HGCA

PROJECT REPORT No. 127

SENSITIVITY OF BARLEY POWDERY MILDEW TO MORPHOLINE FUNGICIDES: I. WORK IN SCOTLAND II. WORK IN ENGLAND AND WALES

JULY 1996

PRICE £8.00

PROJECT REPORT No. 127

SENSITIVITY OF BARLEY POWDERY MILDEW TO MORPHOLINE FUNGICIDES: I. WORK IN SCOTLAND; II. WORK IN ENGLAND AND WALES

by

PART 1. S. ROBERTSON, J. GILMOUR, D. NEWMAN AND J. H. LENNARD
SAC, West Mains Road, Edinburgh EH9 3JG

PART II. D. W. HOLLOMON
IACR Long Ashton, University of Bristol, Long Ashton, Bristol, Avon BS18 9AF

This is the final report of a research project which commenced in August 1989 and lasted for three years. The work was funded by the Home-Grown Cereals Authority as project numbers 0009/3/88A for £21,500 to SAC-Edinburgh (Part I) and 0009/1/88B for £11,178 to the Institute of Plant Science Research, Cambridge Laboratory and 0009/3/88C for £27,900 to IACR Long Ashton (Part II).

CONTENTS

PART I - Figures

PART II

PART II - Tables

PART II - Figures

SENSITIVITY OF BARLEY POWDERY MILDEW ISOLATES TO MORPHOLINE FUNGICIDES

PART I WORK IN SCOTLAND

1 INTRODUCTION

The introduction in the 1970s of absorbed xylem-translocated fungicides (commonly called "systemics") provided barley growers with highly effective means of disease control. In Scotland their use became widespread to control powdery mildew (*Erysiphe graminis* f. sp. *hordei*) on the highly susceptible spring barley cultivar Golden Promise which had quickly predominated following its commercial release in the mid-1960s. The failure of fungicides to control powdery mildew completely had been noted since their introduction, but in many cases poor performance was due to factors such as poor application of sprayed fungicides or poor uptake of seed treatment fungicides. However, in 1971 Wolfe reported the existence of strains of powdery mildew with reduced sensitivity to ethirimol, and in the following years this was confirmed by other workers (Wolfe and Dinoor, 1973; Shephard *et al*, 1975; Hollomon, 1978). From 1976 growers of Golden Promise in south-east Scotland were recommended to adopt programmes of two treatments for mildew control, either a seed treatment followed by a spray where there was a risk of early infection from nearby infected winter barley, or two sprays whether there was less risk of early infection. Winter barley growers were, from 1979, advised to spray mildew-infected crops before nearby spring crops brairded to reduce the carry-over of mildew. To reduce the risk of resistance developing in the mildew population, barley growers were recommended to use unrelated fungicides whenever possible in these three situations.

By the late 1970s, the triazole fungicide triadimefon had become the most widely used spray against barley mildew in south-east Scotland, and a closely related fungicide, triadimenol, was then introduced for use as a seed treatment on both spring and winter barley. During that period several other products based on fungicides which were believed to have similar modes of action were

also in use: nuarimol and triforine as seed treatments, and nuarimol, prochloraz, propiconazole and triforine as sprays. In 1981 on a small number of farms, and in 1982 on a larger number of farms, these fungicides failed to give the expected control of mildew. Associated with these changes were reductions in yield benefit from fungicide application measured in field trials (Gilmour, 1983). A postal survey of spring barley growers in south-east Scotland confirmed that the problem associated with the use of triazoles had been widespread (Gilmour, 1984a). Reduced sensitivity of *Erysiphe graminis* f. sp. *hordei* was reported by Fletcher and Wolfe in 1981 and later by several others (Butters *et al*, 1984; Wolfe, 1985; Andrivon *et al*, 1987).

To avoid mildew control problems in 1983, spring barley growers in south-east Scotland were advised, *inter alia*, to reconsider their commitment to the highly susceptible cultivar Golden Promise; to use two-treatment programmes on susceptible cultivars, either a mildew seed treatment following by a spray, or a two-spray programme; and to use chemically different fungicides for follow-up treatments. A postal survey of spring barley growers in south-east Scotland in 1983 revealed: that Golden Promise retained its popularity (75% overall); that mildew seed treatments were used on 39% of the area grown; that 94% of the crop was sprayed at least once, and 29% sprayed at least twice; that whereas the use of triazoles (triadimefon and propiconazole) and morpholines (fenpropimorph and tridemorph) as first sprays on Golden Promise without seed treatment was similar in 1982 (45% and 44% respectively), in 1983 the use of morpholines predominated at 75% with only 19% of the area being treated with the triazoles; and that where a second spray was applied morpholines were used predominantly in both years (86% and 69% respectively) (Gilmour, 1984b).

It is understandable that growers, following their experiences in 1982, should swing away from the triazole fungicides which lacked a marked eradicant effect and to which the local powdery mildew population may have become less sensitive. There was concern, however, that so much spring barley in 1983 was sprayed, often twice, with the morpholine fungicides fenpropimorph and tridemorph. Growers were specifically warned that this move might well

create new disease control problems if the pathogens became less sensitive to these fungicides.

Several groups of workers have monitored the reaction of *Erysiphe graminis* f. sp. *hordei* to the morpholines and some have noted no significant alteration in sensitivity (Heaney, 1987; Heaney *et al*, 1986; Limpert, 1987). There have, however, been some reports of changes. Mutants insensitive to tridemorph were reported in 1971 (Walmsley-Woodward *et al* 1979a, 1979b). Reduced sensitivity of the pathogen to fenpropimorph was reported by Wolfe *et al* (1984) and by Fletcher *et al* (1987). Similar observations were made by Andrivon *et al* (1987) in respect of a population of isolates from the Champagne region in France.

It was against this background that the present study of barley powdery mildew in Scotland was undertaken as part of the HGCA-funded programme to monitor sensitivity of important cereal pathogens to the fungicides widely used for their control. This report contains details and results of tests on isolates collected during 1988, 1989, 1990 and 1991. One paper giving interim results was published during the study period (Robertson *et al*, 1990).

2 MATERIALS AND METHODS

2.1 Collection method and isolate details

Leaves infected with powdery mildew were collected from barley crops and trial plots in south-east Scotland during 1988-1991. On collection crop details were recorded: crop, site, chemical application history, date etc . The isolates were cultured on detached leaf segments of barley cv. Golden Promise, maintained on 5% water agar containing 80 mg/litre benzimadazole to delay leaf senescence. Each isolate was maintained separately bulked up as required for testing. Results from individual tests were recorded along with the isolate histories in an Oracle data base. All the isolates in this study were collected, maintained and tested as bulk population isolates, designated simply "isolates" throughout this report.

2.2 Basic test method

Three fungicides were used routinely in the fungicide sensitivity screening:

tridemorph, formulated as Calixin (BASF plc), for which the normally recommended field spray concentration is 0.7 litre/ha in 200 litres water/ha;

fenpropimorph, formulated as Mistral (Rhône-Poulenc Agriculture), for which the normally recommended field spray concentration is 1 litre/ha in 200 litres water/ha; and

fenpropidin, formulated as Patrol (ICI), for which the normally recommended field spray concentration is 1 litre/ha in 200 litres water/ha.

Because the fungicides were always used as these formulated commercial products, the product names are used throughout this report to avoid confusion with reports of other work in which technical active ingredients may have been used.

Five fungicide concentrations were used, based on the normally recommended field spray designated "C". For Calixin 1/32C, 1/16C, 1/8C, 1/4C and 1/2C were used giving concentrations of 0.082, 0.164, 0.328, 0.656 and 1.312 g tridemorph per litre. In early experiments Mistral and Patrol were used at concentrations of 0.117, 0.234, 0.469, 0.938 and 1.976 g a.i./litre. In later experiments these concentrations were reduced because of the sensitivity of the screened isolates to the fungicides. The amended concentration range was 1/256C, 1/128C, 1/64C, 1/32C, 1/16C giving 0.015, 0.029, 0.058, 0.117 and 0.234 g a.i./litre. Control plants were sprayed with water.

For each test, seedlings of barley cv. Golden Promise were grown, five to a pot, in a Burkhart Isolation Propagator. Before the ligules were visible on the second leaves (approximately 14 days old), the fungicide solutions were applied to seedlings in two pots in each of a pair of spray cabinets using a Humbrol spray gun: 5 second application time, followed by 15 minutes settling time. The chemicals were applied in ascending order of concentration.

Treated sets of plants were kept apart for 24 hours, to allow sufficient time for fungicide uptake before the preparation of the leaf segments and inoculation of the segments with isolates of powdery mildew. Thirty-two replicate leaf segments were cut from the second leaves of the test plants from each concentration/spray cabinet combination. The leaf segments were placed on 5% water agar containing 80 mg/litre benzimadazole and then inoculated uniformly by tapping heavily infected leaf segments covered with spores of the test isolate over the plates and distributing the spores with a fine paint brush.

Four isolates were screened during each test. The leaf segments were incubated at 18^{o}C in an illuminated incubator with a 12-hour light period in each day.

2.3 Assessment and analysis

Leaf segments were assessed for percentage mildew cover 14 days after inoculation. Several tests were repeated three times to check reproducibility. The results were analysed with the aid of a Genstat 5 programme which fitted symmetrical logistic curves and calculated EC_{50} values (concentration of fungicide which reduced mildew cover to half that of the untreated control), with associated standard errors, both on a log scale. Where calculated values

were obtained from both spray cabinets, the geometric mean was taken and then back-transformed to give the isolate result in terms of the concentration of active ingredient in the spray. Because the standard errors were calculated on the logarithmic scale, they are reported as average percentage of the related EC_{50} value to avoid problems associated with back-transformation. MICs (the minimum concentration at which no visible growth occurred) were also determined. **NB** EC_{50} values and MICs are both reported in terms of the concentration of the active ingredients of the test fungicide products.

2.4 Sensitivity of isolates to fungicide mixtures

Concern over the possible increased risk of loss of efficacy through the extensive use of a limited number of fungicide products has led to the introduction of fungicide products which contain more than one active ingredient, often two fungicides with distinct modes of action. These products often contain lower amounts of the active ingredients than are found in products containing only as the sole active ingredient.

In a pilot study, the influence of the respectively lower application rates on the sensitivity of the powdery mildew population was tested by screening a few isolates for their sensitivity to the product Dorin (Bayer plc), a fungicide mixture containing the morpholine tridemorph (375 g a.i. $litre^{-1}$) and triazole triadimenol (125 g a.i. $litre^{-1}$). The suggestion has been made that the two compounds supplement each other, giving equal control of the whole powdery mildew population with its different races (Schulz & Scheinpflug, 1988). The same isolates were screened for their sensitivity to Calixin and for their sensitivity to Bayfidan, ie two products containing the separate active ingredients of Dorin.

Because the test mixture, Dorin, contained two active ingredients, it was not possible to determine EC_{50} values for the component fungicides by the standard method. Accordingly, these results are presented graphically.

3 RESULTS

3.1 Example test results

Following the test procedure described above, the results were obtained by assessing the percentage mildew cover on the leaf segments 14 days after inoculation. The data collected for each isolate in each test were recorded as shown below.

TEST: BF61 SPRAY DATE: 16-07-91 FUNGICIDE: CALIXIN
RATE: 0.7 litre/ha ASSESSMENT DATE: 30-07-91

Spray	*Spray*	*Replicate leaf segment*							
Conc^n	*Cabinet*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*
0	1	80	80	70	80	50	50	70	60
0	2	80	80	60	40	70	80	75	60
1/32	1	60	60	60	65	80	60	50	20
1/32	2	80	75	60	60	70	65	55	60
1/16	1	70	60	45	30	20	30	20	30
1/16	2	50	60	20	60	40	50	40	20
1/8	1	15	20	30	20	15	60	45	50
1/8	2	15	15	5	15	5	10	5	60
1/4	1	0	0	0	0	0	0	0	0
1/4	2	0	0	0	0	0	0	0	0
1/2	1	0	0	0	0	0	0	0	0
1/2	2	0	0	0	0	0	0	0	0

A Genstat 5 program was used to calculate the median percentage mildew cover for each concentration/spray cabinet combination and then to fit symmetrical logistic curves to the data to facilitate the calculation of EC_{50} values.

Figures 1, 2, 3 and 4 show examples of the final output from this programme, illustrating some of the different patterns obtained. Figure 1 shows the reaction of isolate GP Bush 1990 to five concentrations of Mistral. The graph illustrates the median values of the percentage leaf cover for the eight leaf segments and the computer fitted values. Below the graph, these are tabulated separately for each spray cabinet, together with the EC_{50} values and the mean EC_{50} values for the isolate test.

3.2 Anomalies in the test procedure and their analysis

The logistic curve fitting procedure used was suitable for all "standard" reactions: for example, isolates that can grow on the untreated control and on the two or three lowest concentrations of the test fungicide. A few non-standard reactions exhibited by some of the isolates during the screening require comment.

3.2.1 Extreme reactions

The Genstat program was unable to analyse satisfactorily data from isolates that did not grow on the lowest one or two concentrations of fungicide (ie very sensitive isolates), or from isolates that grew at a steady rate over several concentrations and were killed at a higher concentration with no steady decline in percentage tissue infected. This lower sensitivity reaction had been found several times when isolates were screened for their sensitivity to the triazole Bayfidan (Bayer plc) in previous work, but was also found infrequently when isolates were tested for sensitivity to morpholines.

In the first situation of high sensitivity an EC_{50} value is assigned as "<X", where X= the lowest concentration of chemical sprayed. In the second situation, when the computer program has been unable to fit a curve, an MIC value has been assigned to the isolate and the EC_{50} value omitted.

3.2.2 Growth anomalies

A feature which has been noted occasionally with some isolate/fungicide combinations was that the growth of some isolates was promoted slightly at low fungicide concentrations. To analyse this feature (shown in Figure 5) the programme was modified to "unfix" the untreated control median value enabling the computer to fit a standard symmetrical logistic curve. Where this occurred, the growth anomaly was noted and the EC_{50} value obtained incorporated amongst the standard results.

3.3 Reproducibility of results and stability of isolates

The design of the test procedure required that the screening be reproducible and that the sensitivity of individual isolates to particular fungicides remain stable despite repeated subculture of isolates for maintenance. During the project isolates were tested and re-tested with particular fungicides to check that their sensitivity to the test chemical had remained stable.

Table 1 details some examples of data from the repeated tests carried out during the project, illustrating that the test procedure yields reproducible and consistent results. In Table 1, the sensitivity of two isolates Glf91 and GP91, to the morpholine Calixin are also noted. These two isolates were first tested for sensitivity without sub-culturing on glasshouse plants. The isolates were taken from infected leaf tissue which had been kept under appropriate day length and temperature conditions for one week to optimise sporulation. This initial test gave EC_{50} values of 0.153 and 0.108 g a.i. $litre^{-1}$ respectively. Approximately one month later after three passages through the laboratory host, the EC_{50} values of Glf91 and GP91 to Calixin were 0.161 and 0.105 g a.i. $litre^{-1}$, very similar to the results obtained from the fresh field isolates. Unfortunately it was rare to be able to screen isolates routinely immediately from the field because of the low number of spores available to carry out the inoculation of prepared leaf segments. The test procedure usually required a "bulking up" stage.

Many isolates have been tested repeatedly and the sensitivity of these isolates has appeared stable even after being maintained for two years on Golden Promise in the laboratory. Isolate P8, collected in May 1988 and tested with Calixin in May 1989 and in January 1990 gave EC_{50} values of 0.129 and 0.125 g a.i. $litre^{-1}$. Isolate BSBC was also collected in May 1988 and was tested with Patrol in October and November 1990 giving results of 0.042 and 0.038 g a.i. $litre^{-1}$ respectively. The EC_{50} values determined for each individual isolate were reproducible upon repetition and showed no significant shift over time. A similar result was obtained with one isolate tested with the triazole fungicide triadimenol, formulated as Bayfidan.

3.4 Sensitivity of isolates estimated by EC_{50} values

3.4.1 Variation of sensitivity within years

Table 2 gives, for comparison, EC_{50} values for two "older" isolates, collected in 1973 and 1984. Isolate 2023 was very sensitive to Calixin and Mistral. Isolate JB212 was sensitive to Mistral and Patrol but less sensitive to Calixin.

Tables 3, 4, 5 and 6 detail the EC_{50} values for individual isolates collected during 1988, 1989, 1990 and 1991 respectively. The sensitivities of the isolates collected each year are shown graphically in Figures 6 to 15 with isolates arranged in order of decreasing sensitivity. (NB Each Figure is scaled independently.)

For Calixin there was in 1988, 1989 and 1990 approximately a 4-fold difference between the most sensitive isolate and the least sensitive isolate and the EC_{50} values showed a log-normal distribution over the range of fungicide concentrations tested. The range of sensitivities was less among the smaller number of isolates tested in 1991.

For Mistral and Patrol the isolates showed much greater variations of sensitivity within each year, and much greater variation in range from year to year: for Mistral, 17-fold, 5-fold and 17-fold in 1988, 1989 and 1990 respectively; and for Patrol 44-fold (even omitting the unrepeated high value for isolate BUSB18), 6-fold and 20-fold respectively. Within each year the distributions for Mistral and Patrol over the range of fungicide concentrations tested were skewed with heavy left tails because of the high sensitivity of some isolates to these fungicides.

3.4.2 Variation of sensitivity between years

Annual median and mean EC_{50} values for each fungicide are given in Table 7. Annual variations in mean EC_{50} are illustrated in Figures 16 to 18, and the overall patterns of response to each fungicide shown in Figures 19 to 21. (NB Each Figure is scaled independently.)

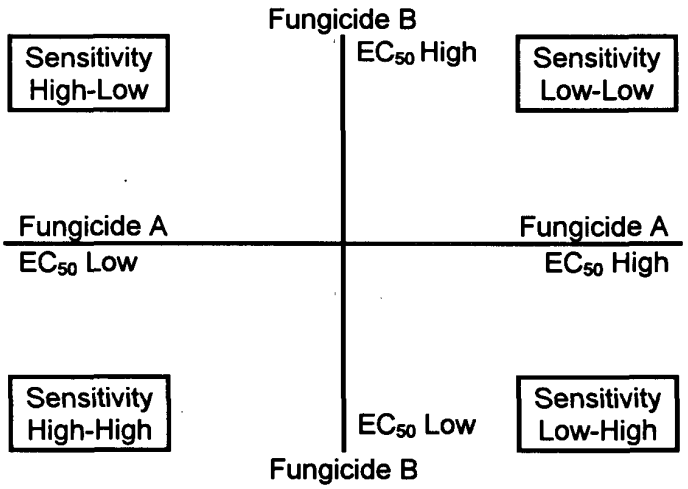
The annual mean sensitivity to Calixin decreased each year from 1988 to 1991, but none of the changes between successive years was statistically significant. The overall decrease between 1988 and 1991 was, however, statistically significant ($t = 2.59$, $P < 0.05$).

The increase in annual mean sensitivity to Mistral between 1988 and 1989 was statistically significant ($t = 2.96$, $P < 0.05$). None of the subsequent annual changes in mean sensitivity to Mistral was statistically significant.

The increase in annual mean sensitivity to Patrol between 1988 and 1989 was statistically significant ($t = 2.84$, $P < 0.05$), as was the subsequent decrease of sensitivity between 1989 and 1990 ($t = 3.54$, $P < 0.01$). No other change was statistically significant.

3.5 Cross-sensitivity of fungicides

Figures 22, 23 and 24 show respectively the distributions of sensitivity to Calixin and Mistral, to Calixin and Patrol and to Mistral and Patrol based on isolates for which both EC_{50} values were available. The axes of these scatter diagrams have been drawn, arbitrarily, approximately half-way between the extremes of sensitivity recorded in these tests. The division of the scatter diagram into four quadrants shows clearly the cross-sensitivity relationships, thus:



NB The designation of sensitivity as "high" or "low" is made only in relation to the range of sensitivity observed.

While for Calixin, isolates were fairly evenly distributed over the range of sensitivity observed, for Mistral and Patrol most isolates had sensitivities in the lower half of the ranges observed.

Taking sensitivity to Calixin and Mistral together, none of the isolates tested could be considered to fall into the low-low category (Figure 22). The isolate which showed the lowest sensitivity to Calixin, P11A (1990, Table 5), was highly sensitive to both Mistral and Patrol. One isolate showed low sensitivity to both Calixin and Patrol (Figure 23). Unfortunately this isolate, PSGB (1990, Table 5), was not tested against Mistral. One 1998 isolate, PB, showed low

sensitivity to both Mistral and Patrol (Figure 24), but its sensitivity to Calixin was high (Table 3).

The two 1990 isolates which were among the least sensitive to Patrol, BH90:32 and PSBK, were highly sensitive to Mistral and were mid-range for Calixin sensitivity. The majority of the isolates tested against Mistral and Patrol fell into the high-high sensitivity category.

3.6 Sensitivity of isolates estimated by MIC values

Tables 8-12 give the MIC values determined in every test undertaken during the course of this project. These data have not been subject to detailed analysis because the spray concentrations used were not selected for MIC determination but rather for EC_{50} calculation. This information should, therefore, be regarded as supplementary rather than definitive.

The annual and overall distributions of MIC values are presented in Table 13. They show broadly the same patterns as the distributions of the corresponding EC_{50} values.

In 1990 two isolates collected from the same trial but from plots that had received different fungicide treatments, TRSBC and TRSBK, were able to grow on leaf segments treated with the highest concentration of Mistral, ie 1/16C. Five other 1990 isolates were able to grow on leaf segments treated with the highest concentration of Patrol, ie 1/16C. Four of these isolates (PSBG, PSBJ, PSBK and PSBW) were from the same trial site, but from plots that had received different fungicide treatments; the fifth isolate (BH90:32) was from a different area.

The MIC values for the three fungicides have not been cross-tabulated because the numbers in some cells of the two-way tables would be too small to be meaningful. Inspection of the data showed, however, that while there was an indication of positive correlation between MIC values for one fungicide and another, there was generally great variation within any one MIC group.

For example; for Calixin, isolates with an MIC of 0.328 had MIC values for Mistral equally spread between 0.014 and 0.234. Similarly isolates with a Calixin MIC of 0.656 had Patrol MIC values ranging from 0.015 to >0.234.

Similar spreads were shown within some of the MIC ranges when Mistral and Patrol were considered together. However the isolates with a Mistral MIC of 0.234 or greater all had a Patrol MIC of 0.234 or greater. Some of these isolates came from each of the main sampling years, 1988, 1989 and 1990.

3.7 Isolate sensitivity and field history of fungicide treatment

The isolates collected each season came from a variety of sources, some from commercial farms, some from chemical trials and some from variety trials. Whenever possible full details were obtained of the fungicide treatments that had been applied to the field or plot. Unfortunately, information for farm fields was often incomplete but comprehensive histories were obtained for trial plots.

Several isolates were collected from fields or plots that had received multiple fungicide treatments. The EC_{50} values of some of these isolates are detailed below, along with the fungicide treatment history.

Isolate PF

Seed Treatment:	Single purpose
Winter Spray:	Dorin (0.75) + Sportak (0.5)
Spring Spray:	Corbel (0.5) + Sportak alpha (1.0)
Summer Spray:	Tilt Turbo (1.0)
Determined EC_{50} Value:	Calixin = 0.086 g a.i. litre^{-1}

Isolate B3

Seed Treatment:	Single purpose
Autumn Spray:	Dorin (1.0)
GS30-31:	Mistral (0.75) + Sportak alpha (1.0)
Determined EC_{50} Value:	Calixin = 0.070 g a.i. litre^{-1}
	Mistral = 0.018 g a.i. litre^{-1}

Isolate B35

Seed Treatment:	Ferrax
GS30-31:	Mistral (0.75) + Sportak alpha (1.0)
Determined EC_{50} Value :	Calixin = 0.122 g a.i. $litre^{-1}$
	Mistral = 0.024 g a.i. $litre^{-1}$
	Patrol = 0.011 g a.i. $litre^{-1}$

Isolate B30

Seed Treatment:	Single Purpose
Spray history:	untreated control (same trial as B35)
Determined EC_{50} Value :	Calixin = 0.079 g a.i. $litre^{-1}$
	Mistral = 0.039 g a.i. $litre^{-1}$
	Patrol = 0.037 g a.i. $litre^{-1}$

Isolate B21

Seed Treatment:	Single purpose
Autumn spray:	Dorin (1.0)
GS30-31:	Mistral (0.75) + Sportak alpha (1.0)
Determined EC_{50} Value :	Calixin = 0.101 g a.i. $litre^{-1}$
	Mistral = 0.031 g a.i. $litre^{-1}$
	Patrol = 0.032 g a.i. $litre^{-1}$

Isolate MD

Seed Treatment:	Single purpose
Spring spray:	Sportak alpha (1.5)
Determined EC_{50} Value :	Calixin = 0.077 g a.i. $litre^{-1}$
	Mistral = 0.095 g a.i. $litre^{-1}$

Isolate ME

Seed Treatment:	Single purpose
Winter spray:	Dorin (0.75) + Sportak (0.5)
Spring spray:	Corbel (0.5) + Sportak alpha (1.0)
Determined EC_{50} Value :	Calixin = 0.102 g a.i. $litre^{-1}$
	Mistral = 0.085 g a.i. $litre^{-1}$

Overall, the data showed no correlation between fungicide treatment before collection of the isolates and sensitivity to the three fungicides used in these tests.

3.8 Sensitivity of isolates to fungicide mixtures

Figures 25, 26 and 27 show the reaction of six isolates to Dorin and to low concentrations of Calixin and Bayfidan. The concentrations of Calixin and Bayfidan were chosen a) to cause an effect on the growth of the powdery mildew isolate and b) to reflect the lower amounts of active ingredient in the mixed product Dorin.

All isolates tested were very sensitive to the mixed product, Dorin, regardless of the sensitivity of the isolates to the component fungicides. Several of the isolates, PGU2, R1 and R14, were less sensitive to Bayfidan (triadimenol), the triazole component of Dorin, and were able to grow on all the spray concentrations tested. All of the isolates were sensitive to Calixin. In all cases the mixed product was more effective than either of the component fungicides, and in some cases the mixed product was markedly more effective. For example isolate R1, which was relatively insensitive to Bayfidan and showed growth promotion at low concentrations of Calixin, was inhibited completely by low concentrations of Dorin.

4. DISCUSSION

The isolates of barley powdery mildew screened in this project displayed a range of sensitivity reactions to the test fungicides. In general the isolates were more sensitive to fenpropimorph and fenpropidin than to tridemorph. This may be due to variation within the local population, an effect of the distinct modes of action of the fungicides or to a decrease of sensitivity to tridemorph which has been used for longer.

The annual mean sensitivity of the screened isolates to Calixin decreased during the study period. The decrease over the four years was statistically significant but was small, changing by only a factor of x1.4. Much larger variation occurred within each year.

The increases of annual mean sensitivity to both Mistral and Patrol between 1988 and 1989 were larger than the overall change for Calixin: factors x2.6 and x3.2 respectively. However there was no clear subsequent trend for Mistral, but for Patrol the 1990 mean sensitivity returned to the 1988 level. The isolate collected in 1973 was the most sensitive of the whole collection to the two fungicides against which it was tested.

The EC_{50} analysis showed that there were very few isolates that could be categorised as having "low-low" sensitivity to any two of the fungicides used in these tests. NB Designation as "low" or "high" is made only in relation to the range of sensitivity observed. Even the lowest value observed may not be of practical significance in the field.

However, the MIC results identified several isolates that had high MIC values to both Mistral and Patrol, ie low-low sensitivities. This difference from the EC_{50} analysis suggests that some of the bulk population isolates used in these tests contained some components that could grow at higher concentrations of both fungicides than would have been expected from the average responses of those isolates. The overall significance of the presence of such components within a population would depend on their fitness, as measured by parameters other than sensitivity to these fungicides, relative to the bulk of

the population. Although the concentrations used in these tests were below those used in the field, especially for Mistral and patrol, the detection of such isolate components may indicate the ability of the powdery mildew fungus to adapt to these two fungicides simultaneously.

The very limited tests undertaken with the mixed fungicide product, Dorin, indicated that such mixtures appear to have an enhancement effect in controlling the fungus compared to the individual component fungicides applied alone. This aspect would undoubtedly repay further study, but more complex test procedures will be required, particularly the use of variable ratios of the components of the mixture.

There was no apparent correlation between field use of fungicides before collection and sensitivity to the test fungicides. Lorenz and Pommer (1984), working with wheat powdery mildew, also found sensitivity to fenpropimorph to be independent of previous fungicide treatment.

In some tests low concentrations of fungicide were found to stimulate the growth and sporulation of the isolates. This peculiarity was reproducible and was not an artefact of the experiment. Similar stimulation has been observed in tests on barley powdery mildew (Williamson, 1984) and on other fungi (eg Boyle *et al*, 1988).

5. CONCLUSIONS

This study took place at a time when barley growers were heavily dependent on fungicides for the effective control of powdery mildew and when the morpholines had been the most widely used for several years in Scotland.

A considerable range of variation of sensitivity was found to all three of the fungicides tested; Calixin (tridemorph), Mistral (fenpropimorph) and Patrol (fenpropidin). Variation of sensitivity to Calixin was least (4-fold) and most consistent over years. Variation of sensitivity to Mistral and Patrol was much greater (up to 17-fold and 44-fold respectively) and the range more variable from year to year.

Mean annual sensitivities to the fungicides changed from year to year, but although sometimes statistically significant these changes were small. Sensitivity to Calixin declined over the four year period. Sensitivity to both Mistral and Patrol increased from 1988 to 1989. Sensitivity to Mistral showed little change thereafter, while sensitivity to Patrol returned to the 1988 level.

Very few isolates showed reduced sensitivity to two fungicides, but a small number of isolates contained components that could grow in the presence of the highest concentrations of Mistral and Patrol used in these tests. This may indicate an adaptive capability of some of the populations of barley powdery mildew in Scotland.

Very limited work with one fungicide mixture, Dorin (triadimenol and tridemorph), suggested that such mixtures may be very much more effective than the component fungicides used alone.

There was no apparent correlation between sensitivity and fungicide used in the field before the isolates were collected.

6. ACKNOWLEDGEMENTS

We are grateful to Mr E. A. Hunter of the Scottish Agricultural Statistics Service for helpful advice, and to the farmers and site managers who gave us access to their fields and plots for the collection of isolates.

7. REFERENCES

Andrivon, D.; Limpert, E.; Felsenstein, F.G. (1987) Sensitivity of French populations of *Erysiphe graminis* DC *f. sp. hordei* Maréchal to triadimenol and fenpropimorph. *Agronomie* **7**: **6**, 443-446.

Boyle, F.; Gilmour, J.; Lennard, J.H. (1988). Sensitivity of cereal brown rust fungi to triadimefon and propiconazole. *British Crop Protection Conference 1988 - Pests and Diseases*, **2**, 379-384.

Butters, J.; Clark, J.; Hollomon, D.W. (1984) Resistance to inhibitors of sterol biosynthesis in barley powdery mildew. *Mededelingen van da Faculteit Landbouwwentenschappen Rijksuniversiteit Gent*, **49:2a**, 143-151.

Fletcher, J.T.; Cooper, S.T.; Prestidge, A. L. H. (1987) The sensitivity of *Erysiphe graminis* f.sp. *tritici* to various fungicides inhibiting sterol biosynthesis. *ISPP Chemical Control Newsletter*, **9**, 30-32.

Fletcher, J. T.; Wolfe, M. S. (1981) Insensitivity of *Eryisphe graminis* F.sp. *hordei* to triadimefon, triadimenol and other fungicides. *British Crop Protection Conference 1981 - Pests and Diseases*, **2**, 633-640.

Gilmour, J. (1983) Performance of barley mildew fungicides in south-east Scotland. *Proceedings tenth International Congress of Plant Protection*, **2**, 643.

Gilmour, J. (1984a) Survey of mildew fungicide use on spring barley in south-east Scotland in 1982. *Proceedings Crop Protection in Northern Britain*, 79-84.

Gilmour, J. (1984b) Comparison of some aspects of mildew fungicide use on spring barley in south-east Scotland in 1982 and 1983. *British Crop Protection Conference - Pests and Diseases*, **1**, 109-114.

Heaney, S. P. (1987) Changes in fungicide sensitivity and strategies for the control of UK barley mildew populations. *Vaxtskyddsrapporter*, **48**, 91-96.

Heaney, S. P.; Hutt, R. T.; Miles, V. G. (1986) Sensitivity of barley powdery mildew populations in England and Scotland; status and implications for fungicide use. *British Crop Protection Conference 1986 - Pests and Diseases*, **2**, 793-800.

Hollomon, D. W. (1978) Competitive ability and ethirimol sensitivity in strains of barley powdery mildew. *Annals of Applied Biology*, **90**, 195-204.

Limpert, E. (1987) Frequencies of virulence and fungicide resistance in the European barley mildew population in 1985. *Journal of Phytopathology*, **119**: **4**, 298-311.

Lorenz, G.; Pommer, E. H. (1984) Investigations into the sensitivity of wheat powdery mildew populations towards fenpropimorph. *British Crop Protection Conference 1984 - Pests and Diseases*, **2**, 489-494.

Robertson, S. M. S.; Gilmour, J.; Newman, D.; Lennard, J. (1990) Sensitivity of barley powdery mildew isolates to morpholine fungicides. *British Crop Protection Conference 1990 - Pests and Diseases*, **3**, 1159-1162.

Schulz, U.; Scheinpflug, H. (1988) Sterol biosynthesis inhibiting fungicides: antifungal properties and application in cereals. **In:** Berg, D.; Plempel, M.; Editors (1988) Sterol biosynthesis inhibitors: Pharmaceutical and agrochemical aspects. Ellis Horwood Ltd, Chichester (England).

Shephard, M. C.; Bent, K. J.; Woolner, M.; Cole, A. M. (1975) Sensitivity to ethirimol of powdery mildew from UK barley crops. *Proceedings 8th British Insecticide and Fungicide Conference*, **1**, 59-66.

Walmsley-Woodward, D. J.; Laws, F. A.; Whittington, W. J. (1979a) Studies on the tolerance of *Erysiphe graminis* f. sp. *hordei* to systemic fungicides. *Annals of Applied Biology*, **92**, 199-209.

Walmsley-Woodward, D. J.; Laws, F. A.; Whittington, W. J. (1979b) The characteristics of isolates of *Erysiphe graminis* f. sp. *hordei* varying in response to tridemorph and ethirimol. *Annals of Applied Biology*, **92**, 211-219.

Williamson, M. B. R. (1983) Studies on the sensitivity of barley mildew to fungicides in south-east Scotland. *MSc thesis, University of Reading*, 64pp.

Wolfe, M. S. (1971) Fungicides and the fungus population problem. *Proceedings 6th British Insecticide and Fungicide Conference,* **3**, 724-734.

Wolfe, M. S.; Dinoor, A. (1973) The problem of fungicide tolerance in the field. *Proceedings 7th British Insecticide and Fungicide Conference*, **1**, 11-19.

Wolfe, M. S.; Slater, S. E.; Minchin, P. N. (1984) Powdery mildew of barley. *Annual Report of the Plant Breeding Institute, 1983.* 87-91.

Wolfe, M. S. (1985) Dynamics of the response of barley mildew to the use of sterol synthesis inhibitors. *EPPO Bulletin*, **15**, 451-457.

Table 1: Test reproducibility and stability of isolates

Isolate	Fungicide	Date of Collection	Test Date	EC_{50}	Average SE %
P8	Calixin	25/5/88	31/5/89	0.129	11.5
			4/1/90	0.125	-
R19	Calixin	14/7/89	11/5/90	0.128	-
			28/8/90	0.124	9.0
R6	Calixin	14/7/89	7/6/90	0.086	-
			5/7/90	0.093	4.5
Glf91	Calixin	5/6/91	12/6/91	0.164	0.6
			16/7/91	0.161	2.4
GP91	Calixin	5/6/91	12/6/91	0.108	30.2
			22/7/91	0.105	-
B21	Mistral	14/7/89	24/7/90	0.030	3.3
			17/11/90	0.031	3.4♦
R17	Mistral	14/7/89	24/7/89	0.022	-
			14/8/90	0.022	4.3
R19	Mistral	14/7/89	24/7/90	0.025	0.2
			14/8/90	0.027	12.5
BSBC	Patrol	18/5/88	4/10/90	0.042	-
			16/11/90	0.038	4.0
B6	Patrol	14/7/89	4/10/90	0.035	56.2♦
			17/5/91	0.034	<0.1
B2	Patrol	14/7/89	17/10/90	0.035	7.4
			16/11/90	0.028	3.4♦
PSBK	Patrol	1/5/90	7/3/91	0.064	7.5
			1/5/91	0.066	-
B2	Bayfidan	14/7/89	14/3/90	0.065	5.5
			17/5/90	0.067	11.8♦

- = no standard error calculated ♦ = standard error for 1 value only

Table 2: EC_{50} values of two older Isolates

Isolate	Calixin	Av SE %	Mistral	Av SE %	Patrol	Av SE %
2023 (1973)	<0.082	-	<0.015	-	*	-
JB212 (1984)	0.075	7.7	0.024	-	0.011	0.3♦

- = no standard error calculated ♦ = standard error for 1 value only

Table 3: EC_{50} values of Isolates collected in 1988

Isolate	Calixin	Av SE %	Mistral	Av SE %	Patrol	Av SE %
BUSB22	*		*		0.087	1.4
CSB9	*		*		0.098	0.7
PK	*		<0.015	-	*	
BSB2:6	*		0.091	-	*	
BUSB2	*		0.118	-	*	
I9	0.031	-	0.007	-	0.071	1.6
BUSB18	0.033	27.5	*		0.226♦	42.3
CSB2:4	0.054	-	*		*	
I32	0.061	-	*		*	
BSB5	0.064	-	0.058	1.3	0.040	4.0
PB	0.070	12.7	0.061	8.5	0.108	22.1
CSB6	0.074	0.7	*		*	
L24	0.074	15.7	*		*	
MD	0.077	0.2	0.095	-	*	
CSB2:2	0.078	0.1	*		*	
CSB8	0.078	0.1	*		0.133	-
J	0.082	0.1	0.015	0.9	0.034	0.3♦
PF	0.086	-	*		*	
BUSB13	0.088	6.6♦	*		*	
L32	0.091	14.8	*		*	
BUSB21	0.094	9.9	0.119	1.0	*	
ME	0.102	32.3	0.085	1.6	*	
I2	0.105	0.3♦	*		*	
I21	0.111	0.3	<0.015	-	0.039	-
P8	0.127	11.5	0.014	8.0	0.003	-
CSB2	0.129	16.7	0.052	1.0	*	
P4	0.171	13.9	*		*	
CSB10	0.194	17.8	*		*	

- = no standard error calculated ♦ = standard error for 1 value only

Table 4: EC_{50} values of isolates collected in 1989

Isolate	Calixin	Av SE %	Mistral	Av SE %	Patrol	Av SE %
R9	*		0.010	2.8	*	
G1122	0.037	-	0.051	10.2	0.050	-
PERTH33	0.048	-	0.019	-	0.012	-
B	0.050	11.9	*		0.024	5.9
PGU1	0.053	4.9♦	0.014	2.0	0.008	-
B1	0.058	1.7	*		*	
B38	0.067	9.6	0.041	1.3	0.009	2.8
B3	0.070	-	0.018	5.7	*	
B26	0.072	1.8	*		*	
B30	0.079	1.4♦	0.039	11.7	0.037	32.4♦
PGU2	0.080	-	0.012	-	*	
B2	0.084	0.1♦	*		0.032	7.4
B48	0.090	-	<0.015	-	0.031	1.2
R6	0.093	1.9	0.031	0.1♦	0.044	-
P1122	0.096	2.5	0.013	2.8	0.017	-
B27	0.098	-	*		*	
R11	0.099	-	0.031	1.3♦	0.030	0.1
R41	0.100	0.2	0.014	12.8	<0.015	-
B21	0.101	0.2	0.031	3.3	0.032	36.7
B36	0.107	-	0.014	0.1♦	0.008	-
R17	0.109	3.7	0.017	2.3	0.018	0.1
R7	0.117	1.3♦	<0.015	-	0.027	2.6
B39	0.117	-	0.015	0	0.045	11.4♦
B32	0.120	7.1	0.035	1.7♦	0.038	1.4
B35	0.122	0.1	0.024	2.8	0.013	2.2
R19	0.124	9.0	0.025	0.2	0.019	<0.1
R15	0.140	4.2	0.026	2.8	0.040	5.9
PGU4	0.141	-	<0.015	-	0.017	1.1♦
R1	0.142	0.1	0.016	-	0.026	7.6
R14	0.161	-	<0.015	-	0.030	4.6♦
B6	0.165	-	<0.015	-	0.034	<0.1

- = no standard error calculated ♦ = standard error for 1 value only

Table 5: EC_{50} values of isolates collected in 1990

Isolate	Calixin	Av SE %	Mistral	Av SE %	Patrol	Av SE %
SBGS37	*		<0.015	-	0.162	-
GPBUSH	*		0.048	4.2	*	
GPUNT	*		0.048	-	*	
TRSBC	*		0.115	63.1♦	*	
PSBW	0.045	8.1	0.072	3.9♦	*	
PSBJ	0.057	12.6	0.044	3.5	0.090	-
90:6	0.064	-	0.016	0.5♦	*	
90:531	0.078	-	*		0.027	12.0
TRSBK	0.084	-	0.056	-	0.073	26.6♦
90:564	0.086	-	0.032	-	0.082	14.9
TRSBG	0.089	-	0.019	10.9	0.052	14.6
P11E	0.102	-	0.030	-	0.081	-
P11B	0.103	-	0.012		*	
CFHD	0.105	0.4	0.011	-	0.008	-
PGU3	0.107	6.3	0.020	0.4	0.048	-
BH90:32	0.120	<0.1♦	0.024	28.1	0.138	-
PSBM	0.125	6.8♦	*		*	
P11L	0.126	3.5	*		0.091	6.1
BH	0.126	-	0.008	-	*	
PSBK	0.135	-	0.032	-	0.139	7.5
WBMEL	0.150	2.2♦	0.011	-	0.015	-
P11A	0.173	-	0.026	-	<0.015	-
PSBG	0.212	23.4	*		0.162	-
TRSBL	0.232	6.2	*		*	

- = no standard error calculated ♦ = standard error for 1 value only

Table 6: EC_{50} values of isolates collected in 1991

Isolate	Calixin	Av SE %	Mistral	Av SE %	Patrol	Av SE %
TRFP6	*		0.118	11.3♦	*	
SHERPA	0.081	19.4	*		*	
CASK	0.090	0.2	0.054	1.2♦	*	
WBBOG	0.097	3.9♦	*		*	
GPUNT	0.108	30.2	0.073	-	*	
91:428	0.122	4.5♦	*		0.057	3.5♦
91:507	0.152	0.1♦	*		*	
BPLWBUNT	0.153	0.1	*		0.122	1.2
GOLFUNT	0.155	-	*		*	
21G	0.170	3.4♦	*		*	
16L	0.701✧	-	*		*	

- = no standard error calculated ♦ = standard error for 1 value only

✧Isolate 16L has not been re-tested. Although the test appeared valid, the very high nature of this result would require repetition for confirmation. This isolate was tested in the last test carried out in this project. This value has been omitted from the related Figures and the calculation of the annual median and mean for 1991.

Table 7: Annual median and mean EC_{50} values

Calixin

Year collected	Number of Isolates	Median EC_{50}	Mean EC_{50}	SE of Mean
1988	23	0.082	0.090	±0.0080
1989	30	0.098	0.099	±0.0063
1990	20	0.106	0.116	±0.0107
1991	9	0.140	0.125	±0.0110

Mistral

Year collected	Number of Isolates	Median EC_{50}	Mean EC_{50}	SE of Mean
1988	13	0.058	0.057	±0.0116
1989	26	0.016	0.022	±0.0021
1990	19	0.025	0.034	±0.0060
1991	3	-	0.082	±0.0190

Patrol

Year collected	Number of Isolates	Median EC_{50}	Mean EC_{50}	SE of Mean
1988	10	0.079	0.084	±0.0202
1989	25	0.027	0.026	±0.0025
1990	14	0.077	0.079	±0.0146
1991	2	-	0.090	±0.0325

Table 8: MIC values of two older isolates

Isolate		Calixin	Mistral	Patrol
2023	(1973)	0.082	0.014	*
JB212	(1984)	0.328	0.058	0.058

Table 9: MIC values of isolates collected in 1988

Isolate	Calixin	Mistral	Patrol
PK	*	*	0.015
BUSB5	*	*	0.117
BUSB22	*	*	0.234
CSB9	*	*	0.234
L32	*	*	0.468
CSB5	*	0.468	*
BSB2-6	*	0.468	*
PF	0.082	*	*
PGU4	0.102	*	*
BSBC	0.117	*	0.234
CSB4	0.164	*	*
CSB2-2	0.164	*	*
I2	0.164	*	*
I9	0.164	0.029	0.029
CSB8	0.164	0.117	0.234
BUSB13	0.328	*	*
CSB2-4	0.328	*	*
I21	0.328	0.015	*
MD	0.328	0.117	*
CSB2	0.656	*	*
ME	0.656	*	*
CSB6	0.656	*	*
CSB10	0.656	*	*
L32	0.656	*	*
P4	0.656	*	0.117
L24	0.656	*	0.468
P8	0.656	0.058	0.015
J	0.656	0.117	0.117
BUSB21	0.656	0.468	*
PB	0.656	0.468	0.234
BUSB18	0.656	0.937	0.468

Table 10: MIC values of isolates collected in 1989

Isolate	Calixin	Mistral	Patrol
R7	*	0.014	*
R15	*	0.058	*
R9	*	0.117	*
R41	*	0.117	0.015
PGU1	*	0.117	0.058
B30	*	0.234	*
R10	0.117	*	*
B1	0.164	*	*
B3	0.164	0.058	0.012
PERTH33	0.164	0.117	<0.029
B39	0.234	0.029	0.015
B36	0.234	0.029	0.029
B35	0.234	0.117	0.058
PGU4	0.328	0.014	0.058
B6	0.328	0.014	0.117
R7	0.328	0.014	0.117
PGU2	0.328	0.029	*
R1	0.328	0.058	*
R19	0.328	0.117	0.117
B30	0.328	0.234	0.234
P1122	0.468	0.029	0.117
R6	0.468	0.054	0.054
B27	0.656	*	*
B26	0.656	*	*
B2	0.656	*	0.117
B	0.656	*	0.117
B48	0.656	0.014	0.117
R14	0.656	0.029	*
R11	0.656	0.058	0.117
B38	0.656	0.117	0.058
R17	0.656	0.117	0.117
B32	0.656	0.117	0.234
B21	0.656	0.234	*
G1122	0.937	0.234	0.234
R15	1.312	0.058	*

Table 11: MIC values of isolates collected in 1990

Isolate	Calixin	Mistral	Patrol
SBGS37	*	0.014	*
B90.6	*	0.058	0.117
GPBSH90	*	0.234	*
TRSBC	*	>0.234	*
TRSBK	*	>0.234	0.234
90:564	0.164	0.058	0.234
TRSBG	0.164	0.234	0.234
P11L	0.328	*	*
P11A	0.328	0.058	0.058
WBMEL	0.328	0.058	0.117
BH90:32	0.328	0.234	>0.234
PSBW	0.328	0.234	>0.234
PSBJ	0.328	0.234	>0.234
PSBM	0.656	*	*
P11B	0.656	0.029	*
FHD	0.656	0.029	0.015
PGU3	0.656	0.058	0.117
P11E	0.656	0.058	0.234
PSBK	0.656	0.234	>0.234
TRSBL	1.312	*	*
PSBG	1.312	*	>0.234
BH90	1.312	0.029	*

Table 12: MIC values of isolates collected in 1991

Isolate	Calixin	Mistral	Patrol
GOLBOG	*	0.058	*
PL6	*	0.234	*
SHERPA	0.328	*	*
91:507	0.328	*	*
91:428	0.328	*	>0.234
CASK	0.328	0.117	*
GPUNT	0.328	0.234	*
GOLFUNT	0.656	*	*
21G	0.656	*	*
WBBOG	0.656	*	*
BPLWBUNT	0.656	*	0.234
16L	1.312	*	*

Table 13: Annual distributions of MIC values

Calixin

	1988		1989		1990		1991		Overall	
MIC	number of isolates	%	number of isolates	%	number of isolates	%	number of isolates	%	number of isolates	%
<0.164	3	12	1	3	0	-	0	-	4	5
0.164	5	21	3	10	2	12	0	-	10	12
0.328	4	17	10	35	6	35	5	50	25	31
0.656	12	50	13	45	6	35	4	40	35	44
1.312	0	-	2	7	3	18	1	10	6	8
TOTAL	24	100	29	100	17	100	10	100	80	100

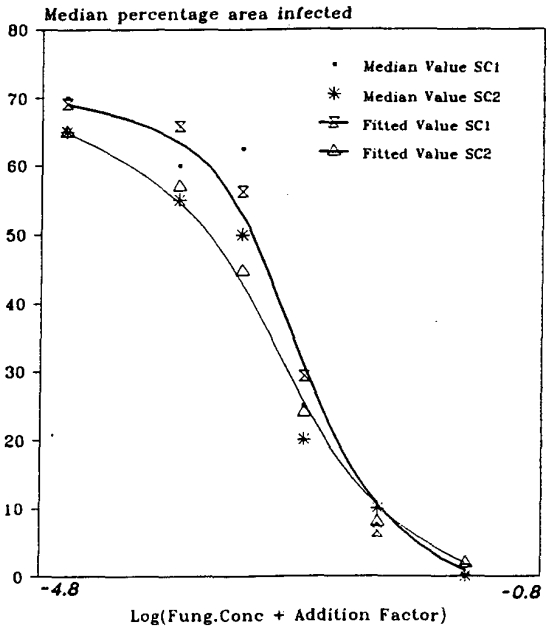
Mistral

	1988		1989		1990		1991		Overall	
MIC	number of isolates	%	number of isolates	%	number of isolates	%	number of isolates	%	number of isolates	%
0.014	0	-	5	17	1	6	0	-	6	10
0.029	2	18	5	17	3	17	0	-	10	16
0.058	1	9	6	21	6	33	1	25	14	22
0.117	3	27	9	31	6	33	1	25	19	31
0.234	0	-	4	14	0	-	2	50	6	10
>0.234	5	46	0	-	2	11	0	-	7	11
TOTAL	11	100	29	100	18	100	4	100	62	100

Patrol

	1988		1989		1990		1991		Overall	
MIC	number of isolates	%	number of isolates	%	number of isolates	%	number of isolates	%	number of isolates	%
0.015	2	14	4	18	1	7	0	-	7	13
0.029	1	7	1	4	0	-	0	-	2	4
0.058	0	-	5	23	1	7	0	-	6	12
0.117	3	21.5	9	41	3	21	0	-	15	29
0.234	5	36	3	14	4	29	1	50	13	25
>0.234	3	21.5	0	-	5	36	1	50	9	17
TOTAL	14	100	22	100	14	100	2	100	52	100

Figure 1: Reaction of isolate GP Bush 1990 to five concentrations of Mistral



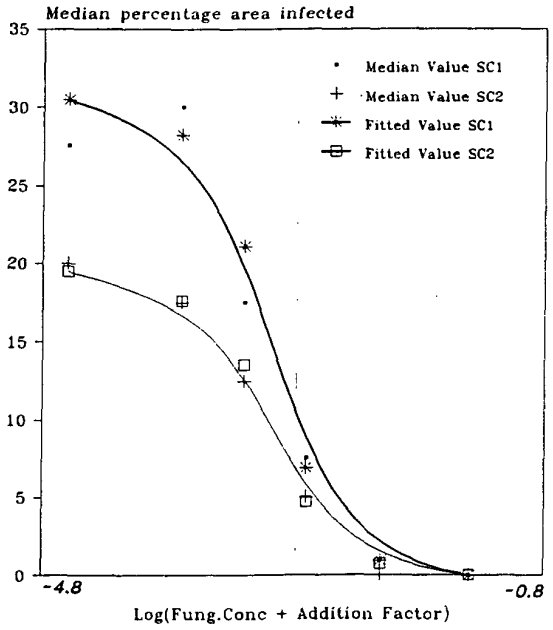
Concentration	0.0	0.015	0.029	0.058	0.117	0.234
Log concentration	-4.6	-3.7	-3.2	-2.7	-2.1	-1.4
Fitted Value SC1	69.1	65.8	56.2	29.3	6.5	0.9
Median % SC1	70.0	60.0	62.5	25.0	7.5	0.0
Fitted Value SC2	64.9	56.9	44.7	24.0	7.9	1.9
Median % SC2	65.0	55.0	50.0	20.0	10.0	0.0

EC_{50} Spray cabinet 1 = 0.052, % Standard error 3.7

EC_{50} Spray cabinet 2 = 0.044, % Standard error 4.6

Mean EC_{50} = 0.048 Geometric mean EC_{50} = 0.048

Figure 2: Reaction of isolate BSBC to five concentrations of Patrol



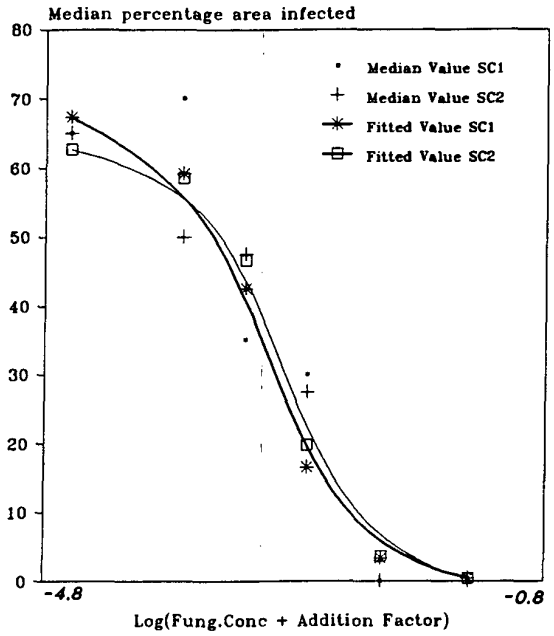
Concentration	0.0	0.015	0.029	0.058	0.117	0.234
Log concentration	-4.6	-3.7	-3.2	-2.7	-2.1	-1.4
Fitted Value SC1	30.5	28.2	21.1	6.9	0.9	0.0
Median % SC1	27.5	30.0	17.5	7.5	1.0	0.0
Fitted Value SC2	19.5	17.6	13.0	4.7	0.7	0.0
Median % SC2	20.0	17.5	12.5	5.0	0.0	0.0

EC_{50} Spray cabinet 1 = 0.039, % Standard error 5.0

EC_{50} Spray cabinet 2 = 0.038, % Standard error 2.9

Mean EC_{50} = 0.038 Geometric mean EC_{50} = 0.038

Figure 3: Reaction of isolate R15 to five concentrations of Patrol



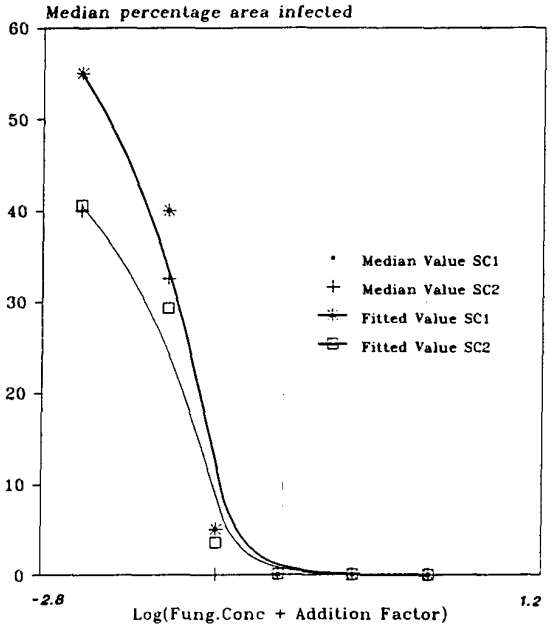
Concentration	0.0	0.015	0.029	0.058	0.117	0.234
Log concentration	-4.6	-3.7	-3.2	-2.7	-2.1	-1.4
Fitted Value SC1	67.3	59.2	42.6	16.5	3.3	0.5
Median % SC1	65.0	70.0	35.0	30.0	0.0	0.0
Fitted Value SC2	62.7	58.5	46.6	19.8	3.6	0.4
Median % SC2	65.0	50.0	47.5	27.5	0.0	0.0

EC_{50} Spray cabinet 1 = 0.037, % Standard error 6.9

EC_{50} Spray cabinet 2 = 0.044, % Standard error 4.9

Mean EC_{50} = 0.040 Geometric mean EC_{50} = 0.040

Figure 4: Reaction of isolate PGU4 to five concentrations of Calixin



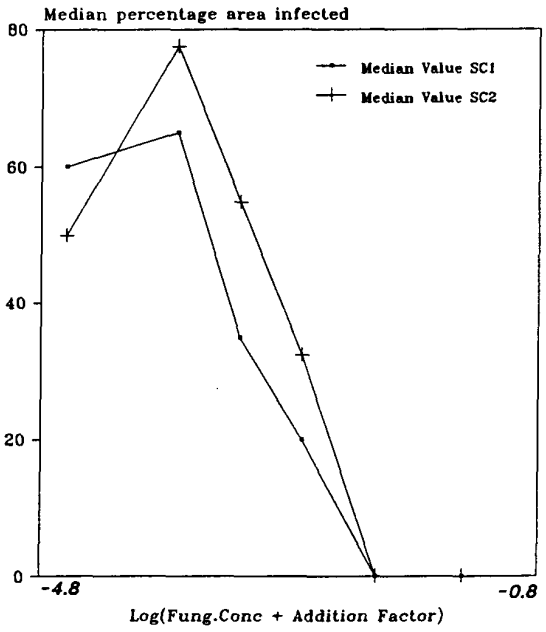
Concentration	0.0	0.082	0.164	0.328	0.656	1.312
Log concentration	-2.5	-1.8	-1.4	-0.9	-0.3	0.3
Fitted Value SC1	55.0	40.0	5.0	0.1	0.0	0.0
Median % SC1	55.0	40.0	5.0	0.0	0.0	0.0
Fitted Value SC2	40.6	29.2	3.5	0.1	0.0	0.0
Median % SC2	40.0	32.5	0.0	0.0	0.0	0.0

EC_{50} Spray cabinet 1 = 0.103, % Standard error 0.1

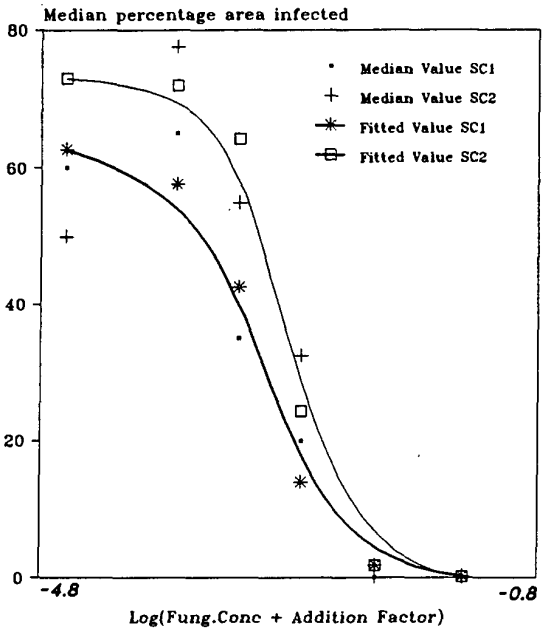
EC_{50} Spray cabinet 2 = 0.102, % Standard error 0.5

Mean EC_{50} = 0.102 Geometric mean EC_{50} = 0.102

Figure 5: Reaction of isolate R6 to five concentrations of Patrol



Observed values note peak at log concentration -3.7



Fitted curves with control "unfixed"

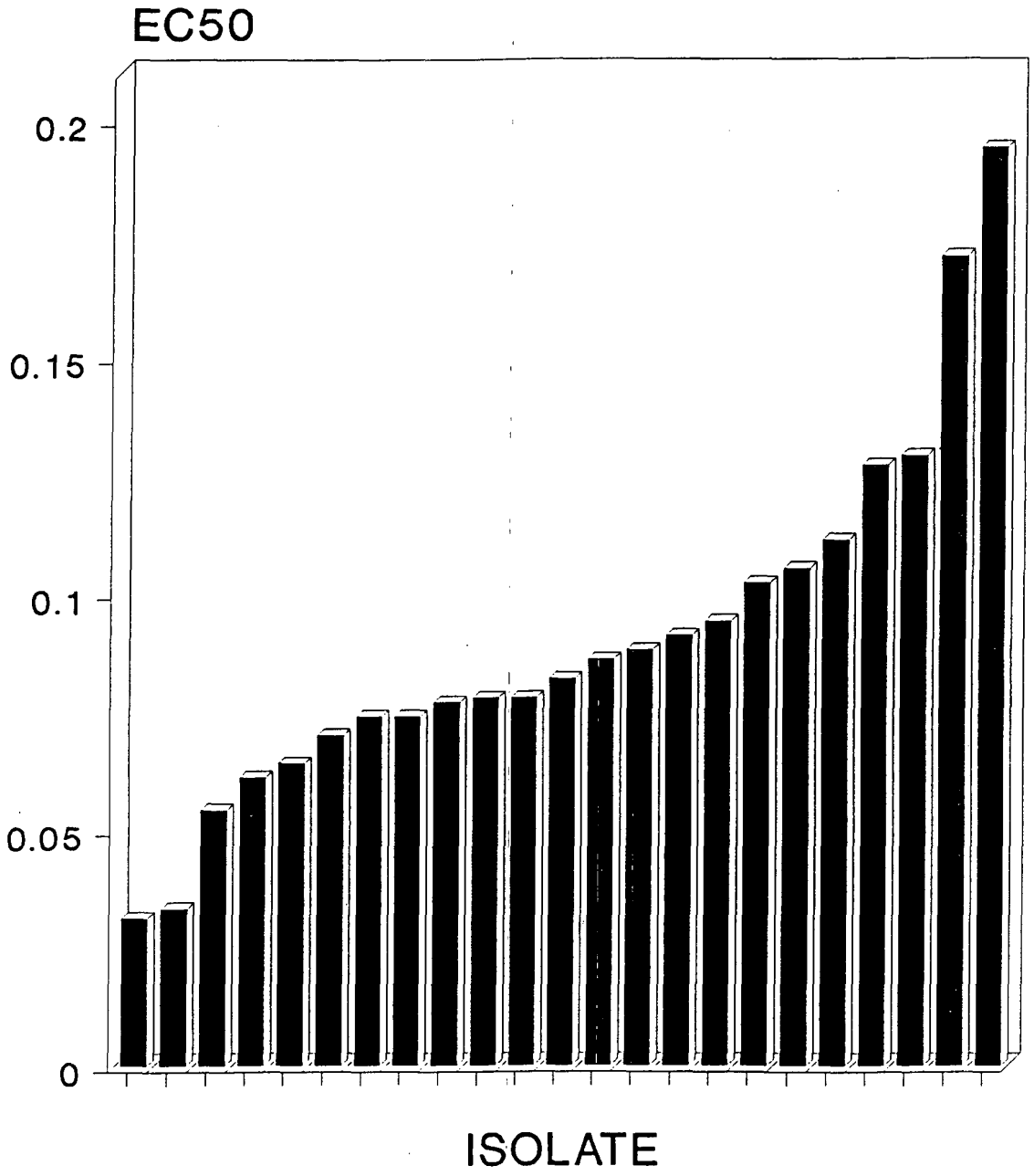
Concentration	0.0	0.015	0.029	0.058	0.117	0.234
Log concentration	-4.6	-3.7	-3.2	-2.7	-2.1	-1.4
Fitted Value SC1	62.7	57.7	42.6	14.0	1.9	0.2
Median % SC1	**60.0**	**65.0**	**35.0**	**20.0**	**0.0**	**0.0**
Fitted Value SC2	72.9	71.9	64.2	24.3	1.8	0.1
Median % SC2	**50.0**	**77.5**	**55.0**	**32.5**	**0.0**	**0.0**

EC_{50} Spray cabinet 1 = 0.038, % Standard error 4.8

EC_{50} Spray cabinet 2 = 0.049, % Standard error 9.5

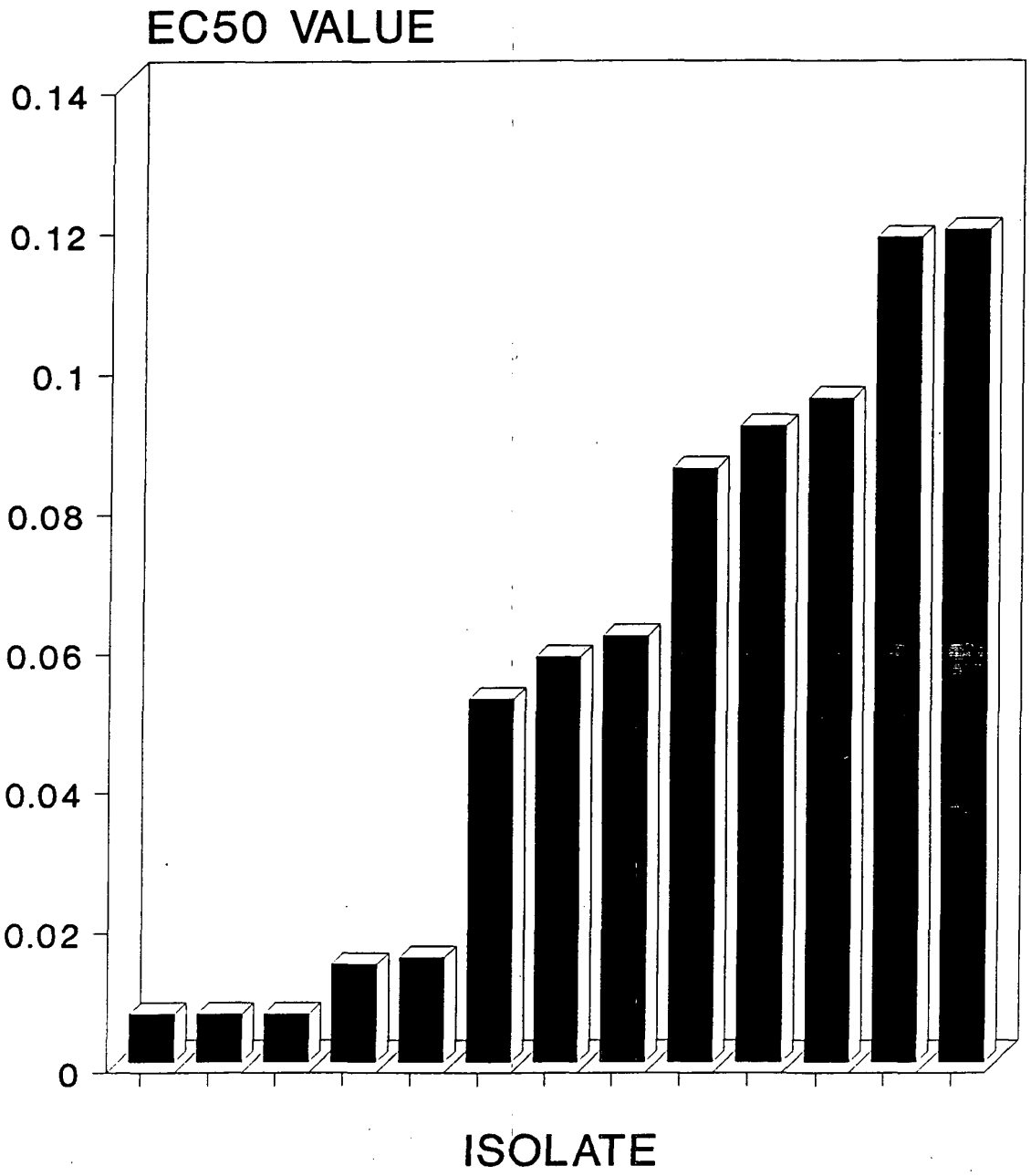
Mean EC_{50} = 0.044 Geometric mean EC_{50} = 0.044

Figure 6: Sensitivity of 1988 isolates to Calixin



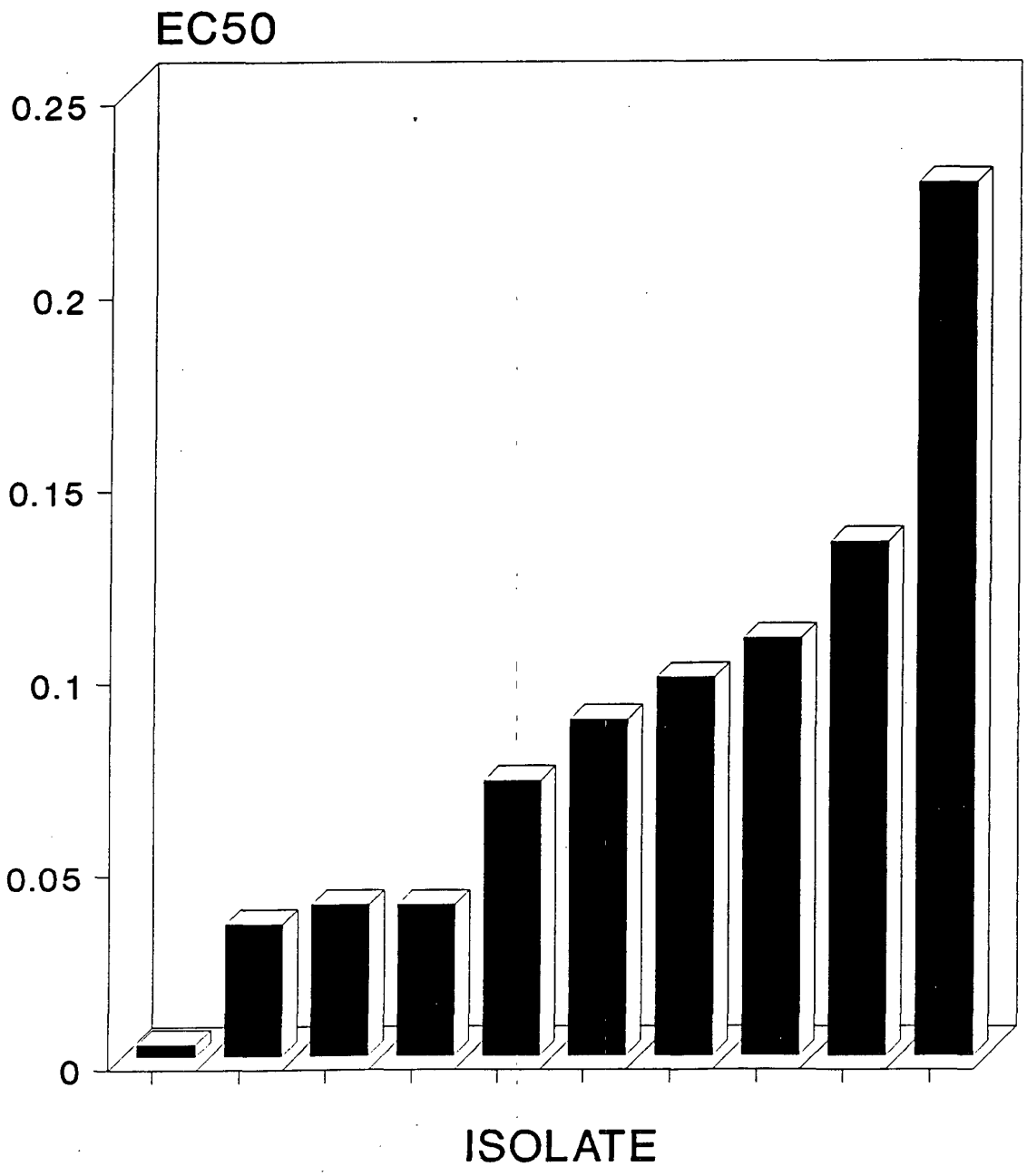
Isolates arranged in order of decreasing sensitivity

Figure 7: Sensitivity of 1988 isolates to Mistral



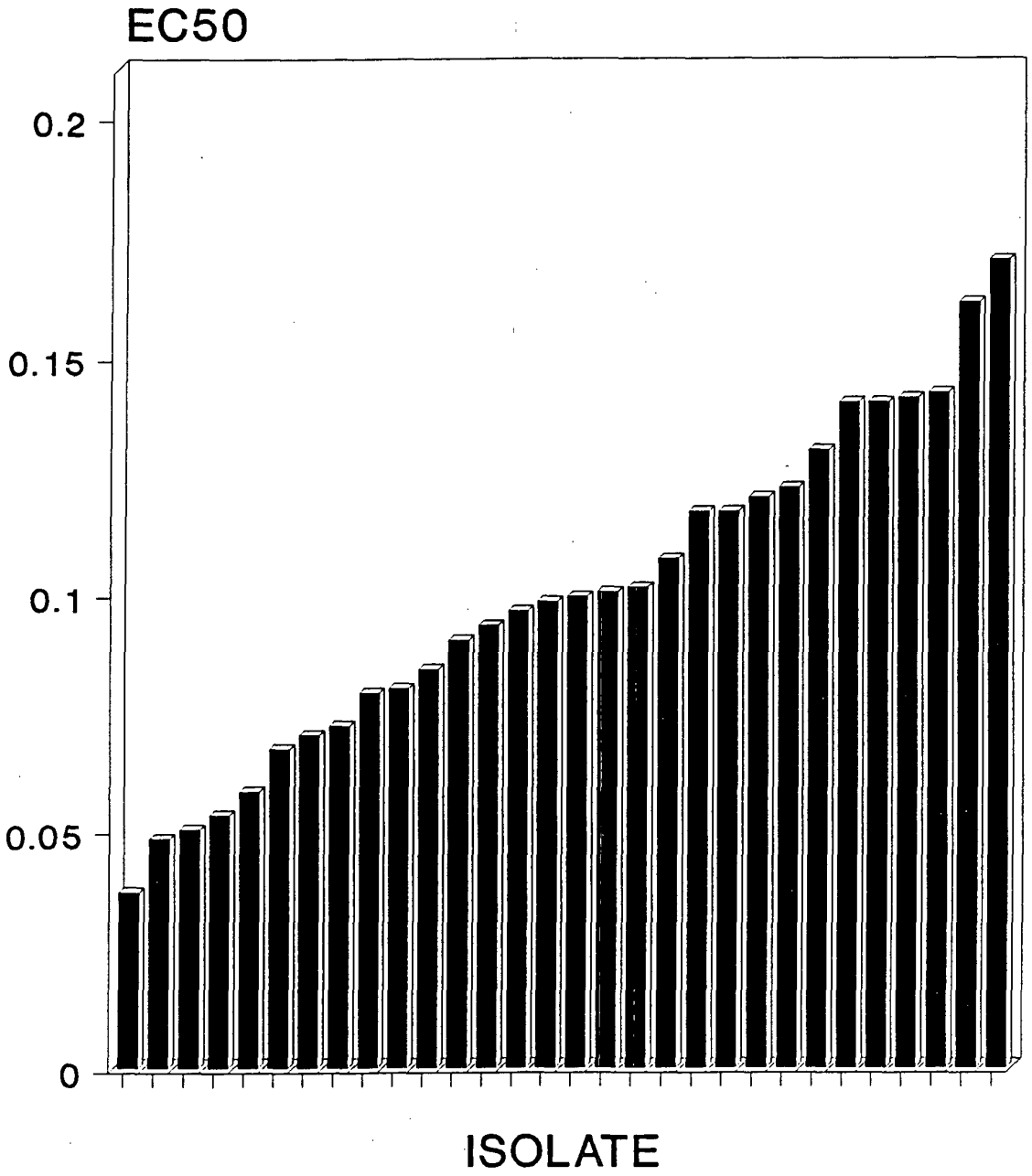
Isolates arranged in order of decreasing sensitivity

Figure 8: Sensitivity of 1988 isolates to Patrol



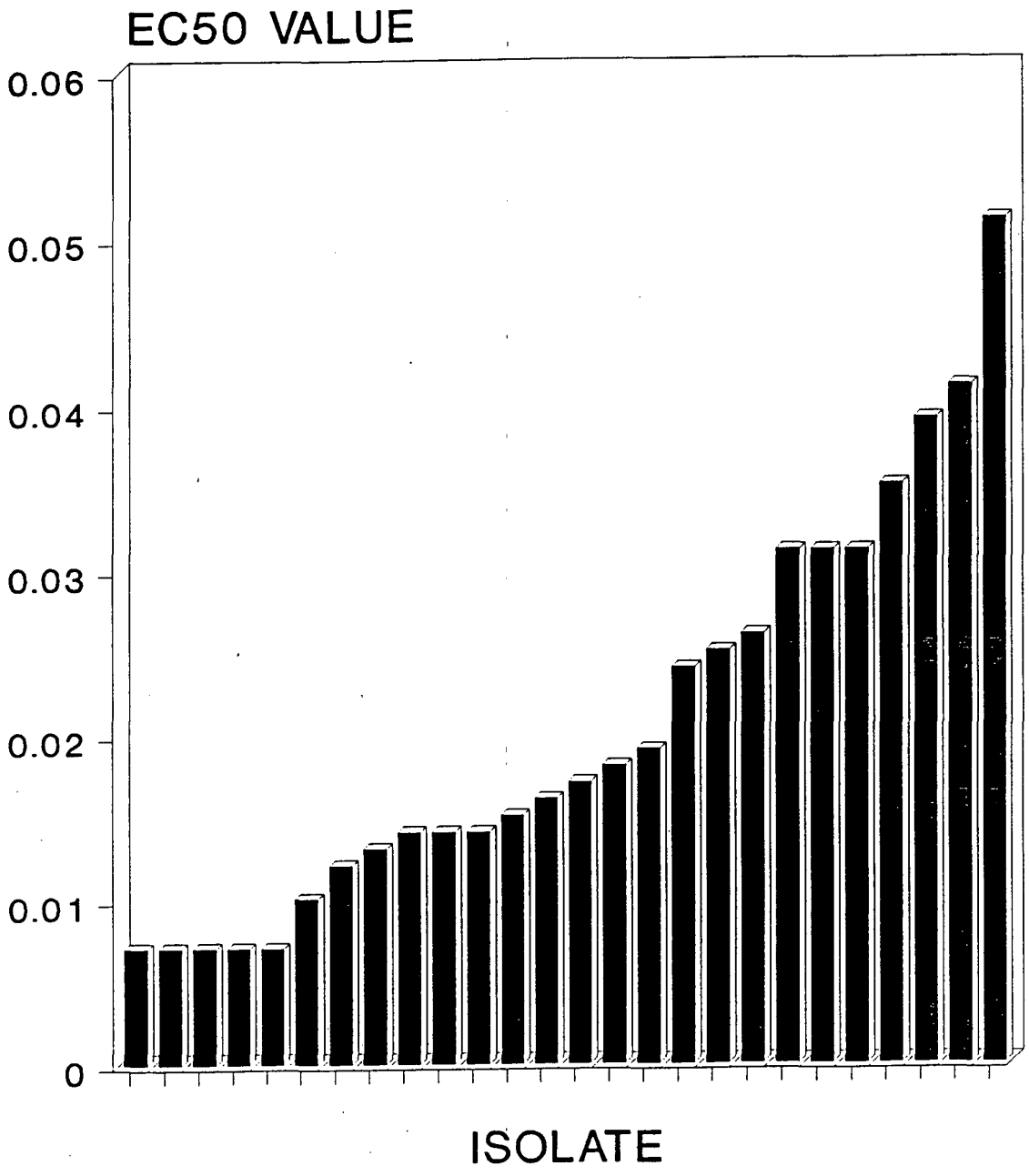
Isolates arranged in order of decreasing sensitivity

Figure 9: Sensitivity of 1989 isolates to Calixin



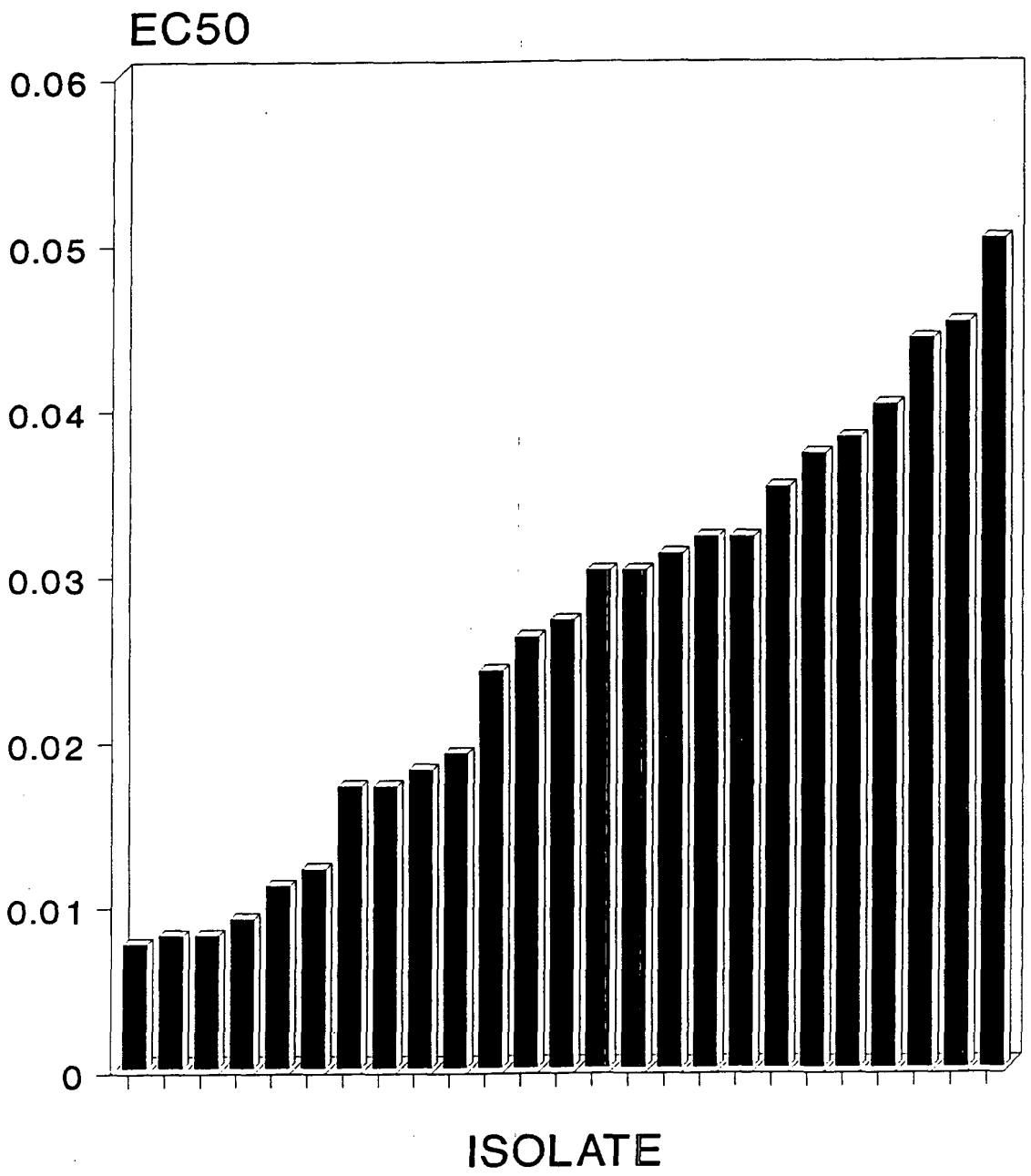
Isolates arranged in order of decreasing sensitivity

Figure 10: Sensitivity of 1989 isolates to Mistral



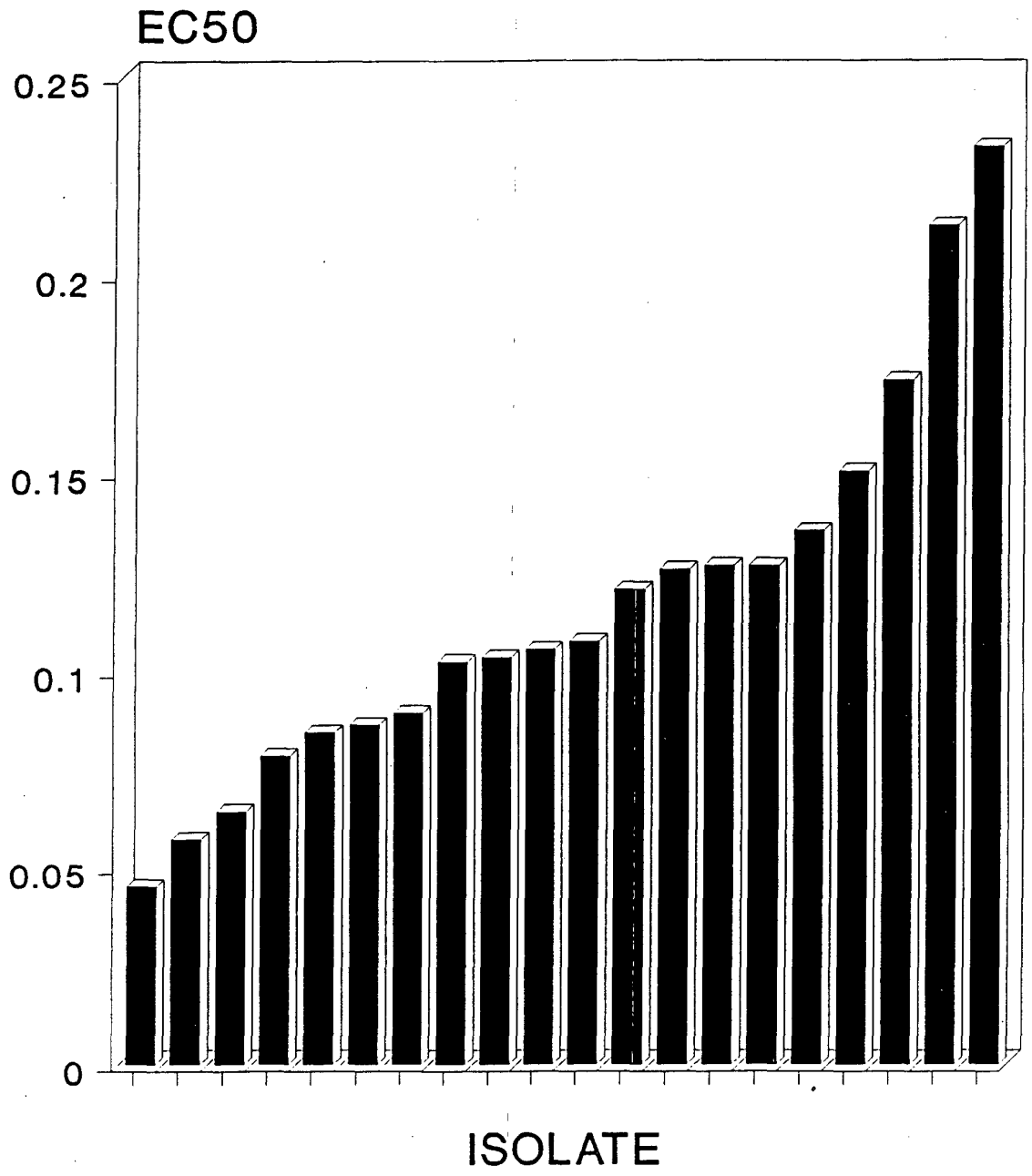
Isolates arranged in order of decreasing sensitivity

Figure 11: Sensitivity of 1989 isolates to Patrol



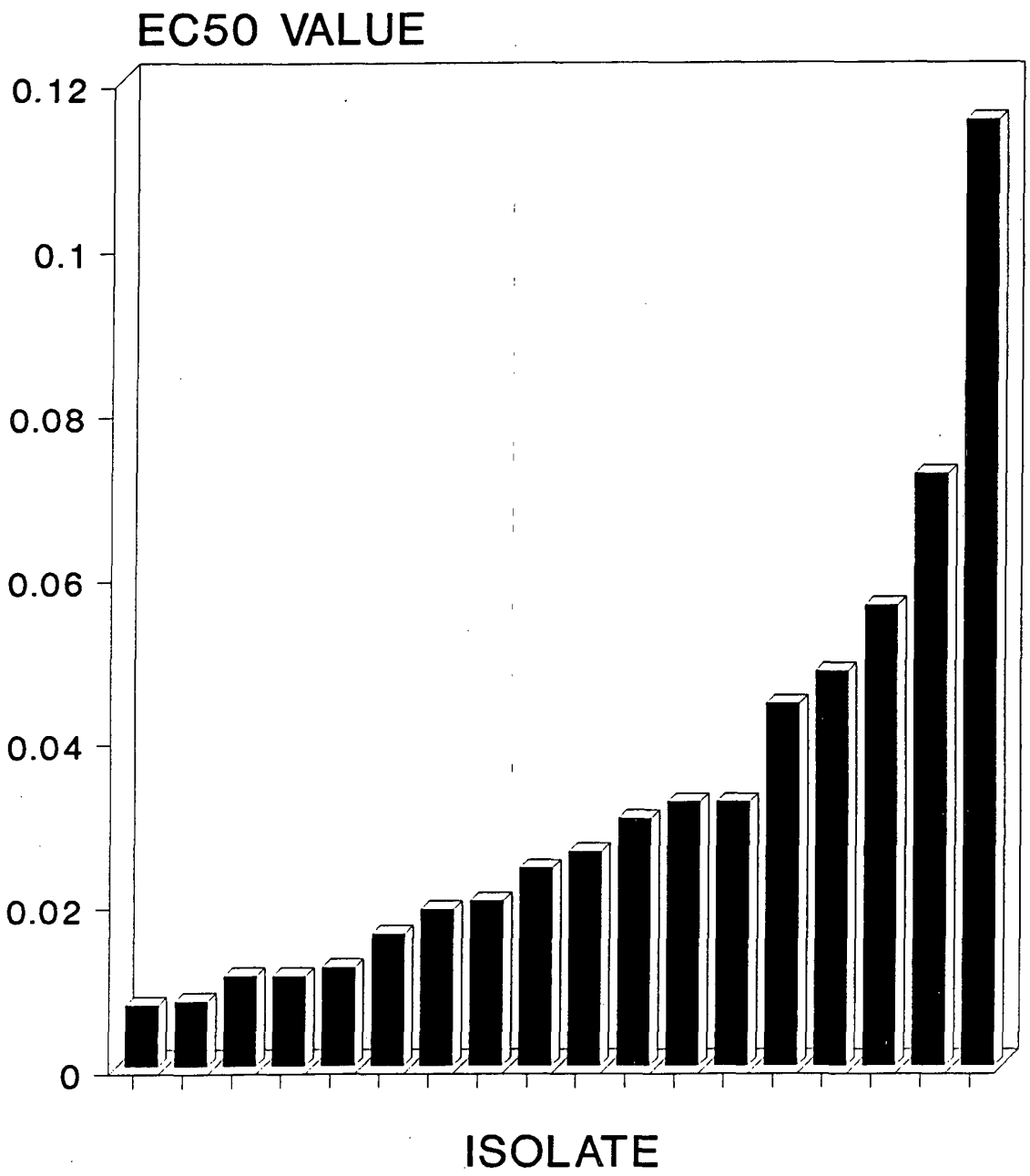
Isolates arranged in order of decreasing sensitivity

Figure 12: Sensitivity of 1990 isolates to Calixin



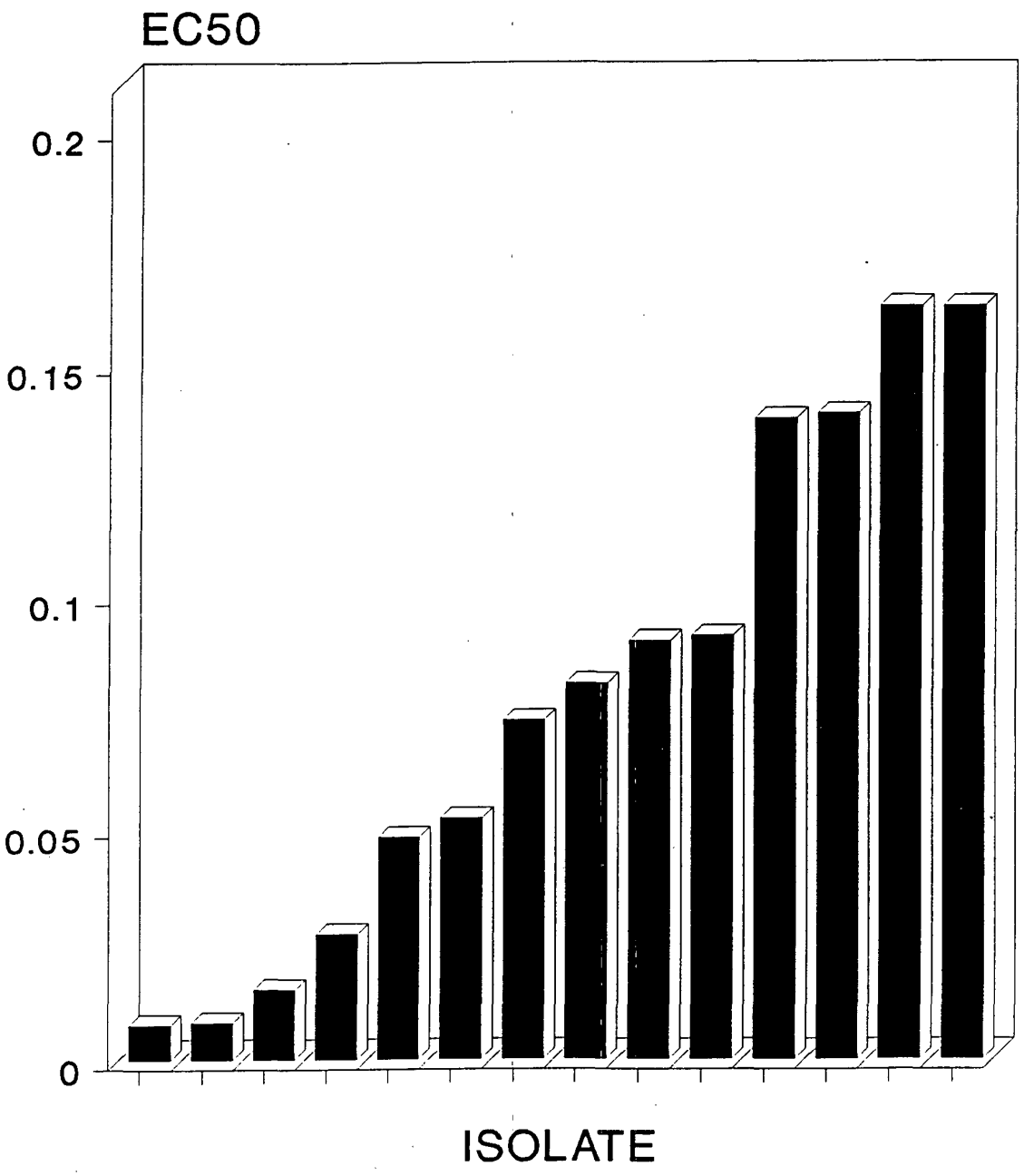
Isolates arranged in order of decreasing sensitivity

Figure 13: Sensitivity of 1990 isolates to Mistral



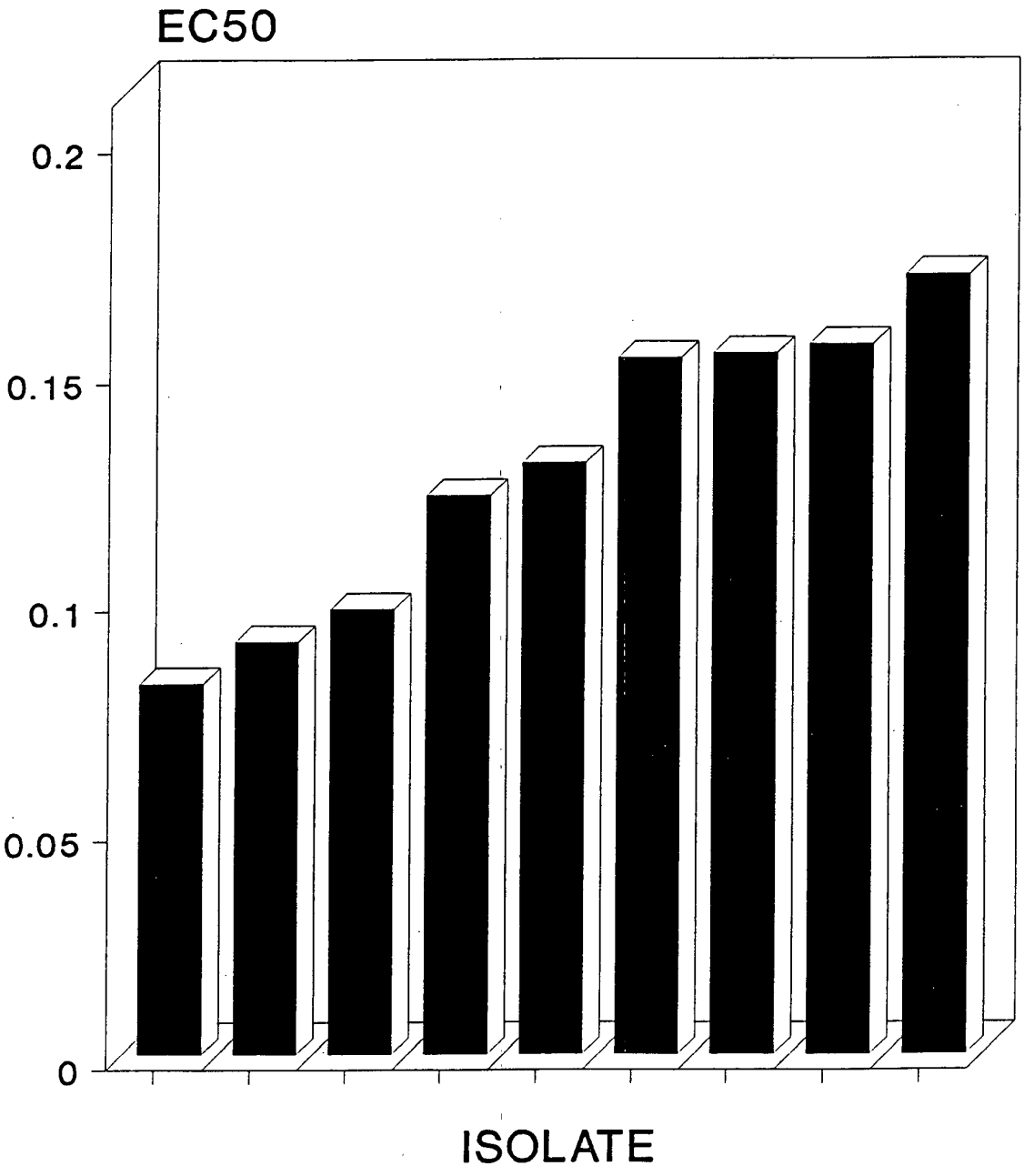
Isolates arranged in order of decreasing sensitivity

Figure 14: Sensitivity of 1990 isolates to Patrol



Isolates arranged in order of decreasing sensitivity

Figure 15: Sensitivity of 1991 isolates to Calixin



Isolates arranged in order of decreasing sensitivity

Figure 16: Annual mean sensitivity of isolates to Calixin

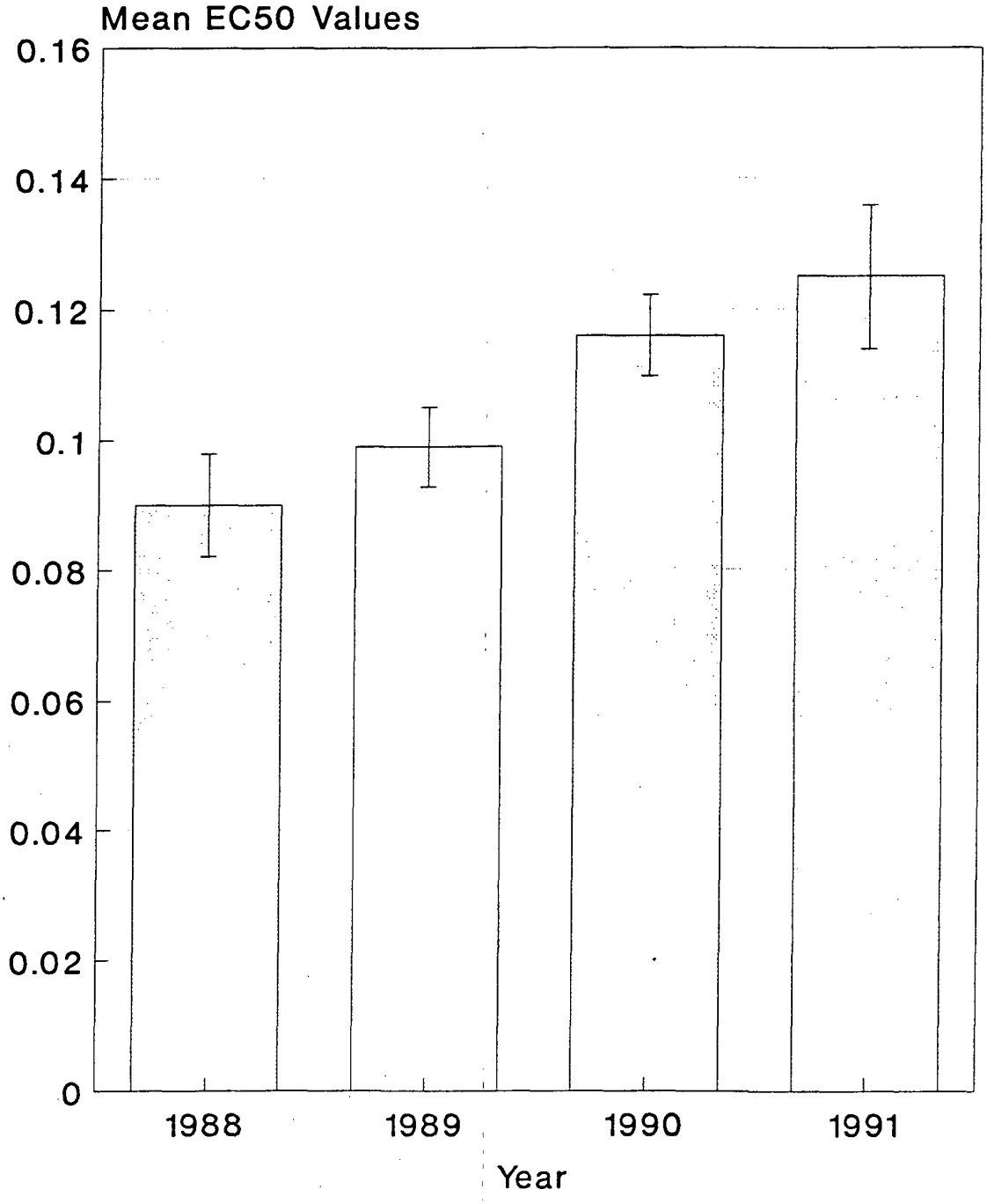


Figure 17: Annual mean sensitivity of isolates to Mistral

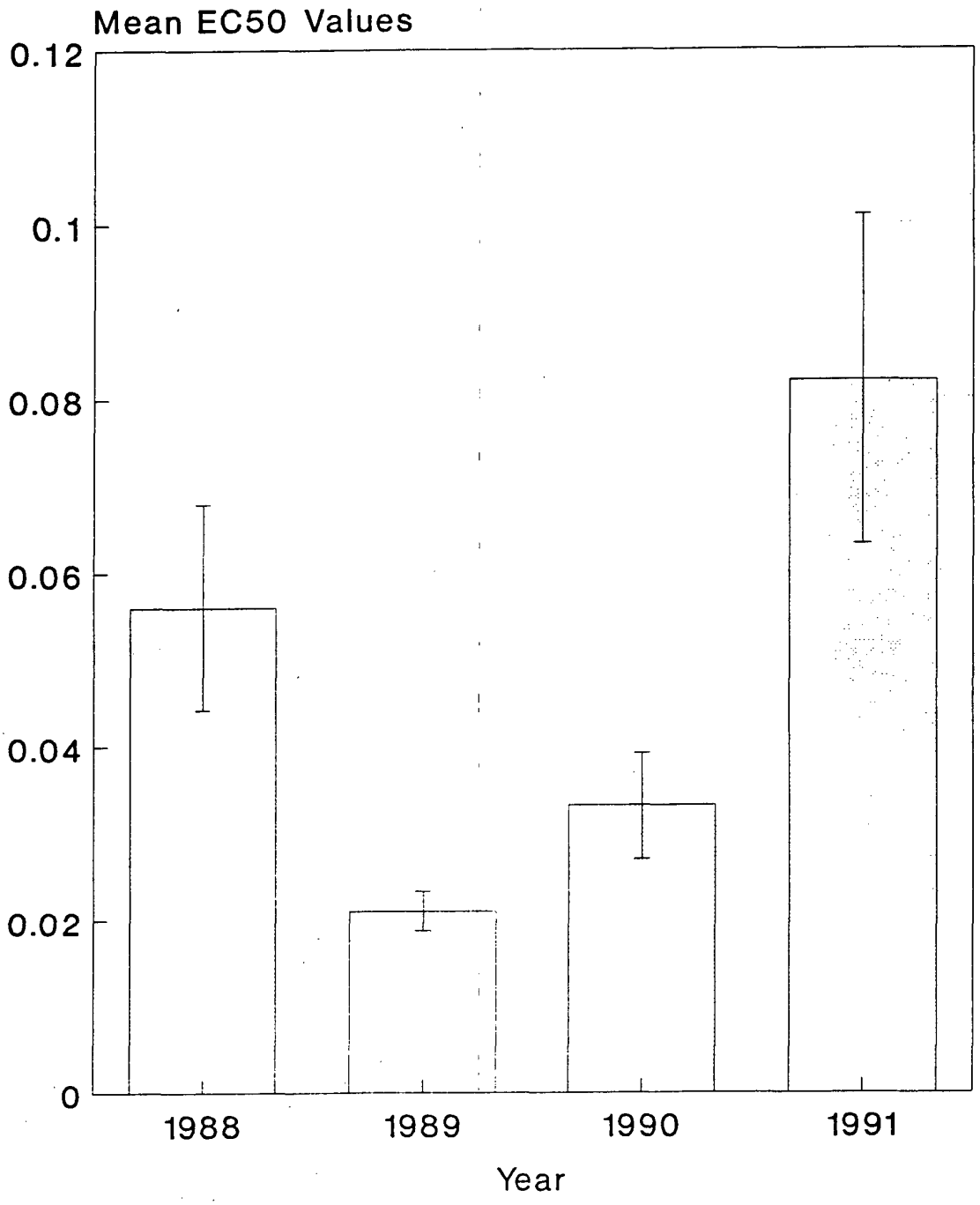


Figure 18: Annual mean sensitivity of isolates to Patrol

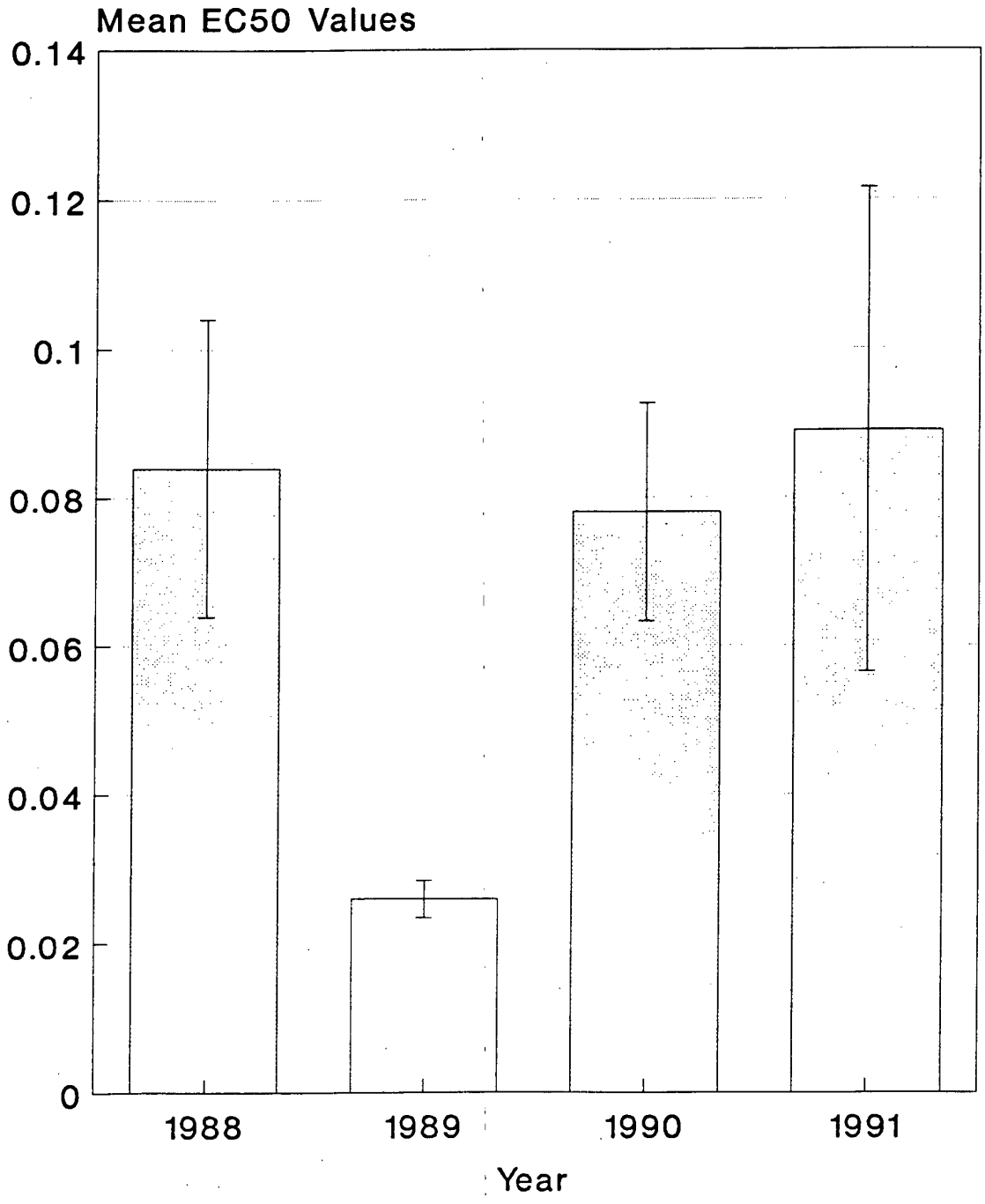


Figure 19: Distribution of sensitivity of isolates to Calixin

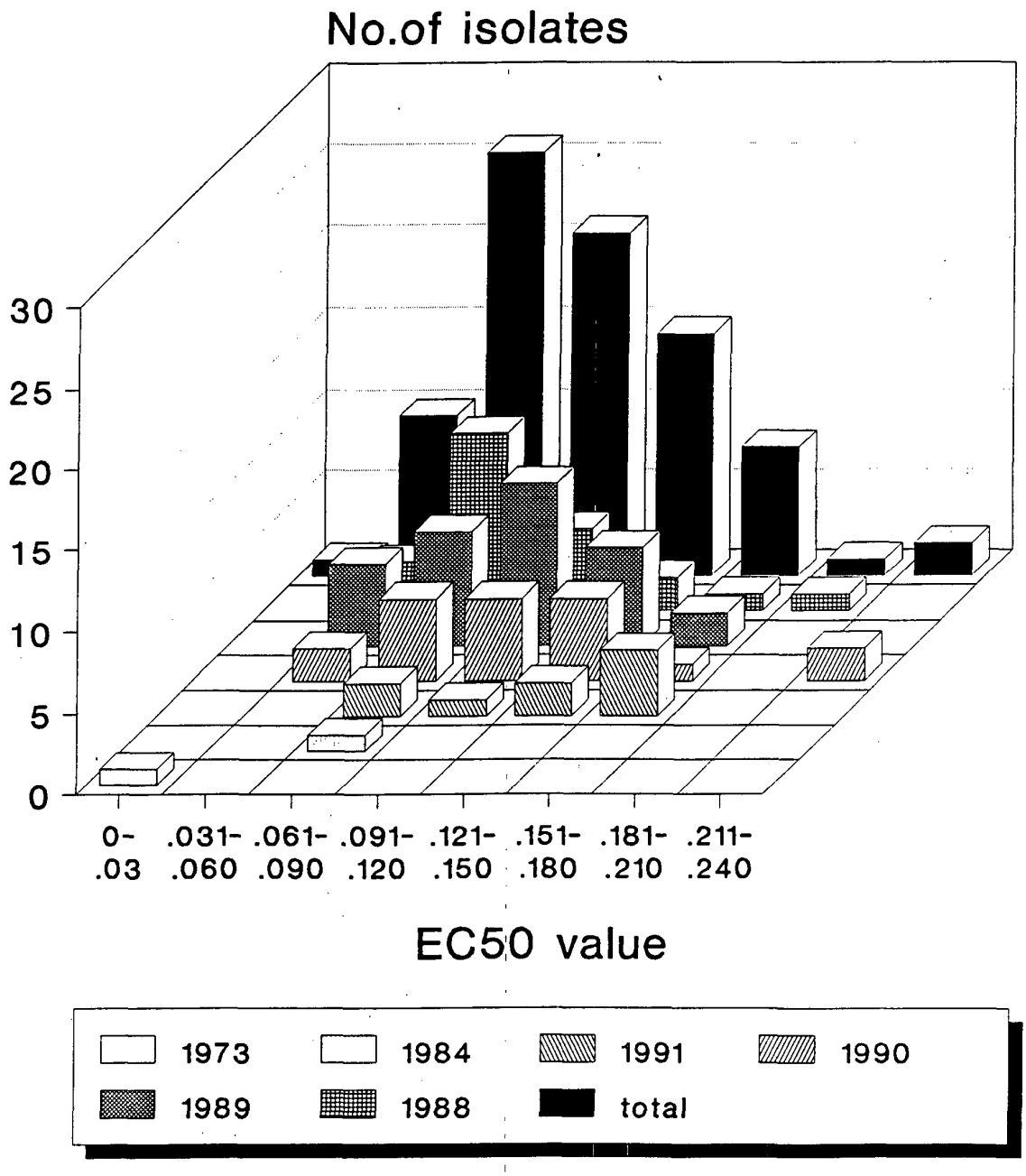


Figure 20: Distribution of sensitivity of isolates to Mistral

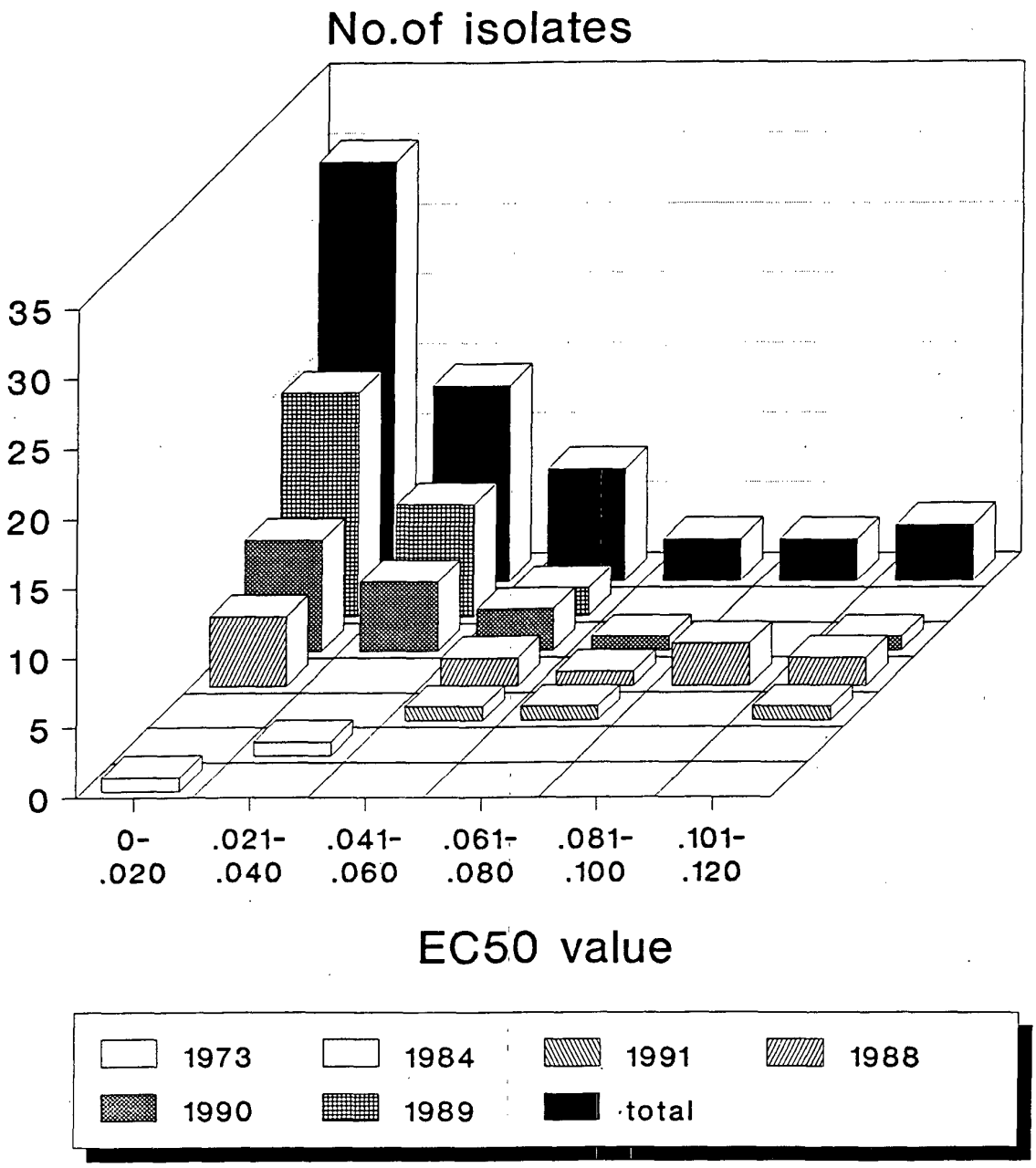
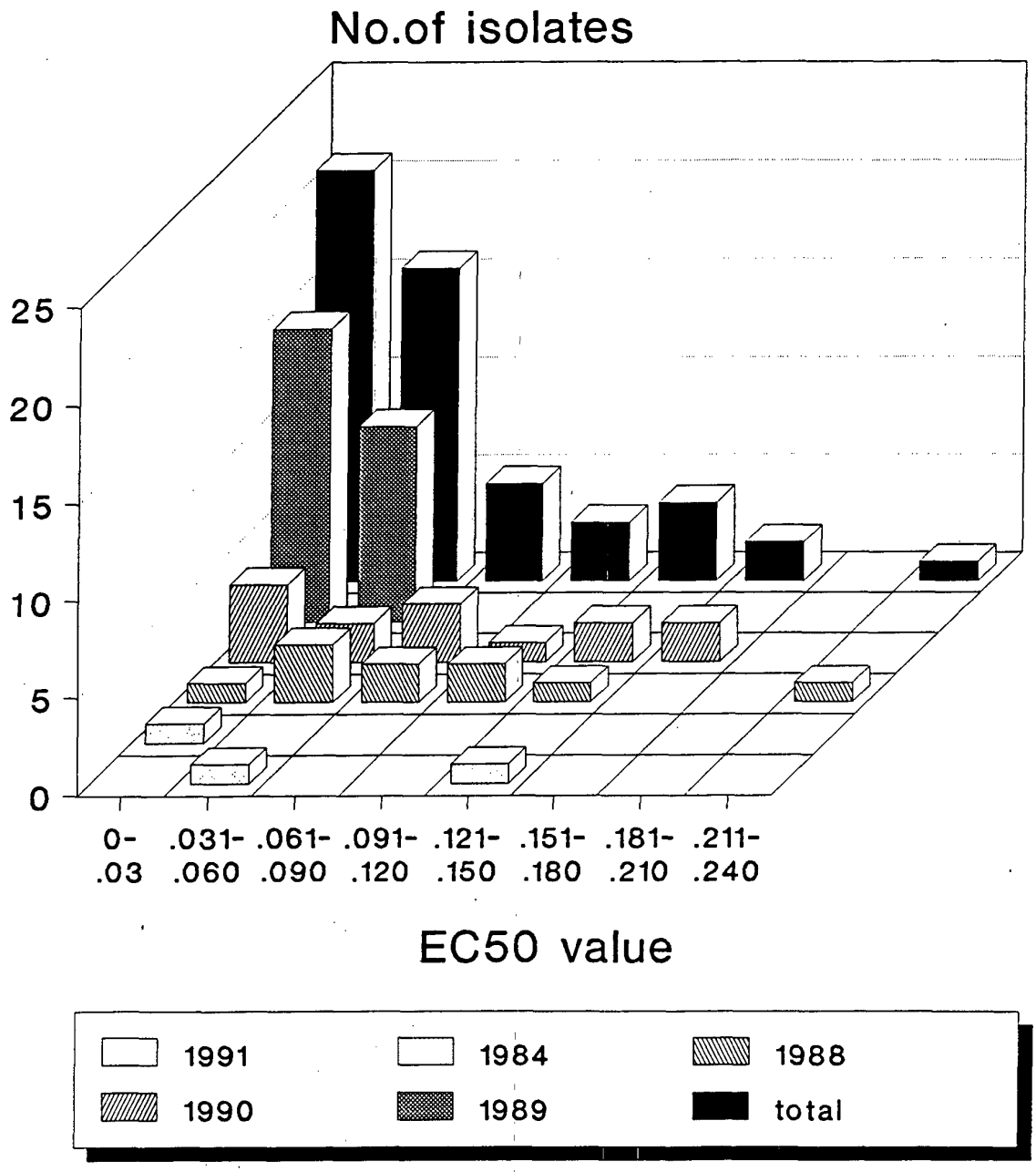


Figure 21: Distribution of sensitivity of isolates to Patrol



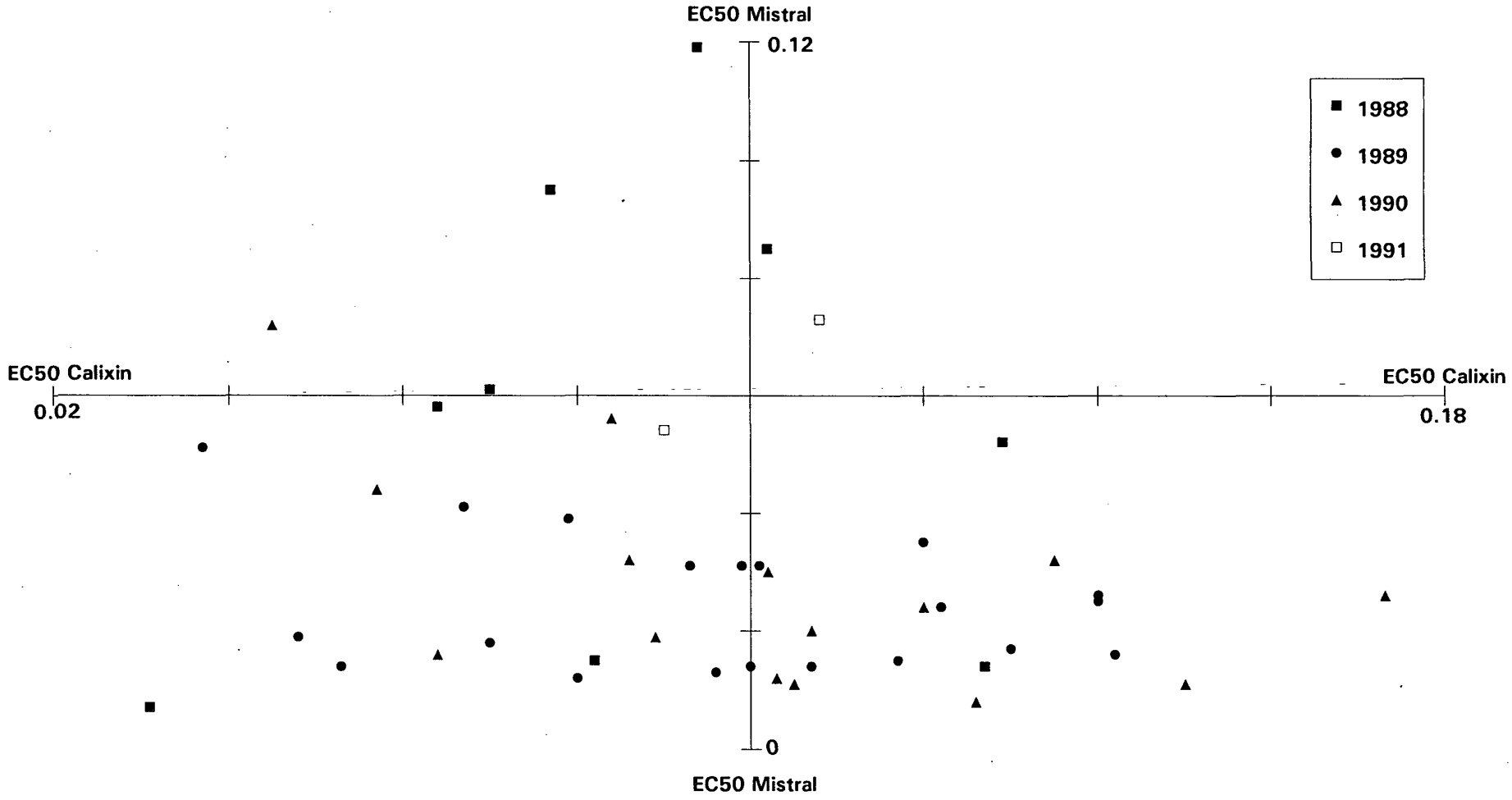


Figure 22: Sensitivity of Isolates to Calixin and Mistral

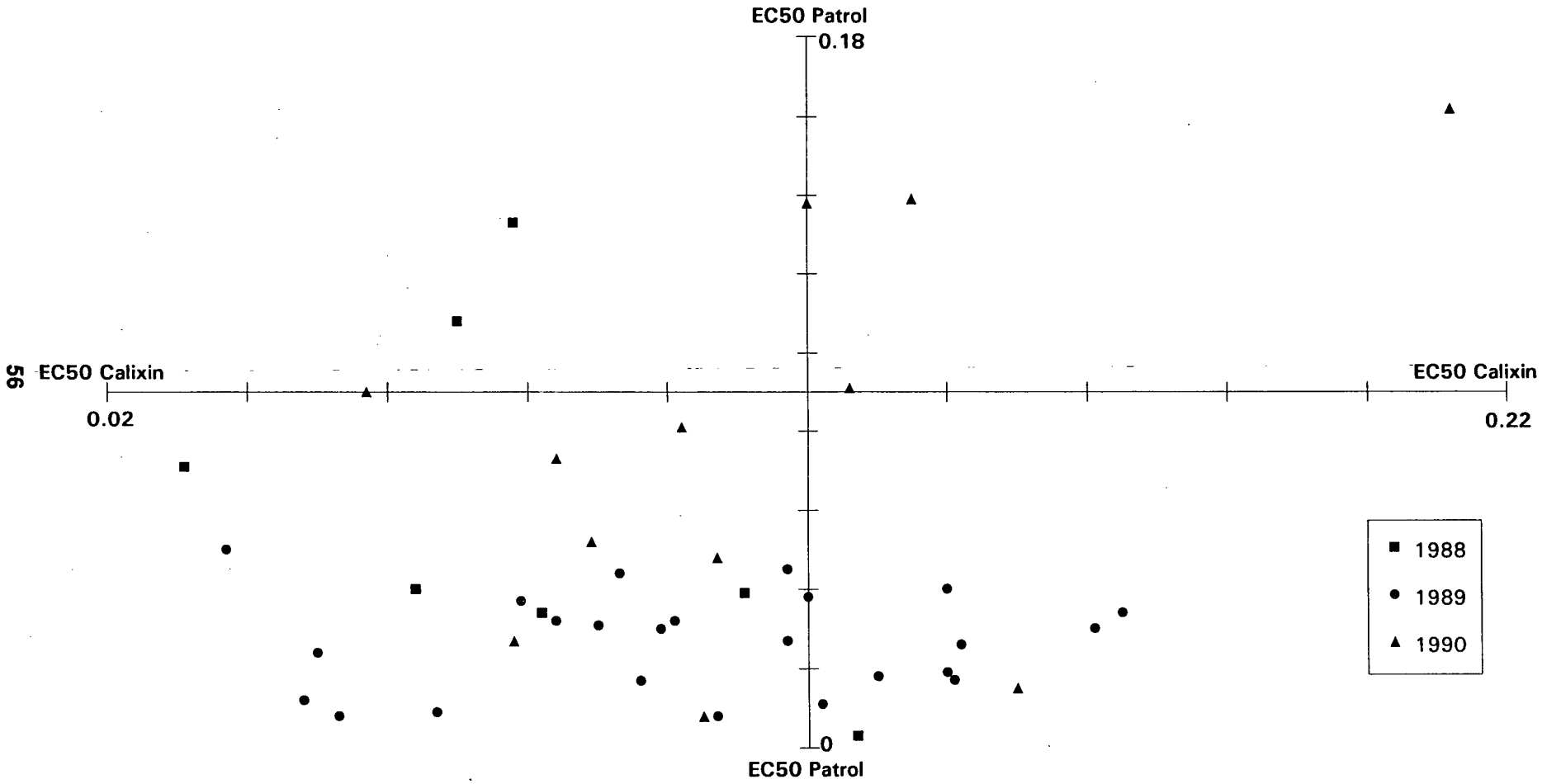


Figure 23: Sensitivity of Isolates to Calixin and Patrol

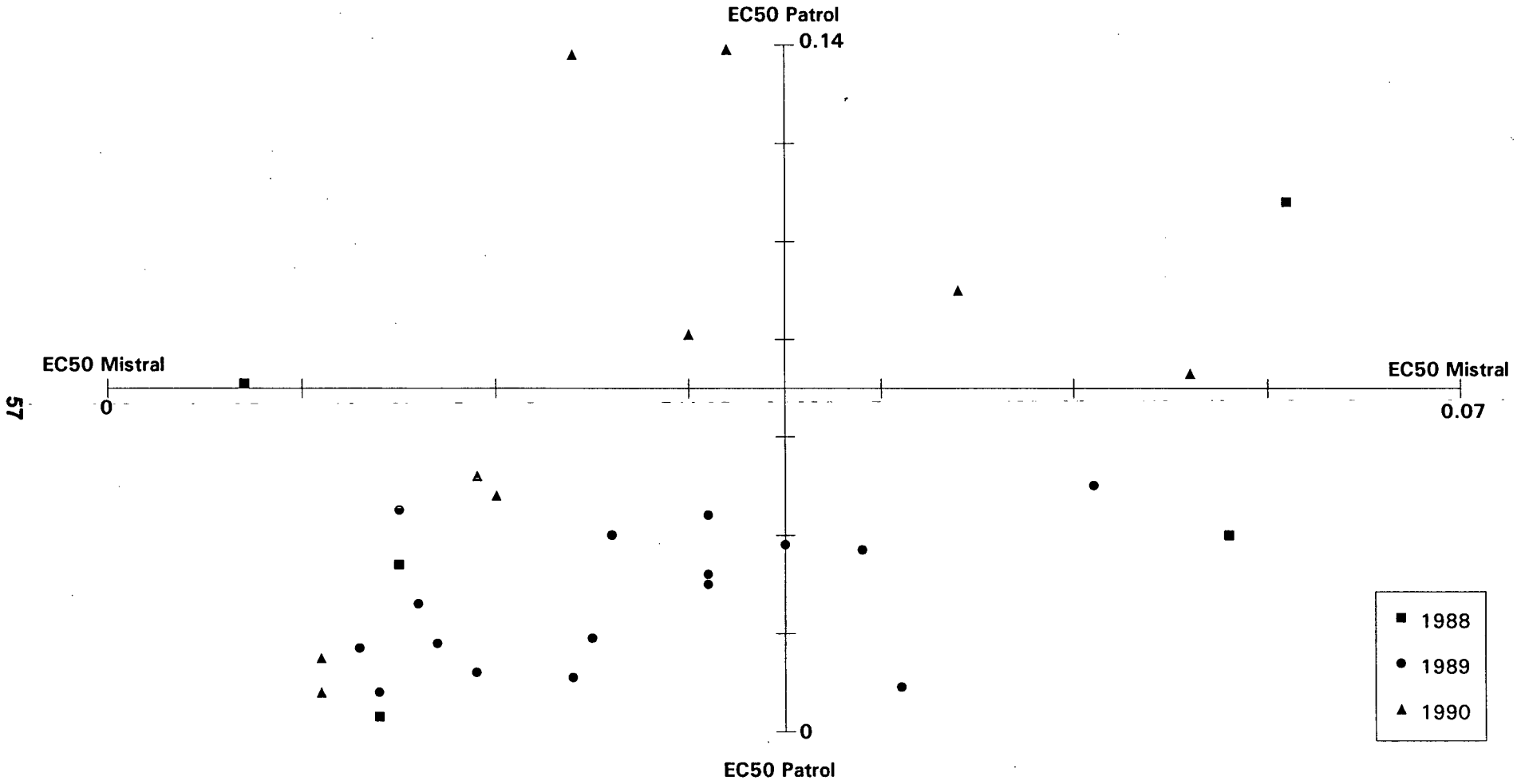
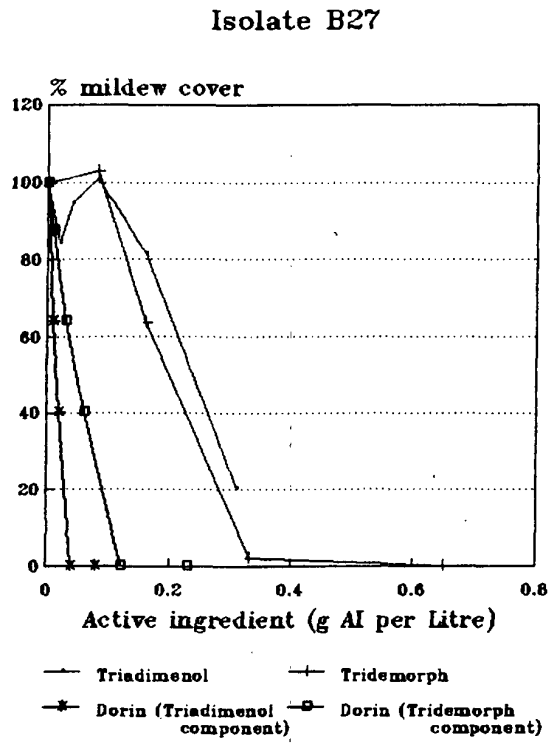


Figure 24: Sensitivity of Isolates to Mistral and Patrol

Figure 25: Reaction of isolates B27 and R1 to Dorin, Bayfidan and Calixin



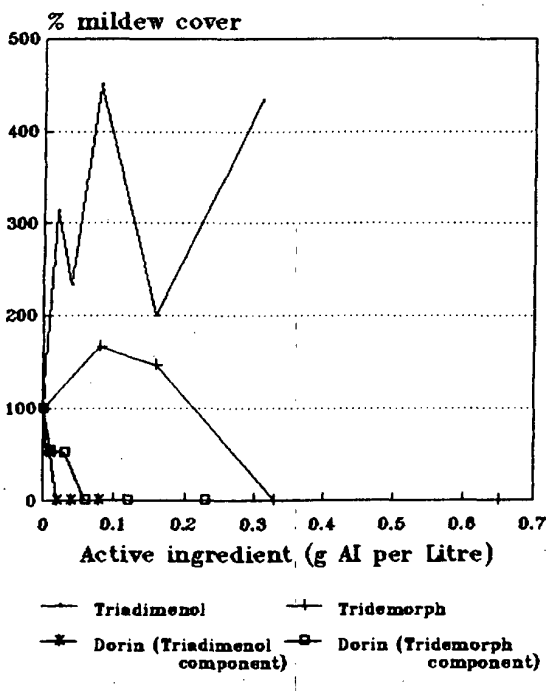
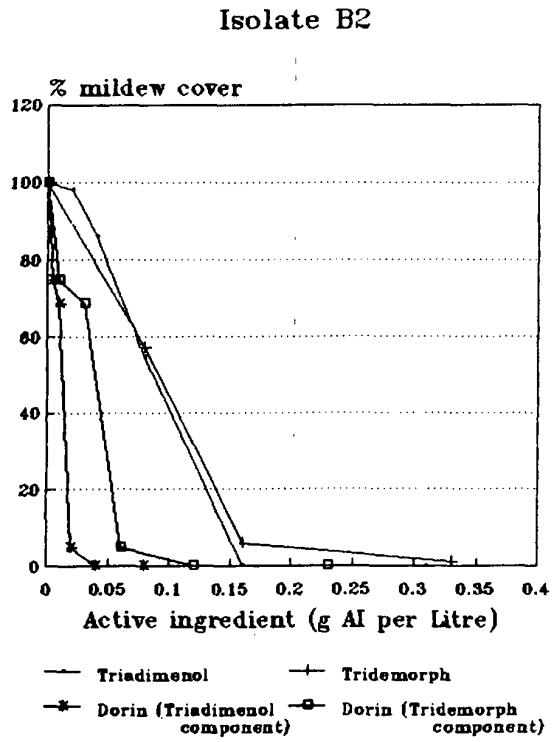


Figure 26: Reaction of isolates B2 and R14 to Dorin, Bayfidan and Calixin



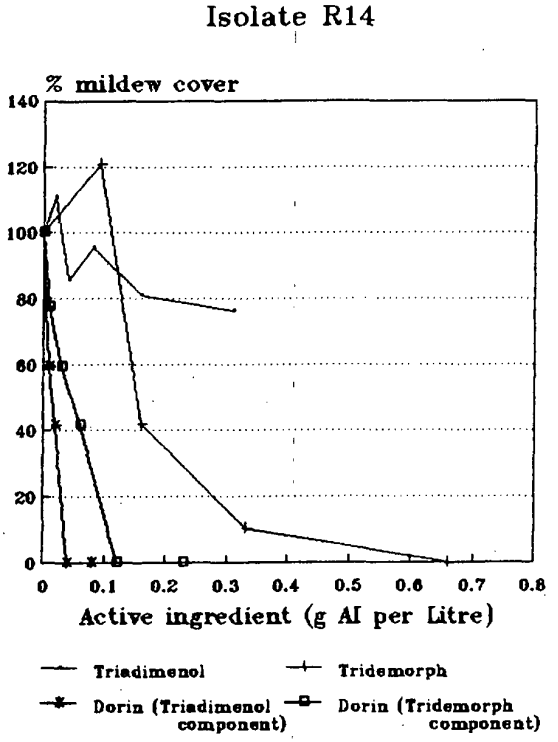
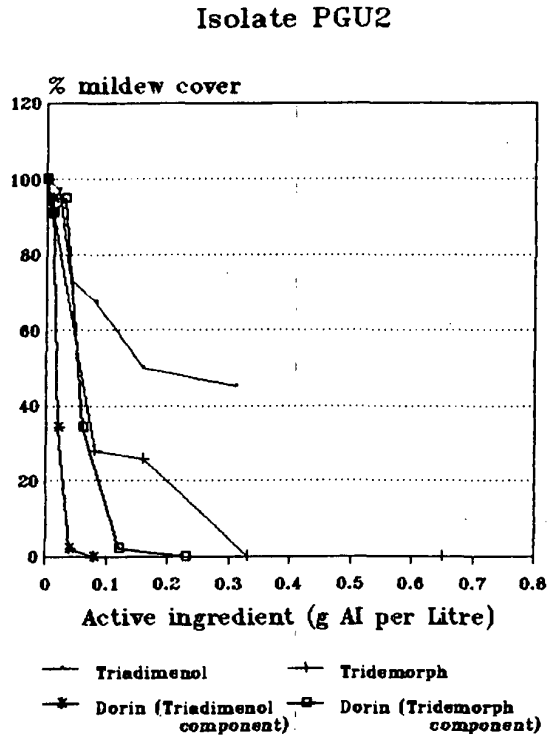
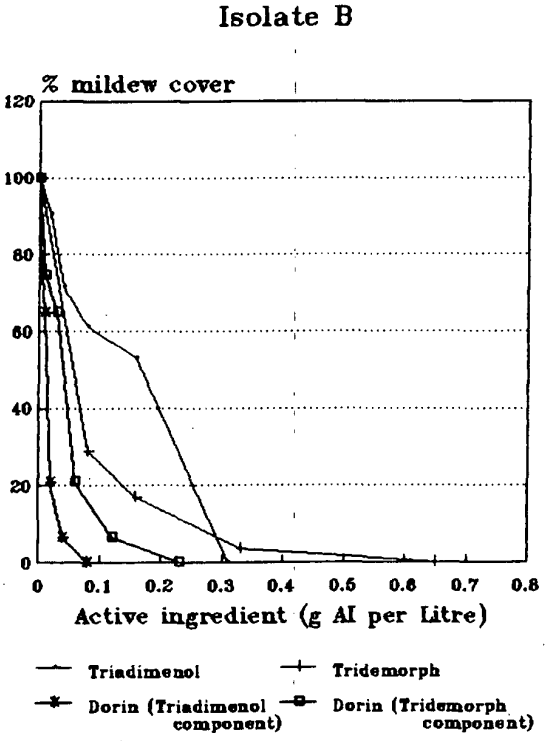


Figure 27: Reaction of isolates PGU2 and B to Dorin, Bayfidan and Calixin





SENSITIVITY OF BARLEY POWDERY MILDEW ISOLATES TO MORPHOLINE FUNGICIDES

PART II WORK IN ENGLAND AND WALES

1 INTRODUCTION

The morpholine fungicide, tridemorph, was introduced for cereal mildew control in England and Wales around 1970. Compared with the acute problem of mildew control on the very susceptible barley cultivar Golden Promise in Scotland, the broader spectrum of disease control needed in cereal crops in England and Wales ensured a different fungicide use pattern. Nevertheless, effective mildew control, coupled with the need to combat the spread of DMI resistance have meant that morpholine fungicides also play a crucial role in cereal disease control in England and Wales.

Although grouped together as "morpholines", the chemistry (Figure 1) and mode of action of individual compounds does differ (Hollomon, 1994). All inhibit a number of steps in the sterol synthesis pathway, but tridemorph interferes primarily with the D^{8-7} isomerase step, whereas fenpropimorph and fenpropidin inhibit the earlier D14-15 reductase step. Because of this possible multi-site action morpholines are considered a low resistance risk.

The first tridemorph-resistant isolates of barley powdery mildew were obtained in 1979 at Stockton, Durham (Walmsley-Woodward et al., 1979). These results were not confirmed by others, and no evidence of resistance problems at that site, or elsewhere in England and Wales have ever been reported. Some shift in sensitivity of wheat powdery mildew, especially to fenpropimorph, has been detected in the Netherlands (DeWaard, 1992), France (Andrivon et al., 1987) and Germany (Lorenz and Pommer, 1984), but field performance was not eroded. Although different assay procedures were used in each of these studies, they revealed the extent of variation in morpholine sensitivity in field populations, and emphasised the potential for selection for resistance.

The main objective of the England and Wales component of this project was to evaluate different methodologies for measuring morpholine sensitivity; to identify the range of variation, and to monitor the changes in sensitivity involved. The first

year included projects based at LARS and IPSR, Cambridge (Professor Martin Wolfe). The second season involved only LARS, but in conjunction with ADAS (Dr David Jones) who carried out two field experiments. Unfortunately, almost no mildew occurred on crops in Southern England in 1990, and the field experiments provided no real measure of fungicide performance.

2 METHODS

Four techniques were evaluated to measure morpholine sensitivity in the first phase of the work:

2.2 Wind Impact Suction Trap (WIST)

This sampled the airspora using an attachment on the car roof. Conidia were impacted onto seedlings, sprayed with a single dose of either tridemorph, fenpropimorph or fenpropidin, as the car travelled along a fixed route which included journeys in Scotland. Pustule numbers were counted seven days later. Single conidial cultures established from these trap-plants were tested on seedlings sprayed with at least five different doses of each fungicide.

At least five dose rates ranging from 1/10th to 1/200th field rate were used for each isolate. Pustule numbers were extremely variable regardless of dose, and were analysed by Principal Component Analysis (PCA). This is a preliminary statistical procedure requiring further analysis before drawing firm conclusions.

2.2 Systemic whole plant assay

Ten-day old barley seedlings (cv Halcyon) were grown in soil-less compost (500 ml) and were drenched with 100 ml fungicide solution. To provide an effective dose-response curve, a range of doses (4.6 - 37.5 mg a.i. per pot) were applied to each pot. Seedlings were inoculated in a large settling tower 4 hours after treatment, and then separated by polythene sleeves to restrict vapour movement between pots. Mildew levels were assessed 7 days later, usually as pustule numbers per leaf, and fungicide sensitivity expressed as the dose needed to reduce mildew infection by half.

2.3 Detached leaf assay

This involves floating inoculated leaf segments on solutions containing different levels of morpholine fungicides. Growth was measured microscopically 72 hours after inoculation. A more detailed account of this method is available in Hollomon (1982). A single isolate assayed on six occasions during 1988 gave a mean ED_{50} for fenpropidin of 0.041 mg ml^{-1} ± 0.025. Values ranged between 0.019 and 0.093

mg ml^{-1} emphasising the variation between assays. This standard reference isolate was included in all assays.

2.4 Vapour action assay

Leaf segments (1 cm) were placed on 0.5% water agar in each well of a 25 well "Repli-plate". 5 ml of each fungicide (technical grade) was applied to the five leaf segments in one row of the plate. Control segments received just methanol. All segments were inoculated in a settling tower and the plates immediately sealed with insulating tape. Mildew infection was assessed as pustule number per segment seven days after inoculation.

3 RESULTS

3.1 WIST

Population surveys showed some regional differences in the number of pustules trapped on treated plants. This was especially so for barley powdery mildew in N. Scotland (Table 1). Test of individual isolates confirmed that barley mildew was more sensitive in England than in Scotland (Table 2).

3.2 Systemic whole plant assay

All ten isolates of barley mildew supplied by Dr Gilmour had ED_{50} values little different from each other (Table 3). One isolate tested on four separate occasions, had an ED_{50} value ranging from 4.3 - 18.0 mg/pot with a mean of 12.2 mg/pot^{-1}. The three standard strains used by Brown *et al.* (1991) were also no different from the other isolates (Table 3).

3.3 Detached leaf assay

Despite variation between tests significant differences between barley mildew isolates were identified using this assay procedure.

The mean ED_{50} value for the sensitivity to fenpropidin of 35 isolates from S.W. England was 0.185 mg ml^{-1} with a range from 0.01 to 0.471 mg ml^{-1}. The three standard isolates CC1, CC139, and CC51 (Brown *et al.*, 1991) were within this range.

3.4 Vapour phase

All three morpholine fungicides exhibited significant vapour phase activity against barley powdery mildew. Although fenpropimorph was the most vapour active, only fenpropidin was used in these tests. As in the other three assay procedures, considerable variation was encountered, and several experiments were carried out to try and identify the sources of variation. Analysis of variance showed signfiicant differences between repli-plates, and wells adjacent to the edge of boxes had fewer pustules than did the central wells. To measure these sources of variation, and to provide a quantitative measure of sensitivity would require at least 25 boxes, and five fungicide doses, just to establish an ED_{50} value for one isolate.

Consequently, it was only possible to qualitatively compare isolates along lines described by Readshaw and Heaney (1994). Fenpropidin sensitivity determined in this way by the vapour test did not rank isolates in the same order as an assay using detached leaf segments (Table 4).

3.5 Changes in fenpropidin sensitivity

A collection of barley mildew isolates has been maintained by IACR since 1973, and data are available for fenpropidin sensitivity from this year until 1993. Very little barley mildew was collected in 1994 and no fenpropidin assays were carried out. Although the numbers tested varied from over 300 strains in 1984 to 10 in 1988, and variation associated with the mean ED_{50} for each year differed, no signficant changes in fenpropidin were observed during this period (Figure 2). This survey also confirmed that there was no cross-resistance between fenpropidin and the DMI fungicide, triadimenol.

3.6 Field performance of morpholine fungicides

Differences in disease levels and cultivars over a period of years complicate attempts to evaluate long-term changes in fungicide performance. Environmental factors such as temperature at time of spraying are also important for the vapour active morpholines. Table 5 shows performance of fungicides against barley powdery mildew based on field trial data from LARS and ADAS. In contrast to the decline in performance of the DMI fungicides triadimenol (Bayleton/Bayton) and propiconazole (Tilt) in the mid-1980s due to resistance, there has been no dramatic fall in either fenpropidin (Patrol) or fenpropimorph (Corbel) performance (Table 5). Tridemorph (Calixin) was not included in many of these trials, but no evidence exists to show any decline in its performance over 25 years of use.

4 DISCUSSION

These studies clarified several aspects of morpholine sensitivity. All four assay procedures generated considerable variation, and attempts to quantify the causes of this variation required unacceptable levels of replication. Principal Component Analysis goes some way to identifying variation, but requires further analysis of the data before firm conclusions can be drawn. Providing standard reference isolates were included in all assays, significant differences between mildew strains in their sensitivity to morpholines were identified by all four assay methods, reflecting a wide range of variation in both barley and wheat mildew populations.

Whether all four assays measure the same performance characteristics of morpholine fungicides is doubtful. The poor correlation between the ranking of isolates using the vapour assay compared with the order obtained using the leaf segment assay, suggest that each method measures a different aspect of performance. The vapour action of morpholines may well account for their rapid "knockdown" activity, whereas assays using whole plants, or detached leaf segments, reflect a more persistent action. Consequently, any decline in the knockdown performance of morpholines will not be detected by assay formats usually used to monitor sensitivity.

Another feature of the results emphasises that, where plant material was treated directly to achieve a dose rate series, these rates were always well below those used to treat field crops. Both wheat and barley mildew were always well controlled on plants sprayed at rates likely to be used in agricultural practice. This agrees with field performance data which shows that morpholines generally perform well on field crops.

Although sample sizes were small compared with the size of natural mildew populations, the surveys of barley powdery mildew show that morpholine sensitivity can vary from year to year. The apparent conflict between the work reported in Part I from monitoring Scottish barley mildew populations, and the results from the WIST survey, particularly in the Moray Firth region, may simply reflect sampling differences, and that the WIST survey was carried out for only one year. Nevertheless, if there has been any decline in sensitivity to morpholines in barley powdery mildew in Scotland, it is small and has not happened in England and Wales. The situation with wheat mildew is less clear, but in 1998-90 this mildew

was less important than barley mildew, and so less effort was directed towards monitoring wheat mildew. That has very largely changed to-day, and HGCA-funded work on fungicide resistance now includes more effort on wheat than on barley mildew.

The risk of resistance to morpholine fungicides seems low, but it remains sensible to only use these fungicides in mixtures to minimise this risk still further. Usually this is with a DMI, but several novel cereal fungicides with different modes of action, will soon be available, and can be used to replace morpholines in these mixtures. Independent evaluation of these mixtures by the HGCA will be needed to help growers formulate treatment strategies.

5 CONCLUSIONS

1. Differences in sensitivity to morpholine fungicides can be detected by different assay methods although the assays may reflect different aspects of morpholine action.

2. The range of variation in sensitivity encountered in both barley and wheat powdery mildew is large, but all isolates were controlled by recommended rates of fungicide. There was no evidence of resistance causing performance difficulties in field crops.

3. Barley mildew populations in England and Wales were possibly more sensitive to morpholine fungicides than those in Northern Scotland.

4. Sensitivity to morpholine fungicides can vary from year to year, but changes were unrelated to fungicide use.

5. Several novel fungicides with new modes of action are becoming available in the UK. These should be used as alternatives to morpholines in mixtures with DMI fungicides.

6 REFERENCES

Andrivon, D.; Limpert, E.; Felsenstein, F.G. (1987) Sensibilité au triadiménol et au fenpropimorphe de populations français d'*Erysiphe graminis* DC. f.sp. *hordei* Marchal. *Agronomie*, **7** 443-446.

Brown, J.K.; Slater, S.E.; See, K.A. (1991) Sensitivity of *Erysiphe graminis* f.sp. *hordei* to morpholine and piperidine fungicides. *Crop Protection*, **10** 445-454.

DeWaard, M.A. (1992) Fungicide resistance strategies in winter wheat in the Netherlands. In: *Resistance 91: Achievements and Developments in Combating Pesticide Resistance*. Eds I. Denholm, A.L. Devonshire, D.W. Hollomon. Elsevier, London, 48-60.

Hollomon (1982) The effects of tridemorph on barley powdery mildew: its mode of action and cross-sensitivity relationship. *Phytopathologische Zeitschrift*, **105**, 279-287.

Hollomon, D.W. (1994) Do morpholine fungicides select for resistance? In: *Fungicide Resistance*. Eds S. Heaney, D. Slawson, D.W. Hollomon, M. Smith, P.E. Russell and D.W. Parry. BCPC Monograph No.60, Farnham, UK. 281-289.

Lorenz, G.; Pommer, E.H. (1984) Investigations into the sensitivity of wheat powdery mildew populations towards fenpropimorph (monitoring programme). Proceedings 1984 *British Crop Protection Conference - Pests and Diseases*, 489-493.

Readshaw, A.E.; Heaney, S.P. (1994) Fenpropimorph sensitivity in *Erysiphe graminis* f.sp. *tritici*: survey of Northern France 1991-1993. In: *Fungicide Resistance*. Eds S. Heaney, D. Slawson, D.W. Hollomon, M. Smith, P.E. Russell and D.W. Parry. BCPC Monograph No.60, Farnham, UK. 297-302.

Walmsley-Woodward, D.J.; Laws, F.A.; Whittington, W.J. (1979) The characteristics of isolates of *Erysiphe graminis* f.sp. *hordei* varying in response to tridemorph and ethirimol. *Annals Applied Biology*, **92** 211-219.

Table 1: Colony counts on wheat and barley seedlings treated with morpholine fungicides

	Treatment of test seedlings (Proportion of field rate)			
	Wheat		Barley	
	A (1/100th)	B (1/50th)	A (1/100th)	C 1/20th)
E. Anglia	28*	0	0	26
E. Midlands	11	0	2	27
N. Midlands	27	24	3	24
Lothians	-	-	19	43
E. Scotland	-	-	16	34
M. Scotland	-	-	75	94

* Pustule numbers as a percentage of those on untreated seedlings

A = Fenpropimorph as Corbel
B = Fenpropidin as Patrol
C = Tridemorph as Bardew

Table 2: Numbers of barley powdery mildew isolates classified as sensitive or resistant to fenpropimorph

	Dose of fenpropimorph (Corbel) on trap plants (proportion of field rate)			
	1/500th		1/50th	
	S*	R	S	R
Northumberland	3	0	3	0
East Lothian/Berwick	0	4	7	1
Moray, Banff, Aberdeen	6	9	6	13

* Sensitivity relative to standard strains CC1 and CC139. For a more detailed description of the methods used see Brown *et al.* (1991).

Table 3: **ED50 values for fenpropidin (Patrol) sensitivity using a systemic whole plant test**

Isolate	ED_{50} mg a.i. per pot Fenpropidin	Isolate	ED_{50} mg a.i. per pot Fenpropidin
BUSB22	12.2 (range 4.3 - 18.0)	CSB2.2	2.6
CSB9	16.0	BUSB13	4.8
BUSB2	4.0	BUSB21	15.3
CSB2.4	5.0	CC1	15.2
CSB2	21.6	CC139	4.3
CSB8	8.0	CC51	5.2
L32	14.8		

Table 4: **Ranking for fenpropidin sensitivity of barley powdery mildew isolates using two different assay methods**

Vapour Assay		Detached leaf assay	
	Isolate	Isolate	ED_{50} mg ml^{-1}
Sensitive	L32	1641	0.01
	BUSB22	BUSB22	0.04
	BUSB20	CSB2	0.06
	23D5	BUSB20	0.08
	CSB2	23D5	0.09
	DH14	DH14	0.13
Resistant	1641	L32	0.14

Table 5: Fungicide efficacy: spring barley 1980-1982

	Mean Disease Control (%)			
	Bayfidan	Tilt	Corbel	Patrol
1980	95*	83	83	–
1984	69	45	79	–
1985	55	20	73	70
1988	21	–	70	66
1990	80	85	66	68
1992	75	82	73	76

* Bayleton

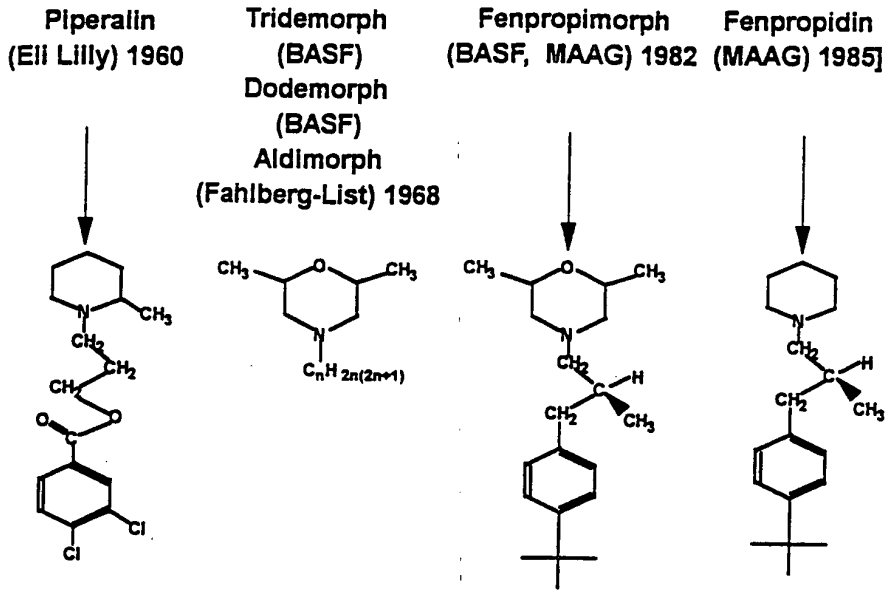


Figure 1: Morpholine and Piperidine fungicides

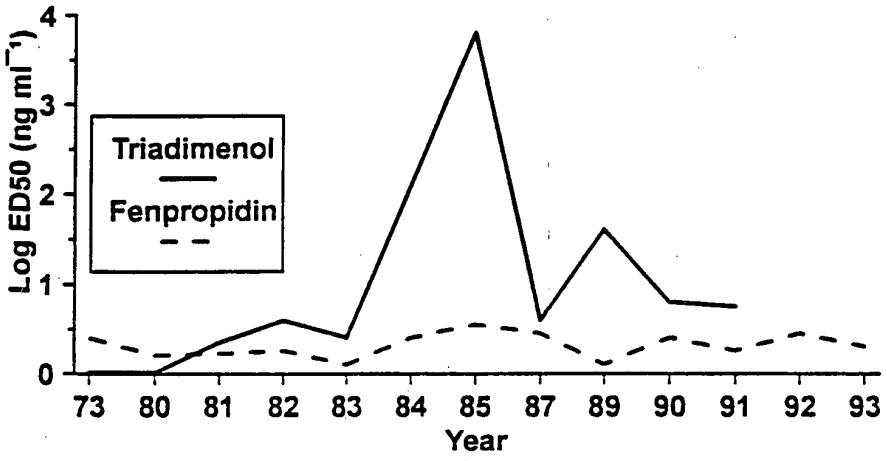


Figure 2: **Changes in fenpropidin and triadimenol sensitivity in barley powdery mildew 1973-1993**