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THE EFFECT OF SYSTEMIC AND OTHER FUNGICIDES ON THE CONTROL OF RED CORE DISEASE AND ON THE YIELD OF TREATED STRAWBERRIES

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Summary Captafol, dichlofluanid and etridiazole applied as soil drenches and metalaxyl, LS 731038, LS 74783, SN 41703 and SN 66752 as soil drenches, root soaks or foliar sprays decreased the severity of red core but pyroxychlor was ineffective. The cumulative effect on yield of annual chemical treatments was assessed for all fungicides except metalaxyl. LS 74783 applied as a pre-plant root soak combined with an annual autumn foliar spray to strawberries planted in the spring was the highest yielding treatment.

Résumé La gravité du 'red core', maladie des fraisiers causée par *Phytophthora fragariae*, fut réduite par un traitement soit au captafol, dichlofluanid ou etridiazole sous forme d'un trempage du sol, ou au metalaxyl, LS 731038, LS 74783, SN 41703 et SN 66752 sous forme d'un trempage du sol ou du système radiculaire avant la plantation ou encore d'une pulvérisation du feuillage. Par contre le pyroxychlor s'est montré être inefficace. L'influence cumulative des applications annuels des produits chimiques sur le rendement fut évaluée pour tous les fongicides utilizés sauf le metalaxyl. Le meilleur rendement fut obtenu après une application du produit LS 74783 sous forme d'un trempage des racines avant la plantation suivi d'une pulvérisation annuelle du feuillage en automne des plantations faites au printemps.

INTRODUCTION

Chemical methods of controlling red core root disease of strawberry caused by *Phytophthora fragariae* Hickman have been sought wherever it has occurred and were reviewed by Montgomerie (1977). In the UK, most modern fungicides have been evaluated for activity against *P. fragariae* in pot experiments (Montgomerie & Kennedy, 1972, 1974, 1975, 1977) and those showing promise tested in the field (Montgomerie, 1969; Montgomerie & Kennedy, 1976; Upstone, 1976). Commercial application of one of these (captafol) has controlled red core in some farms (Anon., 1978). In most field experiments disease severity was decreased by treatment in the year of planting before severe infection had occurred but the results of one trial (Montgomerie & Turner, 1979) suggested that autumn soil drenches of captafol or etridiazole applied to diseased plants would be economic because of the increased yield the following year. This was relevant to fields where areas of affected plants occurred in an otherwise vigorous plantation and the cost of an overall treatment could not be justified. The commercial rates of soil drenches have been derived from manual applications of 284 ml/plant in experiments. At a planting density of 25,000 plants/ha and continuous delivery of fungicide by modified spraying machines the volumes suggested have been 7875-11250 l/ha.

The discovery of systemic fungicides active against Phytophthora spp. opened up the prospect of a more flexible approach to the method and perhaps the rate of application of fungicides and of eliminating possible differences in effectiveness due to soil type. LS 731038 was more effective as a pre-plant root soak than as a soil drench and had promising activity as a foliar spray in pot tests (Montgomerie & Kennedy, 1977).

The work reported here gives more information on the effect of different concentrations of captafol and etridiazole which had given inconsistent results in trials at SHRI; compares systemic and non-systemic chemicals for their prophylactic properties and their effect on yield; and assesses the effectiveness in the field of different methods of application.

MATERIALS AND METHODS

The trials were sited on two infested areas on the farm at SHRI, Invergowrie, which had been cropped continuously with strawberries for over 12 years. The soil in both areas was a sandy loam; Trial 1 was on a slope where the soil had impeded drainage, a pH of 6.0 and 3.47% organic matter (o.m.): all other trials were on a flat site where the soil had good drainage, a pH of 5.75 and 5.18% o.m. Late in 1977 it was discovered that nitrogen fertiliser had been omitted in error and remedial applications of nitrochalk were made in April and September 1978 and May 1979.

Healthy runners of the strawberry cv. Cambridge Favourite were planted in the spring (April/May) at 90 x 45 cm spacing. Six replicates of each treatment were evaluated in a randomized block arrangement. Each plot in Trials 1, 2 and 3 comprised two rows of 18 plants in which two rows of 12 plants remained in the soil for three years to provide data on yield. These were separated by one untreated plant in each row from two rows of five treated plants which remained in the soil for only one year and were then lifted for disease assessment. Immediately after lifting, healthy runners were planted in the same plant stations for disease assessment the following year. Plots were separated from adjoining ones by an untreated row and by an untreated plant within the rows. Plants in the yield plots received the same annual treatments as those in the disease assessment plots except for pre-plant root soaks. Trials 4 and 5 comprised only disease assessment plots with six replicates in randomized blocks. Each plot was a row of five plants separated from adjoining plots by one untreated plant.

The following fungicides were used:

captafol (Sanspor, 50% liquid)
dichlofluanid (Elvaron, 50% w.p.)
etridiazole (Aaterra, 35% w.p.)
metalaxyl (Ridomil, 25% w.p.)
pyroxychlor (Nurelle, 6% e.c.)
SN 41703 (Dynone, 70% aq. soln)
SN 66752 (70% aq. soln)
LS 731038, sodium ethyl phosphonate (EXP.1608, 80% w.s.p. and EXP.1765, 50% aq. soln)
LS 74783, aluminium tris (ethyl phosphonate) (Aliette, 80% w.p.)

Trial 1. Runners were planted in April 1975. Twenty days later plants in the soil mix treatment were lifted and etridiazole wettable powder, mixed with 20 times its volume of sand, incorporated into the soil in each planting hole to give approximately the same amount of fungicide (0.28 g a.i.)/plant as the soil drench (1 g a.i. /1. After replanting, 280 ml water/plant was applied to promote re-establishment. Two weeks later most of the treated plants had died and were replaced in September when the plants grew normally. All other treatments were soil drenches applied on 23rd July 1975. Additional drenches were applied on 3rd November (two and three drench treatments) and on the 2nd February 1976 (three drench treatment). Etridiazole treatments and the water drench were applied at a rate of 284 ml/plant, pyroxychlor and SN 41703 at 500 ml/plant.

The following year applications at the same rates as above were on 2nd August, 1st November 1976 and 3rd February 1977. Plants in the yield plots and runners for disease assessment in the etridiazole soil mix treatment received no fungicide, and as pyroxychlor had been withdrawn for use on edible crops its application was not continued. In the final year, drenches were applied on 27th September, 14th November 1977 and 7 April 1978 at a rate of 250 ml/plant. SN 41703 was not applied because its development for use on edible crops had been discontinued.

Trial 2 was planted in April 1976. Soil drenches were applied at a rate of 284 ml/plant and LS 731038 as a foliar spray to 'run-off' (ca 1125 l/ha) on 17 August 1976. The concentrations of fungicides were the same as were used in 1977 (Table 3) except for captafol (0.6% a.i.) and LS 781038 (0.3% a.i.). The following year treatments were applied on 20 September at 250 ml/plant and in the third year on 8th September at 300 ml/plant. The formulation of LS 731038 used in 1978 was the 50% aq. soln.

Trial 3. Planting was done in April following the pre-plant treatments of immersing roots but not crowns in LS 74783 for four hours. Soil drenches (250 ml/plant) and the foliar spray were applied on 20th September 1977. The next year soil drenches at a rate of 300 ml/plant and the autumn foliar spray treatments were applied on the 12th September 1978. After the first year yield plots in the pre-plant root soak treatment received two foliar sprays, one in the autumn of 1978, the other on the 13th March 1979 while plants for the disease assessment plots received a pre-plant root soak as well as the two foliar sprays.

Trial 4. Runners were planted in April 1977 and all treatments except those with SN 66752 applied before planting, the sprays some with Tween 80 (1 ml/l) applied to 'run-off'. The total immersion treatment consisted of plunging plants into the fungicide until they were completely covered and then withdrawing them. The band sprays of SN 66752 were applied with knapsack sprayer and hand lance with nozzle giving a spray 30 cm wide along the planting line at a rate of 1125 l/ha. One treatment was applied on 19th September, the other on 15th November 1977.

Trial 5 was planted in May 1978 and treatments applied on 19th September, 13th October, 16th November and 14th December. The double applications were applied on the 19th September 1978 and the 13th March 1979. Soil drenches were applied at the rate of 300 ml/plant and foliar sprays to 'run-off'.

The number of roots with red cores was expressed as a percentage of the total number of non-suberized roots. It was impossible to examine each lateral root but where symptoms were absent from the main root and red core was readily seen in any of its laterals, the root was recorded as diseased. Analyses of variance were done on the annual data from each trial and when the variance ratio was significant at the 5% level, Duncan's multiple range test was used to evaluate differences between treatments. In the Tables, numbers followed by the same letter (vertical comparisons only) are not significantly different at the 5% level (Harter, 1960).

RESULTS

Trial 1. The most effective treatments were SN 41703 and the two and three drenches with etridiazole (Table 1). The effects of different times of application suggested that the November drench was the most, and the February drench the least, effective. The shorter time of exposure to infection (September-April) of plants in the etridiazole soil mix treatment could have influenced this result. There were no significant differences in disease severity in 1977 or in 1978 and the data have been omitted from Table 1. A high proportion (46%) of roots had rotted due to unidentified causes in 1976/77 and this may have masked treatment effects. Plants lifted in 1978 were stored in a refrigerator which broke down before disease assessments were done and this may have affected the reliability of red cores as indicators of infection.

Table 1

The effect of etridiazole, pyroxychlor and SN 41703 on the severity of red core disease in strawberries planted in April and lifted one year later (Trial 1)

Treatment	% a.i.	Mean % number of roots with red core (angular transformation) 1975/76
Etridiazole, three soil drenches	0.1	52.1c
Etridiazole, two soil drenches	0.1	53.3c
SN 41703 soil drench	0.56	54.3c
Etridiazole w.p. incorporated in soil	(0.1)	58.2bc
Etridiazole soil drench	0.1	61.2bc
Etridiazole soil drench	0.2	67.8ab
Pyroxychlor soil drench	0.025	71.2ab
Water soil drench	-	78.9a
	S.E.±	4.30

Table 2

Yield from strawberries planted in April 1975 and treated with etridiazole, pyroxychlor, and SN 41703 (Trial 1)

Treatment		Mean weight of fruit/plot (kg)		
		1976	1977	1978
SN 41703 soil drench		3.02a	2.96a	8.13a
Etridiazole soil drench (0.2% a.i.)		2.68ab	1.74b	5.86b
Pyroxychlor soil drench		2.54ab	1.71b	4.82bc
Etridiazole, two soil drenches		2.31ab	1.71b	6.20ab
Etridiazole, three soil drenches		2.18ab	1.80b	6.14ab
Etridiazole soil drench		2.11b	1.53b	5.29bc
Water soil drench		1.82b	1.32b	4.62bc
Etridiazole w.p. incorporated in soil		0.98c	0.96b	3.45c
	S.E.±	0.274	0.316	0.709

With one exception the yields from all treated plants in 1976 were higher than that from untreated ones (Table 2) but the increase was significant only with SN 41703. The low yield after the etridiazole soil mix treatment may have been the result of the later planting date or a residual phytotoxic effect. The following year all yields were lower and only SN 41703 gave a significant increase. The application of nitrogen in the spring of 1978 resulted in higher yields in all plots in the final year. The improvement in yield was again significant only for SN 41703.

Trial 2. In the first year 18% of roots rotted due to causes other than <u>P. fragariae</u> and as there was apparently no effect of any of the treatments these have been omitted from Table 3. In the second year other rots accounted for 2% of roots and all treatments resulted in significant decreases in disease severity the captafol soil drench being the most effective. The two systemic fungicides were equally effective although LS 731038 was used at one third the concentration of LS 74783. The foliar spray of LS 731038 was as effective as the soil drench and was not significantly different from the etridiazole soil drench. In the final year 3% of roots had other rots and only two treatments, soil drenches of captafol and LS 74783, effectively decreased disease severity.

Table 3

The effect of captafol, etridiazole, LS 74783 and LS 731038 on the severity of red core disease in strawberries planted each spring (April/May) and lifted one year later (Trial 2)

Treatment	% a.i.		Mean % number of roots with red core (angular transformation) 1977/78	1978/79
Captafol soil	0.3		29.7c	-
drench	0.18		-	21.2d
Etridiazole soil	0.2		46.5b	-
drench	0.12		-	52.7a
LS 74783	0.3		51.2b	-
soil drench	0.3		-	32.6c
LS 731038	0.1		56.1b	-
soil drench	0.3		-	39.1bc
LS 731038	0.1		58.9b	-
foliar spray	0.3		-	36.4bc
Water soil	-		73.7a	-
drench	-		-	43.9ab
		S.E.±	3.95	3.18

Table 4

Yield from strawberries planted in April 1976 and treated with captafol, etridiazole, LS 74783 and LS 731038 (Trial 2)

Treatment		Mean weight of fruit/plot (kg) 1977	1978	1979
LS 731038 soil drench		3.02	7.18a	10.33a
LS 74783 soil drench		2.97	8.41a	11.03a
Etridiazole soil drench		2.89	4.92ab	3.67c
LS 731038 autumn foliar spray		2.35	5.22ab	7.88b
Captafol soil drench		1.94	6.58a	7.52b
Water soil drench		1.85	2.17b	1.95c
	S.E.±	0.382	1.181	0.806

Although there was a 63% increase in yield following a soil drench with LS 731038 in the first fruiting year it was not statistically significant (Table 4). In the second year, yields from all treatments were increased following the application of nitrogen and soil drenches with the systemic fungicides and captafol resulted in significantly higher yields. The other treatments showed increases in yield over the untreated of 127% and 141% but these were not significant. In the final year yields from all treatments except the etridiazole soil drench and untreated plants were higher than in the previous year. The soil drenches with LS 731038 and LS 74783 resulted in significantly higher yields than any other treatment.

Table 5

The effect of dichlofluanid, LS 74783, and SN 66752 on the severity of red core disease in strawberries planted in the spring (April/May) and lifted one year later (Trial 3)

Treatment	% a.i.	Mean % number of roots with red core (angular transformation) 1977/78	1978/79
LS 74783 root soak (4 h)	0.3	46.5c	-
and autumn foliar spray	0.3	-	25.9d
Dichlofluanid soil drench	0.6	50.0bc	-
	0.6	-	39.0c
SN 66752 soil drench	0.42	52.9bc	-
	0.56	-	56.4b
LS 74783 root soak (4 h)	0.3	60.3b	-
LS 74783 root soak (4 h), autumn and spring foliar sprays	0.3	-	55.5b
Water soil drench	-	75.5a	-
	-	-	72.7a
	S.E.±	3.73	3.85

Table 6

Yield from strawberries planted in April, 1977 and treated with dichlofluanid, LS 74783 and SN 66752 (Trial 3)

Treatment	Mean weight of fruit/plot (kg) 1978	1979
LS 74783 root soak (4 h) and autumn foliar spray	8.30a	14.72a
LS 74783 root soak (4 h)	6.08b	-
LS 74783 root soak (4 h), autumn and spring foliar sprays	-	11.92b
Dichlofluanid soil drench	5.72b	13.36ab
SN 66752 soil drench	5.69b	11.06b
Water soil drench	3.07c	7.21c
S.E.±	0.520	0.821

Trial 3. All treatments resulted in significant decreases in disease severity during 1977/78 (Table 5). The pre-plant root soak combined with an autumn foliar spray of LS 74783 was the most effective and was significantly better than the pre-

plant root soak alone. The following year, the results were similar. The second, spring, application of LS 74783 had no apparent effect but this was probably due to the lack of living leaf tissue. All the over-wintered leaves were moribund following snow and frost and only the earliest emerging leaves were green.

First year yields from all treated plots were significantly higher than from untreated ones the highest yielding being the pre-plant root soak combined with an autumn foliar spray of LS 74783 (Table 6). The root soak alone resulted in yields which were not significantly different from those following soil drenches of dichlofluanid or SN 66752. In the second year all yields were higher with treatment effects similar to those in the first year.

Table 7

The effect of LS 74783 and SN 66752 on the severity of red core disease in strawberries planted in April 1977 and lifted one year later (Trial 4)

Treatment	% a.i.	Mean % number of roots with red core (angular transformation)
None	-	52.0abc
SN 66752	0.7	
band spray (September)		62.6a
(November)		50.1abc
LS 74783		
pre-plant foliar spray	0.05	49.7abc
" " " " + Tween 80		45.4bc
" " " "	0.2	49.5abc
" " " " + Tween 80		41.5bc
pre-plant root soak (4 h)	0.05	54.1ab
	0.1	35.9c
	0.2	45.4bc
pre-plant total immersion (in/out)	0.2	35.7c
	S.E.±	5.07

Table 8

The effect of metalaxyl and LS 74783 on the severity of red core disease in strawberries planted in May 1978 and lifted one year later (Trial 5)

Time of fungicide application	Mean % number of roots with red core (angular transformation)	
	Metalaxyl soil drench (0.03% a.i.)	LS 74783 foliar spray (0.3% a.i.)
September	18.2e	39.9cd
October	7.8ef	44.5cd
November	18.4e	39.5cd
December	59.3b	48.2bc
September and March	0 f	32.9d
September (water soil drench)	78.3a	
	S.E.± 4.38	

Trial 4. None of the treatments controlled the disease although the decrease in severity following total immersion and one of the root soaking LS 74783 treatments approached significance (Table 7).

Trial 5. All treatments effectively decreased the severity of red core (Table 8). The metalaxyl soil drench was significantly better than the foliar spray with LS 74783 except when applied in December. In general, the level of control in 1978/79 by fungicide application in September, October, or November was similar. There was, however, a significantly lower level of control when metalaxyl was applied in December and the results with LS 74783 showed the same trend. Double treatments achieved the highest level of control particularly with metalaxyl.

DISCUSSION

The fungicides which were most effective in decreasing disease severity in each of the trials reported here were metalaxyl (Trial 5), LS 74783 (Trial 3), captafol (Trial 2), etridiazole and SN 41703 (Trial 1). The treatments which brought about the greatest improvement in yield were the SN 41703 soil drench treatment in Trial 1, the LS 74783 soil drench treatment in Trial 2, and the LS 74783 pre-plant root soak and foliar spray treatment in Trial 3. The inconsistencies associated with captafol and etridiazole on these heavily infested sites were not resolved by these experiments and require further investigation.

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USE OF DICARBOXIMIDE FUNGICIDES ON STRAWBERRIES AND POTENTIAL PROBLEMS OF RESISTANCE IN BOTRYTIS CINEREA

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Summary. During field trials carried out in the three seasons 1977 to 1979 the dicarboximide fungicides, vinclozolin and iprodione were as effective as dichlofluanid in reducing pre- and post- harvest infections of strawberries by *Botrytis cinerea*. During post-harvest storage, *Mucor* and *Rhizopus* developed on a greater proportion of berries from fungicide treated plants compared to fruit from untreated plants. In the 1978 season dicarboximide-resistant strains of *B. cinerea* were isolated from lesions of stored fruit. These strains were cross-resistant to both vinclozolin and iprodione, maintained their resistance during sub-culturing for 6 months on fungicide-free media and readily infected detached strawberries at all stages of maturity. Conditions were not favourable for infection of the fruit in the field by *B. cinerea* in the 1979 season and as no resistant strains were detected in that season control of the fungus by the dicarboximide fungicides was maintained.

Résumé. Pendant les épreuves dan le champ mis en train au cours des trois saisons culturales de 1977 à 1979 les fongicides "dicarboximide" vinchlozoline et iprodione se son montrés aussi efficaces que le dichlofluanid à effectuer la reduction d'infection des fraises par le *Botrytis cinerea*, autant avant qu'après la récolte. Tous les trois fongicides ont montré un développement plus avancé pour les espèces *Mucor* et *Rhizopus* pendant l'entreposage des fruits, ce qui ne se manifests pas dans le cas des fruits recueillis des terrains non traités. Au cours de la saison de 1978 on a isolé des souches résistantes aux "dicarboximides" trouvées sur les lesions subies par les fruits entreposés. Celles-ci ont montré une résistance croisée à vinchlozoline et iprodione et la résistance était maintenue au cours d'une culture repetée d'une durée de six mois, sur un milieu sans fongicide. Les fraises detachées des plantes dans toutes les conditions de maturité sont fort susceptibles à l'infection par les souches deja indiquées. Les conditions en 1979 n'ont pas favorisé l'infection des fruits au champ par *Botrytis cinerea*, et pendant cette saison aucune souche résistante a été découverte; donc le fongicide "dicarboximide" était totalement efficace.

INTRODUCTION

The major spoilage fungi of strawberries are the grey mould fungus, *Botrytis cinerea*, and the soft rotting fungi, *Mucor piriformis*, *Rhizopus sexualis* and *Rhizopus stolonifer* (Dennis, 1975; Jordan, 1973). Pre-harvest fungicides are presently used on strawberries to reduce both pre and post-harvest infection by *B. cinerea*, although the soft rotting fungi are insensitive to these treatments (Dennis and Davis, 1977). Recently dichlofluanid has been the most effective fungicide available for the control of B. cinerea on strawberries, especially since the widespread development of resistance in B. cinerea to the benzimidazole fungicides (Dennis, 1974). More recently the dicarboximide fungicides vinclozolin and iprodione have been developed, especially for the control of B. cinerea, on a range of crops including strawberries (Burgaud et al. 1975; Hess and Locher, 1975). Tests using detached strawberries indicated the potential effectiveness of both vinclozolin and iprodione in reducing the level of infection by B. cinerea (Dennis and Davis, 1977). This paper reports the results of subsequent field trials conducted to compare the effectiveness of vinclozolin and iprodione with dichlofluanid in reducing both pre and post-harvest infection of strawberries by B. cinerea, and studies on dicarboximide resistant strains of Botrytis which developed during these trials.

METHODS AND MATERIALS

Evaluation of dicarboximide fungicides in the reduction of pre and post-harvest rots of strawberries

The trials were conducted in 1977, 1978 and 1979 on plots situated at the Food Research Institute, each consisting of a single 9 m row of strawberry plants, cv. Cambridge Favourite. Replicate plots were sprayed three times during the flowering period with 0.05% a.i. iprodione (50% w.p.), 0.0375% a.i. vinclozolin (50% w.p.) and 0.1% a.i. dichlofluanid (50% w.p.) whilst other replicate plots were left unsprayed. The fungicides were applied at the rate of 2250 l/ha using a hand operated ASL Killaspray, commencing at early flower and repeated at 14 day intervals, with the third spray applied 23 days prior to the first harvest in 1977, 12 days in 1978 and 16 days in 1979.

At each harvest all ripe fruit from the plots was picked into 454 g fibre punnets, with fruit showing fungal infection being separated from healthy fruit. The number and weight of healthy and infected berries were recorded and the causal organism of fruit infections determined as described by Dennis (1975).

The fungi associated with freshly harvested healthy fruit and the rate of spoilage of samples of fruit stored at 10°C and 5°C, 95-97% r.h., were monitored at each harvest as described by Dennis and Mountford (1975). The spoilage fungi which developed during storage were determined as described above and in addition the point of initiation of the infection by B. cinerea on the berry was recorded.

Detection and characteristics of dicarboximide resistant strains of Botrytis cincerea

Strains of B. cinerea isolated from lesions which developed on fruit during storage in 1978 season were initially isolated onto Yeast Malt Agar (YMA) pH 4.8 as described by Dennis (1975). These strains were then transferred to Difco Bacto Potato Dextrose Agar (PDA) containing 5 ppm of either vinclozolin or iprodione, a normally lethal concentration of both fungicides (Eichhorn and Lorenz, 1978). Strains of B. cinerea which produced rapidly spreading colonies on this medium after 4 days incubation at 20°C were considered to be resistant. In 1979, the incidence of resistant strains responsible for spoilage was determined by placing infected tissue directly onto PDA and PDA containing 50 ppm of either vinclozolin or iprodione and incubating at 20°C for 4 days.

The degree of resistance of these strains was determined by inoculating each one onto PDA containing 0-10,000 ppm a.i. (50% w.p.) of either vinclozolin or iprodione and incubating at 20°C for 3 days.

The dicarboximide resistant strains of B. cinerea were sub-cultured every 3 to 4 days over a 6 month period onto PDA and PDA containing 5 ppm a.i. of either vinclozolin or iprodione to determine the stability of their resistance to these chemicals. All plates were incubated at 20°C.

The ability to infect strawberries of dicarboximide sensitive and resistant strains of B. cinerea was tested using glasshouse-grown strawberries (cv. Cambridge Vigour) and field-grown strawberries (cv. Cambridge Favourite). Detached berries (10 per treatment) at 3 stages of maturity (green, white and ripe) were sprayed to run-off with a solution containing 0.375 g/l a.i. of either vinclozolin or iprodione, whilst control berries were sprayed with water. The berries were allowed to dry for 1-2 hours before being inoculated with a 4 mm mycelial plug of B. cinerea taken from an actively growing colony on PDA. The fruit was then incubated in humid chambers for 4 days at 20°C.

RESULTS

Fungi associated with field infections

Botrytis cinerea was the predominant spoilage organism of fruit prior to harvest and the level of infection was consistently reduced by all three fungicides (Table 1). The Phycomycete fungi (Mucor piriformis, Rhizopus sexualis and R. stolonifer) infected less than 2% of fruit of all treatments in 1977 and less than 5% in 1978 and 1979. The treatment means and standard errors shown in Table 2 were derived from a non-orthogonal analysis of variance. The non-orthogonality was brought about by the introduction of a factor to take account of gross systematic variation observed in experimental plots.

Fungal flora of freshly harvested fruit

The predominant fungi associated with freshly harvested strawberries were the same as those reported by Dennis and Mountford (1975). The incidence of these fungi was unaffected by the fungicide treatments except B. cinerea, the viable count of which was reduced approximately tenfold by all three fungicides at every harvest in 1977 and 1978. In 1979 the viable count of B. cinerea recorded on the control untreated fruit was approximately tenfold lower than for the previous two seasons and was not consistently reduced by any of the fungicide treatments.

Spoilage during cold storage

Botrytis cinerea and Mucor piriformis were the dominant spoilage fungi of strawberries during storage of fruit at both 10°C and 5°C (Fig 1) in all three seasons. The incidence of spoilage by B. cinerea was consistently decreased by all three fungicides early in the season, however at the final harvest in all three seasons the incidence of B. cinerea was similar on the fungicide treated fruit as on the fruit from the untreated control. Fifteen per cent of Botrytis rots were seen to be initiated from the calyx of the berry whilst 85% developed from other points on the surface of the fruit (Table 3).

However the reduction in Botrytis cinerea achieved by all three fungicides allowed a greater development of rots caused by the Phycomycete fungi, especially M. piriformis, on fruit from these treatments. The incidence of rots caused by M. piriformis was greater in 1978 and 1979, especially early in the season, compared to 1977. Less than 10% of fruit stored at 10°C was infected by Rhizopus sexualis, except for one sample of fruit from the fourth harvest of the vinclozolin treatment in 1977 where 20% was infected. Rotting by R. sexualis was virtually eliminated by storage of the fruit at 5°C in all three seasons.

Table 1

Percentage fruit infected with B. cinerea at harvest

	Unsprayed	Dichlofluanid	Vinclozolin	Iprodione
1977	13	5	6	5
1978	30	9	8	13
1979	5	2	1	1

Table 2

Effect of fungicides on the proportion of healthy and infected berries at harvest

	Proportions				
	Unsprayed	Dichlofluanid	Iprodione	Vinclozolin	S.E.
1977					
Uninfected berries	0.881	0.929	0.941	0.929	0.017
Berries infected with *B.cinerea*	0.118	0.064	0.051*	0.060*	0.015
Berries infected with *Mucor* and *Rhizopus*	0.0013	0.0056	0.0073	0.0107*	0.0019
1978					
Uninfected berries	0.628	0.828*	0.806*	0.866*	0.032
Berries infected with *B.cinerea*	0.328	0.116*	0.138*	0.079*	0.032
Berries infected with *Mucor* and *Rhizopus*	0.031	0.047	0.046	0.050*	0.005
1979					
Uninfected berries	0.896	0.923*	0.959*	0.953*	0.014
Berries infected with *B.cinerea*	0.059	0.028*	0.012*	0.006*	0.009
Berries infected with *Mucor* and *Rhizopus*	0.041	0.047	0.025	0.038	0.006

* Treatment means significantly different from unsprayed at 5% level

Fig. 1 Development of spoilage fungi on stored strawberries - 1978 season

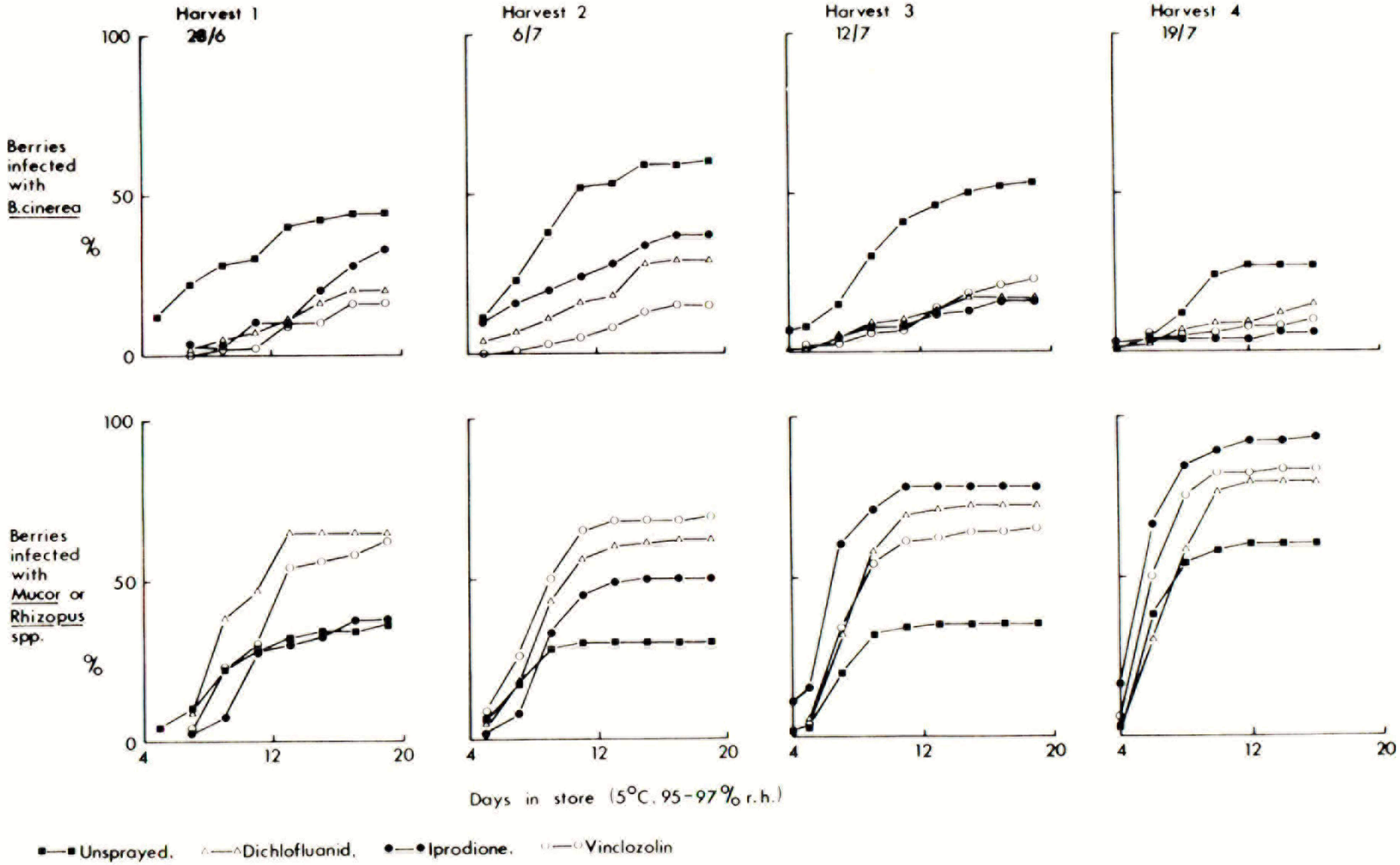

Table 3

Development of *B. cinerea* lesions during storage of fruit (1978)

		% developing from calyx	% developing on berry surface
Unsprayed	10°C	13	87
	5°C	13	87
Dichlofluanid	10°C	15	85
	5°C	25	75
Iprodione	10°C	15	85
	5°C	12	88
Vinclozolin	10°C	15	85
	5°C	15	85

Detection and characteristics of dicarboximide-resistant strains of *B. cinerea*

During storage of fruit from the vinclozolin and iprodione treatments some of the brown lesions, characteristic of infection by *B. cinerea*, developed abnormally slowly and appeared blackened. Isolations from the leading edge of 6 such lesions in 1978 confirmed the causal organism to be *Botrytis cinerea*. Subsequent sub-culturing showed that these 6 strains of *B. cinerea*, 5 from vinclozolin treated berries and 1 from an iprodione treated berry, grew well on PDA containing 5 ppm of either vinclozolin or iprodione whereas sensitive strains were completely inhibited. These 6 strains of *B. cinerea* were therefore considered to be resistant to the dicarboximide fungicides. No dicarboximide resistant strains of *B. cinerea* were isolated in 1979 during routine screening of all *Botrytis* lesions which developed on fruit prior to harvest or during storage.

All 6 resistant strains of *B. cinerea*, isolated from fruit lesions in 1978, were capable of growth on PDA containing up to 10,000 ppm a.i. iprodione, whilst all were completely inhibited by between 1,000 ppm and 5,000 ppm a.i. vinclozolin. Six sensitive strains, isolated from fruit treated with dichlofluanid, were completely inhibited by 1 ppm a.i. vinclozolin and 2 ppm a.i. iprodione.

Four of the 6 strains maintained resistance to the dicarboximide fungicides during 40 sub-cultures over 6 months on fungicide free medium. The other two strains gradually lost viability both in the presence and absence of the fungicide. One strain ceased to grow after 16 transfers and the second after 38 transfers. However, where the 6 isolates were not exposed to dicarboximide fungicides for four sub-cultures after isolation from the fruit only one exhibited resistance.

All 6 dicarboximide resistant strains of *B. cinerea* showed a similar ability to infect green, white and ripe strawberries from a mycelial inoculum, as six sensitive strains. The dicarboximide resistant strains also readily infected fruits sprayed with either vinclozolin or iprodione (both 0.0375% a.i.), whereas none of the sensitive strains infected these fruits.

DISCUSSION

The reduction in the level of rots caused by *B. cinerea* and the consequent improvement in yield of healthy fruits from plots treated with vinclozolin or iprodione was similar to the dichlofluanid treatment, whereas Pappas and Jordan (1977) found that on strawberries grown under polyethylene tunnels both vinclozolin and iprodione gave significantly better control of *B. cinerea* than did dichlofluanid. Trials carried out in the U.S.A. by Sarojak *et al* (1978) and Moore (1977) also showed that vinclozolin was highly effective in reducing the incidence of *B. cinerea* in the field resulting in an improved yield of healthy fruit compared with captan or untreated plots.

During post-harvest storage of the fruit both the vinclozolin and iprodione treatments again showed a similar reduction in the incidence of *B. cinerea* as dichlofluanid. This reduction in the incidence of *B. cinerea* during post-harvest storage of strawberries was also found by Moore (1977) and Defloor and Scholtens (1976). Although all fungicides reduced the level of infection of strawberries by *B. cinerea* during storage they did not affect the ratio of the sites of infection on the berries. The proportion of lesions originating from the calyx or from points all over the surface of the berries from all treatments was similar to that reported by Jarvis (1961) for infections in the field.

Spoilage by *M. piriformis* and *R. sexualis* was of only minor importance in the field, and was not affected by the fungicides. However, in agreement with Dennis and Davis (1977) the reduction of post-harvest infection by *B. cinerea* did allow greater development of rots by the Phycomycete fungi, especially *M. piriformis*. Freeman and Peppin (1976) also reported an increase in spoilage by *Rhizopus* spp. during post-harvest storage of fruit treated with iprodione, whilst Jordan (1973) made similar observation for storage of fruit grown under polyethylene tunnels.

The results of the present trials show that despite the presence of dicarboximide resistant strains of *B. cinerea*, both vinclozolin and iprodione were effective in reducing the level of pre and post-harvest infection of strawberries by this fungus, confirming their potential shown in experiments using detached berries (Dennis and Davis 1977). This is the first situation where strains of *B. cinerea* resistant to the dicarboximide fungicides, vinclozolin and iprodione, have been isolated from infected fruit.

In agreement with the findings of Leroux *et al* (1977) for resistant strains produced in the laboratory, the present strains showed cross-resistance to both fungicides and maintained their resistance over a 6 month period in the absence of the chemical. A similar stability of resistance was also shown for laboratory-produced strains by Schuepp & Kung (1978). However the loss of ability of two of the resistant strains to grow also shown for laboratory-produced strains by Lorenz and Eichhorn (1978), indicates a poor ability to survive when not continuously exposed to the fungicides.

There was no loss of ability by the dicarboximide-resistant strains of *B. cinerea* to infect strawberries, compared to wild type sensitive strains. Even though the resistant strains had been cultured on artificial media for almost a year they readily infected both untreated berries, and berries sprayed with 375 ppm a.i. of either vinclozolin or iprodione; the concentration recommended for field application. Schuepp and Kung (1978) also found that mycelium of laboratory-produced resistant strains of *B. cinerea* readily infected apples, forming lesions similar to wild type sensitive strains, whilst Lorenz and Eichhorn (1978) showed similar results when infecting detached leaves of *Vicia faba* from a spore inoculum. In contrast Gullino and Garribaldi (1979) found laboratory-produced resistant strains were less pathogenic to grape berries than sensitive ones.

Regular surveys by Eichhorn and Lorenz (1979) of commercial vineyards treated with vinclozolin or iprodione since 1976 have not revealed the presence of dicarboximide resistant strains of B. cinerea and control of infection of grapes has not diminished. Similarly reduction in the incidence of B. cinerea was maintained in all three years of our trials conducted on the same strawberry plots. Even though dicarboximide resistant strains of B. cinerea were isolated from berries in the second year, no build up of resistant strains was recorded in 1979. However, compared with 1978, conditions in 1979 were not very favourable for B. cinerea infections which were consequently much lower than for the previous year. The apparent absence of resistant isolates from infected fruit in 1979 may also indicate a poor ability of dicarboximide resistant strains to compete with sensitive strains in the field. Therefore although both vinclozolin and iprodione have shown very good control of B. cinerea on strawberries, the isolation of dicarboximide-resistant strains capable of infecting fruit at all stages of development does reveal the potential for loss of control of the fungus when conditions are favourable for its development.

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