BENZIMIDAZOLE AND DICARBOXIMIDE RESISTANCE IN PATHOGENS OF STORED APPLES AND PEARS

A.M. BERRIE[1], I. KOOMEN[2]

ADAS, Boxworth, Cambridge, CB3 8NN, UK

ABSTRACT

Between 1988 and 1993, during the storage period for apples and pears (December-April), regular visits were made to commercial fruit packhouses in England when fruit was being graded. Samples were taken of the major fungal pathogens causing rotting: *Botrytis cinerea*, *Monilinia fructigena*, *Nectria galligena*, *Gloeosporium* spp., *Penicillium expansum* and *Phytophthora syringae*. Tests for fungicide response were conducted on the cultures established. More than 50% of *B. cinerea* isolates and over 95% of *P. expansum* isolates were consistently found to be resistant to benzimidazole fungicides. No resistance was detected in *M. fructigena* or *N. galligena* isolates. Resistance of *Gloeosporium* spp. to benzimidazole fungicides was detected (approx. 25% of isolates) in the last three years of the study. Resistance of *B. cinerea* to dicarboximide fungicides was detected, but at a low incidence. A very low incidence of *P. syringae* isolates resistant to metalaxyl was also detected. Strategies for minimising the development of fungicide resistance in stored fruit pathogens are discussed.

INTRODUCTION

The harvesting period for most British apples and pears is restricted to the period September to mid October. Efficient storage is therefore essential in order to allow the fruit industry to regulate its supply of fruit onto the UK market for most of the year and enable it to compete with imports of high quality fruit from other EC countries and outside Europe. Losses due to post-harvest rots can seriously affect profitability and their effective control is an integral part of efficient storage. In the 1960's, losses in store were more than 30 per cent from some orchards as a result of infection by *Gloeosporium* spp. (Preece, 1967). Fungicidal control was applied as pre-harvest sprays, principally of protectant fungicides, such as captan. More recent surveys of rotting in stored fruit (Berrie, 1989, 1992a) initially in fungicide-treated fruit (1979-91) and latterly in untreated fruit (1991-93), have indicated that while economic losses (> 2 per cent) occur in most seasons, they are not as high as those recorded previously. This is partly due to improvements in fruit mineral composition and in storage techniques that increase the ability of the fruit to resist fungal attack,

[1] Present address: Horticulture Research International, East Malling, West Malling, Kent ME19 6BJ, UK
[2] Present address: IPO-DLO, Postbus 9060, 6700 GW Wageningen, The Netherlands

but also to the introduction of post-harvest fungicide dips/drenches of initially benzimidazoles in the 1970's and later of metalaxyl (in combination with carbendazim as 'Ridomil mbc 60 wp'). Benzimidazole fungicides were introduced mainly for control of *Gloeosporium* spp. and metalaxyl for the control of *Phytophthora syringae* which emerged as a problem in the 1970's following changes in orchard cultural practices (Edney, 1978; Upstone, 1978). The main fungi responsible for losses in stored apples and pears are shown in Table 1 and the fungicides used to control these rots are given in Table 2.

TABLE 1. Principle fungal species responsible for losses due to rotting in apple (cvs Cox and Bramley) and pear in the U.K.

Apple cv. Cox	Apple cv. Bramley	Pear
Botrytis cinerea	*Monilinia fructigena*	*Botrytis cinerea*
Monilinia fructigena	*Penicillium expansum*	*Monilinia fructigena*
Nectria galligena		
Gloeosporium spp.		
Phytophthora syringae		
Penicillium expansum		

TABLE 2. Fungicides used or previously used as post-harvest dips/drenches on apples and pears in the UK and the fungi controlled.

Active ingredient	Chemical Product	B	M	N	Gl	Ps	Pe
benomyl[1]	'Benlate'	+r	+	+	+	-	+r
carbendazim	e.g. 'Bavistin', 'Derosal'	+r	+	+	+	-	+r
captan	'PP Captan 83'	-	-	-	-	+	-
iprodione[2]	'Rovral Flo'	+	+	-	-	-	-
metalaxyl + carbendazim	'Ridomil mbc 60 wp'	+r	+	+	+	+	+r
thiophanate methyl	'Mildothane'	+r	+	+	+	-	+r
vinclozolin[3]	'Ronilan'	+	+				

Key to fungi

B = *B. cinerea*, M = *M. fructigena*, N = *N. galligena*, Gl = *Gloeosporium* spp., Ps = *Phytophthora syringae*, Pe = *Penicillium* spp.

Notes

\+ Fungi controlled
+r Sensitive isolates only controlled
[1] No longer recommended
[2] Off-label approval on pears only
[3] No longer recommended and use withdrawn in 1990

Between 1979 and 1993 surveys of rotting in stored fruit were conducted, mainly in south-east England. The main objective of these studies was to identify the fungi responsible for rotting in store and to monitor the resistance of these fungi to benzimidazole, dicarboximide and acylalanine fungicides. The results of the surveys of rotting and the earlier study of the incidence of fungicide resistance have been reported elsewhere (Berrie, 1989, 1992a). The purpose of this paper is to report the results of the study on fungicide resistance carried out between 1988 and 1993.

MATERIALS AND METHODS

Source of fungal isolates (1988-93)

Sixteen commercial packhouses and three fruit co-operatives in the south-east and east of England were visited regularly (weekly to some) during the storage period between December and April. Observations were made on fungal rots of apple cvs. Cox and Bramley's Seedling and pear cv. Conference during grading of fruit out of store, by examining one hundred rejected rotted fruit of each cultivar and identifying the rots visually. Samples of fruits with *Botrytis cinerea*, *Monilinia fructigena*, *Nectria galligena*, *Gloeosporium* spp., *Phytophthora syringae* and *Penicillium expansum* were collected. In 1990-93 visits to commercial packhouses in Gloucestershire, Somerset and Herefordshire were also included. Up until 1991 observations on rotting in store were mainly on fruit that had been treated with a fungicide post-harvest. Between 1991 and 1993 samples of rots were also obtained from a more specific survey of rotting in stored Cox apples and Conference pears which were treated or untreated with a fungicide post harvest. In 1991/92 and 1992/93 this survey covered a total of 47 and 76 Cox orchards and 24 and 8 Conference orchards respectively, located mainly in the south-east and east of England, but also from Herefordshire.

Tests for fungicide resistance

Fungal isolates of *B. cinerea*, *M. fructigena* and *P. expansum* were obtained by culturing from rotted fruit onto Potato Dextrose agar (PDA) amended with 100 ppm streptomycin to inhibit bacterial growth. *N. galligena* and *Gloeosporium* spp. were cultured on PDA amended with 2 ppm vinclozolin to inhibit growth of *B. cinerea* and *Penicillium*; and *P. syringae* was cultured on PDA amended with 2 ppm vinclozolin or V8 agar. Fungicide resistance tests were carried out using PDA amended with benomyl, vinclozolin or metalaxyl, all at 2 or 20 ppm. Inoculated plates were assessed after 2 or 7 days depending on the test fungus. Isolates showing no colony growth were regarded as sensitive; those showing normal growth were considered resistant.

RESULTS AND DISCUSSION

The results of the fungicide resistance tests are summarised in Tables 3 (apple) and 4 (pear). The numbers of isolates tested varied from year to year depending on disease incidence and the ease of obtaining uncontaminated isolates for testing.

TABLE 3. Percentage of fungal isolates obtained from stored apples with resistance to 20 ppm benomyl, vinclozolin and metalaxyl (1988-93).

Fungus	Cultivar	Fungicide	1988/89	1989/90	1990/91	1991/92	1992/93
B. cinerea	Cox	benomyl	87 (47)	58 (205)	83 (60)	55 (156)	65 (134)
B. cinerea	Cox	vinclozolin	0 (47)	1 (209)	2 (49)	-	-
B. cinerea	Bramley	benomyl	16 (6)	40 (5)	50 (14)	54 (13)	-
B. cinerea	Bramley	vinclozolin	16 (6)	0 (5)	7 (14)	-	-
P. expansum	Cox	benomyl	97 (30)	92 (99)	88 (49)	75 (32)	-
P. expansum	Bramley	benomyl	100 (24)	100 (26)	79 (38)	-	-
P. syringae	Cox	metalaxyl	0 (2)	-	-	7 (42)	0 (22)
P. syringae	Bramley	metalaxyl	0 (5)	-	-	-	0 (7)
M. fructigena	Cox	benomyl	0 (2)	0 (36)	0 (64)	0 (41)	0 (13)
M. fructigena	Cox	vinclozolin	0 (2)	0 (36)	0 (29)	-	-
M. fructigena	Bramley	benomyl	0 (16)	0 (9)	0 (33)	0 (8)	-
M. fructigena	Bramley	vinclozolin	0 (14)	0 (9)	0 (8)	-	-
N. galligena	Cox	benomyl	0 (69)	0 (33)	0 (28)	0 (151)	0 (180)
N. galligena	Bramley	benomyl	0 (16)	0 (2)	0 (2)	0 (4)	-
Gloeosporium spp	Cox	benomyl	-	0 (13)	27 (11)	41 (58)	27 (102)

Figures in brackets = number of isolates tested

TABLE 4. Percentage of fungal isolates obtained from stored pears with resistance to 20 ppm benomyl, vinclozolin and metalaxyl (1988-93).

Fungus	Fungicide	1988/89	1989/90	1990/91	1991/92	1992/93
B. cinerea	benomyl	80 (99)	74 (31)	82 (60)	68 (97)	71 (38)
B. cinerea	vinclozolin	3 (87)	3 (31)	0 (72)	-	-
P. expansum	benomyl	-	89 (9)	100 (5)	-	-
P. syringae	metalaxyl	-	-	-	0 (1)	0 (2)
M. fructigena	benomyl	0 (3)	0 (4)	0 (2)	0 (7)	0 (4)
M. fructigena	vinclozolin	0 (3)	0 (4)	0 (1)	-	-
N. galligena	benomyl	0 (11)	-	0 (4)	0 (9)	0 (1)
Gloeosporium spp.	benomyl	-	100 (1)	0 (1)	20 (5)	0 (5)

Figures in brackets = number of isolates tested

Fungicide resistance in *B. cinerea*

B. cinerea from Conference pear consistently accounted for 70-80% of rotting over the period of the study and is the main fungus at which post-harvest fungicide treatment is targeted (Berrie, 1989; 1994). Consequently large numbers of isolates were tested, over 70% of which on average were resistant to benomyl (Table 1). The incidence of benomyl-resistant *B. cinerea* was higher (90%) in the early 1980s (Table 5), falling to less than 60% resistance in 1987 before increasing again to over 70%. This incidence reflects fungicide product use. Up until 1983, benzimidazole fungicides were principally used as post-harvest drenches. The high incidence of resistance resulted in significant losses (> 2% on average; 10% losses in some badly affected orchards) which jeopardised the long-term storage of pears (up to June in some years). Vinclozolin was introduced as an alternative treatment in 1984 and was rapidly taken up by pear growers as the principle fungicide treatment. The temporary suspension of approval for vinclozolin in 1990 resulted in a switch back to benzimidazole fungicides and a consequent increase in losses due to *B. cinerea*. In 1993 the granting of an off-label approval for use of iprodione as a post-harvest treatment has again resulted in a major shift in fungicide use. Dicarboximide-resistant *B. cinerea* (Table 4 and 5) has been detected, but at a very low incidence to date, such that good control of rotting is still achieved. The influence of fungicide treatment in selecting resistant isolates of *B. cinerea* is shown in Table 6 where over 70% of isolates from benzimidazole-treated fruit were resistant compared to less than 50% in untreated fruit.

TABLE 5. Incidence of resistance to benomyl and vinclozolin of *B. cinerea* from apple and pear 1980-93.

	% resistance to 20 ppm benomyl		% resistance to 20 ppm vinclozolin	
Year	Cox apple	Conference pear	Cox apple	Conference pear
1980/81		90.3		
1982/83	75.0	90.9		
1983/84	64.3	66.7		
1984/85	70.0	63.6		
1987/88	75.6	56.3	9.8	14.1
1988/89	87.2	79.8	0	3.4
1989/90	58.0	74.2	0.5	3.2
1990/91	83.3	81.7	2.0	0
1991/92	55.1	68.0	-	-
1992/93	64.9	71.1	-	-

B. cinerea from Cox apple is of increasing importance as a cause of rotting (Berrie, 1994) although post-harvest fungicide treatment is mainly targeted at control of *Gloeosporium* spp., *Phytophthora syringae, Nectria galligena* and *Monilinia fructigena*. Consequently, benzimidazole fungicides are the main products used as vinclozolin is ineffective against *Gloeosporium* and *Nectria*. Over 50% of *B. cinerea* isolates were resistant to benomyl and this incidence has remained consistent over the period of study (Tables 3 and 5). The incidence of resistance to dicarboximide was lower than in pear indicating the low or nil usage of these products as drenches for apple.

Fungicide resistance in *M. fructigena*

M. fructigena consistently causes losses in most seasons in both apple and pear, although incidence in pear has declined in recent years (Berrie, 1992a). The fungus is controlled by both benzimidazole and dicarboximide fungicides. Over the ten years of the study, no isolates of *M. fructigena* resistant to dicarboximide or benzimidazole fungicides have been detected. Similar results have been found elsewhere, although resistance of the closely related *Monilinia fructicola*, which causes a brown rot of stone fruit, to both these fungicide groups has been frequently recorded (Penrose *et al.*, 1979; 1985; Ritchie, 1982). A possible explanation for this could be that in *M. fructicola*, which is not found in the UK, the sexual stage is common and forms an important part of the annual disease cycle. In *M. fructigena* occurrence of the sexual stage is rare and the fungus survives asexually in cankers or mummified fruit (Byrde & Willetts, 1977).

Fungicide resistance in *P. expansum*

Many different species of *Penicillium* have been recorded as fruit rots of apple and pear (Rosenberger, 1990), but in the UK *P. expansum* appears to be the most frequently isolated species. The rot commonly occurs on apple and pear every season although actual losses are very low. Almost all isolates tested were found to be resistant to benomyl (Table 3 and 4), which is consistent with results from elsewhere (Kim *et al.* 1989; Rosenberger *et al.*, 1991). In 1991-93, the survey of rotting in treated and untreated fruit has indicated a higher incidence of *P. expansum* in drenched fruit (Berrie, 1993). The use of benzimidazole fungicides as drenches appears to be an effective way of spreading *P. expansum*.

Fungicide resistance in *Gloeosporium* spp.

In the UK three species of *Gloeosporium* have been recorded during the ten years of the survey - *G. album* (*Pezicula alba*), *G. perennans* (*Pezicula malicorticis*) and *G. fructigenum* (*Glomerella cingulata*). Individual species were not identified during the fungicide resistance study. In the 1960's, *Gloeosporium* was the most important cause of rotting in Cox, however the incidence over the ten years of the survey has been very low or absent until the last two seasons. This higher incidence, although actual losses were very low, is probably associated with the poor mineral composition of fruit and consequent poorer quality of cv. Cox apparent in 1992/93 (Sharples, 1980). Resistance of *Gloeosporium* spp. to benzimidazole fungicides has been frequently recorded in Europe (Palm, 1986; van der Scheer and Remijnse, 1988), as a result of reliance on pre-harvest fungicide sprays for storage rot control. The occurrence of such isolates in the UK was thought to be rare or absent. Due to the low incidence of disease, very few isolates were tested until the last three seasons (1990-93). Twenty-five per cent or more of isolates tested were found to be resistant to 20 ppm benomyl (Table 3). Reasons for this are not clear, as the use of benzimidazole fungicides as orchard sprays has been avoided in favour of post-harvest drenching. It is possible that resistance has gradually built-up since the introduction of the benzimidazole fungicides, or that the natural level variation in the population has become evident in the last three seasons when the incidence of *Gloeosporium* was higher and more tests were carried out. Tests to date suggest that either could be true. Table 6 compares the incidence of benomyl resistant isolates from treated and untreated fruit samples. In 1991/92 a higher incidence of resistance is apparent in treated fruit, whereas in 1992/93 the incidence in the two treatments is similar. The situation may become clearer after studies in the current season, when data on the incidence of resistant isolates from fruit treated pre-harvest with carbendazim sprays will be compared to that in untreated fruit. In addition, individual *Gloeosporium* spp. will be identified.

TABLE 6. Percentage resistance to 20 ppm benomyl of *B. cinerea* or *Gloeosporium* spp. from fruit treated or untreated with a benzimidazole fungicide post harvest.

	% resistant isolates			
	B. cinerea		*Gloeosporium*	
Year	Treated	Untreated	Treated	Untreated
1991/92	68.1	46.3	72.7	11.5
1992/93	73.2	47.2	21.9	27.4

Fungicide resistance in *Nectria galligena*

The incidence and importance of *Nectria* fruit rot varies according to the occurrence of *Nectria* canker. In orchards where canker is present, losses due to rotting can be severe (up to 30%) following wet summers (Berrie, 1992a). Only benzimidazole fungicides are effective in rot control (Berrie, 1992b) although they appear to be less effective when applied as post-harvest treatments. In culture, *Nectria galligena* is variable, isolates being very pale, almost white in colour, to dark orange with similar variation in fluffiness and growth rate. No isolates have been detected which are resistant to 20 ppm benomyl (Table 3 and 4), during the ten years of the survey (Berrie, 1989) or from other studies on control of *Nectria* canker, even after the application of 48 sprays of carbendazim to an orchard over four seasons (Berrie, 1992b). However, isolates do vary in sensitivity to benomyl, some being sensitive to 2 ppm and some being resistant to 2 ppm but sensitive to 20 ppm (Table 7). This would appear to be a feature of natural variation in the population with incidence varying from season to season, and not apparently influenced by post-harvest treatment (Table 8).

Fungicide resistance in *P. syringae*

Phytophthora fruit rot is mainly a problem of cv. Cox, although occasionally it can be serious on Conference pears and Bramley apples, associated with wet weather at harvest. The occurrence of the rot is thus sporadic and particularly associated with

TABLE 7. Sensitivity of *Nectria galligena* isolates to benomyl (1988-93).

Crop	Cultivar	% isolate sensitive to benomyl 1988/89 2 ppm	1988/89 20 ppm	1989/90 2 ppm	1989/90 20 ppm	1990/91 2 ppm	1990/91 20 ppm	1991/92 2 ppm	1991/92 20 ppm	1992/93 2 ppm	1992/93 20 ppm
Apple	Cox	42	58	76	24	96	4	33	68	68	32
Apple	Bramley	38	63	100	0	100	0	0	100	-	-
Pear	Conference	9	91	-	-	50	50	22	78	-	-

TABLE 8. Sensitivity to benomyl of *Nectria galligena* isolates from fruit treated or untreated post harvest with benzimidazole fungicide.

Post harvest Treatment	% isolates sensitive to benomyl 1991/92 2 ppm	1991/92 20 ppm	1992/93 2 ppm	1992/93 20 ppm
Treated	33	67	57	44
Untreated	13	87	69	31

modern intensive orchard systems. The rot is effectively controlled by the use of metalaxyl (in combination with carbendazim as 'Ridomil mbc 60 wp'). Numbers of isolates tested have been low, but a low level of resistance to metalaxyl at 20 ppm was detected in 1991/92 (Table 3). This is of particular concern as at present there are no other effective products available for use on fruit.

STRATEGIES FOR FUNGICIDE USE TO CONTROL STORAGE ROTS

Up until the introduction of post-harvest treatments in the 1970's, control of storage rots had relied on the use of pre-harvest sprays of captan or benzimidazole fungicides. Once the efficacy of post-harvest treatments was established, use of benzimidazole fungicides in the orchard was actively discouraged, to preserve the efficacy of these treatments for storage rots. A similar policy was later adopted for metalaxyl. In Europe, post-harvest treatments were never developed and in some countries their use was prevented by law. Repeated pre-harvest use of benzimidazoles for control, in particular of *Gloeosporium* spp., has resulted in resistant isolates developing and becoming widespread. In the UK, it would appear that the adopted strategy has at least delayed the appearance of benzimidazole-resistant *Gloeosporium*.

To develop a strategy of fungicide use for resistance management of storage rots is difficult when the availability of effective products is limited. Preserving the products for post-harvest use only was and is a sound strategy, however this is now threatened by the future of post-harvest treatments, not for scientific or environmental reasons but because of consumer concerns over such uses. Alternative, less effective, fungicides such as captan are available as pre-harvest fungicide sprays to control *Nectria*, *Gloeosporium* and possibly *Monilinia*. However, for control of *Botrytis* and *Phytophthora*, alternative fungicide options are limited and much less effective. For control of *Phytophthora* fruit rot, an alternative orchard treatment is the use of metalaxyl + mancozeb (as an off-label approval) as an orchard spray to the soil and low hanging fruit (Harris, 1979; Edney & Chambers, 1981). Should post-harvest treatments be withdrawn, such a use of metalaxyl would become routine in high risk *Phytophthora* orchards (modern intensive Cox orchards) and would probably greatly accelerate the development of resistance in both *P. syringae* (fruit rot) and *P. cactorum* (the cause of crown rot and collar rot in apple).

For fungi, such as *Penicillium*, chemical control offers few solutions and attention must be focused on cultural procedures, enhancing the ability of the apple to resist rotting and attention to hygiene. The possibility of using chlorine as a water disinfectant treatment should also be considered.

Any strategy for control of storage rots must be based on an integrated approach, relying on orchard cultural treatments, good storage conditions and methods, such as ensuring the correct fruit mineral composition, that will enhance the ability of the fruit to resist rotting. Such cultural methods can be combined with a system of determination of rot risk to ensure that fungicide treatment is only used when necessary, and preferably applied post-harvest, when all the factors relating to rot-risk are known. The continued availability of post-harvest treatments is, therefore, essential both to ensure the long-term and economic storage of UK apples and pears, and to preserve the effectiveness of the limited range of available fungicide products.

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SELECTION IN POPULATIONS OF THE EYESPOT FUNGUS IN CONTINUOUS WHEAT BY REPEATED APPLICATIONS OF CARBENDAZIM AND PROCHLORAZ

G.L. BATEMAN

Institute of Arable Crops Research, Rothamsted Experimental Station, Harpenden, Herts., AL5 2JQ

ABSTRACT

The effects of repeated applications of carbendazim, prochloraz or a mixture of the two fungicides, on eyespot, grain yield and population structure of the eyespot fungus (Pseudocercosporella herpotrichoides) were compared in nine years of consecutive winter wheat crops. Failure of control by carbendazim occurred within two years because of its selection for resistance in the fungus population. Prochloraz was moderately effective in most years, but selected for the R-type of the eyespot fungus and, slowly, for carbendazim sensitivity in the R-type; discriminating concentration tests showed carbendazim-resistant isolates to be slightly more sensitive than carbendazim-sensitive isolates to prochloraz. Prochloraz applied with carbendazim was sometimes more effective than prochloraz applied alone. Determination of EC50s after eight years showed R-type isolates from plots treated with prochloraz, either alone or with carbendazim, to be slightly less sensitive to prochloraz than were isolates from untreated plots, but no resistance to prochloraz was found.

INTRODUCTION

Changes in population structure of the eyespot fungus (Pseudocercosporella herpotrichoides) and in the performance against eyespot of the fungicides carbendazim and prochloraz, applied each year, were monitored from 1984 in a field experiment on continuous winter wheat. The detailed results of population and disease monitoring up to 1989 have been reported (Bateman et al., 1990; Bateman & Fitt, 1991).

This paper summarises previous findings and reports the most recent results on the long-term effects of the fungicides on eyespot and pathogen population structure. Data on fungicide sensitivity in fungal populations are used to attempt to explain variability in the performance of the fungicides when used separately and as mixtures.

METHODS

A field experiment was established in a third successive crop of winter wheat in 1984. Details of the design and treatments are reported elsewhere (Bateman et al., 1990). Some plots had inoculum applied artificially to create large populations with known initial proportions of carbendazim-sensitivity and resistance and of the R-type and W-type of the fungus. However, results only from the uninoculated plots are described here; the background population in these in the summer of 1984 was 85% W-type and 15% R-type, and 97% carbendazim-sensitive and 3% carbendazim-resistant. The fungicides, carbendazim, prochloraz or prochloraz + carbendazim, were applied in November or December and March or April in each year. Cv. Avalon was grown each year up to 1990, and cv. Mercia subsequently. The site was shallow tine-cultivated after each harvest to prevent the burial of inoculum, except in 1989 and 1990 when it was ploughed to control grass weeds.

Samples were taken in July for eyespot assessments and fungus isolations, using methods described previously (Bateman et al., 1990; Bateman & Fitt, 1991). Grain yields were measured in all years except 1987.

Sensitivity to carbendazim was determined in each year, except 1990 and 1991, by a single discriminating concentration (1 mg l^{-1}) in agar. Sensitivity to prochloraz was determined in 1988 by two discriminating concentrations (2 and 5 mg l^{-1}) and in 1992 by dosage-response tests (0.0008-0.4 mg l^{-1}) in which EC50s were estimated by fitting logistic curves, using log-transformed concentrations, and compared by Wald tests.

RESULTS

Effects of fungicides on eyespot and grain yields

Carbendazim was moderately effective against eyespot for one year only, after which resistance developed causing control failure (Fig. 1). Prochloraz was usually partially effective but failed to decrease eyespot in 1988. Prochloraz and carbendazim together decreased disease more than prochloraz in six years out of nine, the difference being significant in 1988 and 1992.

Yields were greater when carbendazim was applied with prochloraz than when prochloraz was applied alone in seven years out of eight (Fig. 2). Although these differences were not significant, a maximum difference of 1.41 t ha^{-1} occurred in 1985, before the selection of carbendazim-resistance, and the average difference was 0.63 t ha^{-1}.

Effects of fungicides on populations of P. herpotrichoides

Carbendazim selected rapidly for resistance (Fig. 3) but did not select for the W-type or R-type (Fig. 4). Prochloraz selected for the R-type and, when applied alone, for sensitivity to carbendazim in this type. After four years, in 1988, discriminating concentration tests showed carbendazim-sensitive R-type isolates to be less sensitive to prochloraz than were those that were carbendazim-resistant, regardless of whether or not they were from plots treated with prochloraz (Table 1); some

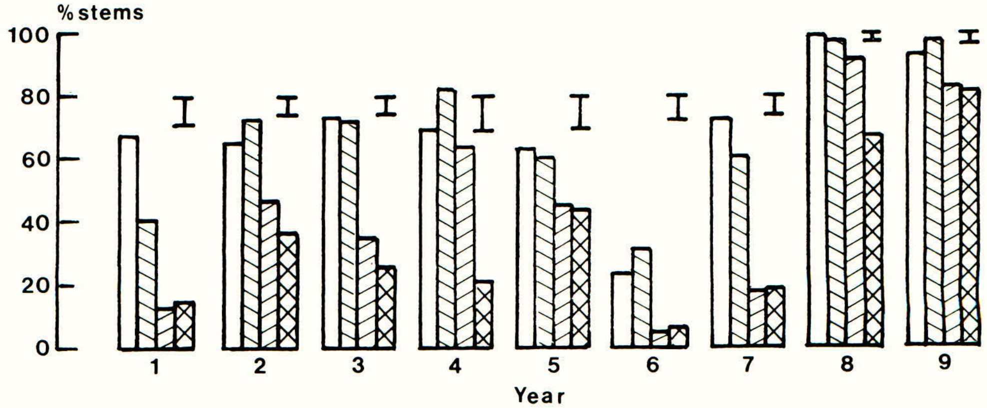

FIG. 1. Effects of fungicides applied each year on percentage of stems with eyespot in July in successive wheat crops, 1985-1993. □, untreated; ⧅, carbendazim; ⧄, prochloraz; ⊠, carbendazim + prochloraz. Vertical bars, SED (24 DF).

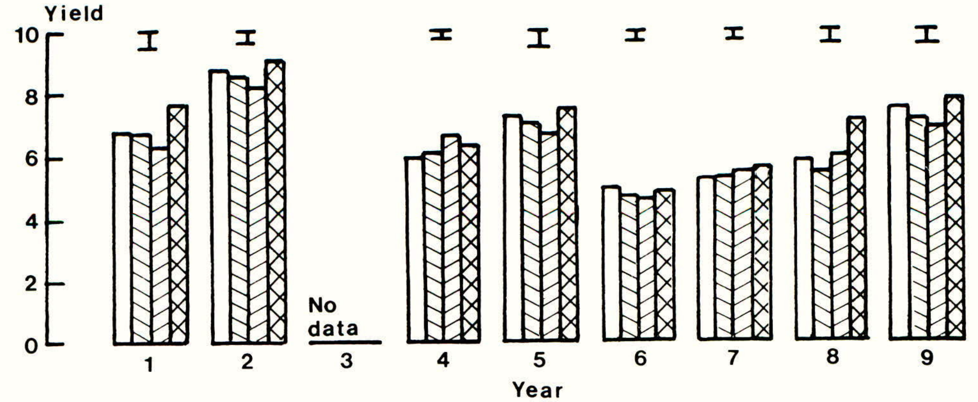

FIG. 2. Effects of fungicides applied each year on grain yields (t ha^{-1}) in successive wheat crops, 1985-1993. Key: see Fig.1.

plots yielded too few isolates to allow a full comparison of levels of sensitivity to prochloraz in relation to previous treatments. After eight years, in 1992, dosage-response tests to determine EC50s for prochloraz showed that R-type isolates from prochloraz-treated plots were less sensitive ($P<0.05$) than those not exposed to prochloraz (Table 2). There was no evidence on this occasion of differences between carbendazim-sensitive and carbendazim-resistant isolates. The range of EC50s for prochloraz was greater in R-type isolates (0.006-0.2 mg l^{-1}) than in W-type isolates (0.010-0.074 mg l^{-1}).

DISCUSSION

The most rapid selection in populations of the eyespot fungus was for carbendazim-resistance. The build-up from 3% to almost 100% resistant isolates in the population resulted in disease control failure within two years. Moderately rapid selection for the R-type by prochloraz occurred apparently because this type naturally has a greater range of sensitivities than the W-type. Where selection by prochloraz treatment occurred, the R-type fungi sampled were less sensitive than the unselected R-type or the W-type fungi to prochloraz. Europe-wide surveys have shown consistently that R-type fungi are, on average, slightly more sensitive than the W-type to prochloraz (Birchmore & Russell, 1990), suggesting that the populations

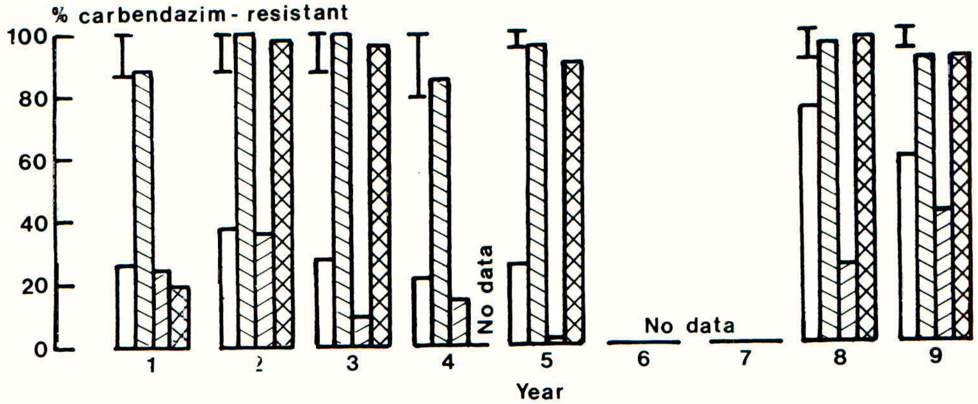

FIG. 3. Effects of fungicide treatments applied each year on percentages of carbendazim-resistant isolates in populations of the eyespot fungus in July in successive wheat crops, 1985-1993. Key: see Fig. 1.

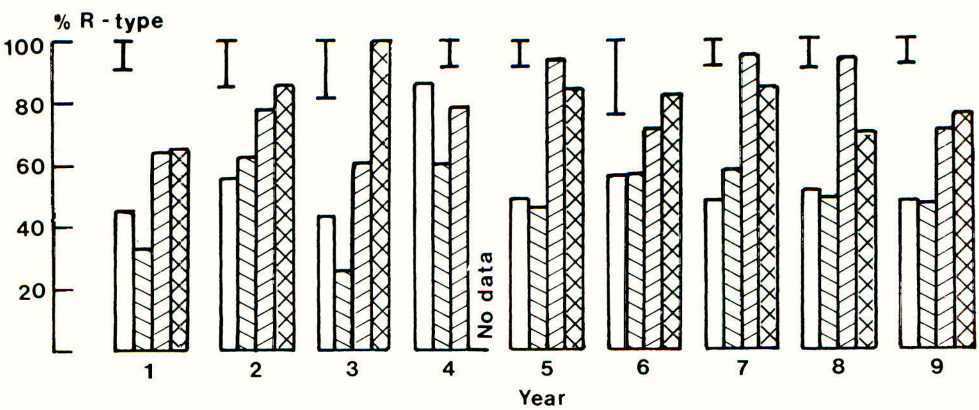

FIG. 4. Effects of fungicide treatments applied each year on the percentages of R-type isolates in populations of the eyespot fungus in July in successive wheat crops, 1985-1993. Key: see Fig. 1.

sampled in those surveys were largely unselected by prior treatments with prochloraz. Our results showed selection for decreased sensitivity to prochloraz and less control by that fungicide. Decreased sensitivity to prochloraz has been reported

TABLE 1. Sensitivity to prochloraz of isolates of <u>Pseudocercosporella herpotrichoides</u> taken in 1988, determined by discriminating concentration tests in agar plates.

Test	Carbendazim-sensitivity	No. of isolates	Percentage showing growth* 2 mg l^{-1}	5 mg l^{-1}
1	Sensitive	22	90.9	13.6
	Resistant	50	32.0(2.0)	2.0(2.0)
2	Sensitive	36	91.7(91.7)	25.0(25.0)
	Resistant	12	16.7	0.0
3	Sensitive	60	61.8	28.1
	Resistant	29	17.2	3.4

* The numbers in brackets are the percentages that were from prochloraz-treated plots.

TABLE 2. Sensitivity to prochloraz (EC50, back-transformed from $\log_{10}$) of isolates of the eyespot fungus taken in July 1992 from wheat in plots treated each year since 1985 with different fungicides.

Treatment to plots	W-type	R-type
None	0.037	0.029
Carbendazim	0.023	0.016
Prochloraz	No data	0.067
Carb. + proc.	0.039	0.088

also in northern France, with evidence of some decreased performance in field trials (Migeon <u>et al</u>., 1992), but selection was not detected elsewhere in small population samples (Birchmore <u>et al</u>., 1992).

The experiment consistently produced evidence of selection against carbendazim-resistance by prochloraz in the R-type fungus. The elimination of this by carbendazim probably accounts for the sometimes better eyespot control by the mixture of fungicides than by prochloraz alone. There was convincing evidence in 1988 that this resulted from greater tolerance to prochloraz in the carbendazim-sensitive fungus; this strain may be more fit and able to cope with the fungicide. This was not confirmed by a different testing procedure in 1992, perhaps because either the carbendazim-resistant strain had been further selected for tolerance to prochloraz in the intervening four years, or the high discriminating concentrations used in 1988 were better able to detect small differences than were the EC50 tests used in 1992. Evidence for the former explanation came in 1993 when carbendazim failed to add significantly to the performance of prochloraz, although the relative performances of the fungicides may differ from year to year as environmental conditions change.

Wheat in continuous cultivation, and the consequent need for annual applications of fungicides to control stem base disease, are becoming increasingly uncommon in Europe as attempts are made to reduce the surplus of grain. Even so, applying mixtures of fungicides may be a way of preventing slow selection of decreased sensitivity to a fungicide as reported here. Mixed formulations of prochloraz and benzimidazoles, however, are not currently an important part of the armoury against eyespot. Alternating fungicides were not tested in this experiment. The interactions between prochloraz and carbendazim, fungicides with different modes of action and selectivity, may be relevant to diseases in other crops grown continuously in which fungicides can be used separately or as mixtures.

ACKNOWLEDGEMENTS

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