SESSION 4C

CONCURRENT RESISTANCE TO FUNGICIDES AND INSECTICIDES: RESULTS OF RECENT LABORATORY AND FIELD STUDIES

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THE SURVIVAL OF PHENYLAMIDE-RESISTANT STRAINS OF PHYTOPHTHORA INFESTANS IN POTATO TUBERS

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ABSTRACT

Of eight isolates of Phytophthora infestans obtained in 1986, three were phenylamide-resistant in vivo and in vitro, four were phenylamide-sensitive in both cases, whilst one (isolate 67) was resistant in vivo but sensitive in vitro. The sensitive isolates and isolate 67 grew more slowly than the resistant isolates at 5° and 10°C on agar and on potato slices of cvs Kerr's Pink and Bintje, but not on cv Home Guard or at higher temperatures. In an overwintering experiment using inoculated whole tubers of cv Kerr's Pink, the resistant isolates infected more tubers than did the sensitive ones or isolate 67, but fewer of these tubers survived to produce plants the following season due to their invasion during the winter by soft-rotting bacteria. The implications of these results for survival of resistant and sensitive strains of P. infestans are discussed.

INTRODUCTION

In 1980, phenylamide-resistant (PR) Phytophthora infestans was isolated from foliage blight lesions on potato crops in the Republic of Ireland (Dowley & O'Sullivan 1981), after the phenylamide metalaxyl had failed to control the disease. PR P. infestans was also obtained from infected tubers of the 1980 Northern Ireland crop, where a metalaxyl+ mancozeb formulation had given good control of potato blight (Cooke 1981).

In the Republic of Ireland, Dowley and O'Sullivan (1985) reported that, following withdrawal of metalaxyl from the market, PR isolates of P. infestans detected in annual surveys declined from 75% in 1981 to 6% in 1983. In N. Ireland, despite widespread use of phenylamides+mancozeb, the proportion of PR isolates showed no consistent increase between 1981 and 1985 (Cooke 1986). The proportion of PR strains detected was lower at the start of each season than at the end of the preceding one (Cooke unpublished data). It has been suggested that PR strains may not overwinter as successfully as phenylamide-sensitive ones (PS) (Cooke 1986). This paper reports investigations into the effect of temperature on PR and PS strains of P. infestans and on their ability to overwinter within the tuber.

MATERIALS AND METHODS

Isolates of P. infestans and their maintenance

The eight isolates of P. infestans used in these studies were obtained from different potato crops grown in N. Ireland in 1986. All were from foliage infections, except T10 which was from a tuber. The isolates were tested on leaf discs for their sensitivity to phenylamides

(Cooke 1986). For production of sporangia and zoospores, isolates were inoculated onto chick peas and incubated (15°C, 21 d). Sporangia were washed from these cultures, chilled at 5°C (1.5 h) and allowed to warm to room temperature to stimulate zoospore release. Sporangial concentration was adjusted to 10^5 sporangia/ml for all experiments.

Determination of sensitivity in vitro to metalaxyl

Solutions of technical grade metalaxyl (94.1% wt/wt, Ciba-Geigy) in acetone were added to clarified V8 agar (Ribeiro 1978) to give a range of concentrations. Mycelial plugs of each isolate were used to inoculate Petri dishes containing a series of six concentrations (eight dishes per concentration) plus a control (1% acetone in agar). Mycelial growth was measured after 7 and 10 d incubation (20°C) and the ED_{50} (the metalaxyl concentration which reduced growth by 50%) determined by a computer-fitted optimisation of log-logistic curves of concentration versus zone diameter.

Effect of temperature on in vitro growth of isolates

Mycelial plugs of each isolate were used to inoculate Petri dishes of clarified V8 agar. Ten replicates of each isolate were incubated at each temperature (5, 10, 15, 20, 25 and 30°C). Diameters of mycelial growth zones were measured daily after four days' incubation and the rate of growth during the linear growth phase determined.

Potato tubers

Tubers from mancozeb-sprayed crops of cvs Kerr's Pink and Bintje and unsprayed cv Home Guard were used in studies on the effect of temperature. For the overwintering experiment, the tubers used were from two crops of cv Kerr's Pink; one had received mancozeb alone (Crop A) for blight control, the other metalaxyl + mancozeb (Crop B).

Effect of temperature on growth of isolates on tuber slices

Surface-sterilised tubers were cut into 5 mm thick slices. Eight slices were placed on each of ten mesh screens and one slice per screen inoculated with each *P. infestans* isolate (10 µl spore suspension) in a fully randomised block design. The screens were incubated at 100% r.h. and at 5, 10 or 15°C. Areas of growth were measured after between 4 and 35 d, depending on the growth rate.

Overwintering experiment

Tubers from Crops A and B were inoculated with each isolate (80 tubers/crop/isolate) using filter paper discs dipped into spore suspensions and placed on eyes at the rose end of the tuber (2 discs/tuber). After 48 h at 100% r.h. (10°C), the tubers were removed and stored in net bags in the dark at 10°C to simulate seed storage. A fully randomised block design with eight replicates of ten tubers was used. Four months after inoculation, external blight symptoms were recorded. Half the tubers were then cut open; where infection was present, isolation of *P. infestans* was attempted and isolates tested on leaf discs for resistance to metalaxyl. The remaining tubers were sprouted and planted on 9 May, in plots maintaining the randomised design. Emergence and disease symptoms were assessed at intervals.

RESULTS

Sensitivity of isolates to metalaxyl

Isolates 27, 49, 51 and 96 were sensitive to metalaxyl *in vivo* and had low ED_{50}'s for mycelial growth on agar (Table 1); they are subsequently referred to as PS isolates. Isolates T10, 64 and 82 proved resistant to metalaxyl both *in vivo* and *in vitro* and were designated PR. Isolate 67, which was resistant *in vivo*, had a low ED_{50} *in vitro*. However, the dose-response of this isolate was shallow; some growth occurred even at 64 mg metalaxyl/l, unlike the PS isolates which were totally inhibited by 5 mg/l.

TABLE 1

Sensitivity of *Phytophthora infestans* isolates to metalaxyl.

Isolate	Resistant (R) or Sensitive (S)*	ED_{50} (mg metalaxyl/l) 7d mycelial growth on agar
T10	R	108
64	R	232
82	R	185
67	R	0.04
27	S	0.01
49	S	0.03
51	S	0.04
96	S	0.06

* R sporulated on 100 mg metalaxyl/l-treated discs, S only on untreated

Effect of temperature on in vitro growth of isolates

The PR isolates grew faster than the PS ones at 5 and 10°C, but not at 15°C or higher (Table 2). Isolate 67 behaved similarly to the PS isolates.

TABLE 2

The effect of temperature on growth rates of *Phytophthora infestans* isolates on agar.

	Rate of growth (mm/day)					
Isolate	5°C	10°C	15°C	20°C	25°C	30°C
T10	2.1	4.0	6.2	8.3	3.7	0.4
64	2.4	4.2	6.6	7.4	3.8	0.5
82	1.7	3.5	4.4	8.0	3.2	0.4
67	0.7	1.3	4.9	8.2	5.9	0.7
27	0.4	0.4	2.7	4.7	2.7	2.5
49	0.8	1.4	5.6	9.7	6.4	0.4
51	0.8	2.8	4.8	7.0	4.8	0.4
96	0.5	0.6	1.9	7.1	1.8	0.4
S.E. (56 D.F.)	0.07	0.10	0.14	0.15	0.13	0.17

Effect of temperature on growth of isolates on tuber slices

At 5 and 10°C on cv Kerr's Pink tuber slices, the PR isolates grew faster than the PS ones and isolate 67 (Table 3). However, after 28 d at 10°C, extensive bacterial development on the slices inoculated with the PR isolates had destroyed the growth of P. infestans, whilst the PS isolates continued to grow and sporulate. At 15°C, there were no significant differences between the resistant and sensitive isolates after 3 d and bacterial colonisation prevented further measurement. With Bintje tuber slices, similar results were obtained, growth of the PR isolates being greater than that of the PS ones at 5 and 10°C, but not at 15°C. However, with slices of cv Home Guard, no distinct differences between the PR and PS isolates were observed.

TABLE 3

The effect of temperature on growth of Phytophthora infestans isolates on potato slices.

Isolate	Area of growth (cm^2)								
	Kerr's Pink			Bintje			Home Guard		
	5°C[a]	10°C[b]	15°C[c]	5°C[d]	10°C[e]	15°C[f]	5°C[d]	10°C[b]	15°C[e]
T10	6.9	22.4	5.4	1.0	1.6	5.4	1.0	15.1	14.7
64	12.0	20.9	4.9	1.1	1.9	7.3	0.5	1.3	2.8
82	8.4	20.4	4.7	1.2	1.7	4.0	1.4	17.1	17.3
67	2.1	3.5	4.0	0.2	0.5	4.0	0.4	1.1	1.6
27	1.1	2.9	3.3	0.3	0.4	1.3	0.6	0.8	1.1
49	3.6	6.9	3.3	0.3	0.9	3.9	0.6	16.5	12.5
51	2.9	4.2	4.2	0.4	0.9	3.9	0.7	11.7	11.9
96	3.0	7.7	5.3	0.4	0.8	5.0	2.1	16.3	17.8
S.E.[g]	1.07	1.11	0.35	0.10	0.15	0.37	0.29	0.94	1.04

measured after a 35d; b 18d; c 3d; d 21d; e 7d; f 4d; g 63 D.F.

Overwintering experiment

All isolates produced some tuber infection, but the PR ones gave significantly more infected tubers than the PS ones or isolate 67 on both crops (Table 4). Bacteria tended to rot the blight-infected tubers causing them to disintegrate or fail to sprout. Consequently, few of the PR-inoculated tubers survived for planting. Emergence of planted tubers was greater than 80%, except for those from Crop B inoculated with T10. Overall, of the tubers inoculated with the three PR isolates, 1.8% of those from Crop A were visibly infected and suitable for planting and all emerged, and 4.3% from Crop B with blight were planted and 0.8% emerged. However, of the tubers inoculated with the PS isolates and isolate 67, 10.5% of those from Crop A were visibly infected and planted and 8.5% emerged, whilst the corresponding figures for Crop B were 7.0 and 5.5%. No blight symptoms were observed on any plants up to 19 July. Re-isolation of P. infestans from the cut tubers with symptoms of blight proved difficult due to bacterial colonisation. Successful isolations were made from only three tubers, these had been inoculated with isolates T10, 82 and 27. The first two proved to be PR, whilst the last was PS.

TABLE 4

Development of blight, survival and emergence of inoculated tubers of cv Kerr's Pink stored overwinter.

Isolate	Blight-infected tubers (%)	Soft-rotted tubers discarded (%)	No.[a] planted tubers (no. emerged[b])		No.[a] planted tubers with blight (no. emerged[b])	
Crop A[c]						
T10	98.8	87.5	0.1	(0.1)	0.1	(0.1)
64	83.4	75.0	0.8	(0.8)	0.1	(0.1)
82	97.5	91.3	0.1	(0.1)	0.0	(0.0)
67	6.3	0.0	5.0	(5.0)	0.4	(0.4)
27	10.0	0.0	5.0	(4.9)	0.4	(0.3)
49	20.5	10.3	4.3	(4.3)	0.3	(0.3)
51	18.7	0.0	5.0	(4.8)	0.8	(0.8)
96	13.8	1.3	4.9	(4.5)	0.9	(0.5)
S.E.(49 D.F.)	4.16	2.41	0.14	0.19	0.18	0.18
Crop B						
T10	98.8	85.0	0.5	(0.1)	0.4	(0.0)
64	92.5	83.5	0.6	(0.5)	0.3	(0.1)
82	91.3	86.3	0.3	(0.3)	0.0	(0.0)
67	8.8	2.5	4.9	(4.6)	0.6	(0.5)
27	2.5	0.0	5.0	(5.0)	0.1	(0.1)
49	39.5	29.0	3.5	(3.5)	0.4	(0.4)
51	3.7	2.5	4.9	(4.9)	0.0	(0.0)
96	23.7	7.5	4.5	(4.0)	0.6	(0.4)
S.E.(49 D.F.)	3.48	2.76	0.21	0.20	0.23	0.17

a mean of 5 tubers/plot, 8 replicate plots b assessed 22 June
c Crop A was sprayed with mancozeb, Crop B with metalaxyl+mancozeb

DISCUSSION

The three PR isolates grew faster than the PS ones at low temperatures on agar and potato slices. Piganeau and Clerjeau (1985) reported that at low temperatures, PR Plasmopara viticola strains were stimulated compared with PS ones, due to differences in germination and sporulation. In the present work, there were no consistent differences in the sporulation per unit area between the PR and PS isolates both on tuber slices and leaf discs (Walker, unpublished data). In contrast, in the Republic of Ireland, Dowley (1987) found that five PR P. infestans isolates produced fewer sporangia per unit leaf area than five PS ones. However, both studies used relatively few isolates, so the results may not be representative of the whole P. infestans population. Alternatively, they may reflect populations selected under different conditions. Isolate 67, which showed phenylamide-resistance in vivo but not in vitro, behaved like the PS isolates in its growth rates on agar and potato slices.

Differences in growth rates between PS and PR P. infestans at 10° C and below will only influence the mycelial phase within the tuber. A faster growth rate at low temperature might appear to favour survival of

PR rather than PS strains. However, in the overwintering trial, although the PR isolates infected more tubers, fewer of them survived due to bacterial colonisation. In fact, more visibly-infected tubers inoculated with PS isolates produced plants. In this trial, no blight lesions developed on plants from infected mother tubers; this was not unexpected since very few produce infected plants (Hirst & Stedman 1960). Nonetheless, after the winter there was a greater potential inoculum source amongst plants from PS rather than PR-inoculated tubers.

Two factors complicate this interpretation. First, the behaviour of isolates on cv Home Guard, which differed from that on the other cultivars, suggests a cultivar effect. Second, the ability of metalaxyl to protect tubers directly from PS P. infestans (Bruin et al. 1982), may reduce tuber infection by these, but not by PR strains. In the present study, the PS isolates infected the metalaxyl+mancozeb and mancozeb-sprayed crops to the same extent, but much less than the PR isolates. Subsequent enquiry revealed that Crop A had received one spray of metalaxyl+mancozeb 20 d before desiccation. Further investigation is needed to ascertain whether the lower level of infection by the PS isolates was caused by metalaxyl in the tubers or the effect of temperature.

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RESISTANCE RISK EVALUATION OF PHENYLAMIDE AND EBI FUNGICIDES

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ABSTRACT

The overall resistance risk of fungicides is composed of inherent and management risks. The inherent risk comprises parameters linked to chemical and biological behaviour of the fungicide in specific disease and environmental situations, whereas the management risk includes all factors related to the fungicide use strategy. The resistance risk of fungicides belonging to the phenylamides and EBIs is evaluated based on a new scheme. High risk fungicides (e.g. phenylamides), when used with proper strategies, may not cause more overall risk than low risk fungicides (e.g. EBIs) used improperly. Also, resistance risk is low if a fungicide is little effective or if the disease pressure is low. For every anti-resistance strategy, a separate risk evaluation is required.

INTRODUCTION

Fungicide resistance describes both the level of sensitivity of individuals and the increase of such individuals in a population through selection. Failure of disease control in the field may arise from an increase in frequency rather than the absolute number of resistant strains in the population. Therefore, besides detection of resistant strains, the description of the genetic variation in wild-type populations is essential. The overall resistance risk of fungicides is composed of inherent and management risks. The **inherent resistance risk** comprises properties like efficacy of the chemical, behaviour of the pathogen, environmental conditions as well as genetics of the interactions between chemical and pathogen. The **management resistance risk** includes all factors related to the frequency and strategy of the fungicide applications. In this paper we give examples of how to estimate the inherent as well as the management and the overall resistance risk of fungicides belonging to the phenylamides and the ergosterol biosynthesis inhibitors (EBIs). The evaluation is based on a new scheme described in more detail recently by Gisi and Staehle-Csech (1988a).

ELEMENTS OF INHERENT RESISTANCE RISK

Twelve elements of inherent risk are considered to be important for the risk evaluation. Although some of the elements are closely related to each other, we preferred to list them independently rather than giving specific weightings. Each element is estimated to bear a low, medium or high level of inherent risk yielding 1, 2 or 3 risk points, respectively. The total inherent risk is the sum of the risk points of all elements (Table 1).

1. Cross resistance and resistance mechanisms

Decreased sensitivity of a given fungus strain against a fungicide A also occurs against a fungicide B if resistance is caused by the same genetic factor, or if A and B interfere at the same site in the metabolic pathway of the fungus. This pattern, called (positive) cross resistance, occurs typically within a chemical class of related structures. Differences in sensitivity of strains in a population may vary within a factor of at least 10 and should therefore not necessarily be related to resistance phenomena. Cross resistance is always reciprocal and the two compounds cannot substitute for each other in case one fails to control the resistant subpopulation. There are

examples of fungicide resistance related to changes at the site of action (e.g. phenylamides), but others like DMIs have resistance mechanisms related to changes in the fungicide uptake-export balance or to other effects.

2. Search for resistant (r) strains in the field

The sensitivity of the population is tested with a range of fungicide concentrations. Treated plants are exposed for a short period to the environment and then taken back to the greenhouse for further incubation and sensitivity tests. Significant variation in sensitivity of different strains to the test fungicide is an important precondition to the selection process of a less sensitive subpopulation.

3. Selection of r-strains with fungicides in the laboratory

A simulated selection of resistant strains out of bulk populations is made under laboratory conditions using repeated exposure to increasing concentrations of the fungicide. Several generations are treated with sublethal concentrations of the fungicide. The surviving individuals are used for inoculation of the next test series. Such training experiments normally yield resistant strains fairly rapidly in case of monogenic resistance but rather slowly for polygenic resistance.

4. Production of r-strains with a combination of fungicide and mutagen treatment in the laboratory

If fungicide exposure is combined with or follows mutagenic treatment such as UV-radiation or NTG (N-methyl-N'-nitro-N-nitrosoguanidine), resistant strains can be produced at much higher frequencies than without mutagens. The degree of resistance (resistance factor) rather than the mutation frequency should be considered as a resistance risk parameter. The higher the resistance factor, the higher the probability that resistance is controlled by one or only few genes.

5. Selection of r-strains in the field

Selection of r-strains in the field may be possible through repeated fungicide applications with short intervals. Selection trials should be made under controlled conditions and in fields far away from areas where the corresponding disease represents a major problem.

6. Survey programs

Survey programs can reveal a decrease in field performance of a fungicide, only if it was sprayed according to label recommendations and is compared with standard fungicides having different modes of action. Surveys of fungicide performance and resistance monitoring can be done in preselected trials ("base-line monitoring") or as an emergency service ("complaint monitoring"). Resistance as a possible reason for decreased efficacy should be considered only if significant reductions of disease control and product failure can be correlated with an increase of the resistant subpopulation.

7. Level and stability of resistance in a population

If fungicide treatments of the original population result in a separation into distinct subpopulations containing either sensitive or extremely resistant strains, resistance probably originates from one or at most a few mutations; the type of resistance is monogenic (single-gene) and the selection process is disruptive. The monogenic type of resistance is considered to have a high inherent risk of resistance. If fungicide treatments of the original population result in a new but unimodal distribution of strains with different sensitivities, resistance is probably controlled polygenically and the selection process is continuous. This progressive change in sensitivity causes a "shifting" of the population (Georgopoulos and Skylakakis 1986).

8. Genetics of resistance

Crossing of parental strains with different sensitivities to a fungicide result in the production of F1 progeny. If pathogens are haploid and have a sexual stage that can be manipulated in the laboratory (e.g. Erysiphe), classical genetic analysis may reveal how many genetic factors are involved in resistance. Where the sexual stage is lacking protoplast fusion techniques may be used instead. For diploid (e.g. Phytophthora) or dikaryotic (e.g. rusts) pathogens, dominance relationships will also be required. Segregation of progeny will either follow Mendelian rules, suggesting only a few genes are involved (e.g. Phytophthora treated with phenylamides), or will produce a unimodal distribution indicating the involvement of many genetic factors with various degrees of additivity (e.g. Erysiphe treated with DMIs).

9. Relative fitness of s- and r-strains in the absence of fungicide

Fungicide resistant strains may be either less fit or equally or even more fit compared to sensitive strains. Fitness and relative competitiveness of resistant strains are measured in the absence of the fungicide and includes observations of different steps in the infection cycle like disease efficiency, latent period, sporulation capacity and intensity. Resistant strains may differ in one specific development step but not in another one.

10. Dynamics of resistance build-up

Mixtures of two strains that differ in sensitivity to the fungicide are treated repeatedly with the fungicide resulting in a permanent selection of the resistant subpopulation which can be measured by changes in the frequency of resistant individuals. Such experiments reveal the potential risk and speed of resistance build-up in relation to the number of fungicide applications, the level of initial resistance in the population and the competitive abilities of s- and r-strains in the population (Gisi and Staehle-Csech 1988b).

11. Persistence of fungicide selection pressure

It is generally accepted that fungicides providing very high levels of disease control over a long period of time do not permit sufficient "escape" of fungal propagules, and therefore cause higher selection pressures than fungicides with shorter persistence and intermediate levels of activity.

12. Fungus biology

Two additional properties of the fungus contribute to the inherent risk of fungicide resistance, namely a short generation time (or high apparent infection rate) and a low migration rate. Further increase of resistance risk is provided by favourable conditions for disease development. Resistance risk evaluation for a given fungicide has to be done separately for every individual pathogen and disease situation.

ESTIMATION OF INHERENT AND MANAGEMENT RESISTANCE RISK

The level of inherent risk for every single element is estimated independently (Table 1). For example, if relative fitness of resistant strains is higher, equally high or lower than that of sensitive strains, the level of risk is considered to be 3, 2 or 1, respectively. If cross resistance is always present, we assigned 3 points; if cross resistance is present in some but not all cases, we assigned 2 points; if cross resistance is absent we assigned 1 point. If all twelve elements are estimated to bear a low level of risk, the total inherent risk results in 12 points and the fungicide is classified as "low risk". On the other hand, if all twelve elements are estimated to be of high risk, the total inherent risk is 36 points and the fungicide is classified as "high risk". For a specific fungicide, single elements may be

rated with 3 points, others with 1 point. Therefore, we assigned ranges rather than single figures of risk levels resulting in 12-20 and 29-36 points of inherent risk, respectively, for low and high risk fungicides. The estimation of the management resistance risk is determined by factors like number and timing of applications, spray intervals, size of treated area. The development of the disease in treated plots, and the relative changes of the s- and r-subpopulations will determine the validity of a fungicide use strategy. If no significant increase of the resistant subpopulation is observed, the management risk is estimated to be low (1 risk point). If resistance build-up occurs after a specific use strategy but can be controlled by suitable use limitations, the management risk is medium (2 risk points). A high management risk (3 risk points) results with a use strategy causing a significant increase of resistant strains and unacceptable disease levels that may occur after many applications with short intervals of a high risk fungicide against a disease with a short generation time.

ESTIMATION OF OVERALL RESISTANCE RISK

The estimation of the overall risk is a combined analysis of inherent and management risks (Table 1).For every single anti-resistance strategy (e.g. use of mixtures versus alternation; number of applications in relation to the generation time of the fungus), separate evaluations of the overall resistance risk will be required.The overall risk can be estimated by multiplying the total inherent risk with the management risk (Table 1). With appropriate use strategies (1 risk point), the overall resistance risk will not increase resulting in 12-20 and 29-36 overall risk points, respectively, for low risk and high risk fungicides (Table 1). In contrast, a high risk fungicide may result in serious problems with 108 overall risk points, when handled inappropriately (3 risk points). More important is the conclusion, that a high risk fungicide, when used with proper strategies, may not cause more overall risk (36 points) than a low risk fungicide used improperly (36 points).

TABLE 1

Estimation of the inherent resistance risk of three different fungicide types (A, B, C) against a specific pathogen (left) and estimation of the overall resistance risk of a specific antiresistance strategy against a specific disease by combining inherent with management risk factors (right)

Elements of **inherent** risk	level of **inherent** risk		
	low = 1	medium=2	high = 3
1 cross resistance	A	B	C
2 field r-strains	A	B	C
3 selection r-strains	A	B	C
4 production r-strains	A		B C
5 selection in field	A B		C
6 field performance	A	B	C
7 level/stability of r	A B		C
8 genetics of resistance	A B		C
9 fitness of r-strains	A B		C
10 r-build-up	A B		C
11 selection pressure	A	B	C
12 fungus biology	A		B C
total **inherent** risk	12-20	21-28	29-36

Overall resistance risk			
level of **management** risk	total **inherent** risk (from left side)		
	12-20	21-28	29-36
low = 1	12-20	21-28	29- 36
medium = 2	24-40	42-56	58- 72
high = 3	36-60	63-84	87-108
level of overall risk: low = 12-43, medium = 44-75, high = 76-108			

OVERALL RESISTANCE RISK OF PHENYLAMIDE FUNGICIDES (Tables 2 and 3)

The inherent risk of two phenylamide fungicides, oxadixyl and a standard compound (M), is estimated for two diseases P. infestans on potato and P. viticola on grapes (Table 2). It is obvious that both fungicides result in a high level of inherent risk for P. infestans (34 to 36 risk points) as well as for P. viticola (31 to 33 risk points). Therefore, both are considered to be high risk fungicides. The risk for the compounds against P. viticola is somewhat lower than for P. infestans, because the stability of resistance, the fitness of resistant strains, and the biology of P. viticola represent only a medium risk (Hassan 1987). The two fungicides may cause slight differences in the dynamics of resistance build-up.

TABLE 2

Estimation of the inherent resistance risk of phenylamide fungicides against Phytophthora infestans (PI) on potato or Plasmopara viticola (PV) on grape

Elements of inherent risk	level of risk for			
	oxadixyl PI	oxadixyl PV	Comp. M PI	Comp. M PV
1 cross resistance	3	3	3	3
2 field r-strains	3	3	3	3
3 selection r-strains	3	3	3	3
4 production r-strains	3	3	3	3
5 selection in field	3	3	3	3
6 field performance	3	3	3	3
7 level/stability of r	3	2	3	2
8 genetics of resistance	3	3	3	3
9 fitness of r-strains	3	2	3	2
10 r-build-up	2	2	3	3
11 selection pressure	2	2	3	3
12 fungus biology	3	2	3	2
total of inherent risk	34	31	36	33
level of risk: low = 12-20, medium = 21-28, high = 29-36				

TABLE 3

Estimation of the overall resistance risk of different use strategies of oxadixyl (A) alone or in mixture with a contact fungicide (e.g. mancozeb, B) and cymoxanil (C), against Phytophthora infestans on potato

	total inherent risk (from table 2)				
	34	34	34	34	34
level of management risk	4 appl. A,A,A,A	4 appl. A+B,A+B A+B,A+B	4 appl. A+B+C,A+B+C A+B+C,A+B+C	6 appl. B, A+B, B, A+B, B, A+B	6 appl. B, B, A+B, A+B, A+B, B
low = 1			34		51
medium = 2		68		85	
high = 3	102				
level of risk: low = 12-43, medium = 44-75, high = 76-108					

In table 3, the overall risk of five different use strategies of oxadixyl containing products are compared. Four consecutive sprays of oxadixyl alone (A) exhibit a strong selection pressure and represent a risky use strategy (3 points) yielding 102 overall risk points. Also very risky (2.5 points) is alternation of contact fungicide B (e.g. mancozeb) and the mixture (A + B) giving a total of six sprays and an overall risk of 85 points. Only medium overall risk may be attributed to the use of four consecutive applications of two-way mixtures of oxadixyl and a contact fungicide (A+B) yielding 68 risk points or a strategy like B, B, A+B, A+B, A+B, B with 51 risk points. A low risk may be produced by four consecutive applications of the three-way mixture oxadixyl+contact+cymoxanil (A+B+C) yielding 34 risk points. These estimations are based on experimental data of Samoucha and Gisi (1987).

OVERALL RESISTANCE RISK OF EBIs (Tables 4 and 5)

The inherent risk of cyproconazole (SAN 619 F, Gisi et al. 1986) is evaluated compared to that of imidazole or morpholine standards for five different diseases (Table 4). The control of E. graminis represents a potentially high risk for all three fungicides (3 risk points for fungus biology) because of its short generation time. The morpholine produces only a low level of risk (14 risk points), since no resistant strains have been found so far (elements 1 to 6 rated as 1 point each) and all other criteria indicate only minimum resistance risks (elements 7 to 11 rated as 1 point each). On the other hand, both the imidazole and cyproconazole may represent a medium level of risk, the imidazole being somewhat less active and less risky (21 points) than cyproconazole (26 points) or any other triazole. DMI-resistant strains

TABLE 4

Estimation of the inherent resistance risk of EBIs against different wheat diseases (I - V) [a]

Elements of inherent risk	level of risk for							
	Cypro-con-azole I	Imid-azole I	Mor-pho-line I	Cypro-con-azole II	Cypro-con-azole III	Imid-azole III	Cypro-con-azole IV	Cypro-con-azole V
1 cross resistance	3	3	1	1	2	2	1	1
2 field r-strains	2	1	1	1	1	1	1	1
3 selection r-strains	2	2	1	1	1	1	1	1
4 production r-strains	3	3	1	1	3	3	1	1
5 selection in field	1	1	1	1	1	1	1	1
6 field performance	1	1	1	1	1	1	1	1
7 level/stability of r	2	1	1	1	1	1	1	1
8 crossing s/r strains	2	2	1	1	1	1	1	1
9 fitness of r-strains	2	2	1	1	1	1	1	1
10 r-build-up	2	1	1	1	1	1	1	1
11 selection pressure	3	1	1	3	1	3	2	1
12 fungus biology	3	3	3	3	1	1	2	1
total inherent risk	26	21	14	16	15	17	14	12
level of risk:	low = 12-20,		medium = 21-28,		high = 29-36			

a) **I:** Erysiphe graminis; **II:** Puccinia recondita; **III:** Pseudocercosporella herpotrichoides; **IV:** Leptosphaeria nodorum; **V:** Fusarium sp.

can be found and cross resistance between all triazoles and imidazoles is common (3 risk points each for cross resistance), but resistance factors and stability are not very high and may vary among different DMIs. Resistant field strains are, in most cases, less fit (Buchenauer et al. 1984) and resistance build-up is only slow. Cyproconazole controls P. recondita extremly well (Gisi et al. 1986) and may, therefore, induce a strong selection pressure during the short generation cycles (3 risk points each for selection pressure and fungus biology); all other risk elements are considered to be low resulting in a total of only 16 inherent risk points.

Control of P. herpotrichoides with cyproconazole represents only a low level of inherent risk (15 risk points), because cyproconazole shows medium eyespot control and the fungus produces only few generations per season. Cyproconazole does not control all strains equally well, especially when R-strains (slow strains) dominate the population. This results from the low efficacy against R-type strains rather than selection of cyproconazole resistant strains. Production of resistant strains under laboratory conditions is possible (3 risk points for this element for both cyproconazole and the imidazole) but resistant strains have not been found in nature so far. Cross resistance in P. herpotrichoides is present but significant differences exist between single molecules. The inherent risk of cyproconazole for the control of L. nodorum is very low (14 risk points). All attempts to find cyproconazole resistant strains have so far been unsuccessful, but the sensitivity of field strains varied at least tenfold (SANDOZ internal results, unpublished). Selection pressure and fungus biology may cause only medium risk (2 points each). Cyproconazole is not active against Fusarium sp. and, therefore, the resistance risk is nil. It is trivial but important to say, that a fungicide which is not active against one disease, but very active against another disease in the same crop, represents no resistance risk at all for the first disease but a certain risk for the other.

In table 5, the overall risk of different cyproconazole uses are compared. If four consecutive applications with cyproconazole alone are made at the stages seed/stem base/leaf/ear, the overall risk for powdery mildew control is considered to be medium (52 risk points), whereas a low overall risk is produced (26 risk points) by using the following program: 1) seed treatment with cyproconazole plus non-azole molecule (N); 2) a mixture of cyproconazole plus an anti-eyespot standard (O) as stem base application; 3) an application of a morpholine (P) or a mixture of cyproconazole + P as leaf

TABLE 5

Estimation of the overall resistance risk of different use strategies of cyproconazole (cypr) alone or in mixture with fungicides N, O, P or R against different diseases (I - V)

level of management risk	total inherent risk (from table 4) (I) 26	(I) 26	(II) 16	(III) 16	(IV/V) 14/12
	4 appl. cypr single	4 applications; seed: cypr+N/stem base: cypr+O/ leaf: P or cypr+P/ear: cypr+R			
low = 1 medium = 2 high = 3	52	26	16	24	28/18
level of risk: low = 12-43, medium = 44-75, high = 76-108					

treatment; 4) a mixture of cyproconazole plus contact fungicide (R). Cyproconazole, used at normal rate for control of seed-borne diseases, does not control early powdery mildew attack on the first leaves and, therefore, does not select for resistant powdery mildew strains as occurs with some other triazoles. Furthermore, the use of morpholines or morpholines plus cyproconazole as third treatment further decreases the risk of selection for resistant powdery mildew strains. The same arguments can be used for the risk evaluation of rust control, which causes only a very low level of risk (16 risk points). The overall risk of four fungicide applications will not increase significantly for the other three diseases like eyespot (1.5 points), Leptosphaeria (2 points) and Fusarium (1.5 points), yielding 24, 28 and 18 overall risk points, respectively. The overall risk may further decrease by the addition of companion fungicides O, P or R.

DISCUSSION

Resistance risk has to be evaluated as seriously as, for example, pesticide residues in food. Based on experimental data and a new evaluation scheme, we have estimated the overall risk of different phenylamide and DMI use strategies knowing that we have done the analysis with one of several possible approaches. The value of such evaluations is a better prediction of possible trends in resistance build-up rather than the precise assignment of risk points. Nevertheless, we would not recommend a use strategy with a high level of overall risk. Strategies yielding medium levels of overall risk may be used, only if product performance and resistance build-up are observed closly, e.g. by monitoring programs. A specific use strategy may represent a medium overall risk for one country but a low risk for another one depending on factors like climatic conditions and agricultural practices. It is equally counterproductive to ignore the resistance risk of certain fungicide use strategies or to generalize individual cases of fungicide resistance.

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STRATEGIES TO COMBAT FUNGICIDE RESISTANCE IN BARLEY POWDERY MILDEW

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ABSTRACT

A comparison of fungicide strategies to combat the development of barley powdery mildew resistant to triazole fungicides was conducted in field experiments over two seasons. Spray programmes of a triazole alone, a triazole plus morpholine mixture or an alternation of morpholine and triazole fungicides, were tested in replicated plot field trials. Spring barley (cv. Golden Promise), seed-treated with a triazole fungicide was used throughout.

The sensitivity to propiconazole of the mildew collected from plots treated with each fungicide strategy was related to mildew control, green leaf retention and yield. Significant differences in fungicide sensitivity were recorded between spray programmes, and these differences correlated with disease control and yield. The triazole only strategy reduced fungicide sensitivity significantly relative to untreated plots; mildew control and grain yields were lower than from plots treated with the mixture or an alternation of triazole and morpholine fungicides. The fungicide mixture programme was the only strategy that did not reduce the sensitivity of mildew relative to the untreated plots.

INTRODUCTION

Barley mildew (Erysiphe graminis f sp. hordei) sensitivity to triazole fungicides has been monitored in GB since 1981 (Fletcher & Wolfe, 1981). A decline in sensitivity has been observed, this has been linked with widespread commercial use of products based on this chemistry (Wolfe et al. 1984, Heaney et al. 1986). Previously unpublished survey data by Ciba-Geigy Agrochemicals confirmed this for mildew populations sampled from widely varying locations in England and Scotland, and at various times during the season. The decline, measured as an LC_{50} to propiconazole, was more or less linear over the 5 year period from 1982 to 1986 (see Figure 1) but seemed to reverse in 1987, perhaps due to an increased use of morpholine fungicides relative to triazole products for the mildew control.

The widespread use of triazole-based fungicides is, nevertheless, likely to continue throughout Western Europe, as no new fungicides with a different mode of action seem close to commercialisation. Clearly the sensitivity of mildew to the triazoles has decreased generally and there is a risk that this phenomenon will be repeated with other diseases. In such a situation it is vitally important to know whether the problem can be countered and if so, how.

Mathematical models have predicted that an alternation of fungicides with different modes of action, or a mixed product combining two fungicides of different modes of action, will provide an effective anti-resistance strategy (Kable & Jefferies, 1979). One or other of these strategies was predicted to be superior in particular circumstances depending upon such factors as the pathogen's generation cycle time (Skylakakis, 1981), but this has never been demonstrated satisfactorily in the field. The object of the work reported here was to demonstrate whether either strategy could reduce, or prevent, the decline in triazole sensitivity of barley mildew, and if so, to identify the most effective strategy. Trials were initiated in the seasons 1984-87, all of which showed similar trends but only the 1986 + 1987 trials were statistically satisfactory and are reported here.

MATERIALS AND METHODS

Identical trials were conducted in 1986 and 1987, using spring barley (cv. Golden Promise), treated with triadimenol + fuberidazole + imazalil (37.5 + 4.5 + 4.95 g ai/100 kg seed) as "Baytan IM" seed treatment, sown at Whittlesford, Cambridge. Foliar sprays were applied to 18 m x 24 m plots, replicated 8 times, by tractor mounted sprayer in 200 l water/ha at GS 31 and again at GS 53-57.

Propiconazole as "Tilt 250EC" or triadimenol as "Bayfidan" (both at 125 g ai/ha), were applied twice to represent an intensive triazole only programme; propiconazole + tridemorph as "Tilt Turbo" was applied twice at 125 + 350 g ai/ha in 1986 and at 125 + 250 g ai/ha in 1987 to represent the mixture strategy; and tridemorph as "Calixin" at 525 g ai/ha (GS 31), followed by propiconazole, at 125 g ai/ha (GS 53) represented the alternating product strategy.

Mildew levels were 5% overall at GS 31 and at 2-5% active disease in the propiconazole + tridemorph plots at GS 53-57. Mildew control was assessed every 7 days as total % leaf area infected. Grain yield per plot was assessed at normal harvest by a small plot combine harvester.

Fungicide sensitivity was measured 21-23 days after the first spray and 25 days after the second spray using the method described by Staub and Sozzi, 1981. This involves inoculating barley plants (cv. Golden Promise)grown in glass tubes on vermiculite, watered with Hewitt's solution amended with propiconazole at a rate of 0, 3.0, 10.0 or 30.0 ppm. For each strategy, bulked mildew samples were collected from from each of the eight field replicates. Assays of each mildew sample were replicated three times. Seedlings were assessed after 10 days for % leaf area infected, from which the LC_{50} and LC_{90} values were calculated by probit analysis.

RESULTS

Mildew levels

In both years mildew developed rapidly from 5% leaf area infected at the first application to a final aggressive attack of 80-90% leaf area infected. Fifteen days after the first application there was a clear difference between triazole only sprays which had 6-13% leaf area infected and the mixture or alternating product strategies which had a 1-2% infection (table 1). This order of difference continued until 13-14 days after the second application. At this stage 80-85% of the leaf was infected in untreated plots; 27-44% in the triazole only treatments; 12-25% in the alternating product strategy plots and 5-12% in the mixed product strategy plots (table 1). The performance of the straight triazole programmes was disappointing giving only 50-75% control, whereas the alternating and mixed product strategies performed satisfactorily. The mixture strategy was superior to all other strategies towards the end of the epidemic.

TABLE 1

Mildew: % leaf area infected per plot

Strategy	15 days after first application		13-14 days after second application	
	1986	1987	1986	1987
untreated	28.1	37.7	80.0	85.0
propiconazole	6.2	6.6	43.8	26.8
triadimenol	12.8	11.1	40.0	35.9
propiconazole + tridemorph mixture	0.6	1.2	11.8	4.7
tridemorph, propiconazole alternation	0.7	0.5	24.9	11.9
LSD p = 0.05	3.75	7.26	9.0	7.86

The relative performance of the disease control strategies was reflected in the retention of green leaf area (table 2). 95% senescence was recorded in the untreated plots at 21 days after the second application whereas the mixed product strategy gave a green leaf area of 66-71%. This was a significant improvement over that of the alternating and straight triazole strategies at 52-61% or 32-50% respectively.

Yield

Grain yields correlated well with mildew control and green leaf area assessments (table 3). Triazoles alone gave a 12-26% yield response over untreated, and the alternating strategy increased yields by 28-39%. The best yield followed use of the mixture strategy with an increase of 44-47% above the untreated.

Fungicide sensitivity

After the first application, no significant differences in mildew sensitivity were detected. After the second application, the sensitivity from the untreated plots was virtually unchanged at an LC_{90} value of 10.8-13.4 ppm propiconazole. The straight triazole strategy significantly reduced sensitivity to 18.9-19.6 ppm propiconazole. The alternating product strategy slightly increased the LC_{90} to 13.6-15.4 ppm but in both years this was not significantly different from the untreated control. The mixed product strategy did not reduce the sensitivity of the mildew, but in fact marginally increased the LC_{90} to 9.6-12.6 ppm relative to LC_{90} values for mildew from untreated plots. However, these increases in sensitivity were not significant at the 95% probability level. Differences in mildew sensitivity correlate well with differences in mildew control, green leaf retention and grain yield described above.

Both the mixture and the alternating product strategies did therefore, prove to be effective anti-resistance strategies relative to repeated use of triazole alone. Of the two, the mixed product strategy was the best, giving a significant reduction in mildew sensitivity relative to the straight triazole programme and a non-significant reduction relative to the untreated population in both years of testing.

TABLE 2

% green leaf area at senescence

Strategy	1986	1987
untreated	5.4	4.9
propiconazole	48.8	50.0
triadimenol	31.9	29.9
propiconazole + tridemorph mixture	70.6	65.7
tridemorph, propiconazole alternation	51.9	61.4
LSD p = 0.05	9.87	-

TABLE 3

Grain yield (% of untreated)

Strategy	1986	1987
untreated	100(3.4 t/ha)	100(5.4 t/ha)
propiconazole	120	126
triadimenol	116	112
propiconazole + tridemorph mixture	147	144
tridemorph, propiconazole alternation	128	139
LSD p = 0.05	25.6	10.5

TABLE 4

Sensitivity of mildew to propiconazole 21-23 days after first fungicide application.

Strategy	LC_{90} ppm propiconazole 1986	1987
untreated	11.1	7.2
propiconazole	11.2	15.2
triadimenol	11.8	18.4
propiconazole + tridemorph mixture	12.3	15.3
tridemorph, propiconazole alternation	9.5	15.7
LSD p = 0.05	NS	NS

TABLE 5

Sensitivity of mildew per treatment 25 days after second application

Strategy	C_{90} ppm propiconazole 1986	1987
untreated	10.8	13.4
propiconazole	19.5	19.6
triadimenol	19.4	18.9
propiconazole + tridemorph mixture	9.6	12.6
tridemorph, propiconazole alternation	13.6	15.4
LSD p = 0.05	3.6	6.17

DISCUSSION

Previous workers have shown that barley mildew populations have become increasingly less sensitive to triazole fungicides, particularly where persistent triazole seed treatments have been used (Wolfe et al. 1984). Hunter et al. (1984) showed that where ethirimol or triadimenol seed treatments were followed by foliar fungicides from the same chemical group, mildew sensitivity declined. If such seed treatments were followed by a mixture of fungicides with a different mode of action, the decline in sensitivity was not recorded.

The field experiments reported here and previous ones conducted in 1984 and 1985 at Whittlesford, confirm the danger of combining foliar sprays and a seed treatment with the same biochemical mode of action. This practice is still widespread in Great Britain as it represents a cost effective disease control system for intensive barley production. Farmers are primarily motivated by a desire to improve the level and reliability of disease control in cereals. The evidence presented here is that a mixed product containing active ingredients with different biochemical modes of action was an effective anti-resistance strategy and gave added security to the intensive use of today's highly active products.

The need to develop effective anti-resistance strategies is not limited to mildew and the triazole fungicides. The morpholine based products must also be vulnerable fungicides and it is known that such pathogens as Rhynchosporium secalis, Septoria spp and Pseudocercosporella herpotrichoides have the genetic potential to develop resistance to triazole and MBC generating fungicides. No other effective chemical groups of major importance have been reported to be under development by the agrochemical companies in recent years. Thus the need to preserve those currently available now becomes paramount. Both the alternating and mixed product strategies were confirmed to be effective in the work presented here with a clear advantage by the mixed product in both performances and anti-resistance properties.

How far this work can be extrapolated to other crop situations, fungicide combinations and geographical locations is not known. Staub and Sozzi (1984) showed that in controlled environment conditions, a mixture of metalaxyl and mancozeb was an effective anti-resistance strategy for Phytophthora infestans in potatoes. Delp (1981) reported that the use of mixtures of benzimidazole with unrelated companion fungicides had delayed and prevented resistance problems.

Little hard evidence from controlled field trials has been reported however, and thus it is believed that the work reported here is particularly valuable. We believe that it is now important to repeat such work in other situations to establish with confidence, how to use the disease control tools at our disposal; this should ideally include resistant varieties as well as pesticides to allow advice to be given confidently within an integrated crop management framework.

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VARIATION IN SENSITIVITY OF ERYSIPHE GRAMINIS F.SP. TRITICI TO SBI FUNGICIDES IN WESTERN HUNGARY

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ABSTRACT

Fungicides which inhibit sterol biosynthesis (SBI) have been used in Hungary for control of wheat powdery mildew (Erysiphe graminis f.sp. tritici) since 1978. Mildew populations were tested during 1986 and 1987 for their sensitivity to several SBI inhibitors, and isolates with reduced sensitivity to prochloraz and triadimefon were detected. In some isolates this reduced sensitivity, which was stable in the absence of fungicide, was correlated with poor control by these two fungicides under glasshouse conditions. Cross-sensitivity to other SBI fungicides varied, and cross-resistance to propiconazole and flutriafol was not always found in the isolates tested. These cross-resistance patterns suggest that several resistance mechanisms operate against SBI fungicides in wheat powdery mildew, and that some of these mechanisms do not operate against all SBI fungicides.

INTRODUCTION

Disease control in winter wheat in Hungary usually involves one or two foliar appliations of fungicides, or a seed treatment. The first sterol biosynthesis inhibitor (SBI) fungicide, the triazole triadimefon, was introduced in 1978. Before then only sulphur and MBC-generating fungicides were applied at all widely. Since 1980 other SBI's (diclobutrazol, propiconazole, fenpropimorph, prochloraz, flutriafol, 4-dodecyl-2,6-dimethylmorpholine ("Falimorph"), N-(2,2,2-trichloro-1-morpholinoethyl formamide) ("Fademorph") have been used especially in areas where wheat yields regularly exceed 5-6 t/ha.

Biotypes with decreased sensitivity to some SBI fungicides were found in the Netherlands after several years use of this type of fungicide (de Waard et al. 1986). This prompted a similar investigation of Hungarian wheat mildew populations. In 1986 randomly selected crops were sampled throughout western Hungary, but in 1987 efforts were concentrated in "Tolna" county, an area where the intensity of fungicide use on wheat is above the Hungarian average, and in the neighbouring county "Somogy", where it is slightly below average.

MATERIALS AND METHODS

Plants

Wheat seedlings, cv."Mv-4"[(Mironovskaja 808 x Bezostaja 1) x Bezostaja 1] were used for both maintenance of mildew populations, and foliar spray tests of fungicide sensitivity.

Fungicides

In all cases formulated commercial products were used (flutriafol -

"Impact" 125 EC; prochloraz - "Sportak" 450 EC; propiconazole - "Tilt" 250 EC; tebuconazole (HWG 1608) - "Folicur" 250 EC; triadimefon - "Bayleton" 125 EC; triadimenol - "Bayfidan" 250 EC).

Collection and maintenance of mildew isolates

About 50 mildew infected plants were collected from each field sampled. These were used to inoculate 200-300 wheat seedlings at the first leaf stage. These "mini-populations" were kept in the greenhouse, and subcultured onto healthy seedlings every fortnight. Isolates were tested as soon as possible after collection.

Fungicide sensitvity tests

Batches of five pots, each containing 10-15 seedlings, were sprayed to run-off with one of a range of concentrations of each fungicide. After drying, seedlings were inoculated with the test isolate by shaking infected plants over them. Eight days after inoculation the leaf area infected with mildew was measured, and the mean value for each fungicide concentration expressed as a percentage of that on unsprayed plants. Percentages were transformed to probits and both EC50 and EC95 values calculated using probit analysis (Finney, 1971). EC50 and EC90 values are given as μg/ml active ingredient. A standard isolate, which had not been exposed to SBI fungicides, was used as a standard in all tests.

RESULTS

Sensitivity changes

The range of EC95 values obtained for four SBI fungicides indicated a shift in sensitivity from 1986 to 1987 (Table 1). This shift may have resulted from many factors, including the favourable weather conditions for powdery mildew spread in 1987. Compared to 1986, more of the isolates tested in 1987 originated from areas where the intensity of fungicide use was above the Hungarian average. However, these shifts in sensitivity could not be attributed to only these factors. The largest shift in sensitivity (20 - 40-fold) appeared to be to prochloraz, which was hardly used on the sampled crops, whereas the shift in sensitivity to propiconazole, which was widely used, was no more than 4-fold. Consequently, some isolates were examined in more detail to establish cross-sensitivity patterns amongst these isolates.

Cross-sensitivity

Some isolates showed a 100-fold decrease in sensitivity to triadimefon when EC50 values were compared with that of the standard isolate (Tables 2 & 3). Under the test conditions, triadimefon gave some phytotoxicity above 200 μg/ml, yet EC95 values were above this level. These isolates were not controlled by triadimefon at rates equivalent to those used in the field, where phytotoxicity is not a problem, whereas the standard isolate was compltely controlled at these rates. Cross-resistance patterns depended on whether EC50 or EC95 values were compared, but were not consistent amongst the different SBI fungicides examined (Tables 2 & 3). In some isolates, cross-resistance extended to triadimenol and prochloraz, but not to propiconazole or flutriafol. Resistance to tebuconazole was intermediate between these two extremes. No isolate tested showed high levels of resistance to all six SBI fungicides used in this study.

TABLE 1

Sensitivity to SBI fungicides of Hungarian populations of Erysiphe graminis f.sp. tritici.

Fungicide	Range of EC95 values (µg/ml) 1986	1987
Flutriafol	6 - 43	11 - 56
Prochloraz	2 - 27	83 - 540
Propiconazole	0.7- 43	3 - 95
Triadimefon	12 - 154	60 - 892

TABLE 2

Sensitivity to SBI fungicides of three isolates of E. graminis f.sp. tritici collected in Hungary.

Fungicide		Standard	Isolate Kethely	Decs K2/3
Flutriafol	EC50*	0.32	2.3	1.4
	EC95	7.2	47.8	50.5
Prochloraz	EC50	0.30	12.2	12.5
	EC95	4.1	99.0	255.4
Propiconazole	EC50	0.14	0.4	0.7
	EC95	3.7	12.8	6.5
Tebuconazole	EC50	0.34	1.4	4.0
	EC95	8.6	10.7	82.8
Triadimefon	EC50	0.3	48.1	41.2
	EC95	12.2	892.2	225.4
Triadimenol	EC50	0.7	n.d.**	21.7
	EC95	4.4	n.d.**	138.4

* = value in µg/ml ** = not determined

Stability of triadimefon resistance

The stability of triadimefon resistance was tested with two isolates. One isolate lost its original resistance level after 18 generations on untreated seedlings. It was still, however, more than 50 times less sensitive than the standard isolate. The other isolate retained its starting EC50 value during this period.

TABLE 3

Cross-sensitivity between triadimefon, propiconazole and flutriafol in E. graminis f.sp. tritici isolates collected in Hungary.

Isolate	Triadimefon EC50*	Triadimefon EC95	Propiconazole EC50	Propiconazole EC95	Flutriafol EC50	Flutriafol EC95
Standard	0.3	12.2	0.14	3.7	0.32	7.2
Nagyatad '86	0.4	56.1	0.03	2.4	0.05	2.4
Kethely '86	1.4	19.5	0.37	4.0	0.40	25.3
Segesd	1.7	61.6	0.06	3.4	0.08	7.1
Balatonkenese	2.2	35.0	0.15	13.6	0.51	5.5
Rojtokmuzsaly	2.8	47.7	0.98	42.6	1.67	42.7
Decs S/6	8.7	60.1	0.23	75.0	0.48	13.4
Decs B/8	8.8	163.6	0.33	3.2	0.92	30.3
Fadd 1	9.7	140.1	1.37	15.4	0.49	12.2
Kethely '87	13.1	221.6	1.46	19.6	0.43	33.9
Fadd 2	13.4	116.6	1.31	26.7	0.36	10.6
Nagyatad '87	30.0	237.9	0.20	2.9	0.54	56.1
Decs K2/3	41.2	225.4	0.71	6.5	1.38	50.5
Kethely	48.1	892.1	0.42	12.8	2.32	47.8

* μg/ml

TABLE 4

The stability of triadimefon resistance in the absence of fungicides

	Isolate			
	Kethely		Decs K2/3	
	EC50*	95% confidence limits	EC50	95% confidence limits
1st generation	48.1	37.6 - 61.5	41.2	33.5 - 50.6
18th generation	18.4	13.8 - 24.5	46.2	38.6 - 55.0

* EC50 values given as μg/ml

Two other resistant isolates were selected with triadimefon (up to 200 μg/ml) for six generations (Table 5). Some decrease in sensitivity to all fungicides tested occurred as a result of selection, but in general the changes were small and not consistent.

TABLE 5

Selection of two E. graminis f.sp. tritici isolates with increasing doses of triadimefon.

Fungicide	Sensitivity (EC50 ug/ml) after selection with triadimefon (µg/ml) 0	50	100	200
Kethely '87				
Flutriafol	0.4	3.6	1.4	1.4
Prochloraz	12.2	50.4	6.1	49.6
Propiconazole	1.5	1.9	2.3	1.3
Triadimefon	13.1	18.6	34.3	24.8
Tebuconazole	1.4	1.0	1.6	2.5
Decs K2				
Flutriafol	1.6	2.5	5.6	4.6
Prochloraz	12.8	9.7	16.8	27.1
Propiconazole	1.0	4.1	4.0	3.1
Triadimefon	73.3	139.5	160.7	161.9
Tebuconazole	3.9	3.8	4.5	4.8

DISCUSSION

Control of wheat powdery mildew with SBI fungicides in Hungary has so far been adequate. However, the results reported here suggest that there is sufficient variation in fungicide sensitivity to indicate a possible change towards less sensitive populations, at least for some fungicides. The relative efficacy of four of the fungicides used in this investigation would seem to be: propiconazole = flutriafol > prochloraz = triadimefon. This does not correlate with the use of these fungicides, because in the sampled area it was the triadimefon = propiconazole > flutriafol > prochloraz.

Triadimefon resistant E. graminis f.sp. tritici biotypes were clearly demonstrated from the Hungarian fields sampled. The different cross-sensitivity patterns in these Hungarian isolates to five SBI fungicides are consistent with those observed in wheat mildew isolates from the Netherlands (de Waard et al. 1986) and barley mildew isolates in the UK (Hollomon 1982). A lack of any close correlation between triadimefon and tebuconazole sensitivity has also been reported (Berg et al. 1987). Studies with other pathogens eg. Cladosporium cucumerinium, Sphaerotheca fuliginea and Pyrenophora teres have shown similar inconsistencies in cross-resistance patterns amongst SBI fungicides (Kendall, 1986). The fact that triadimefon resistance is not always correlated with sensitivity to propiconazole and flutriafol suggests that there are probably several mechanisms of resistance to SBI fungicides in E. graminis f.sp. tritici. Some of these fungicides may have more than one mode of action (Berg et al. 1987).

Further investigations are required to clarify these possible mechanisms of resistance to SBI fungicides and establish their heritability as has been reported for that in E. graminis f.sp. hordei

(Butters et al. 1986) and Venturia inaequalis (Stanis & Jones 1985). More information is also required on the fitness of these resistant isolates in order to assess the practical consequences of the variation present in wheat mildew populations in Hungary.

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SENSITIVITY OF CEREAL BROWN RUST FUNGI TO TRIADIMEFON AND PROPICONAZOLE

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ABSTRACT

The sensitivities of isolates of barley and wheat brown rust, collected during 1987, to triadimefon and propiconazole were determined (EC_{50}) in tests with detached leaf segments and compared with those of some isolates collected before 1987. Both pathogens showed a 20-fold range of sensitivity to triadimefon, but only a 6 to 10-fold range to propiconazole. The sensitivity rankings of the wheat isolates to the two fungicides were similar, with the earlier isolates the most sensitive, but the rankings of the barley isolates differed and the earlier isolates were not the most sensitive to triadimefon. The growth of some barley isolates was apparently stimulated by low concentrations of both fungicides. There was no apparent relationship between sensitivity and virulence.

INTRODUCTION

Brown rust is an important disease of winter and spring barley and winter wheat in the UK, causing significant losses of yield in some seasons, especially in southern England (Cook et al 1981). Some currently popular cultivars of barley and wheat are susceptible to brown rust and control is highly dependent on systemic triazole and morpholine fungicides. To date, no problems of field control of the fungi Puccinia recondita (= P. triticina) and P. hordei, have been reported, but insensitivity to triazole fungicides has developed in cereal mildew populations, leading to loss of control in the field (Fletcher & Wolfe, 1981; Wolfe, 1985). There have been few studies on the sensitivity of rust fungi to fungicides, but insensitivity to oxycarboxin was recorded in Puccinia horiana where the fungicide failed to control chrysanthemum rust (Abiko et al, 1977).

This paper presents preliminary results from a survey of fungicide sensitivity of UK isolates of barley and wheat brown rusts, funded by the Home-Grown Cereals Authority.

MATERIALS AND METHODS

Leaves infected with brown rust were collected from barley and wheat crops, mainly from southern England during 1987, and sent to Aberystwyth where the fungi were cultured on susceptible cultivars of the respective host. These isolates were screened against a standard set of differential cultivars and their virulence characteristics determined in accordance with the procedures used in the UK Cereal Pathogen Virulence Survey (Jones & Clifford, 1980; Jones & Clifford, 1988). For comparison, some earlier isolates were included. Freeze-dried spores of the isolates were sent to Edinburgh where they were bulked as required on plants in a Burkhart Isolation Propagator (barley isolates on cv Midas, wheat isolates on cv

Fenman) to produce fresh inoculum for the fungicide tests. When necessary, bulked inoculum was stored for a few days under refrigeration. For each test, seedlings were grown, three per pot, in an air-filtered cabinet in a glasshouse. When the second leaves were just fully expanded (GS12), 15 ml of fungicide solution were applied as a fine spray for 5 seconds to four pots of seedlings in an enclosed cabinet. Plants were left for 15 minutes to allow the fungicide cloud to settle. Six fungicide concentrations were used: 1/16 C, 1/8 C, 1/4 C, 1/2 C, C, 2 C, where C is the concentration of normally recommended field spray. For triadimefon (0.5 kg Bayleton/ha in 200 litres water/ha) this series gave: 0.039, 0.078, 0.156, 0.312, 0.625, 1.250 g a.i. per litre. For propiconazole (0.5 litres Tilt/ha in 150 litres water/ha) concentrations were: 0.052, 0.104, 0.208, 0.416, 0.833, 1.666 g a.i. per litre. Control plants were sprayed with water.

Twenty-four hours after spraying, 16 replicate leaf segments (2.5-3.0 cm long) were cut from the second leaves of the test plants. The proximal end of each segment was inserted in 80 ppm benzimadazole water agar (0.4%) in 10 x 10 cm culture dishes, each dish holding eight leaf segments. All 14 dishes required for one test were inoculated together in a large settling tower to ensure uniformity. Spores (0.00625 g) were diluted with talc (1:3). Dishes were removed after 15 mins and incubated at 18°C in an illuminated incubator with a 12 hour light period in each day. The number of pustules on each leaf segment was counted at the onset of sporulation which occurred 10 days after inoculation, when pustules were discrete and easily identifiable. A further count, 16 days after inoculation, was made in some tests. Pustule numbers were standardised to segment areas of 1 cm^2 and analysed with the aid of Genstat 5 program which fitted symmetrical logistic curves and calculated EC_{50} values (concentration of fungicide which reduced pustule number to half that of the untreated control).

RESULTS

Table 1 lists barley brown rust isolates in order of decreasing sensitivity to triadimefon when assessed 10 days after inoculation. Assessments at 16 days generally followed the same pattern as those at 10 days. There was a 23-fold difference between the most sensitive and the least sensitive isolate, although most fell within an eight-fold range. There was a 10-fold range of sensitivity to propiconazole among the smaller number of isolates tested. Some isolates showed broadly similar sensitivity to both fungicides, while others were much more sensitive to one fungicide than the other (eg BBRF-87-9, BBRF-87-19). The earlier isolates (Race A and Octal 11) were among the most sensitive to propiconazole, but not to triadimefon.

The 1987 isolates showed few differences in virulence: races 673 and 1673 differ only in virulence on cv Triumph; races 1653 and 1673 differ only in virulence on cv Quinn (Pa_5). The earlier isolates are virulent on only two differential cultivars, whereas most of the recent isolates are virulent on seven or eight. There was no apparent relationship between EC_{50} and virulence in this small sample.

Differences in response across the six concentrations of the fungicides are illustrated in Table 2. Race A did not grow beyond the lowest concentrations, while BBRF-87-9 grew at all but the highest concentrations, producing seven pustules per square cm on the 1C concentration of propiconazole. Pustule production of some isolates was stimulated at the

lowest fungicide concentrations: BBRF-87-7 and BBRF-87-19 by triadimefon; BBRF-87-14 and BBRF-87-9 by propiconazole.

TABLE 1
Sensitivity of barley brown rust to triadimefon and propiconazole (EC_{50} in g/l) assessed at 10 and 16 days after inoculation, virulence race and source.

Isolate	triadimefon 10 days	triadimefon 16 days	propiconazole 10 days	propiconazole 16 days	Race	Source
BBRF-87-22	-	-	0.034	0.044	1673	Northumberland
BBRF-87-11	0.013	0.016	-	-	673	Oxfordshire
BBRF-87-17	0.021	0.051	0.068	0.078	1673	Oxfordshire
BBRF-87-15	0.025	-	-	-	1673	Hampshire
BBRF-87-24	0.034	0.028	-	-	1653	Hampshire
Race A ESA	0.035	-	-	-	11	Unknown
Octal 11 WPBS	0.037	-	-	0.029	11	Unknown (1970)
BBRF-87-9	0.058	0.059	0.258	0.286	1653	Wiltshire
BBRF-87-3	0.069	-	-	-	1653	Hampshire
BBRF-87-14	0.090	0.134	0.204	0.211	1673	Oxfordshire
BBRF-87-7	0.100	-	-	0.247	1653	Dyfed
BBRF-87-19	0.297	0.274	0.051	0.056	1653	Cambridgeshire
Average S.E.	4.5%	6.4%	5.7%	7.3%		

TABLE 2
Median pustule numbers on leaf segments treated with six concentrations of triadimefon or propiconazole for selected barley brown rust isolates 10 days after inoculation

Isolate	EC_{50} g a.i./l	Untreated pustules per cm^2	Percentage pustule number relative to untreated control 1/16C	1/8C	1/4C	1/2C	1C	2C
<u>triadimefon</u>								
BBRF-87-22	-	15	7	0	0	0	0	0
BBRF-87-11	0.013	14	10	6	5	0	0	0
Race A	0.035	10	28	0	0	0	0	0
BBRF-87-9	0.058	26	78	30	8	1	3	0
BBRF-87-3	0.069	14	59	50	42	0	0	0
BBRF-87-14	0.090	11	71	68	23	9	0	0
BBRF-87-7	0.100	14	110	64	20	3	0	0
BBRF-87-19	0.297	15	115	79	75	45	0	0
<u>propiconazole</u>								
Race A	-	26	10	0	0	0	0	0
BBRF-87-22	0.034	36	20	2	0	0	0	0
BBRF-87-17	0.068	22	54	33	16	0	0	0
BBRF-87-14	0.204	12	178	56	53	7	0	0
BBRF-87-9	0.258	44	114	66	58	40	17	0

In Table 3, wheat brown rust isolates are listed in order of decreasing sensitivity to triadimefon when assessed 10 days after inoculation. The order of sensitivity to the triadimefon and propiconazole among these isolates was broadly similar, but the EC_{50} values of the least sensitive to triadimenol were substantially higher.

TABLE 3
Sensitivity of wheat brown rust isolates to triadimefon and propiconazole (EC_{50} in g/l) assessed at 10 and 16 days after inoculation, virulence factors and source.

	triadimefon	propiconazole			
Isolate	10 days	10 days	16 days	WBV Factors	Source
WBRS-77-22	0.025	0.033	-	1,2,5,8	Norfolk
WBRS-74-22	0.032	0.049	-	2,5	Lincolnshire
WBRF-87-9	0.068	0.091	0.108	2,6	Suffolk
WBRF-87-5	0.142	0.051	0.053	6,7	Hertfordshire
WBRF-87-8	0.300	-	0.187	2,6	Hertfordshire
WBRF-87-2	0.489	-	0.164	6	Hampshire
Average S.E.	4.7%	2.8%	5.1%		

There was a 20-fold difference of sensitivity to triadimefon between the most sensitive and the least sensitive isolates, but only a six-fold difference of sensitivity to propiconazole. The earlier isolates were the most sensitive to both fungicides. Both the earlier, most sensitive isolates carried WBV5, while the 1987, less sensitive isolates did not, but carried WBV6.

TABLE 4
Median pustule numbers on leaf segments treated with six concentrations of triadimefon or propiconazole for selected wheat brown rust isolates 10 or 16 days after inoculation.

Isolate	Days	EC_{50} g a.i./l	Untreated pustules per cm^2	Percentage pustule number relative to untreated control					
				1/16C	1/8C	1/4C	1/2C	1C	2C
<u>triadimefon</u>									
WBRS-77-22	10	0.025	19	8	0	0	0	0	0
WBRS-74-2	10	0.034	12	18	0	0	0	0	0
WBRF-87-5	10	0.142	5	104	112	30	26	0	0
WBRF-87-8	10	0.300	18	72	92	88	44	18	0
WBRF-87-2	10	0.489	7	190	388	143	150	0	0
<u>propiconazole</u>									
WBRS-77-22	10	0.033	18	24	5	0	0	0	0
WBRS-74-2	10	0.049	11	6	0	0	0	0	0
WBRF-87-5	10	0.051	24	44	33	0	0	0	0
WBRF-87-2	16	0.164	4	96	109	0	0	0	0
WBRF-87-8	16	0.187	10	62	101	16	0	0	0

Differences in response across the six concentrations of the fungicides are illustrated in Table 4. The two earlier isolates did not grow beyond the lowest concentration of triadimefon and WBRS-77-22 showed only a little growth at a higher concentration of propiconazole. WBRF-87-8 grew at all except the highest concentration of triadimefon, but was more sensitive to propiconazole. Pustule production of some isolates was stimulated at the lower fungicide concentrations: WBRF-87-5 by triadimefon; WBRF-87-2 by triadimefon and propiconazole.

DISCUSSION

Fewer than half the isolates collected in 1987 have been tested, and only against two fungicides, so any conclusions must be regarded as preliminary. The isolates showed a 20-fold variation of sensitivity to triadimefon, but only a 6 to 10-fold variation to propiconazole. These ranges are wider than those reported by Buchenauer et al (1984) for barley mildew, but within those suggested by Hollomon et al (1984). The earlier isolates of both barley and wheat brown rust, with no known exposure to fungicides, were the most sensitive to propiconazole, as were the earlier isolates of wheat brown rust to triadimefon. However, the earlier isolates of barley brown rust were not the most sensitive to triadimefon. The sensitivity rankings of wheat brown rust isolates to the two fungicides were similar, but those of the barley isolates differed quite markedly. There was no apparent relationship between sensitivity and virulence among the barley isolates, while the sample of wheat isolates so far tested is too small to draw any conclusion.

The production of pustules appeared to be stimulated in some isolates by low concentrations of fungicide. It is not possible at this stage to say whether this is an effect on the pathogens or on the host leaf segments. No differences were noted among the leaf segments in the triadimefon tests, but those treated with the lower concentrations of propiconazole frequently remained green for longer than the untreated leaf segments. This may point to a host effect. However, similar stimulation has been reported in tests on other fungi: Botrytis cinerea (Miller & Fletcher, 1974); Fusarium oxysporum f.sp. tulipae (Duineveld & Beijersbergen, 1983); Erysiphe graminis f.sp. hordei (Williamson, 1983); Pseudocercosporella herpotrichoides (Corrigan, 1984).

In the tests reported here assessments were made at 10 and 16 days after inoculation. Generally after this time a significant proportion of the leaf segments senesced so that later assessments would have been less reliable. Where segments did remain green for up to 21 days after inoculation, sporulation was noted on segments treated with the highest concentrations (2 C) of the fungicides. This may indicate that a small proportion of spores can remain viable and be capable of causing infection as the concentration of fungicide within the plant tissue declines.

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EFFECTS OF FENPROPIDIN ON DMI-RESISTANT STRAINS OF ERYSIPHE GRAMINIS F.SP. HORDEI AND RHYNCHOSPORIUM SECALIS

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ABSTRACT

Analysis of sterols from Erysiphe graminis f.sp. hordei conidia and Rhynchosporium secalis mycelium showed that fenpropidin inhibited $\Delta^{14(15)}$-reduction in both fungi. Tridemorph, another morpholine fungicide, did not inhibit this step in barley mildew, but appeared to affect $\Delta^{8}\rightarrow\Delta^{7}$-isomerase activity. Despite these changes in sterol composition caused by tridemorph, mildew conidia still infected normally, whereas fenpropidin reduced the viability of the conidiospores. The DMI fungicide propiconazole inhibited sterol 14-demethylation in both DMI-sensitive and -resistant strains. No cross-resistance was observed between DMI and morpholine fungicides in either pathogen. These results indicate that the two groups of SBI fungicides are appropriate partners in mixtures to combat the spread of DMI resistance in these two pathogens.

INTRODUCTION

Fungicides known to inhibit the 14-demethylase step in sterol biosynthesis (DMIs) feature prominently in cereal disease control. Significant variation in sensitivity to DMIs has been encountered in cereal powdery mildews, and repeated use over several years has eroded their performance against these important pathogens (Brent & Hollomon 1988). Strategies to combat the spread of resistance use fungicides with different modes of action, either in mixtures or in alternating sequences. At present, only fragmentary evidence exists to suggest that these strategies do counteract the spread of fungicide resistance in cereal crops (Hunter et al. 1984; Bolton & Smith 1988). Morpholine fungicides are common components in these strategies, especially against barley powdery mildew (E. graminis f.sp. hordei). Although morpholines and SBI fungicides inhibit sterol biosynthesis, morpholines do so at different steps in the pathway (Mercer 1988). This may account for the lack of cross-resistance between these two fungicide groups (Hollomon 1982; de Waard et al. 1986). As part of our work on DMI resistance in cereal pathogens, we have examined the effects of some DMI and morpholine fungicides, on both E. graminis f.sp. hordei and R. secalis. We report here on cross-resistance patterns and effects on sterol composition.

METHODS

Erysiphe graminis f.sp. hordei

Mildew strains derived from single pustules of field isolates were cultured on detached barley leaves (cv. Halcyon). Fungicide assays were carried out on barley leaf segments floating on solutions containing

different fungicide concentrations, as described by Hollomon (1982). For extracting sterols from conidia, leaves of 10 day old seedlings, grown in 12.5 cm (1 litre) pots in an isolated propagator, were inoculated with 10 μl conidial suspensions in "Fluorinert" (FC 43, MMM Company, Manchester, UK; 10 mg/ml). After 8 days, when sporulation was beginning, the surface of the compost was drenched with either 100ml "Patrol" (46 mg fenpropidin/pot) or "Calixin" (52 mg tridemorph/pot). Conidia were collected 48 h later using a small cyclone spore collector. Some of the conidia were suspended in FC 43 (2 mg/ml), and 5 μl of this suspension was applied to detached leaves in order to check viability; the remaining conidia were freeze dried before sterol extraction and analysis.

Rhynchosporium secalis

Strains originating as single spore isolates from field samples collected during a UK survey of fungicide sensitivity in R. secalis were maintained on Czapek-Dox agar with the addition of 0.5% (w/v) mycological peptone, which greatly improves the long-term viability of R. secalis. A more quantitative measurement of fungicide sensitivity than MIC (Hollomon 1984) was required to establish cross resistance patterns. Fungicides were added, from stock solutions in methanol, to medium containing 3.34% (w/v) Czapek-Dox liquid (Oxoid CM 95), 0.5% (w/v) mycological peptone (Oxoid L40) and 0.4% (w/v) purified agar (Oxoid L28). Propiconazole and triadimenol were added prior to autoclaving (122°C for 15 min); tridemorph and fenpropidin when media had cooled to 40°C. Fungicide concentrations covered a two-fold dilution series encompassing the expected ED_{50} concentration; five replicate tubes were included for each concentration. Medium (2 ml) was dispensed into 12 mm o.d. test tubes with aluminum caps. Conidial suspensions (2×10^6 spores/ml) were prepared in sterile water from inoculum plates as described by Hollomon (1984). Tubes were inoculated with 100 μl of this suspension, mixed thoroughly on a Vortex shaker, and the initial turbidity measured using a ratio turbidimeter (Hach. model 18900, Camlab Instruments, Cambridge, UK). Tubes were incubated at 19°C vortexing thoroughly every two days to disperse the growing fungus and aerate the medium. After 10 - 14 days turbidity was again measured. The increase in turbidity during growth was used to calculate ED_{50} by fitting a logistic curve of turbidity change against the logarithm of dose, using the method of least squares.

Material for sterol analysis was produced in liquid glucose-malt-yeast medium with added mycological peptone (0.5% w/v). Spore suspensions (not less than 5×10^4/ml) were used to inoculate 50 ml medium which was then shaken continuously at 18°C for 7 - 10 days. Cells were collected by filtration, washed, and freeze dried. Growth inhibition was calculated from dry weight of samples.

Sterol extraction and analysis

Freeze-dried conidia or mycelium (50 mg), and cholesterol (internal standard, 50 μg), were heated under reflux with ethanolic KOH (20 ml) under N_2 for 1 h. After dilution with water, the pH was adjusted to 8 with 1M phosphoric acid, and neutral lipids were extracted three times with hexane. The combined extracts were washed twice with water, dried by passing through phase-separating filter paper, and the solvent evaporated off. The residue was dissolved in ethanol (5 ml) and its u.v. spectrum determined. Ethanol was removed and samples, in toluene

(200 µl), were silylated [N,O-bis(trimethylsilyl)acetamide, 60°C, 1 h] prior to glc analysis. Samples (0.5 µl) were injected via an on-column injector on to an OV1-BP capillary column at 80°C, the oven temperature rapidly increased to 260°C and then programmed at 3°/min to 290°C; the carrier gas was H_2 (5 psi). For gc-ms an Alltech SE-52 capillary column was used on a Kratos MS80 mass spectrometer. Samples were injected using a split/splitless injector; carrier gas was He (ca. 1 ml/min), and the temperature was programmed from 180°C to 280°C at 5°/min. Retention times were calculated relative to cholesterol as internal standard.

Chemicals

Technical grade fungicides supplied by the relevant manufacturers were used for all laboratory studies. Barley seedlings were treated with "Patrol" (Fenpropidin, ICI Agrochemicals, Fernhurst, UK) or "Calixin" (BASF, Hadleigh, UK). Hexane and ethanol were redistilled before use; all other solvents and reagents were analytical grade.

RESULTS

Cross-resistance

Routine monitoring in our laboratory of fungicide sensitivity in E. graminis f.sp. hordei has failed to detect cross-resistance between DMI and morpholine fungicides. Values shown in Table 1 are for a small, representative sample of strains tested over several years. Significant variation in sensitivity to both triadimenol and fenpropidin does occur, but differences were not correlated between the two groups. These results extend earlier ones (Butters et al. 1984), which showed small differences in sensitivity to another morpholine fungicide, fenpropimorph, but no links with differences in triadimenol sensitivity.

TABLE 1

Sensitivity of Erysiphe graminis f.sp. hordei and Rhynchosporium secalis to DMI and morpholine fungicides.

Strain	Year of isolation	Sensitivity (ED_{50}, µg/ml) Triadimenol	Propiconazole	Fenpropidin	Tridemorph
E. graminis f.sp. hordei					
DH14	1976	0.002 a*		0.095 a	
JB 6	1980	0.004 a		0.23 ab	
JB 1351	1984	0.024 b		0.50 bc	
JB 1355	1985	0.06 b		1.05 c	
JB 1398	1973	0.50 c		3.46 c	
JB 1743	1988	1.33 cd		0.032 a	
MK 6	1985	2.88 d		0.069 a	
R. secalis					
ACP	1986	0.094 a	0.008 a	0.057 a	0.030 a
218.02	1986	0.032 a	0.012 a	0.251 b	0.176 a
243.02	1986	13.95 b	0.105 b	0.231 b	0.078 a

*For each pathogen, values in vertical columns followed by the same letter are not significantly different (P = 0.05).

Data available for R. secalis are more limited at present. Cross-resistance occurred between triadimenol and propiconazole, but "resistance factors" were different for each DMI fungicide. There was no evidence of cross-resistance between DMI and morpholine fungicides in this experiment.

Sterol composition

Erysiphe graminis f.sp. hordei

Sterols were identified by relative retention times and comparison with standard mass spectra. Identification of the sterol found in fenpropidin treated cultures as ergosta-8,14,24(28)-trienol was based on the following evidence: the relative retention time (RRT) was consistent with the predicted value based on comparisons of RRTs of other sterols; u.v. absorbance at 250 nm in the fenpropidin treated sterol extract indicated conjugated double bonds at C8 and C14; a 24(28) double bond was indicated by a peak at m/e = 369 [M-(Me+C_6H_{12})] in the mass spectrum. Neither fenpropidin nor tridemorph inhibited conidia production at the concentrations used (data not shown), but fenpropidin reduced conidial viability by 82%; tridemorph had little effect on viability. Sterol analysis showed that the percentage of the main sterol in E. graminis f.sp. hordei, 24-methylene-cholesterol (Loeffler et al. 1984), was reduced by treatment with both morpholine fungicides, but, because of the increase in overall sterol content, its absolute amount was almost unchanged. Episterol content was not affected by fenpropidin, but markedly increased in tridemorph treated conidia. The main additional sterols that accumulated were fecosterol in the presence of tridemorph and ergosta-8,14,24(28)-trienol in the presence of fenpropidin.

TABLE 2

Effect of fenpropidin and tridemorph on viability and sterol composition of conidia of Erysiphe graminis f.sp. hordei*.

Sterols found in conidia	RRT (rel. to cholesterol)	% total sterols		
		Untreated	Fenpropidin mg a.i./pot 46	Tridemorph 52
24-methylene-cholesterol	1.085	85	66	61
Ergosta-8,14,24(28)-trienol	1.095	-	16	-
Fecosterol	1.100	-	-	8
Episterol	1.120	8	8	23
Unidentified		7	10	8
Total sterols (µg/mg dry weight)		2.42	2.73	3.22
Conidial viability (pustules/leaf)		24.7±7.6	5.5±2.9	19.8±6.5

*Strain 23D5. Sensitive to triadimenol (ED_{50} = 0.062 µg/ml).

R. secalis

Sterol composition in R. secalis was more complex than in barley mildew. Few differences in sterol composition were observed between DMI sensitive and resistant strains and, although ergosterol was not always the major sterol, desmethyl sterols accounted for more than 80% of the total (Table 3). Treatment of the DMI sensitive strain with a propiconazole concentration close to its ED_{50} value, led to the expected accumulation of methylated sterols. At this concentration less methylated sterol was found in the DMI resistant strain; only at higher concentrations were these desmethyl sterols replaced by methylated ones (Table 3). In contrast, the effects of fenpropidin were identical on both strains. Desmethyl sterols were replaced with Δ^{14} sterols indicating inhibition of Δ^{14-15} reductase. There was also some accumulation of Δ^{8} sterols.

TABLE 3

Effect of propiconazole (Pc) and fenpropidin (Fp) on sterol composition in Rhynchosporium secalis.

Sterols found	Percentage composition						
	Strain 218.02			Strain 243.02			
	None	Pc 0.04	Fp 0.4	None	Pc 0.04	Pc 0.2	Fp 0.4
Ergosta-5,22-dienol	21	3	1	9	2	1	1
Erg-5,8,22-trienol	14	2	2	22	6	3	2
Ergosterol	25	9	10	37	22	8	14
Ergost-5-enol	17	6	0	9	15	11	0
Erg-8,14,24(28)-trienol	0	0	21	0	0	0	17
Ignosterol	0	0	33	0	0	0	28
Ergost-8-enol	0	0	8	0	0	0	9
Ergosta-5,7-dienol	1	5	2	1	16	13	4
Episterol	5	2	3	3	4	4	4
Ergost-7-enol	1	1	5	1	1	2	6
Obtusifoliol	tr.	5	1	tr.	2	4	1
24-Methylenedihydro-lanosterol	5	45	2	7	18	31	1
Other sterols	11	20	14	8	12	22	15
Sterol content (μg/ml dry weight)	4.9	4.8	4.8	4.0	2.8	2.6	4.0
Growth inhibition (%)	0	62	82	0	17	55	57

Pc=propiconazole; Fp=fenpropidin; tr.=< 0.5%. Fungicide concentrations in μg/ml. Sterols listed in order of increasing retention time.

DISCUSSION

These results confirm that the mode of action of the morpholine fungicides, fenpropidin and tridemorph, differs from that of DMI's. Fenpropidin primarily inhibits Δ^{14-15}-reductase in both pathogens, whereas tridemorph inhibits $\Delta^{8}\rightarrow\Delta^{7}$-isomerase in powdery mildew at least. This agrees with results already described for Ustilago maydis and Saccharomyces cerevisiae (Baloch et al. 1984). However, accumulation of

abnormal sterols in mildew conidia after tridemorph treatment did not appear to reduce viability significantly.

DMI resistance in R. secalis is not associated with alterations in sterol composition. Propiconazole inhibited the 14-demethylation step as expected; accumulation of methylated sterols occurred in the resistant strain but at higher fungicide concentrations than in the DMI-sensitive strain. Propiconazole also appeared to affect sterol biosynthesis in R. secalis beyond the 14-demethylation step, as evidenced by changes in the relative amounts of desmethyl sterols, especially in the DMI-resistant strain 243.02. This may account for the lower level of resistance to this fungicide compared to that for triadimenol. Cross-resistance patterns and sterol composition clearly indicate that morpholine fungicides differ from DMI fungicides in mode of action. Therefore, morpholine fungicides are likely to be good partners in mixtures with DMI fungicides.

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