Session 3

Benzimidazoles and Dicarboximides

Chairman	D SLAWSON
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EFFECT OF FUNGICIDE MIXTURES AND ALTERNATIONS ON DICARBOXIMIDE RESISTANCE DYNAMICS

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ABSTRACT

Botrytis cinerea populations initiated from spores, 5% of which were resistant to the dicarboximide fungicide vinclozolin, were cycled at 10 day intervals over a 30 day period on excised *Pelargonium* leaf tissue that had been sprayed once with single or mixtures of fungicides. Vinclozolin, whether used alone or in alternations or in mixtures with chlorothalonil, maintained the selection pressure in favour of resistance as a consequence of its long residual activity. It was concluded that once resistance is present, dicarboximide fungicide use should cease in greenhouses, particularly when disease pressure is high.

INTRODUCTION

Botrytis cinerea infects a wide range of greenhouse plants as a result of conidiospore germination and penetration or hyphal growth from infected tissue that has fallen onto healthy tissue. Spores do not necessarily germinate immediately and can remain dormant on plant tissue for weeks before germinating and penetrating (Salinas *et al.*, 1989). The few fungicides effective against *Botrytis* in greenhouses are used repeatedly to protect crops, particularly when disease pressure is high. When populations of *B. cinerea* are repeatedly exposed to dicarboximide fungicides, selection for resistance can be rapid (Gullino *et al.*, 1983, 1984; Locke and Fletcher, 1988; Northover and Matteoni, 1986). The presence of significant numbers of dicarboximide resistant *Botrytis* spores has been documented to occur in greenhouses where dicarboximides have never been used (Northover and Matteoni, 1986; Moorman and Lease, unpublished). It is presumed that this is a result of the movement of apparently healthy plants, especially vegetatively propagated plants such as *Pelargonium* harbouring fungicide-resistant spores, from greenhouses where dicarboximides were employed.

It appears that carrying dicarboximide resistance is not significantly detrimental to the overall fitness of the fungus (Faretra et

al., 1989; Gullino *et al.*, 1982; Moorman and Lease, 1992; Vali and Moorman, 1992). While the dicarboximide resistant portion of the *Botrytis* population in the greenhouse persists even after use of this class of fungicides ceases (Locke and Fletcher, 1988), there can be fluctuations in the fungicide resistant:sensitive ratio in the spore population during the year. Katan and Ovadia (1985) noted that in greenhouses during the summer dormant season, when few plants are grown in Israel, the dicarboximide resistance proportion in the population declined and was relatively low early in the subsequent growing season. This allowed the successful use of dicarboximides once or twice late in the growing season. Although this practice fostered an increase in the proportion of resistant spores, it was postulated that conditions which were not defined, would suppress the resistant portion of the population during the following summer thereby allowing growers to apply dicarboximides late the next season. A similar decline in the dicarboximide resistance frequency was found to occur during the winter dormancy of strawberries grown in greenhouse-like plastic tunnels (Hunter *et al.*, 1987). Thus, there appear to be as yet undefined environmental selection pressures either against the resistant or in favour of the sensitive portion of the population.

Since populations with high proportions of resistance can be correlated with the frequency of fungicide use (Northover, 1988), the goal of management practices has been to limit the exposure of the fungus to the resistance selection pressure of the fungicide by applying fewer sprays per season or mixing or alternating dicarboximides with fungicides that have different modes of action. The effectiveness of alternations depends on selection pressures against the slightly less fit resistant portion of the population to reduce the frequency of resistance. The efficacy of mixtures depends on the dicarboximide to reduce or eliminate the sensitive portion of the population while the partner chemical holds in check the resistant portion. However, Gullino and Garibaldi (1987) and Vali and Moorman (1992) demonstrated that once dicarboximide resistance is present, dicarboximides should not be used. Neither mixtures nor alternations with non-dicarboximide fungicides significantly delayed the build-up of the dicarboximide-resistant portion of the population. In our previous work (Vali and Moorman, 1992), methods to cycle a population of *Botrytis* on plant tissue involved inoculation as soon as the spray had dried. Spores subsequently developing on this treated tissue were harvested and assayed to determine the percentage of resistant spores and were also used to inoculate newly treated plant tissue. Those experiments tested the situation where the fungus was continually exposed to freshly applied active ingredient. We have since modified the procedures to determine the effects of various chemicals when inoculation is done 0,

10, or 20, or 30 days after spraying in order to simulate the situation where inoculum lands on the treated tissue after the activity of the fungicide has begun to decline. In this way, the effect of the declining residual efficacy on the selection for resistance in the population was examined.

METHODS

To identify the combined effects of fungicide use patterns and declining residual activity on the population dynamics of resistance in *Botrytis*, the previously described *Pelargonium* leaf disk technique (Vali and Moorman, 1992) was modified slightly. Briefly, 12 wk old seed geraniums were sprayed only once with water, a fungicide, or a mixture of fungicides. Leaf disks were excised the day following treatment (day 0) and inoculated with a population of *Botrytis* spores, 5% of which were resistant to vinclozolin. After a 10 day incubation period, the number of infected disks was recorded as a measure of disease and the resistant:sensitive ratio in the spore population was ascertained by plating some spores from each treatment on fungicide-amended (20 µg vinclozolin/ml) and fungicide-free agar and counting the number of germinating spores. Spores were then used to inoculate leaf disks excised from the remaining plants that had been treated at the beginning of the experiment. In this manner, tissue was inoculated 0, 10, 20, and 30 days after spraying with spores harvested from the previously inoculated leaf disks. Fungicide-free tissue was inoculated with spores from each treatment on each inoculation date to be certain that the pathogenicity of the populations being carried in each treatment did not change. The experiment was done twice.

RESULTS AND DISCUSSION

As in previous research (Vali and Moorman, 1992), the use of vinclozolin alone and in a mixture with another fungicide favoured the rapid increase in the proportion of resistant spores (Table 1). The long residual activity of vinclozolin maintained the selection pressure favouring resistance over the 30 day period after treatment as indicated by the level of disease that developed (Table 2) and the high percent of resistant spores in all treatments involving vinclozolin. The 10 day interval between exposures to vinclozolin afforded in the alternation with chlorothalonil did not allow a significant decline in the percentage of resistant spores. Thus, these experiments indicate that under conditions of high disease pressure, once resistance is present in the population, dicarboximides should not be used in the greenhouse.

Beever *et al.* (1991) postulated that with a particular fungicide use pattern, the frequency of resistance will stabilize. This theory is based on the assumption that the selection for resistance exerted by the fungicide is balanced by the selection against the slightly less environmentally fit resistant part of the population. The problem indicated by the experiments reported here is that the selection pressure of dicarboximides is so strong, the high residual activity so prolonged, and the differences in fitness between resistant and sensitive strains of *Botrytis* so slight (Moorman and Lease, 1992a , 1992b; Vali and Moorman, 1992) that the frequency of resistance may stabilize at a level too high for dicarboximides to be effective.

It remains to be determined whether there is a proportion of resistance to dicarboximides in *Botrytis* populations at which dicarboximides can still be useful in greenhouses. While some (Beever and Brien, 1983; Locke and Fletcher, 1988) report good control despite a resistance frequency of 38%, others (Gullino and Garibaldi, 1987) report relatively poor control associated with a 10% frequency. If an acceptable resistance level can be identified, along with specifications of the conditions or treatments that force the resistant portion of the population below that level, it may be possible to use dicarboximide fungicides to a limited extent, despite the presence of resistance. The grower must clearly understand, however, that the resistance frequency will sharply increase after treatment. Where growers are unable or unwilling to use dicarboximides judiciously, it may be wisest to avoid dicarboximide use and recommend the application of mixtures of fungicides to which *B. cinerea* is not resistant and which have been demonstrated to provide the initial protection and long residual activity approaching that obtained with dicarboximides (Moorman and Lease, 1992a, 1992b, 1993).

Table 1. Mean percent of *Botrytis cinerea* spores carrying resistance to vinclozolin (dicarboximide) fungicide harvested from leaf disks inoculated the indicated number of days after spraying and incubated 10 days. Five percent of the initial population of spores used on day 0 were resistant to vinclozolin.

Treatment	Days after spraying			
	0	10	20	30
No fungicide (water)	9	2	1	1
vinclozolin[1]	99	99	98	95
chlorothalonil[2]	4	13	44	24
mancozeb[3]	59	98	99	96
vinclozolin[1] + chlorothalonil[2]	91	95	99	99
Alternation of chlorothalonil[2] (c) and vinclozolin[1](v)	(c) 4	(v) 96	(c) 98	(v) 99

1 vinclozolin, 0.6 g AI/litre (Ornalin 50% WP)
2 chlorothalonil, 1.2 g AI/litre (Daconil 2787, 4.17F)
3 mancozeb, 0.5 g AI/litre (Dithane F-45)

Table 2. Mean percent disease based on the number of leaf disks infected per 50 inoculated with *Botrytis cinerea* in each of two experiments.

Treatment	Days after spraying			
	0	10	20	30
No fungicide (water)	91	97	66	100
vinclozolin[1]	62	83	62	95
chlorothalonil[2]	30	17	22	47
mancozeb[3]	52	60	64	90
vinclozolin[1] + chlorothalonil[2]	10	28	19	61
Alternation of chlorothalonil[2] (c) and vinclozolin[1](v)	(c) 30	(v) 33	(c) 14	(v) 96

1 vinclozolin, 0.6 g AI/litre (Ornalin 50% WP)
2 chlorothalonil, 1.2 g AI/litre (Daconil 2787, 4.17F)
3 mancozeb, 0.5 g AI/litre (Dithane F-45)

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RESISTANCE OF *HELMINTHOSPORIUM SOLANI* TO THIABENDAZOLE IN RELATION TO DIFFERENT STRATEGIES OF FUNGICIDE USE DURING SEED POTATO PRODUCTION.

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ABSTRACT

A single application of thiabendazole to seed tubers resulted in 54% of *Helminthosporium solani* isolates from daughter tubers being resistant (IC50 >100 mg/l) to the fungicide. A further application of thiabendazole to these tubers resulted in 24% of isolates from the progeny crop being resistant at the Aberdeen site and 32% at the Edinburgh site. Application of thiabendazole in a mixture with imazalil appeared to delay the formation of resistant isolates.

INTRODUCTION

Thiabendazole has been widely used since the mid 1970's in the UK as a treatment for both ware and seed potatoes. Applied to tubers immediately after harvest the incidence of silver scurf (*Helminthosporium solani*), skin spot (*Polyscytalum pustulans*), gangrene (*Phoma foveata*) and dry rot (*Fusarium* spp.) in stored ware or seed tubers is reduced (Hide and Cayley 1983; 1985; 1987). When used as a seed tuber treatment, applied either as a spray during storage or as a dust pre-planting the incidence stem canker and black scurf (*Rhizoctonia solani*), silver scurf and skin spot in the growing crop is reduced (Hide *et al.*, 1980). However, since 1985 resistance to thiabendazole has been found in isolates of both *H. solani* and *P. pustulans* (Hide et al., 1988; Carnegie and Cameron, 1992; Burgess et al., 1993).

Other fungicides are available for the treatment of seed potato tubers. However, the availability of treatments for stored ware potatoes is limited to thiabendazole in the UK. Thus thiabendazole remains widely used despite problems with resistance in economically important pathogens.

The present study aims to determine the effect of fungicide applications to seed tubers on the level of control of silver scurf and the incidence of resistance to thiabendazole over three generations of seed production.

MATERIALS AND METHODS

Field trials

On 15 September 1990, a stock of seed potatoes (cv. Pentland Squire, VTSC2 Scottish Seed Potato Classification Scheme) was machine harvested at Tillycorthie Farm, Aberdeenshire and obtained for use in this study. This seed stock had been multiplied from supplied clones supplied by The Scottish Office Agriculture and Fisheries Department (SOAFD) at Tillycorthie Farm and had been treated with 2-Aminobutane during previous storage seasons. However, no other fungicide seed treatments had been applied to the seed tubers of this stock, or others, during multiplication at Tillycorthie Farm.

The crop was sized over square riddles and a quantity of the seed fraction (35-55 mm diameter tubers) was used for these trials. The next day liquid fungicides were applied as a hydraulic spray (2.2 litres diluted product applied per tonne) as tubers passed on a roller table. 2-Aminobutane was applied as a fumigant some four to five weeks later.

The fungicides applied were :-

1. Untreated (UT). No fungicide applied to tubers.
2. Thiabendazole (TBZ). 90 ml/t (40.5 mg AI kg^{-1}) 'Storite Flowable' (MSD Agvet, Hoddeston Herts.)
3. Thiabendazole + imazalil (TBZ+IM). 100 ml/t (30 + 10 mg AI kg^{-1}) 'Extratect' (MSD Agvet, Hoddeston, Herts.)
4. Imazalil (IM). 100 ml (10 mg AI kg^{-1}) 'Fungazil 100SL' (Rhone Poulenc Agriculture Ltd, Brentwood Essex)
5. 2-Aminobutane (2AB). 200 mg 2AB kg^{-1} (Chemical Spraying Co. Ltd., Perth)

Half the tubers of each treatment were stored at Tillycorthie Farm with the remainder transported to Boghall Farm, The Scottish Agricultural College, Edinburgh. At each site, the tubers were stored under commercial storage conditions in closed paper potato sacks. The temperature was maintained at *c.* 4°C throughout the storage period at both sites.

The tubers were planted as unreplicated large plots at each site in May 1991 (Table 1). The husbandry of plots was as recommended for the production of a specialist seed potato crop under local conditions. The plots were individually machine harvested and graded before treatment with the same fungicide as applied to the mother tubers. Samples of tubers were removed before treatment for resistance testing (Aberdeen only) and disease assessment. Treatments were applied by hydraulic spray at Aberdeen. At Edinburgh an electrostatic sprayer (Microstat, Horstine Farmery) was used to apply fungicide treatments (1.1 l of diluted product was applied per tonne). Treated seed tubers were stored in closed paper bags at each site under commercial storage conditions (*c.* 4°C) until planting as replicated block field trials in May 1992 (Table 1). Husbandry of the plots at each site was as recommended for a specialist seed potato crop.

After harvest in September 1992 samples from each plot were removed for disease assessment and resistance testing. Further fungicide treatments were applied and tubers were stored and planted as a

TABLE 1. Trial details and timetable of operations at Aberdeen and Edinburgh sites.

Growing season	Operation	Aberdeen	Edinburgh
	Harvest of initial seed stock	15/9/90	-
	Treatments applied	16/9/90	-
1st Growing season	Trial Planted	10/5/91	1/5/91
	Site	Udny Green Aberdeenshire	Boghall Farm Midlothian
	Plot size	45m x 2 drills	20m x 8 drills
	Replication	one	one
	Harvest	26/9/91	4/10/91
	Treatments applied	27/9/91	21/10/91
2nd growing season	Trial planted	25/5/92	7/5/92
	Site	Udny Green, Aberdeenshire	Boghall Farm, Midlothian
	Plot size	7.5m x 4 drills	7 m x 4 drills
	Replication	five	six
	Harvest	26/9/92	9/10/92
	Treatments applied	9/10/92	13/10/92
3rd growing season	Trial planted	7/5/93	5/5/93
	Site	Longside, Aberdeenshire	Boghall Farm, Midlothian
	Plot size	5m x 4 drills	6m x 4 drills
	Replication	six	six
	Harvest	18/10/93	21/10/93

replicated block field trial in May 1993. The husbandry of each site was as recommended for the production of a ware potato crop. Samples of tubers were removed after harvest for disease assessment.

The yield and number of tubers in each plot was measured for each replicated field trial.

Disease assessment

At harvest samples of tubers from each plot were washed and the percentage surface area infected with silver scurf was estimated by visual assessment. The results are presented as the % incidence (% tubers infected with silver scurf) and the % severity (mean % of the surface area infected with silver scurf). Assessments were also conducted on samples of tubers stored (as harvest) in closed paper sacks at 4°C for *ca.* 6 months. Samples of 100 (1991), 50 (1992) or 25 (1993) tubers were assessed from each plot.

Sensitivity of *H. solani* to thiabendazole

Samples of tubers were stored, in sealed paper bags, from harvest at 8°C until isolations of *H. solani* were made between 2 and 4 months later. Isolates were taken from each treatment at the Aberdeen site

after harvest 1991 and from both the Aberdeen and Edinburgh sites after harvest 1992. Infected tubers were washed under tap water then incubated in humid conditions at 15°C for 14 days. After this period, conidia of *H. solani* could be removed using a sterile needle. These were streaked onto malt extract agar containing streptomycin and penicillin (2700 and 626 mg/l respectivley). The plates were incubated at 20°C for 7 days, after which time single spore isolates of *H solani* were selected and transferred onto buffered (pH 7) plates of malt extract agar and incubated (20°C) for 21 days before inoculation onto test media. Only one isolate was obtained from each infected tuber. Each isolate was tested for sensitivity to TBZ (as 'Storite Flowable') at 0 mg/l, 5 mg/l and 100 mg/l, using the method of Hide *et al.* (1988). The results are expressed as the concentration of TBZ required to reduce the colony diameter by 50% when compared to colonies growing on agar without TBZ (IC50).

Residues of thiabendazole present on tubers

Samples for residue analysis were stored at 4°C until analysis was commenced. Analysis for TBZ was by the method of Martindale (1988). Sub-samples of whole tubers were extracted with acetone/methanol and the extracts subjected to mild acid hydrolysis. After solvent extraction clean-up, the extracts were made alkaline and TBZ extracted into dichloromethane. After concentration, TBZ was determined by high performance liquid chromatography using a 25 cm CPS column and fluorescence detection.

RESULTS

Residues of thiabendazole on tubers after treatment

Residues of TBZ ranged from 1.01 to 6.20 mg/kg (Table 2). This represents between 2.5 and 16.6% of the target dose. The residues obtained after harvest in 1992 were generally less than those obtained in 1991. Residues at Aberdeen and Edinburgh sites were similar despite the different application methods used at each site (hydraulic and electrostatic respectively).

TABLE 2. Results of analysis of tuber samples for residues of thiabendazole after treatment in 1991 and 1992.

Year	Site	Treatment	Thiabendazole (mg/kg)	Percentage of target dose
1991	Aberdeen	TBZ	4.00	9.9
		TBZ+IM	4.20	14.0
	Edinburgh	TBZ	6.20	15.3
		TBZ+IM	5.00	16.6
1992	Aberdeen	TBZ	2.33	5.8
		TBZ+IM	1.77	5.9
	Edinburgh	TBZ	1.01	2.5
		TBZ+IM	1.61	5.3

Silver scurf on progeny tubers

Aberdeen

After storage of tubers harvested in 1991 the only treatment that appeared to reduce silver scurf were those that included imazalil (Table 3). However, there was no replication at this stage of the trial. After harvest 1992, silver scurf levels were generally higher. Treatment IM significantly ($P<0.05$) reduced both the incidence and severity of disease when compared to TBZ and UT treatments. There was no difference in the amount of silver scurf observed on TBZ and UT tubers. Application of TBZ+IM resulted in intermediate levels of disease control. There was a large and significant increase in the amount of disease following 2AB treatment. The levels of disease present on tubers did not increase during storage and there were no significant differences between treatments after storage. After harvest 1993 only low levels of disease were observed.

TABLE 3. Percentage incidence and severity of silver scurf at harvest and after storage at 4°C, Aberdeen site

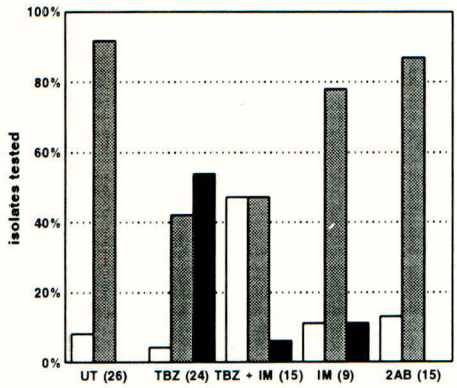
Treatment	After storage 1991-1992		Harvest 1992		After storage 1992-1993		Harvest 1993	
	Incidence	Severity	Incidence	Severity	Incidence	Severity	Incidence	Severity
UT	72	6.0	50	14.9	20	7.5	4	1.1
TBZ	88	9.8	54	15.4	45	12.7	13	2.1
TBZ+IM	26	2.1	38	11.0	27	7.8	13	1.6
IM	60	4.2	17	5.3	32	10.9	11	1.6
2AB	80	8.5	73	33.6	42	14.5	4	0.2
LSD ($P<0.05$)	-	-	11.6	9.01	NS[1]	NS	NS	NS

[1] NS Not significant

Table 4 Percentage incidence and severity of silver scurf at harvest and after storage at 4°C, Edinburgh site

Treatment	After storage 1991-1992		Harvest 1992		After storage 1992-1993		Harvest 1993	
	Incidence	Severity	Incidence	Severity	Incidence	Severity	Incidence	Severity
UT	60	3.3	0	0	19	3.1	77	9.4
TBZ	26	1.3	0	0	16	2.2	78	10.9
TBZ+IM	50	7.1	0	0	14	1.7	67	5.9
IM	30	2.4	0	0	8	0.9	69	7.2
2AB	34	2.5	0	0	1	2.1	62	5.1
LSD ($P<0.05$)	-	-	-	-	6.1	0.99	18.5	3.60

(a)



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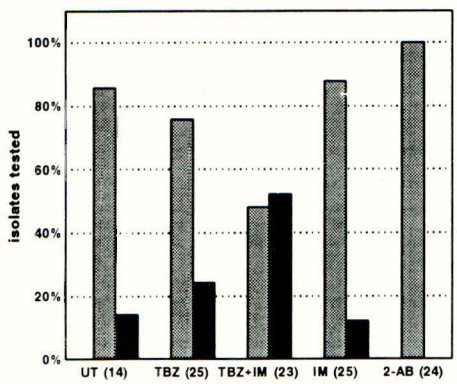

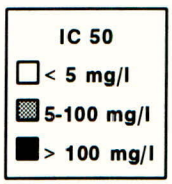

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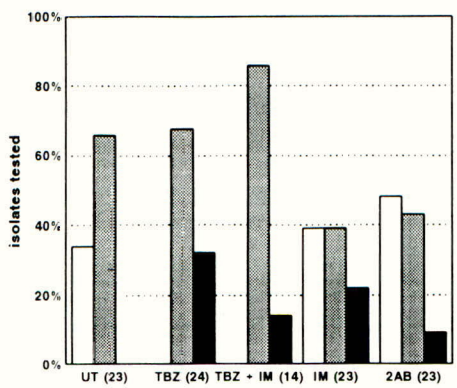

FIGURE 1. Percentage of isolates from each treatment with an IC 50 of < 5 mg/l, 5-100 mg/l or > 100 mg/l. (a) Aberdeen after harvest 1991 (b) Aberdeen after harvest 1992 and (c) Edinburgh after harvest 1992. Number of isolates tested given in parenthesis.

Edinburgh

At the Edinburgh site disease levels have remained generally low throughout the trial (Table 4). After storage 1991-1992, TBZ treatment appeared to result in least disease (no replication at this stage of the trial). At harvest 1992, no silver scurf was observed. However, during the subsequent storage period, disease did develop but remained at low levels. After harvest 1993, higher levels of disease were observed. There was least disease where mother tubers had been treated with TBZ+IM or 2AB. Treatment with TBZ resulted in no difference from the UT control.

Sensitivity of *H solani* isolates to thiabendazole

Aberdeen site after harvest 1991

After a single growing season, most isolates from untreated tubers were of intermediate sensitivity to TBZ (IC50 5-100 mg/l) (Figure 1a). After treatment of mother tubers with TBZ 54% of isolates were fully resistant (IC50 >100 mg/l). The sensitivity of isolates from 2AB and IM treatments were similar to the untreated control. After application of TBZ+IM a single isolate (6%) was found to be fully resistant to TBZ.

Aberdeen site after harvest 1992

After harvest 1992, no isolates (from a total of 107 tested) fully sensitive to TBZ were recovered from tubers of any treatment (Figure 1b). Isolates fully resistant to TBZ were recovered from TBZ (24% of isolates), TBZ+IM (52%) and IM (12%) treated tubers. The proportion of isolates with an IC50 >100 mg/l had decreased in TBZ treated tubers but increased in those treated with TBZ+IM when compared to the previous season. A small proportion (12%) of isolates from imazalil treated tubers had an IC50 >100 mg/l.

Edinburgh site after harvest 1992

In contrast to results from the Aberdeen site a proportion of isolates from all treatments in which TBZ had not been included were fully sensitive to TBZ (IC50 <5 mg/l) (Figure 1c). However, resistant isolates (IC50 >100 mg/l) were obtained from all treatments except the UT control. The range of sensitivities was greater for IM and 2AB treatments. Treatment with TBZ resulted in 32% of isolates being fully resistant to TBZ. The proportion was reduced to 14% when TBZ+IM was applied.

Yield and tuber numbers

Assessments of yield and tuber number were made only after harvest of replicated field trials (1992 and 1993), the results are given in Table 5. At Aberdeen in 1993, both TBZ+IM and IM treatments significantly ($P<0.05$) increased the total yield when compared to the UT control. However, TBZ applied alone did not increase the yield. At Edinburgh (1993), treatment IM significantly reduced the total yield when compared to the UT control. However, the yield of other treatments was not significantly different from the UT control. At this site in both 1992 and 1993, significantly ($P<0.05$) less tubers were produced following treatment with IM than TBZ.

TABLE 5. Yield (t/ha) and tuber number (1000's/ha) at Aberdeen and Edinburgh sites in 1992 and 1993.

Treatment	Aberdeen				Edinburgh			
	1992		1993		1992		1993	
	t/ha	000's/ha	t/ha	000's/ha	t/ha	000's/ha	t/ha	000's/ha
UT	43.9	449	54.6	458	58.6	367	49.6	349
TBZ	47.2	457	57.0	447	61.5	393	52.5	395
TBZ+IM	45.9	437	60.8	445	59.4	365	51.6	368
IM	46.7	479	61.4	432	58.0	343	45.7	344
2AB	41.0	425	59.1	432	55.0	329	51.2	388
LSD (P>0.05)	5.48	51.8	5.13	49.7	3.70	37.70	3.56	35.1

DISCUSSION

In these trials a large proportion of *H. solani* isolates, across all treatments and at both sites were found to be intermediate in their sensitivity (IC50 5-100 mg/l) to thiabendazole. Although no isolates were taken from the initial mother crop (1990 harvest), the majority of isolates (92%) from the untreated plot after one season were intermediate in their sensitivity to TBZ. The remaining isolates were fully sensitive to TBZ (IC50 <5 mg/l). Hide *et al.* (1988) also reported isolates of intermediate sensitivity. This result would appear to confirm that there is a large range of sensitivity to TBZ within populations of *H. solani*, which is unusual in fungal resistance to benzimidazole fungicides (Hide *et al.*, 1988).

A single treatment of mother tubers with TBZ resulted in 54% of isolates being fully resistant to the fungicide at the Aberdeen site. This is a similar proportion to that reported in previous studies (Hide *et al.*, 1993). Treatment with a mixture of TBZ and imazalil resulted in fewer resistant isolates than treatment with TBZ alone. The increased sensitivity to the fungicide was also reflected in the degree of silver scurf control observed after treatment with the mixture of TBZ and imazalil.

In the second year of TBZ treatment, a smaller proportion of isolates were resistant to TBZ (24% at Aberdeen and 32% at Edinburgh). This result contrasts with that of Hide *et al.* (1993) who recorded almost complete resistance to TBZ after two treatments. Treatment of tubers with a mixture of TBZ and imazalil resulted in a higher proportion of resistant isolates at Aberdeen but fewer at Edinburgh.

Residues of TBZ on tubers after treatment were recorded after treatment in 1991 and 1992. In both years, the residues achieved were low (2.5% to 16.6% of target dose). These low residues may be the reason that the levels of resistance did not increase in the second season of these trials. Hide *et al.* (1993) dipped tubers in differing concentrations of fungicide and concluded that resistance was most likely to develop where higher concentrations of TBZ had been used. The work reported in this paper would seem to support this conclusion. The residues achieved on tubers in this study are similar to those achieved in commercial practice. Thus over the two years of these trials the

degree of resistance observed may be similar to that present in commercial practice.

The higher than expected degree of sensitivity to TBZ could also be explained by an inherent inability of the *H. solani* population to become resistant to TBZ. Alternatively, it could be due to introduction of 'wild-type' *H. solani* from outside sources, although efforts were made throughout this trial series to reduce such sources of contamination. Some cross-contamination of treatments would appear to have occurred in these experiments with resistant isolates being isolated from treatments to which no TBZ had been applied. Cross-contamination would also occur in commercial practice and thus this study may give a realistic picture of the restsistance situation in commercail stores.

TBZ treatment of mother tubers resulted in no appreciable control of disease, despite the fact that not all the isolates obtained from daughter tubers were resistant to the fungicide. In general, treatments containing imazalil performed better. Similar results were reported by Hall and Hide (1992).

The results reported here indicate that resistance to TBZ by *H. solani* is perhaps not as widespread as previously considered due to the fact that in commercial practice good coverage of tubers with fungicides is often not achieved. However, it does appear from these results that the level of silver scurf control achieved with TBZ is not sufficient to justify its inclusion in seed production. Based on these results and those of Hide *et al.* (1993), the use of formulations of TBZ and imazalil reduce the build-up of resistance but does not prevent it developing entirely.

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