MONITORING STUDY OF THE RESISTANCE OF *BOTRYTIS CINEREA* TO BENZIMIDAZOLE AND DICARBOXIMIDE FUNGICIDES IN GRAPES IN HUNGARY

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ABSTRACT

According to monitoring studies, which began in 1981, resistance to benzimidazole fungicides has developed in strains of *B. cinerea* in grapes. Negative cross-resistance to phenylcarbamates can be detected. Since 1988, a decrease in sensitivity to dicarboximides has also been observed. A simulation model was developed to predict the development of resistance. The model has been compared to the results of monitoring studies and is considered to be suitable for choosing spray programmes to prevent the build-up of resistance to dicarboximides.

INTRODUCTION

Grapevines are grown in Hungary on an area of 110,000 ha. There are three important regions (Northern Hungary, Transdanubia and the Plain) and these include several wine districts. One dangerous pathogen occurring in the vineyards is *B. cinerea*, which causes severe damage in some years depending on weather conditions. Infection may occur before and during flowering, at closing of the bunch growth stage and at the start of ripening. Treatments are made with multi-site inhibitors (e.g. chlorothalonil, dichlofluanid, folpet and thiram) and dicarboximides (e.g. iprodione, procymidone and vinclozolin). Dicarboximide fungicides are mainly used during the second half of the growing season; earlier application is only justified by extreme rainy weather. In the past, benzimidazole compounds were also used (e.g. benomyl and thiophanate-methyl). They were introduced in 1972 and were widely used from the second half of the decade. A decrease in the field efficacy of benzimidazoles was first observed in 1981. In laboratory studies, it was confirmed that this loss in efficacy was due to a decrease of pathogen sensitivity to benzimidazole fungicide (Kaptás & Dula 1984). This prompted the start of the monitoring studies for resistance, which were mainly performed in the Northern grapevine growing districts of Hungary but which included other parts of the country.

The main objectives of study were: to detect occurrence and frequency of resistance to various fungicides; to determine properties of resistance, cross-resistance, duration of

resistance; and to study any correlation between the frequency of treatments and the change of sensitivity level of pathogen to fungicides.

MATERIALS AND METHODS

Ten infected bunches were collected randomly at vintage from each field. *B. cinerea* was isolated onto 2% malt-czapek agar and was tested for carbendazim (as 'Kolfugo 25 FW') and iprodione (as 'Rovral') sensitivity at rates of 0, 1, 5, 10, 50 and 100 mg AI/l. Results are expressed as the concentration of carbendazim and iprodione required to reduce colony diameter by 50% (EC_{50}) when compared to colonies growing on agar without fungicide. Recommendations of literature are studied when using the method (Leroux *et al*, 1982; Leroux & Besselat, 1984). The factor of resistance (FR) was determined based on EC_{50} values compared to a sensitive (reference) isolate.

RESULTS

Resistance of *B. cinerea* to benzimidazole fungicides on grapevine is common in Hungary. Based on various studies, resistance to benzimidazoles is persistent and because of this, they are no longer used. No positive cross-resistance has been observed to other fungicide groups. However, a negative cross-resistance to phenylcarbamates (e.g. diethofencarb) was detected (Kaptás & Dula, 1988; Kaptás *et al*, 1990). Phenylcarbamates are not yet registered against *B. cinerea* in Hungary.

Dicarboximides were first registered in Hungary in 1978 and their became widespread in the 1980's. No general resistance to dicarboximides has developed up to now in Hungary. However, a decrease in sensitivity has been observed since 1988 (Table 1). The frequency of the resistant strains changes with years. The decrease of benzimidazole + dicarboximide sensitivity occurring at the same time is typical (Kaptás *et al*, 1990).

Table 1. Distribution of grapevine *B. cinerea* strains according to Factor of Resistance (FR) in Hungary, between 1987 and 1992.

Years	Distribution of *B. cinerea* strains in %					
	classes of FR[x]					
	1	1-5	5-10	10-15	15-20	20
1987	78	22	0	0	0	0
1988	65	21	0	14	0	0
1989	0	86	7	7	0	0
1990	0	93	0	7	0	0
1991	24	57	5	14	0	0
1992	16	54	6	3	15	6

[x]FR = $\frac{\text{EC50 of field strain}}{\text{EC50 of sensitive (reference) strain}}$

Comparison of the above results with the simulation model

Results of the monitoring studies have been compared with values calculated by a simulation model, which was modified from an earlier general resistant model (Josepovits & Dobrovolsky, 1985; Josepovits, 1989).

Calculation with the simulation programme shows the decrease of resistance level in crops not treated with dicarboximides. More than two treatments a year induce an increase in the frequency of resistance. This increase is the highest if the dicarboximide treatment is made at the risky period of flowering and early ripening. The monitoring data confirm the reliability of the calculation, i.e. the frequency of resistance ranges between 7 and 30% during 5 years, while the average number of the dicarboximide treatment was annually 1 and 2. For the evaluation of the increase in 1992, it should be considered that most resistant isolates are from the same crop varieties of one county. No close correlation between the treatments and the resistance frequency could be made from the monitoring data because of the spread of conidia by wind.

Results obtained with the simulation model show also that the application of the mixture of multi-site fungicides and dicarboximides does not offer a real alternative for a rotation of treatments because of their greatly different action. To control *B. cinerea*, experimental data are available for carbendazim + diethofencarb among fungicides of similar or better action. At the application of this combination, it should be considered that the effect of their components is not added according to the Abbott's formula (Abbott, 1925) and that it offers satisfactory efficiency at a concentration in which each component has adequate activity to the strains which are more sensitive to them. The simulation model suggests that a triple combination of the above products with a dicarboximide could be used positively during the period of the rapid spread of the pathogen when the use of a dicarboximide alone is to be avoided because of the risk of resistance.

With this paper on the resistance of *B. cinerea* to various fungicide, the authors wanted to contribute to the picture known in the Western European countries' grapevine growing districts with their results obtained in Hungary.

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BENZIMIDAZOLE RESISTANCE IN *RHYNCHOSPORIUM SECALIS* IN NORTHERN IRELAND AND ITS IMPLICATIONS FOR DISEASE CONTROL

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ABSTRACT

Before 1990, there was little evidence of benzimidazole resistance in the Northern Ireland *Rhynchosporium secalis* population. By 1992, resistance had become common and widespread. In field trials in 1993, carbendazim controlled *R. secalis* well at a site where the majority of *R. secalis* isolates recovered were benzimidazole-sensitive. At another site where most *R. secalis* isolates proved resistant, carbendazim, both alone and with propiconazole, apparently increased the severity of *R. secalis* infection, relative to the untreated control and the propiconazole treatment respectively.

INTRODUCTION

Rhynchosporium secalis, which causes leaf blotch, is the most prevalent and damaging disease of winter barley in N. Ireland (Mercer & Easson, 1987). Fungicides offer the only effective means of control in autumn sown cultivars. Recently a decline in the sensitivity of *R. secalis* to DMI fungicides in the British Isles has increased interest in the benzimidazole fungicide carbendazim for control of leaf blotch (Kendall *et al.*, 1993). Although benzimidazole resistance has not until recently been detected in field populations of *R. secalis* in the British Isles, highly resistant and fit strains of other cereal pathogens, e.g. *Pseudocercosporella herpotrichoides* (King & Griffin, 1985), are widespread. This paper reports the results of surveys of benzimidazole resistance in *R. secalis* in Northern Ireland (1990-1993) and the results of 1993 field trials to investigate the effect of resistance on disease control in the field.

MATERIALS & METHODS

Survey of benzimidazole resistance in *R. secalis*

R. secalis isolates were collected from barley crops (mainly autumn sown) throughout Northern Ireland from 1990 until 1993. The isolates were assayed on yeast malt agar containing different concentrations of technical grade carbendazim, added from stock solutions in dilute hypophosphorous acid before autoclaving. Fungal growth was assessed after ten days at 18°C and sensitivity to carbendazim expressed as the minimum inhibitory concentration (MIC) value (the lowest fungicide concentration which completely prevented fungal growth).

Field trials

Two sites were chosen, both commercial crops of winter barley, cv. Fighter, near Portaferry and Strangford, Co. Down, respectively. A randomised block design, with four blocks and five treatments, was used at both sites. The treatments are summarised in Table 1. Fungicide sprays were applied on two occasions, growth stages 33 and 57 (as defined by Zadoks *et al.*, 1974).

R. secalis isolates were recovered from plots before treatments were applied and tested, *in vitro*, using the method described above, for their sensitivity to carbendazim. Foliar disease assessments were made on several occasions during the growing season, using the key described by James (1971). Grain yields were recorded at the end of the growing season.

TABLE 1. Field trial treatments

Proprietary name	Manufacturer	Active ingredients	Application rate (g/ha)
'Derosal WDG'	AgrEvo	carbendazim	250
'Tilt 250 EC'	Ciba Agriculture	propiconazole	125
'Hispor 45 WP'	Ciba Agriculture	propiconazole + carbendazim	125 + 100
'Legend'	Zeneca	propiconazole + fenpropidin	125 + 562.5

RESULTS

Survey of benzimidazole resistance in *R. secalis*

All isolates tested in 1990 proved benzimidazole-sensitive (MIC values 1 mg/l or less), but since 1991 substantial proportions of benzimidazole-resistant (MIC values >10 mg/l) isolates have been obtained (Figure 1). All isolates with MIC values of >10 mg/l grew well at higher carbendazim concentrations (25 mg/l). Resistant isolates were not restricted to certain regions, but were recovered from all cereal growing areas (Table 2) and in many cases both resistant and sensitive isolates were recovered from the same site (Table 3).

FIGURE 1. Sensitivity of *Rhynchosporium secalis* to carbendazim in N. Ireland, 1990-93

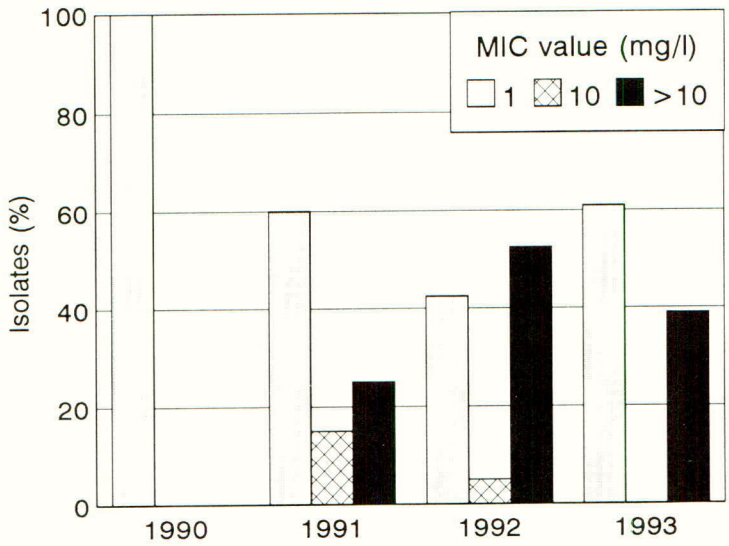

TABLE 2. Geographical distribution of benzimidazole-resistant *Rhynchosporium secalis* isolates collected in 1993

County	Number		% resistant
	sensitive	resistant	
Antrim	7	5	42
Armagh	2	9	82
Down	18	4	18
Londonderry	17	10	37
Total	44	28	39

TABLE 3. Distribution of benzimidazole-resistant *Rhynchosporium secalis* isolates within sites in 1992-93

Year	Number of sites with			Total number of sites
	Sensitive only	Resistant only	Resistant+ sensitive	
1992	8	6	8	22
1993	5	2	14	21

Field trials

Only 7.6% of the isolates recovered from the Portaferry site were benzimidazole-resistant (MIC value >10 mg/l), whereas 85.7% of isolates from the Strangford site were resistant. The numbers of isolates recovered and assayed from the two sites were 156 and 119, respectively.

At the "sensitive" Portaferry site all fungicides controlled *R. secalis* (Table 4). There was no indication that propiconazole + carbendazim, or propiconazole + fenpropidin provided better control than propiconazole alone. However, propiconazole controlled *R. secalis* so effectively that any benefit from carbendazim would have been difficult to detect. At the "resistant" Strangford site only the propiconazole and propiconazole + fenpropidin treatments gave adequate disease control. The severity of *R. secalis* infection was greater with carbendazim than with the control and greater with propiconazole + carbendazim than with propiconazole alone. There were no significant differences in yield between treatments at either site.

TABLE 4. Infection of winter barley cv. Fighter by *Rhynchosporium secalis*, 1993

Treatment	*Rhynchosporium secalis* infection (arcsin %) 21 June		Yield (t/ha) at 16% moisture
	Leaf 1	Leaf 2	
Portaferry			
none	24.3	25.3	5.9
carbendazim	7.0	9.6	5.8
propiconazole	5.8	9.6	6.1
propiconazole + carbendazim	8.6	10.1	5.8
propiconazole + fenpropidin	4.0	5.4	6.9
S.E. (12 D.F.)	3.17	2.13	0.32
Significance[a]	**	***	ns
Strangford			
none	31.7	19.6	4.1
carbendazim	29.7	33.7	3.8
propiconazole	8.1	9.2	5.4
propiconazole + carbendazim	15.1	25.4	5.0
propiconazole + fenpropidin	7.2	10.1	5.3
S.E. (12 D.F.)	3.74	2.70	0.61
Significance[a]	***	**	ns

a Significance, ns = $P>0.05$, ** = $P<0.01$, *** = $P<0.001$

DISCUSSION

In Northern Ireland surveys of 1988-89 (Cooke, L.R., unpublished data) as well as in 1990, no isolates of *R. secalis* were confirmed as benzimidazole-resistant. In 1989, one isolate grew on carbendazim at 1 mg/l in an initial test, but died before it could be re-tested. No benzimidazole-resistant isolates of *R. secalis* were obtained in ADAS surveys in England and Wales in 1987 and 1989 (Jones, 1990). However, the 1991-93 surveys reported here clearly demonstrate that benzimidazole resistance is now both common and widespread in the *R. secalis* population of Northern Ireland. During the same period, further surveys carried out by ADAS have confirmed the occurrence of benzimidazole-resistant *R. secalis* in commercial winter barley crops in England and Wales (Locke, 1994).

In the field trials in 1993, carbendazim alone gave good control of *R. secalis* at the "sensitive" site, but no control at the "resistant" site, providing strong evidence that benzimidazole-resistant *R. secalis* is associated with poor disease control by carbendazim. A more surprising finding was that the use of carbendazim, either alone or in combination with propiconazole, apparently increased the severity of *R. secalis* infection at the "resistant" site. Some degree of disease control by carbendazim would have been expected, even at a site where the majority of the *R. secalis* population was benzimidazole-resistant. The reasons for the apparent stimulation by carbendazim of *R. secalis* at the "resistant" site are unclear and will be investigated further.

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RESISTANCE TO IPRODIONE IN *ALTERNARIA LINICOLA*

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ABSTRACT

Resistance by *Alternaria linicola* to iprodione was first discovered in linseed in the UK in 1986 and spread rapidly to affect 85% of all seed samples by 1988, leading to the abandonment of the treatment. However, in 1993, resistance could no longer be detected. The maximum incidence of resistance within a sample was estimated at 24% in 1987.

INTRODUCTION

The Department of Agriculture for Northern Ireland (DANI) acts as the Official Seed Tester for seed-borne disease of linseed in the UK. In 1986, when the most commonly used seed-treatment was the fungicide iprodione ('Rovral', Rhône-Poulenc), several treated samples were received with levels of the pathogen *Alternaria linicola*, which exceeded the official 5% limit and consequently failed the test. These samples were further treated with iprodione in case there had been a failure to cover the seed adequately, but on retesting, *A. linicola* was again isolated at levels which resulted in failure. As previous research (Mercer *et al.*, 1985) had shown iprodione to be highly effective in the control of *A. linicola* on linseed, a programme of work was started to investigate possible resistance to the fungicide by *A. linicola*. Results are reported below.

TESTS FOR RESISTANCE

In 1986, seven isolates of *A. linicola*, from seed samples treated with iprodione and where there had been problems with disease control, were compared for sensitivity to iprodione with three isolates from seed samples where there was no obvious control problem. Isolates were plated out on 2% malt agar to which a suitable range of iprodione concentrations in acetone had been added as the agar was cooling (10 ml stock solution/litre agar). At least five concentrations and a control (acetone only added to the agar) were used for each isolate. There were five replicates. ED_{50} values (mg/l) for mycelial growth after nine days' growth in the dark at 20°C were estimated using logarithmic probability paper. Two types of responses were observed:

1. Completely sensitive (the three isolates from samples where there was no disease control problem and two isolates from seed samples where resistance was suspected): ED_{50} values in the range 0.7 - 1.4 mg/l.

2. Resistant (five isolates from the suspect samples): moderate inhibition of mycelial growth (*ca.* 24%) at 0.8 mg/l but inhibition only increased to *ca.* 36% at 500 mg/l. One isolate was tested at 1000 mg/l but inhibition was still only 48% . Repeat tests of resistant isolates, maintained on malt agar without the addition of iprodione, showed no change in resistance over five years.

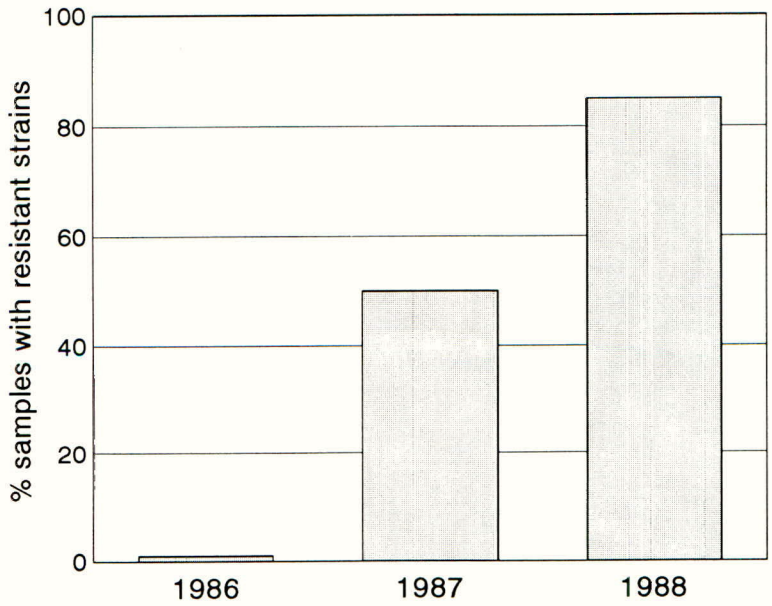
In 1987, 17 further isolates (from seed samples where resistance was suspected) were tested as in the previous season, except that iprodione concentrations were restricted to 10 and 100 mg/l. One isolate proved to be sensitive and fourteen of the others resistant in a similar way to those in found to be resistant in 1986. However, there was also a third type of response (two isolates) in which growth was increased by iprodione at 10 mg/l (0.4% and 72.4%) and more so at 100 mg/l (28.4% and 84.1%).

In 1993, 25 isolates of *A. linicola*, taken from samples where there was an incidence of the pathogen of over 15%, were tested on agar containing 10 mg/l of iprodione. All isolates were sensitive.

INCIDENCE OF IPRODIONE RESISTANCE

A dramatic increase in the detection of iprodione-resistant *A. linicola* isolated from linseed samples received by DANI occurred between 1986 and 1988 (Figure 1). By 1988, 85% of all seed samples yielded some isolates of *A. linicola* showing resistance to iprodione.

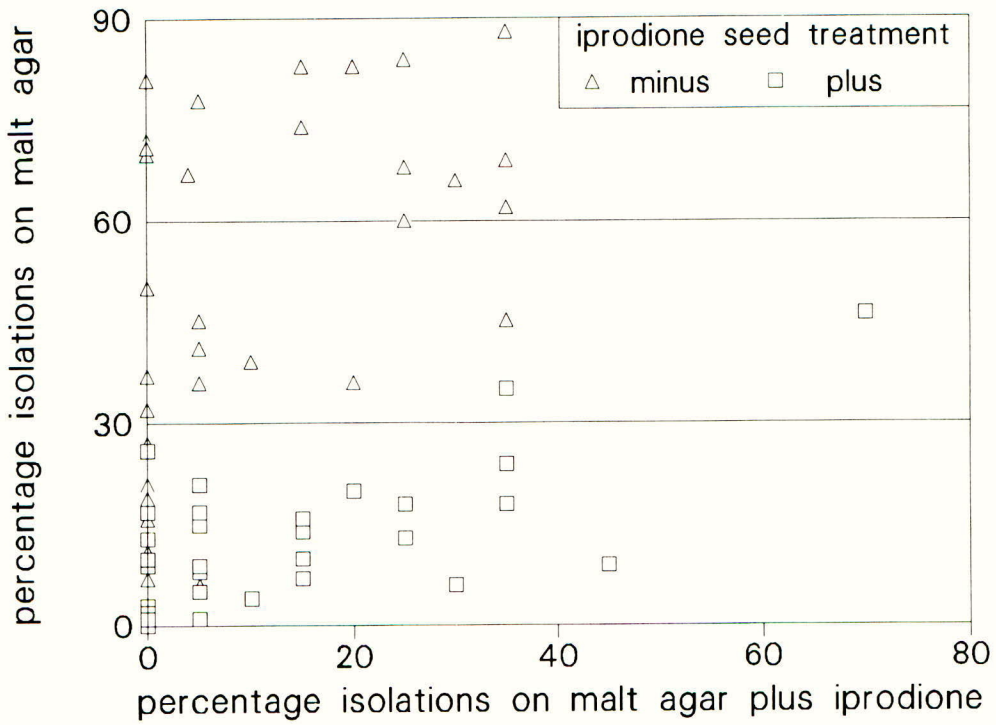
FIGURE 1. Percentage of linseed samples received by DANI from 1986-88 in which iprodione-resistant *A. linicola* was detected.



In 1987, estimations were made of iprodione-resistant and -sensitive populations of *A. linicola* in untreated and iprodione-treated seed-samples received by DANI for seed-testing. Samples of untreated and of iprodione-treated seed (37 and 34 respectively) were selected at random. One hundred seeds from each sample were plated out on 2% malt agar and a further 100 on 2% malt agar containing 10 mg/l iprodione, incubated for 7 days at 20°C and 12 h light, 12 h n.u.v. light before being examined for the presence of *Alternaria linicola*. The percentage of seed from which *A. linicola* was isolated when plated out on malt agar was graphed against that obtained when plated out on malt agar containing iprodione.

All samples of seed, apart from one treated sample, yielded isolates of *A. linicola* (Figure 2). Fifty-one percent of samples of untreated seed and 65% of samples of iprodione-treated seed yielded isolates of *A. linicola* resistant to iprodione. Samples of seed treated with iprodione yielded fewer isolates than samples of untreated seed, when grown on untreated malt agar - 12% of seed compared with 46%, indicating that many of the iprodione-sensitive strains had been suppressed. Treatment also reduced the percentage of samples yielding only sensitive isolates - 35% compared with 49%. A possible reason for the existence of **any** sensitive isolates following treatment may lie in poor coverage of the seed in some samples.

FIGURE 2. Percentage isolations of *A. linicola* from seed samples treated or untreated with iprodione and plated out on agar either containing or not containing 10 mg iprodione/l.



Estimates of the percentage of isolates showing resistance were obtained from counts of isolates from treated or untreated seed plated out on agar containing iprodione. Results from both sets of seed were similar, showing that when resistant strains were detected within a sample they were found in 18.6 - 20% (average 19%) of the seeds. A similar estimate obtained from counts from untreated seed plated out on untreated agar showed that when resistant strains were present within a batch, 59% of seeds yielded isolates of either types, *i.e.* approximately 19% resistant and 40% sensitive. If isolates on individual seeds were always either resistant or sensitive, a maximum ratio of resistant to sensitive isolates of 1:2.1 is obtained. However, if both resistant and sensitive isolates were present in the same seed, the ratio would be reduced as the number of sensitive strains would be underestimated. In any untreated or untreated sample, therefore, the maximum chance of isolating a resistant isolate of *A. linicola* would have been 24% or 31% respectively (51% or 65% x 1/2.1).

INTERACTION OF CULTIVAR AND IPRODIONE-TREATMENT ON INCIDENCE OF *A. LINICOLA*.

In 1989, an survey was made of the incidence of *A. linicola* in a random selection of iprodione-treated and untreated seed of three cultivars, Antares, Atalante and Lidgate. Results showed 78% fewer isolates of *A. linicola* from treated than from untreated samples of Antares but 99% and 96% fewer of Atalante and Lidgate, respectively, indicating a very low level of iprodione-resistance in *A. linicola* from these two cultivars.

DISCUSSION

After iprodione-resistant *A. linicola* was first detected in 1986, its incidence increased rapidly, resulting in the virtual abandonment of iprodione and its replacement by prochloraz (Mercer *et al.*, 1988). Resistance appeared to be higher in cultivar Antares, probably because this was the single most popular cultivar in the early to mid 1980s. Recent testing of isolates detected no iprodione-resistant strains, suggesting that the resistant isolates were less fit than their sensitive counterparts. However, if iprodione treatments were to be reintroduced on a large scale, resistance might well recur.

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CARBENDAZIM RESISTANCE IN *RHYNCHOSPORIUM SECALIS* IN ENGLAND AND WALES

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ABSTRACT

A survey of winter barley crops in England and Wales in 1993 found that 45.9% contained carbendazim resistant isolates of *Rhynchosporium secalis*. Of the 639 isolates tested 16.6% were resistant to carbendazim at 1.0 µg/ml.

INTRODUCTION

Rhynchosporium secalis is a common disease of barley in England and Wales (Polley *et al*, 1993) and can cause considerable yield losses as a result of leaf infection (James *et al*, 1968). Methyl bendizimidazole carbamate (MBC) fungicides have given useful control of this pathogen since being introduced for use on barley in 1975. Monitoring of the *R. secalis* field population from 1980-1989 failed to detect any strains resistant to MBC, but laboratory mutants possessing resistance can be produced readily (Kendall *et al*, 1994).

In 1991 ADAS confirmed that MBC-resistant isolates of *R. secalis* were present in a crop of winter barley in Dyfed, Wales and in 1992 an *ad hoc* survey was carried out of winter barley crops in England and Wales. This survey of 19 crops found that 47.4% contained MBC-resistant isolates of *R. secalis* and that 14.2% of the isolates examined were resistant at 1 µg/ml. The following year ADAS undertook a stratified survey of winter barley crops to determine the level of resistance in England and Wales.

MATERIALS AND METHODS

Ninety-five winter barley crops were sampled at GS 30-31 and the pathogen was isolated on to Czapek Dox agar amended with bacteriological peptone and streptomycin. A maximum of 10 pure cultures from each crop were then tested for carbendazim sensitivity at 1 µg/ml, alongside standard isolates known to be sensitive and resistant at this level of fungicide incorporation. Cultures were incubated for 14 days in darkness at 17°C and then scored for spore germination and growth. Isolates making no growth on the carbendazim-amended agar were classed as sensitive, whilst those making similar growth on amended and unamended agar were classed as resistant.

TABLE 1. Geographical and cultivar distribution of winter barley crops from which *Rhynchosporium secalis* was isolated

Location	CULTIVAR						
	Pastoral	**Pipkin**	**Fighter**	**Puffin**	**Marinka**	**Halcyon**	**Others#**
Cumbria/Northumberland	6	0	2	0	0	1	3
Yorkshire/N.Humberside	1	2	4	2	0	0	1
Norfolk/Lincs./Cambs./S.Humberside	1	1	1	3	0	2	3
Suffolk/Essex	0	7	0	0	0	0	0
Kent/Sussex	2	1	1	3	1	0	1
Shropshire/Staffs./Herefords./Oxon.	0	3	4	0	0	0	1
Cornwall/Devon/Somerset/Avon/ Dorset/Hants./Berks.	2	0	0	0	1	0	2
Wales	2	0	1	3	4	1	1
England and Wales	14	14	13	11	6	4	12

Other cultivars were 2 each crops of Sprite and Kira, one each of Willow, Magi, Bambi, Bronze, Frolic, Maris Otter, Igri and one crop consisting of a mixture of cultivars

TABLE 2. Incidence of carbendazim resistance in *R. secalis* by crop and isolate according to cultivar

Winter Barley cultivar	CROP		ISOLATE	
	Number sampled	% with resistant isolates present	Number tested	% resistant to carbendazim
Marinka	6	83.3	53	47.2
Pastoral	14	64.9	114	36.0
Fighter	13	46.2	121	13.4
Halcyon	4	50.0	40	10.0
Puffin	11	36.4	96	9.4
Pipkin	14	14.3	113	2.7
Other cultivars	12	33.3	102	7.8
All cultivars	74	45.9	639	16.6

TABLE 3. Geographic distribution of carbendazim - resistance in *R. secalis*, by crop and isolate

Location	CROP		ISOLATE	
	Number sampled	% with resistant isolates present	Number tested	% resistant to carbendazim
Cumbria/Northumberland	12	66.7	103	24.3
Yorkshire/N.Humberside	10	10.0	89	1.1
Norfolk/Lincs./Cambs./ S.Humberside	11	27.3	102	15.7
Suffolk/Essex	7	0	54	0
Kent/Sussex	9	66.7	67	20.9
Shropshire/Staffs./ Herefords./Oxon.	8	25.0	80	5.0
Cornwall/Devon/Somerset/ Avon/Dorset/Hants./Berks.	12	75.0	109	31.2
Wales	5	100	35	34.3
England and Wales	74	45.9	639	16.6

RESULTS

R. secalis isolates were successfully cultured from plants from 74 crops (Table 1) and 639 were screened for carbendazim resistance (average 8.6 isolates per crop). Resistance was detected in 16.6% of isolates, and 45.9% of the 74 crops had such isolates present. The level of resistance varied according to the cultivar from which the isolate was obtained (Table 2) and geographic location of the crop (Table 3).

DISCUSSION

The incidence of carbendazim resistance detected in the 1993 survey was very similar to that found in 1992 and therefore probably represents an accurate picture of the situation in England and Wales. More resistance was found in the South West of England and in Wales - areas of higher rainfall and higher rhynchosporium leaf blotch incidence in most years. Crops in these areas are likely to have more frequent applications of fungicides and hence the selection pressure for resistance would be greater. The situation concerning variations in resistance levels in different cultivars needs to be explored further.

The geographic distribution of the cultivars sampled in the survey was variable, with cv. Pipkin being the only type encountered in Suffolk/Essex. As this cultivar had the lowest incidence of recovery of carbendazim-resistant isolates of *R. secalis*, some caution is needed in interpreting the survey finding that no resistance was confirmed in those counties.

The low incidence of resistance in cv. Pipkin suggests that a genetically-based disassociation may exist between virulence for host resistance factor Rh_4 in that cultivar and sensitivity to carbendazim. As a consequence of these findings advice to farmers on their strategy for the control of *R. secalis* will have to be examined.

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DETECTION OF BENZIMIDAZOLE-RESISTANT DRY ROT IN POTATO SEED LOTS USING A MODIFICATION OF THE "BAG TEST."

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ABSTRACT

Benzimidazole resistance in the potato dry rot fungus (*Fusarium sambucinum*) has been encountered with increasing frequency in Idaho and other potato-producing areas of the United States in the last five years. The traditional "bag test" is a simple, on-farm test that can be performed by growers and fieldmen to test for the dry rot potential within a given seed lot. A modification of the bag test referred to as the "modified bag test," has been developed to include the identification of benzimidazole resistance. Information gathered from modified bag test results has been employed as a resistance monitoring tool to estimate the frequency and distribution of benzimidazole resistance in the Idaho potato industry. The test also serves in an educational capacity by providing growers with information on benzimidazole resistance and effective alternative seed piece treatments for control of Fusarium dry rot.

INTRODUCTION

Benzimidazole resistance in the dry rot fungus (*Fusarium sambucinum*) was first confirmed in Idaho seed potatoes in 1990 (Nolte, 1993-a,b) . A seed lot which had been cut, treated with a benzimidazole fungicide, and stored for five weeks before planting displayed virtually 100 percent incidence of dry rot decay with severities that averaged 50 percent or more. In the following two growing seasons, several additional instances were encountered in seed lots cut, treated with benzimidazoles 'Topsin' (thiophanate-methyl) or TBZ (thiabendazole) and stored for several weeks before planting. Because of the threat of benzimidazole resistance, growers who make a practice of cutting and storing their seed before planting have been warned that the practice can be very risky (Nolte, 1993-a).

The resistance problem in cut seed has an even greater significance in view of the fact that thiabendazole is the only fungicide approved for post-harvest application on potatoes in the USA. In many cases, it appears that the resistant strains of *Fusarium sambucinum* can be brought in on the seed and be transmitted to the daughter tubers. This is thought to occur because benzimidazole-treated seed will decay in the rhizosphere providing resistant inoculum to infect daughter tubers during the rigours of harvest and handling (Author, unpublished). If thiabendazole is applied post-harvest, selection pressure again favours the resistant strains. The use patterns of this class of chemicals in Idaho would seem to be a classic case of over-reliance on a single-site action fungicide for control of a fungal disease. In some areas the benzimidazoles have been used for both seed treatment application and post-harvest fungicide application for several years in a row. The development of resistance under this kind of selection pressure seems almost inevitable.

Effective management strategies depend heavily on grower awareness of the problem. The modified bag test is a tool that educates growers about fungicide resistance and provides them with information about the potato seed lots that they are selling or purchasing (Nolte, 1993-b). The kit also contains information on fungicide resistance management including the use of alternate and benzimidazole-combination products for seed decay control as well as Integrated Pest Management techniques for disease control (Brent, 1988; Delp, 1988; Wade, 1988).

MATERIALS AND METHODS

Several chemical companies, including Snake River Chemical, Caldwell, Idaho; Wilbur-Ellis, Twin Falls, Idaho and Plant Health Technologies, Boise, Idaho have produced and made pre-packaged test kits available to Idaho growers who want them. The pre-packaged kits are very convenient and may be one reason for the large number of growers (hundreds of kits were distributed in 1993) who are performing these tests. General instructions for performing the modified bag test as they were provided to Idaho growers are included below.

The Modified Bag Test

The familiar "bag test" was developed to enable growers to determine whether or not there is a potential for dry rot problems in their seed potatoes (Secor, *et al*, 1985, 1992). The modified bag test employs exactly the same methods but has some simple added features for fungicide resistance determination. The traditional bag test will be described first, followed by the modified test.

The Traditional Bag Test

"To do the bag test you will need a knife, a large paper shopping bag, a large plastic trash bag (large lawn bags are ideal), and at least twenty-five 6 to 10 oz seed tubers from the storage or seedlot that you wish to test. Pick the tubers at random but don't include any that have obvious decay problems.

Once you have all these items gathered, the test is simple to perform. First, cut all of the tubers into seed pieces with the knife and place them in the large paper shopping bag. Cutting the tubers into quarters with a longitudinal cut followed by a cross section cut exposes a lot of wounded tissue. Seed pieces made this way are ideal for the test. Next, roll the top down on the paper bag and gently shake the bag for at least 30 seconds. You may wish to invert the bag several times during this process. The shaking will serve to inoculate the freshly-cut surfaces with any Fusarium spores that might be present. Don't worry if the top of the bag unrolls when you are finished.

Then put the paper bag (with the cut seed still inside) into the large plastic trash bag. DO NOT SEAL THE PLASTIC BAG! Instead, just allow the top of the bag to droop over so that the seed in the paper bag is not subjected to direct air currents. The aim here is to trap most of the humidity from the cut seed in the plastic bag while still allowing for some oxygen exchange. Without some humidity, the seed pieces will simply dry out and without oxygen (if the trash bag were sealed) they will probably decay due to soft rot.

Now store the bag test in a place where you can leave it undisturbed for 3 to 4 weeks at a reasonably warm temperature. Room temperature (72 F) is about the upper limit but the temperature should be at least 65 F. A corner of your office or under a bench in a heated shop is an ideal location.

Examine the cut seed after 3 to 4 weeks for decay. If the seed pieces are whole and there is little or no dry rot present, you should have no problems with your seed. If there is a lot of dry rot, however, your seedlot may have a high dry rot potential. This factor will have to be taken into account when planting or selling the seed next season.

The bag test is easy to perform and provides growers with important information about their seed. Growers should perform this simple test on their seedlots every year."

Determining Benzimidazole Resistance

"Now, we'll make one minor modification to the traditional bag test which will turn it into a tool for determining benzimidazole fungicide resistance. Gather the materials for doing a bag test, only this time we'll triple the number of tubers and the number of shopping bags. This means three shopping bags and at least 75 tubers. Divide the 75 tubers into three equal groups. Do the bag test exactly as it is described above for one of the three groups of tubers. Consider this to be the control.

With the other two groups we will do almost the same thing but we'll make one modification: *After the cut seed has been shaken in the paper bag to inoculate it, open the bag and add a seed treatment.* Use a benzimidazole treatment such as TBZ or 'Topsin' in one bag and either a combination or an EBDC such as mancozeb in the other. It would be best if you used a small scale to weigh the cut seed to determine the proper amount of treatment. Here's a simple formula you can perform on a hand calculator to determine the amount of seed treatment you'll need: 16 X (the weight of your seed in lb. /100) = __ oz of seed treat for the test.

If you don't have this equipment, you can still get close enough to the right amount to do a valid test. The following values are very close to the proper amount: 25 tubers X 8 oz (avg.) = 12.5 lb. of cut seed. Most seed treatments go on at the rate of 1 lb. (16 oz) / cwt., so 16 x (12.5 / 100) = 2.0 oz of seed treat. To make things even easier, we have determined that 1 oz of seed treat is just about exactly 3 level tablespoons. If you use an actual tablespoon, take steps to ensure that no one uses it for preparing food afterwards.

After you add the seed treatment, tightly roll the top of the bag down and shake it gently for at least 30 seconds to coat the seed pieces with the treatment. You may wish to turn the bag upside down several times during this procedure to ensure good coverage. Let the roll at the top of the bag relax.

Now place your control and your bags of treated seed in the plastic trash bag and store as described above. At the end of 3 -4 weeks examine both treated and untreated seed for decay. If the benzimidazole treated seed has as much or more decay than the control and the combination or EBDC treatment has little or no decay, you probably have a resistant strain of *Fusarium* in your seedlot. Call your County Agent or Extension Specialist if you find, or think you have found, this problem. Once the problem has been identified, it is relatively easy to control."

RESULTS AND DISCUSSION

Results of all of the hundreds of modified bag tests performed in 1993 were not provided to University of Idaho investigators. Apparently many who performed the test preferred to keep the information for their own use. Results from tests on 68 seed lots from all over the state were reported to or personally evaluated by the Author. Of these, only 15 showed sensitivity to benzimidazole with the other 53 showing resistance (Nolte, 1993-a,b). In other words, 78 % of the growers who performed the modified bag test and reported their results to us had some level of benzimidazole resistance in their seed lots. Modified bag tests have been distributed to growers all over the state of Idaho for the 1994 season. Results will be collected and compared to 1993 data. Hopefully, with increased awareness of the problem and the adoption of resistance management techniques, over the next few years we will see a reduction in the amount of resistance reported. The modified bag test has played and should play a major role in this educational effort. There are additional problems, however. The role of soil-borne *Fusarium* that may carry resistance remains an unknown quantity. If soil-borne fungi provide a significant source of resistant inoculum, then the wisdom of the continued use of unprotected (not in combination with a fungicide of alternative activity) benzimidazoles for control of potato dry rot both in storage and on cut seed should be seriously questioned.

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