RESISTANCE OF *UNCINULA NECATOR* TO DMI FUNGICIDES IN CALIFORNIA VINES

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ABSTRACT

This paper discusses interactions between environmental conditions, pathogen life-cycle and application strategy, and their effects on disease severity and development of Demethylation Inhibitor (DMI) resistance in *Uncinula necator* in Californian vines.

A programme of screening for DMI resistance in isolates from 19 vineyards in different regions of California was initiated in 1990. Isolates were tested for sensitivity to triadimefon, myclobutanil and fenarimol using a leaf disc test. Frequency distribution curves indicated higher levels of resistance to triadimefon and to lesser extent to myclobutanil in most regions. Increased levels of resistance found to fenarimol were not as evident compared to wild-type sensitivity.

A time-course experiment was conducted to follow the development of resistance to the three chemicals in a vineyard subjected to multiple applications of triadimefon. Resistance levels increased during the growing season. Resistance levels to myclobutanil and fenarimol in ascospore populations collected prior to and after the growing season were similar, but resistance levels to triadimefon were found to be increased in the latter ascospore population. Cross-resistance relationships are discussed.

INTRODUCTION

Grape production in California utilizes 300,000 ha of land in virtually every agricultural production area. Coastal regions, particularly Central and South are prone to severe powdery mildew pressure annually, while some North Coast regions, the Sacramento Valley and most of the San Joaquin Valley deal with heavy pressure from *Uncinula necator* less frequently. Though varietal differences exist between these areas and certainly can contribute to disease development, differences in optimum and minimum temperatures are more important. Under optimum temperature conditions, *U. necator* can complete its life-cycle and reproduce in as little as 5 days. At 33°C, reproduction may require up to 15 days and colonies may be killed when temperatures exceed 37°C for only a few hours.

It is in this context that resistance to DMI fungicides has been somewhat ignored in *U. necator* populations and quite possibly in other pathogen systems where single site action fungicide resistance occurs.

The effect of genetic recombination in fungicide-resistant populations should be addressed. Though cleistothecia are produced and ascospores function in the disease cycle in nearly all California vineyards, there are production areas in which cleistothecia are produced in much higher numbers, thus increasing the risk of increased resistance due to genetic recombination. In California, the greatest number of cleistothecia are produced in the cool coastal areas and in the Delta area and southernmost areas of the San Joaquin Valley. Germination and infection efficiency of ascospores in California are very high. In the aforementioned production areas, viable ascospores have been released from cleistothecia in late summer and fall, as well as from overwintered cleistothecia from the bark in early spring. We suspect that two ascospore-derived populations per year can occur in these areas.

Resistance to the DMI fungicides in California was suspected in 1985, three years after the introduction of triadimefon. In 1985, control failures were reported from nearly every production area, but were more severe in vineyards along the coast. In 1986, disease pressure was extremely high statewide and control failures were prevalent. Isolates collected in 1986 ranged in LC50 values from 2.5 to 39 mg/l. Because no baseline sensitivity data existed for triadimefon for any *U. necator* population, attempts were made to show resistance by investigating whether isolates with differing LC50 values could differentially attack leaves of seedling grapevines after they had been treated with 154 mg/l of triadimefon. Though these tests were successful, in that isolates with the highest LC50 values were able to infect and colonize leaves seven to ten days earlier than isolates with lower LC50 values, poor performance of triadimefon in California was assigned to coverage problems, rate problems or fast tractor speeds during application, rather than resistance. In 1987 and 1988, temperatures statewide were above normal and no problems with powdery mildew spray programmes occurred except in some coastal vineyards. In 1989 and again in 1990, large scale losses occurred in many of the production areas.

MATERIALS AND METHODS

Pathogen sampling was initiated in 1990 from 19 vineyards in California, including one, 15 year old, isolated vineyard (Renaissance) which had never been treated with DMI fungicides. Because the latter vineyard was treated with sulfur only, we designated isolates from this vineyard as wild types.

Thirty mass isolates were collected from 30 individual vines in each vineyard. The isolates were transferred to individual Carignan grapevine seedlings and maintained in isolation tubes held at 23-25°C. Isolates were screened for resistance, using leaf discs treated with a concentration range of each of the three DMI fungicides; triadimefon, myclobutanil and fenarimol. Discs were inoculated

with conidia brushed from colonized leaves at the top of a 1.5 m settling tower, then incubated at 24°C for 10 days. Discs were rated for percent surface area covered by the fungus using a Horsefall-Barratt system and LC50 values were calculated by probit transformation of the percentages of inhibition and regression against the logarithm of the concentrations used.

RESULTS AND DISCUSSION

Results of the 1990 survey showed that all nineteen vineyards contained isolates of *U. necator* resistant to triadimefon (ie. exceeding maximum LC50 values obtained from Renaissance vineyard), while resistance to myclobutanil and fenarimol was observed in ten and three vineyards, respectively. For the purposes of this paper, isolates from these 19 vineyards have been regionalized and Table 1 shows the patterns and levels of resistance found in the previously discussed regions of California, i.e. North Coast, Central Coast, Central Valley, South San Joaquin Valley and Renaissance vineyard, where the *U. necator* population has been designated as representing wild type.

TABLE 1. Mean, minimum and maximum LC50-values of *U. necator* populations to triadimefon, myclobutanil and fenarimol in different regions of California, 1990.

Region in California	Fungicide	Number of isolates	Mean LC50 (mg/l)	Minimum LC50 (mg/l)	Maximum LC50 (mg/l)
Renaissance	triadimefon	61	1.40	0.70	3.91
	myclobutanil	62	0.15	0.01	0.80
	fenarimol	62	0.13	0.01	0.59
North Coast	triadimefon	120	4.33	0.62	16.70
	myclobutanil	121	0.67	0.01	6.80
	fenarimol	121	0.22	0.01	1.29
Central Coast	triadimefon	103	16.48	0.49	65.51
	myclobutanil	102	2.83	0.05	6.44
	fenarimol	101	0.44	0.06	1.19
Central Valley	triadimefon	131	5.32	0.10	43.12
	myclobutanil	133	0.58	0.01	1.82
	fenarimol	133	0.19	0.01	1.14
South San Joaquin Valley	triadimefon	105	6.02	0.29	45.83
	myclobutanil	108	0.69	0.01	3.54
	fenarimol	106	0.20	0.01	0.64

The mass isolates collected from Renaissance vineyard demonstrated LC50 values not exceeding values of 3.91 mg/l (mean 1.40 mg/l), 0.80 mg/l (mean 0.15 mg/l) and 0.59 mg/l (mean 0.13 mg/l) for triadimefon, myclobutanil and fenarimol, respectively.

The highest mean levels of resistance (expressed as LC50 values), to all three fungicides were observed in vineyards in the Central Coast, and also the widest ranges of sensitivity levels were found in this area. For triadimefon, and to a lesser extent for myclobutanil, frequency distribution curves range far into levels of higher resistance. This effect is much less pronounced for fenarimol. The frequency distribution curves for the Central Valley, South San Joaquin Valley and North Coast isolates showed a less pronounced occurrence of higher levels of resistance. However occurrence of higher levels of resistance to triadimefon and to a lesser extent myclobutanil is evident. Mean and highest levels of resistance to fenarimol in the latter regions were similar to those found in Renaissance vineyard.

Overwintering of resistance has been documented to occur in ascospore populations which reside in cleistothecia on the bark of cordons, spurs, canes and trunks of grapevines. This phenomenon occurs prevalently in coastal production areas and it is not uncommon to find 600-700 cleistothecia in 10 grams of bark. In addition, ascospore germination and infection efficiency is approximately 50% in these same areas. This phenomenon, coupled with heavy disease pressure starting early in the growing season and continuing through the season, partially explains the high levels of resistance observed in these production areas. Table 2 lists the results of a time course sampling experiment from one vineyard in Monterey County. Ascospores were released from cleistothecia collected in January 1990 from cordon bark of cv. Chardonnay. Thirty ascospore-derived colonies were screened for sensitivity to triadimefon, myclobutanil and fenarimol and mean LC50 values were 21.35 mg/l, 3.16 mg/l and 0.41 mg/l, respectively. After one application of triadimefon (Bayleton 50WP, 285g/ha), thirty mass conidial isolates were collected on May 4 and screened. The average LC50 values were found to have increased with respect to the ascospore-derived population to 33.35, 3.27 and 0.69 mg/l for triadimefon, myclobutanil and fenarimol, respectively. The mean level of sensitivity was found to have decreased further in conidial samples collected on June 25, July 24 and September 3, after the second, third and fourth application of triadimefon, respectively (Table 2). On October 23, a second ascospore population of thirty isolates was collected from cleistothecia residing on leaves. These overwintering resistant isolates appeared to be quite fit and survived well in nature. The above mentioned ascospore samples were also examined for sensitivity to myclobutanil and fenarimol but development of resistance was not as severe for these fungicides and the average LC50 values found in October were not significantly different from those of January. Distributions for the sampled conidial populations showed bimodal curves for both triadimefon and myclobutanil, whereas population curves for fenarimol were normal with only slight movement towards higher levels of resistance from May to September.

TABLE 2. Mean, minimum and maximum LC50-values of 30 samples of a *U. necator* population in Lone Oak vineyard, Monterey County to triadimefon, myclobutanil and fenarimol between January 22, 1990 and October 23, 1990.

Fungicide	Sampling Date	Population derived from:	Mean LC50 (mg/l)	Minimum LC50 (mg/l)	Maximum LC50 (mg/l)
triadimefon	January 22	ascospores	21.35 A	2.27	49.34
	May 4	conidia	33.35 B	8.04	124.15
	June 25	conidia	34.01 B	15.65	66.21
	July 24	conidia	42.93 BC	9.22	66.61
	September 3	conidia	54.41 C	7.21	86.33
	October 23	ascospores	32.20 B	7.22	61.92
myclobutanil	January 22	ascospores	3.16 A	0.44	15.77
	May 4	conidia	3.27 A	0.21	12.08
	June 25	conidia	3.77 A	0.13	13.83
	July 24	conidia	4.51 AB	0.75	9.69
	September 3	conidia	7.94 B	1.33	36.92
	October 23	ascospores	3.52 A	0.38	11.58
fenarimol	January 22	ascospores	0.41 A	0.16	1.65
	May 4	conidia	0.69 A	0.13	2.51
	June 25	conidia	0.73 AB	0.12	2.09
	July 24	conidia	0.85 AB	0.22	2.23
	September 3	conidia	0.94 B	0.25	2.61
	October 23	ascospores	0.43 A	0.13	0.93

Mean values followed by different letters indicate significant differences at the 5% probability level.

The effect of temperature on pathogen increase and development of resistance to DMI-fungicides has largely been ignored. When resistance to DMI fungicides first appeared in *U. necator* populations, the common thinking was that economic loss due to resistance was unlikely to occur because of the gradual movement of the population towards resistance. However, our data indicate that directional shifts in resistance can mimic disruptive shifts which can result in large scale disease control failures. This is primarily due to population increase as effected by environmental conditions. For example, when a *U. necator* population with sufficiently high levels of resistance to triadimefon is subjected to optimal environmental conditions, selection for these high levels of resistance occurs rapidly, due to maximum reproduction rate of the pathogen and selection by the fungicide. This leads to the increased occurrence of members of the population

capable of attacking leaves and fruit on the same day the spray is applied. Under these conditions, economic losses have occurred in vineyards throughout California. On the other hand, a resistant population can be controlled easily when temperatures are high and reproduction rates are decreased, because selection for and increase of the resistant population do not take place at a rate fast enough to cause economic losses.

From the presented data, it is evident that resistance of *U. necator* to fenarimol in California vineyards has remained relatively low, indicating that cross resistance between fenarimol and other DMIs may not be wholly representative of situations with other pathogens on other crops. The fact that population curves for DMI fungicide-resistant populations of *U. necator* do not exhibit the same distribution or shape could indicate that development of resistance to fenarimol is at an early stage in California. However, more recent monitoring over a three year period in all production areas has not demonstrated increased levels of resistance to fenarimol even in years conducive to disease development. If no further increase in resistance is observed in future years, fenarimol could possibly be used in integrated control programs to prevent an increase in strains resistant to the other DMI fungicides.

CONCLUSIONS

This research documents the overwintering of resistance to the DMIs, triadimefon, myclobutanil and, to a lesser extent, fenarimol, in ascospores of *U. necator*.

In vineyards where this occurred a time course sampling experiment demonstrated that the mean sensitivity of conidial populations to triadimefon and myclobutanil declined through the growing season as successive applications of triadimefon were made to control disease. Changes in sensitivity to fenarimol were only slight and not significant.

From other studies carried out since 1986 but not reported here, we would like to pass on the following conclusions or observations:

- Resistance to DMI fungicides has occurred more prevalently in California vineyards where triadimefon was used exclusively for disease control between 1982 and 1985.

- Vineyards having the highest levels of resistance in 1986 have the same ranking in 1993, indicating that while control programmes based on sulfur and DMI fungicides appeared to have stabilized the development of resistance, they have had little impact on reverting populations to wild type.

- Resistance has been consistently more severe in vineyards in which cleistothecia are produced. In addition there appears to be a correlation between numbers of cleistothecia produced and levels of resistance in a particular vineyard or region.

- Environmental conditions, primarily temperature, can influence the rate of development of resistance by influencing the reproduction rate of a population of *U. necator*. Under conditions of rapid increase of a population in which high levels of resistance occur, DMI fungicides remove sensitive isolates and allow for a rapid increase in the frequency of the most resistant isolates. Under these conditions, directional shifts in resistance can mimic disruptive shifts with respect to disease control failure.

SENSITIVITY OF EYESPOT TO PROCHLORAZ

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ABSTRACT

Monitoring carried out between 1985 and 1991 revealed no evidence of changes in sensitivity to prochloraz of French populations of Pseudocercosporella herpotrichoides. However, sampling during 1992 and 1993 identified a number of less-sensitive, slow-growing, R-type isolates from sites in Northern France. The level of reduced sensitivity was relatively low, in common with other reported instances of altered sensitivity to sterol biosynthesis inhibitor fungicides. Prochloraz efficacy was reduced at some, but not all sites where less-sensitive strains were found. However, yield increases were obtained at the majority of sites studied. There was no consistent evidence of prochloraz applications selecting for the R-type at sites studied in detail.

INTRODUCTION

Prochloraz has been the leading fungicide for control of the cereal eyespot pathogen, Pseudocercosporella herpotrichoides, since the mid-1980s, when resistance to carbendazim became widespread in European populations. One of the strengths of prochloraz is its equal activity against both W and R strains of the pathogen under normal circumstances.

The sensitivity of European populations of P. herpotrichoides to prochloraz has remained remarkably stable since the introduction of the compound in the early 1980s. However, there have been several recent reports of the occurrence of isolates with reduced sensitivity to prochloraz in the North of France (Leroux and Marchegay, 1991, Cavelier et al, 1992, Migeon et al, 1992). Initially these authors reported the detection of a reduction in sensitivity under laboratory conditions only, but Leroux and Migeon (1993) concluded that in some instances there was a correlation between in vitro insensitivity and reduced field efficacy.

As part of a programme of work to investigate these findings a large number of trials were carried out at sites in France during 1992 and 1993. This paper will present the results of these trials as well as reviewing recent literature on this subject.

MATERIALS AND METHODS

Field trials were established across N. France, all in winter wheat with known cropping histories. The sites were chosen as being representative of prochloraz use,

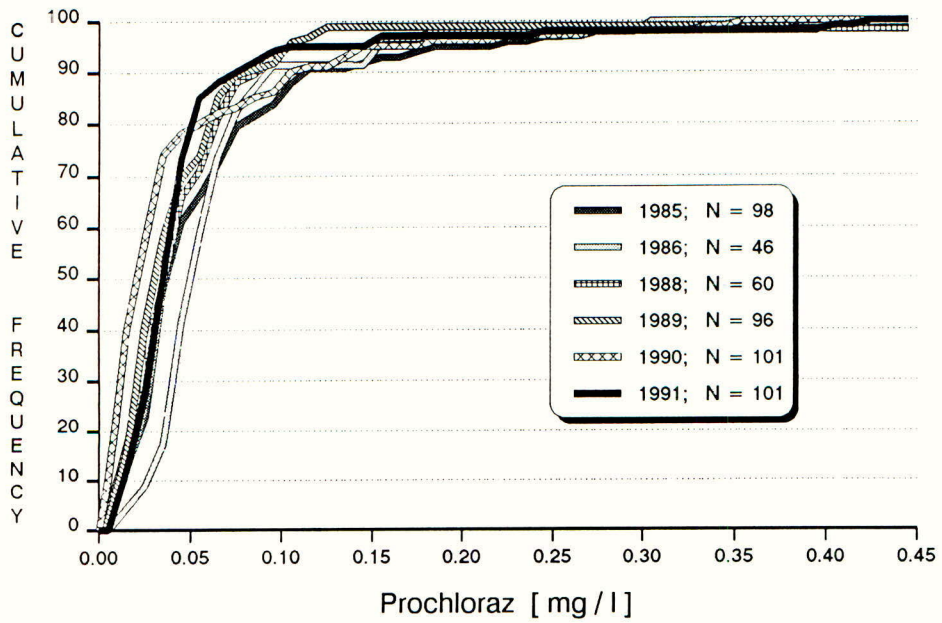
but included ones identified as having less sensitive eyespot populations which were studied in detail. Trials were arranged as random block designs and prochloraz applied in 200 l water/ha at decimal growth stage 31 to 32 (Tottman, 1987). Levels of eyespot were assessed regularly throughout the season and samples of infected stems were removed at intervals for isolation of the pathogen at Chesterford Park.

Sampling, and subsequent sensitivity testing of the isolates, were carried out according to the published method (Birchmore, 1991), using a range of concentrations of prochloraz from 0.0075 mg/l to 1.0 mg/l. The colony diameters obtained after 14 days growth at 20°C were used to calculate an IG_{50} value for each isolate, by applying linear regression. The IG_{50} value is defined as the concentration of prochloraz which is required to give 50% inhibition of growth (IG) of each isolate on potato dextrose agar (PDA, Oxoid).

RESULTS AND DISCUSSION

The results of sensitivity-monitoring carried out at Chesterford Park between 1985 and 1991 are shown in Fig 1. During these years the monitoring process consisted of a 0.5 mg prochloraz/l single dose-rate test for all isolates, followed by a dose-response test carried out on a random-selection of approximately 100 isolates per year from each country. The data show that no changes were detected in the populations sampled during these seven years.

Figure 1 Cumulative frequency distributions of prochloraz IG_{50} values from dose-response testing of P. herpotrichoides isolates from French trials between 1985 and 1991.



This conclusion is confirmed by the single dose-rate data which have also been published (Birchmore et al, 1992). However, during 1991 the detection of isolates of P. herpotrichoides with reduced sensitivity to prochloraz was reported (Leroux and Marchegay, 1991). These isolates were obtained during 1990 from two sites north of Paris, in the Oise department, and one site further south in Essonne. All of the isolates were of the type II, which can be loosely correlated with the R, or slow-growing type and were designated type IIp. At that time there were no indications of any effect on the efficacy of prochloraz. However, a later report by Leroux and Migeon (1993) indicated that there were instances of reduced efficacy at the end of the season at sites where isolates with reduced sensitivity to prochloraz were found.

During 1992 and 1993 the monitoring programme at Chesterford Park was expanded so that dose-response testing was carried out on all isolates. Sampling during 1992 was concentrated on a relatively small number of sites in Oise, including Jamericourt and Ressons, the two locations reported by Leroux and Marchegay (1991) to have less-sensitive populations. In addition, a more random selection of sites was sampled, spread over a wider area. An increased total of 378 isolates was fully tested in 1992, in comparison with 101 in 1991. Substantial numbers of the R type or slow growing isolates were found to give IG_{50} values above the previous limit of approximately 0.5 mg prochloraz/l. However, the majority of these less-sensitive isolates were derived from a small number of sites which were intensively sampled, including those at Jamericourt and Ressons (Fig 2). Even at these sites, 76.3% of isolates were found to have IG_{50} values below the 0.5 mg prochloraz/l threshold level. Fig 3 shows the population derived from the sites where less-sensitive isolates were not found.

Figure 2 Results of dose-response testing of 329 eyespot isolates obtained during 1992 from French "less-sensitive" sites out of a total of 378 isolates

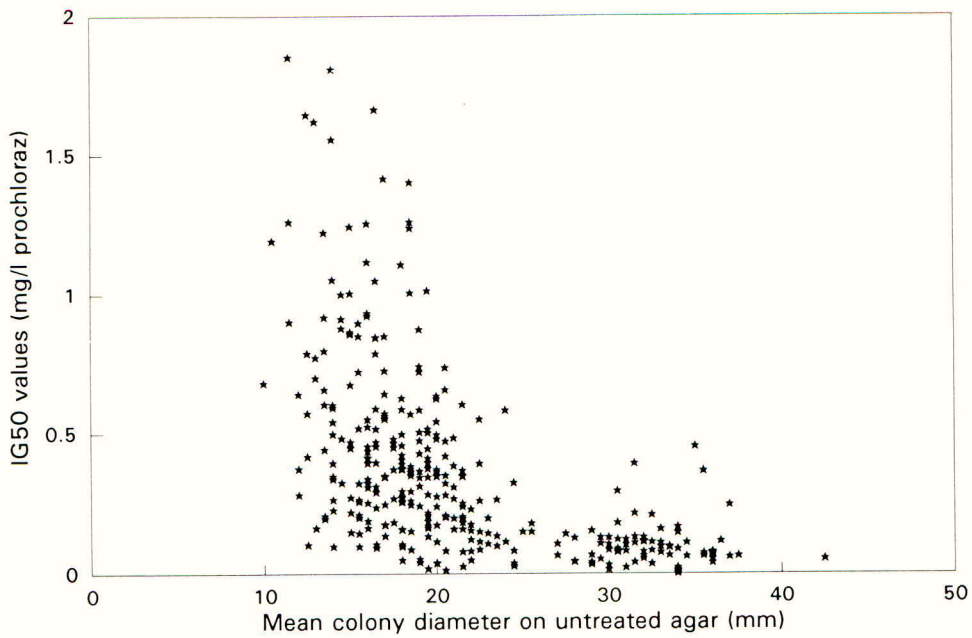


Figure 3 Results of dose-response testing of 49 eyespot isolates from "sensitive" sites

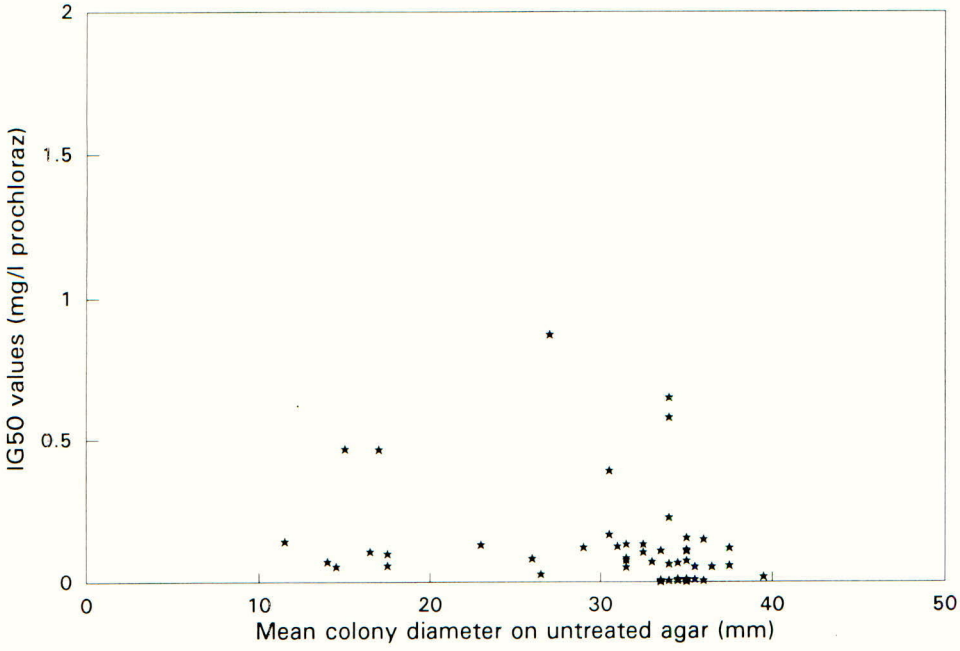
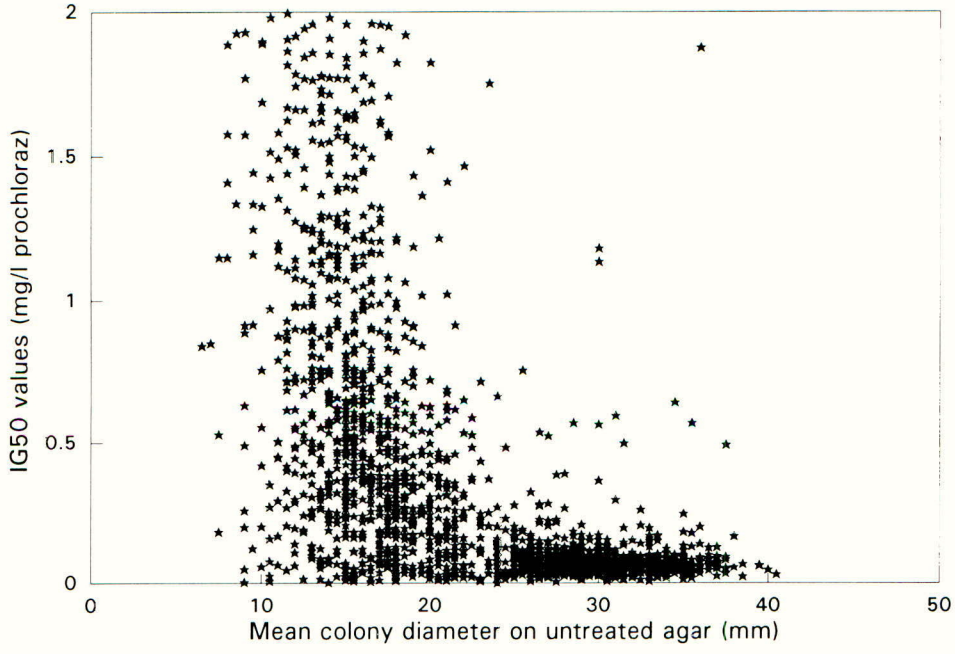


Figure 4 Results of dose-response testing 2000 eyespot isolates obtained during monitoring of French sites in 1993



A similar pattern to 1992 (Fig 2) was seen in 1993 (Fig 4) and again the emphasis was placed on studying sites where less sensitive isolates were thought to be present.

An even larger number of samples was monitored than in 1992, with 2000 isolates being tested. The results showed that, while a high proportion (25%) of less-sensitive, R-type isolates were detected, nevertheless, the majority (75%) of the overall population was prochloraz sensitive, that is, had an IG_{50} value of less than 0.5 mg/l.

A very small number of fast-growing isolates were also found to be less-sensitive (Fig 4). These are currently under investigation, as the possibility exists that these could be fast-growing sectors of R types, as reported by Julian (1990).

Our results suggest that the margin between sensitive and less-sensitive isolates was relatively modest and that there still appears to be a continuous distribution. This may indicate a limited, step-wise movement towards reduced sensitivity, as seen with other SBI inhibitors, rather than selection of a mutation with high levels of resistance.

Occurrence of less-sensitive isolates

Migeon et al (1994) reported the results of a large scale monitoring programme, carried out during 1993. This covered 25 departments in the north of France, with samples being taken from over 230 plots and 6,000 isolates of P. herpotrichoides being evaluated for sensitivity to prochloraz. They concluded that W or fast-growing types were in the majority and were not implicated in prochloraz "resistance". However, the proportion of W type isolates which were resistant to triazoles had risen over recent years, to become more common than the sensitive portion of the population. In agreement with our findings in 1992 and 1993, the type IIp isolates were found to be "limited to a few North Western Departments where their distribution is irregular". In the absence of a field-by-field mapping exercise it is not possible to say with precision where and why these isolates occur.

Efficacy and Yield

Trials were carried out in France during 1993 with the objective of examining the relationships between the occurrence of less-sensitive isolates, the efficacy of prochloraz applications and the subsequent yield.

In each of the trials 450g AI/ha of prochloraz was applied during early April at GS 31-32 to winter wheat crops. The efficacy of this application was assessed during the first two weeks of June, when the crops were at GS 74-75. The yields from treated and untreated plots were measured at the time of harvesting and the increase in yield given by the treatment calculated.

Despite variability in efficacy at the end of the season, cost-effective yield increases, above 1.0 decitonne/ha, were obtained at 85% of the 48 sites examined. It was possible to identify 6 sites which had a higher than normal proportion of less-sensitive isolates. The frequencies of occurence of these, together with disease control and yield are shown in Table 1.

Table 1 Effect of 450g AI/ha prochloraz applied at GS 31 on eyespot levels, assessed 9/6/93 at GS 74-75.

Site location (department)	Mean Eyespot Index			% Control	Yield % increase over untreated	% of R types classed as insensitive at harvest
	Untreated	Treated	SE ±			
1 Oise (60)	0.56	0.10	0.13	81.5	2.6 NS	40
2 Oise (60)	2.21	1.27	0.21	42.5	7.9 •	33
3 Oise (60)	1.16	1.68	0.13	0	0	37
4 Oise (60)	1.54	0.76	0.14	50.7	8.8 •	48
5 Oise (60)	0.65	0.89	0.08	0	6.7 •	30
6 Somme (80)	1.55	1.21	0.10	41.0	3.7 •	40

The maximum eyespot index = 4.0

•, significantly different from untreated at P = 0.05

NS, not significantly different from untreated at P = 0.05

The six sites were all located in the north of France, in the Oise and Somme departments. Levels of eyespot at the beginning of June varied from low at two of the sites (1 and 5) to moderate at sites 3, 4 and 6 and high at site 2. The levels of control given by prochloraz at this time also varied, from 81.5% at site 1, to moderate efficacy at sites 2, 4 and 6 and no apparent control at sites 3 and 5.

Yield increases were seen at 5 of the 6 sites and all but one of these increases was significant. The single case, site 1, where the increase was found not to be significant, despite excellent efficacy, could have been due to the levels of disease being so low that there was only limited potential for improvement.

These wide variations in levels of infection, efficacy and yield were seen despite the relative uniformity of the proportions of less-sensitive R type isolates, which ranged from 30% to 48% of the eyespot populations obtained from the sites. The presence of these isolates did not, therefore, appear to be the major factor determining yield and efficacy at these sites.

Similarly, Migeon et al (1994) concluded that at certain sites, prochloraz gave good efficacy, despite the presence of significant proportions of less sensitive isolates.

Effects of prochloraz application on eyespot populations

Bateman et al (1986) reported that prochloraz selected for R-types when applied to trial plots artificially inoculated with mixtures of W and R-types. In vitro assessments of sensitivity, however, have indicated that the R-type is slightly more sensitive to prochloraz than the W-type, on average (Birchmore et al, 1986, Gallimore et al, 1987).

Analysis of the data from the trials conducted during 1993 indicates that there is no clear relationship between prochloraz application and the proportions of R-types (Table 2).

Table 2 Effects of an application of 450g prochloraz/ha on proportions of R-types isolated from stem samples

Department	% R types isolated		
	April Pre-treatment	June	
		Untreated	Prochloraz treated
Eure (27)	70	75	97
Seine-et-Marne (77)	15	20	0
Marne (51)	15	60	15
Marne (51)	13	20	25
Oise (60)	30	30	50
Somme (80)	44	70	70
Oise (60)	67	58	74
Oise (60)	84	57	61

Any differences seen may not be due to one type being more susceptible than another but to differences in the timing and speed of infection of the cereal stem. W-types have been found to infect and develop in stem-bases much more rapidly than R-types and may, therefore, be more susceptible to fungicide applied early in the season than the later, slower developing R-type (Goulds and Fitt, 1990).

CONCLUSIONS

Extensive investigations carried out by both independent researchers and ourselves have confirmed the presence of isolates with decreased sensitivity to prochloraz at a limited number of sites in northern France. Research has shown that at individual trial sites, such isolates can form a significant proportion of the R-type population. However, prochloraz can provide useful efficacy and yield benefits even in their presence.

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