorous educational programme for growers by direct advice, through publications and other forms of publicity.

Unlike some of the fungal diseases, an additional problem in blackleg control stems from the fact that hygiene is the only means preventing re-infection. Skin spot and gangrene, for instance, can be controlled by the systemic fungicides and by fumigation with sec-butylamine, so that combining by chemical treatment with hygienic practices, re-infections can be cut to a minimum. At present, there is no known chemical treatment for blackleg but a systemic bactericide active against the blackleg bacterium would undoubtedly be a great help in maintaining health.

Nevertheless, it seems likely that, in the long term, the stem cutting procedure will make a great impact on tuber health in Scotland and contribute significantly towards a reduction in the cost of potato production throughout the United Kingdom.

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SEED DRESSING TRIALS TO CONTROL BACTERIAL BLIGHT OF COTTON (XANTHOMONAS MALVACEARUM) IN THE NORTHERN STATES OF NIGERIA

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Summary The seed borne disease bacterial blight is the only serious disease of cotton in Nigeria. By 1967 about 70 per cent of the seed used for sowing was being dressed against it, either with organo-mercurial or with copper formulations, but because both these types had disadvantages, an intensive search was begun for alternative seed dressings. Trials between 1967 and 1970 showed that bronopol was the only one of the less poisonous dressings tested to possess the required bactericidal properties, and so this compound has now been recommended as an alternative to the mercurial and copper dressings currently in use. It has recently been shown that at least three other dressings are as efficient in controlling bacterial blight.

INTRODUCTION

The only serious disease of cotton (Gossypium hirsutum) in the six Northern States of Nigeria is bacterial blight (Xanthomonas malvacearum). The disease is most damaging in its primary phase on seedlings; the symptoms consist of water-soaked lesions on the cotyledons produced by bacteria which have been carried over from the previous season on the seed coat. Under conditions favourable to the pathogen, the bacteria can also infect the growing point of the seedling and destroy it. Maximum development of primary symptoms normally occurs about three weeks after sowing.

Subsequent spread of the disease to the true leaves (the angular leaf spot phase) is by means of wind-blown rain, and it follows that if the primary cotyledon symptoms do not occur, the crop is likely to remain free of blight throughout its life, provided the disease is not present on other cotton in the vicinity. Observations have suggested that secondary infection will only markedly affect the yield of seed cotton if the bolls are seriously affected by the bacterial boll blight phase.

The disease is currently being controlled in northern Nigeria by resistance breeding and by the use of chemical seed dressings. This paper considers the latter aspect only, although in practice both methods are of equal importance.

The widespread use of dressed seed is made easier because cotton growing in the Northern States of Nigeria is under the control of the Northern States Marketing Board, which issues seed free of charge to farmers and purchases the seed cotton at a guaranteed price. This means that the seed can be dressed centrally with approved chemicals at standard rates under the supervision of the sole agent for cotton ginning in Nigeria, the British Cotton Growing Association (BCGA), before the farmer receives it.

Over sixty different seed dressings have been tested at Samaru since 1953 (Dransfield, 1968) to find chemicals that will control bacterial blight at reasonable cost without being phytotoxic to the cotton plant, and that can be applied mechanically at the ginnery immediately the cotton has been ginned so that additional handling costs are avoided. A seed dressing of low mammalian toxicity is desirable, but until recently all the seed dressings that would effectively control X. malvacearum were also rather poisonous.

As a result of these earlier trials, a mixture of phenylmercury acetate and ethylmercury chloride containing five per cent of organic mercury (Agrosan 5W), applied to the seed at a rate of 1:150 w/w, was adopted as the standard commercial treatment. The general blight level in the cotton crop began to fall in those areas where dressed seed was being sown, and during the next few years the dosage rate was gradually reduced until in 1967 Agrosan 3W (three per cent mercury) was being used at a rate of 1:170 w/w.

Two ginneries had been fitted with continuous action seed dressers (Plantector), and by this time they were dressing about 70 per cent of the planting seed. To augment the continuous machines, batch drum mixers (Booth) had been installed in five other ginneries, but because mercurial dressings were thought to be unsafe the rather less efficient copper dressings, containing 45 per cent copper as cuprous oxide, were recommended for these machines at a rate of 1:150 w/w.

Copper dressings were increasing in cost and the disadvantages of organo-mercurial dressings were well known to the authorities in Nigeria. In 1967 an intensive search was started for suitable formulations containing other active ingredients. This paper deals mainly with the results of testing one such alternative, bronopol, discovered by The Boots Company Ltd., and developed by that firm in conjunction with Fisons Ltd. (Spooner and Wakerley, 1971).

METHODS AND MATERIALS

The testing of a new seed dressing at Samaru takes at least three years. If it proves suitable in all respects after these three years of trials, the Institute can then recommend it for approval by the Marketing Board.

In the first year of testing, small scale field trials are set up to compare it with standard recommended dressings. Each new formulation is applied to heavily infested cotton seed at a range of accurately measured dosage rates, usually between 1:250 and 1:100. Lower rates are difficult to apply evenly to the heavily fuzzed Nigerian seed and at more than 1:100 dressing is often lost from the seed during subsequent handling. Control plots of undressed seed, and also seed dressed at the standard rates with a recommended mercurial and copper dressing, are also included. Between one and two thousand seeds are used for each treatment, and the trial is sown in duplicate (in mid-June and in mid-July) in a randomised block layout of between four and eight replications. Frequent germination counts are made to detect any phytotoxicity.

Disease scoring is normally made three weeks after sowing, or as soon as about 80 per cent of the seedlings grown from undressed seed are showing good disease symptoms. All seedlings are uprooted, washed to remove mud splashes and to enhance the clarity of the lesions, and immediately graded in the field for primary blight symptoms using a 0-5 scale. In these first year's trials, no seedlings are left in the ground for further observations because plot-to-plot spread of the disease would soon mask any differences imposed by the various seed dressing treatments.

Any promising formulation from the first season's trials is tested in similar, but larger, trials using more dosage rates so that its optimum application rate can be determined. The plots are generally larger, and sufficient seedlings are left to grow on after blight scoring in order to confirm that there is no delayed reaction of the cotton plant to the chemical.

If the new formulation is satisfactory in controlling primary symptoms of blight, other aspects are considered in the third year. Yield trials are carried out using larger plots of up to 200 m^2, arranged in a 4 x 4 latin square and separated from each other by 5m barriers of a resistant variety of cotton to delay plot-to-plot spread of blight. Most trials are sprayed against insect damage six times at weekly intervals, starting nine weeks after sowing. Attempts had been made to grow the different treatments on separate sites, using a standard resistant cotton variety on each site as a yield indicator to compensate for the inevitable variation between sites, but they were abandoned because, although secondary spread of the disease from infected to healthy plots was eliminated, there were too many variables between sites from the yield data to be meaningful.

Also in this third year of testing, a 25-50 kg sample of the new seed dressing is obtained from the manufacturer to test whether it will pass satisfactorily through both types of dressing machine without clogging the mechanism and without liberating an excessive amount of dust into the atmosphere, and that a constant application rate can be maintained. In addition, samples of dressed seed are stored for at least nine months (the maximum commercial storage period) and then sown to ensure that there is no loss of germinative power during this period.

In all trials reported here the then current commercial variety of cotton - Samaru 26J - was used.

RESULTS AND DISCUSSION

The results of several identical trials have been combined in each of the following tables of results. Standard errors are therefore not quoted but each individual trial has been analysed statistically and indications of any statistical significance in the results are given. References to bronopol in this paper refer to the dressing containing 12 per cent bronopol, now known as Bronocot (Plant Protection Ltd.).

Bronopol was first included in two trials in 1967 (Dransfield, 1969a). The results of both trials (sown in mid-June and mid-July) are combined in Table 1.

Table 1

Bacterial blight in small scale trials at Samaru in 1967

Seed dressing *	Dosage rate (dressing: seed w/w)	Per cent germination	Per cent diseased plants**	Mean disease score**
Agrosan 5W	1:150	69	3.8	0.07
Cuprocot	1:150	65	13.2	0.27
Bronopol	1:150	68	3.9	0.07
"	1:200	66	7.7	0.14
"	1:250	68	24.1	0.46
LF 651	1:150	67	37.4	0.70
Oxine copper 15%	1:150	71	45.8	1.01
"	1:250	71	44.6	0.91
Untreated control	-	70	98.3	3.64

* Cuprocot contains 45% copper as cuprous oxide; the a.i. in L.F.651 was not disclosed; the formulation of oxine copper used was Quinolate 15.

** The first four treatments gave significantly superior bacterial blight control to the last five treatments.

These results showed that bronopol was as effective as an organo-mercurial dressing at similar dosages.

Over the three seasons 1968-70, bronopol was tested more intensively (Dransfield, 1969b, 1970, 1971). Further field trials were laid down to confirm its efficiency in controlling primary symptoms under different climatic and soil conditions, and to obtain an idea of the optimum application rate. In each of the three years there were two trials, sown in June and July as usual, and Table 2 summarises the results from the six trials.

Table 2

Bacterial blight in larger-scale trials at Samaru in 1968-70

Seed dressing	Dosage rate (dressing: seed w/w)	Per cent germination	Per cent diseased plants	Mean disease score
Agrosan 3W	1:150	67	0.7	0.01
Bronopol	1:150	59	1.3	0.03
"	1:160	59	2.2	0.05
"	1:170	60	2.5	0.05
"	1:180	59	3.0	0.06
"	1:190	58	3.9	0.09
"	1:200	57	4.5	0.10
"	1:225	60	6.9	0.17
"	1:250	60	8.2	0.19
Untreated control	-	61	69.2	2.05

Germination was rather variable in the different trials and there were no statistical differences in the germination percentages among the various treatments in any of the six trials. The percentages of diseased seedlings rose slowly as the dosage rates of bronopol were decreased, but in no trial was there any statistical difference between the bacterial blight figures for any of the first five treatments in the table.

This implies that application rates of bronopol as low as 1:180 would give satisfactory blight control, subject to the dressing having been applied evenly - something that is difficult to achieve in large-scale application of seed dressings. Application rates below 1:190 were generally less effective in all trials although even at 1:250 bronopol gave a useful measure of blight control.

The long-term storage trial carried out in 1968 was satisfactory (Dransfield, 1969b), and the larger scale application trials of bronopol through both types of dresser at the ginneries satisfied the B.C.G.A. that there would be no difficulties in handling the new dressing.

Yield trials were made in 1968 and 1969 at several sites in the Northern States (Dransfield, 1969b, 1970, 1971). The majority of these trials were sown in June and were sprayed against insects; a few were sown in July and were left unsprayed as is the typical farmer's practice.

Table 3

Yields of seed cotton in kg/ha in 1968 and 1969 trials (sown mid-June and sprayed against insects)

Site	SEED DRESSING			
	Untreated control	Mercurial 3% Hg	Copper 45% Cu	Bronopol
Samaru	1402	1596	1600	1523
Mokwa	828	924	966	986
Kontagora	603	631	659	735
Gusau	650	734	758	730
Tumu	989	1055	1012	951
Mean yields	894	988	999	985
% increase over control		10.5	11.7	10.2

On each site in the June-sown trials (Table 3), all three seed dressings generally gave highly significant yield increases over the undressed control, but no one seed dressing was consistently the best.

Table 4

Yields of seed cotton in kg/ha in 1968 and 1969 trials (sown mid-July and not sprayed against insects)

Site	SEED DRESSING			
	Untreated control	Mercurial 3% Hg	Copper 45% Cu	Bronopol
Samaru	398	403	403	415
Gusau	551	593	551	579
Tumu	154	118	118	160
Mean yields	368	371	357	385
% increase over control		0.9	- 2.8	4.6

As expected, yields in the July-sown trials (Table 4) were very poor compared with the June-sown sprayed trials, and there were no significant differences among treatments on any of the three sites.

At present, the cost of dressing cotton seed in Nigeria is about £0.15 sterling per ha, and the farmer currently is paid about £0.06 per kg for his seed cotton. This means that a yield increase, through sowing dressed seed, of as little as 3 kg/ha is sufficient to cover the cost of dressing the seed. In practice, much greater yield increases can consistently be obtained, but only if insects are controlled. The serious losses caused by insect damage in the unsprayed trials masked any potential yield increases that might have resulted from the use of dressed seed.

Testing of other seed dressings is still in progress, and although yield trials and application trials have not yet been made, the following three seed dressings under test all appear to be as effective as bronopol in controlling primary symptoms of bacterial blight: SN 5978 (The Boots Co., Ltd.); JF 2912 (Plant Protection Ltd.); cuframeb (Cufram Z - Universal Crop Protection Ltd.).

Bronopol cotton seed dressing is apparently as effective in all respects as the organo-mercurial formulations in controlling seed-borne bacterial blight; it is also safer (Spooner and Wakerley, 1971) although possibly slightly more expensive. It shows no phytotoxicity towards cotton.

Accordingly, in 1969, the Institute for Agricultural Research, Samaru, advised that bronopol cotton seed dressing should be added to the list of cotton seed dressings approved by the Northern States Marketing Board, and this advice has been accepted by the Board.

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