SENSITIVITY TO MORPHOLINE FUNGICIDES IN YELLOW RUST OF WHEAT (*PUCCINIA STRIIFORMIS)*

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ABSTRACT

Isolates of *Puccinia striiformis* collected between 1961 and 1990 were tested for sensitivity to fenpropimorph using wheat seedlings sprayed with low doses of fungicide. Isolates varied widely in their sensitivity, but there was no evidence that sensitivity had declined over the thirty year period. There were indications that sensitivity was related to the geographical origin and specific virulence of isolates. Isolates from the south of the U.K., and those without virulence for the wheat cultivar Hornet, tended to be less sensitive than isolates from the north and those possessing virulence for cv. Hornet. Sensitivity appeared to be unrelated to fungicide applications to the crop prior to sampling. The ranking of a number of isolates for sensitivity to fenpropidin was similar to their ranking for fenpropimorph.

INTRODUCTION

Puccinia striiformis, the causal agent of yellow rust, is a major pathogen of wheat in the U.K. Epidemics of the disease occur on average once every four to five years, depending largely on the susceptibility of cultivars dominating the wheat acreage. Although fungicides of the triazole and morpholine groups have been widely used to control yellow rust, there have been no substantiated reports of loss of disease control in the field.

The main aims of this investigation were to establish a base-line measure of sensitivity of *P.striiformis* to triazoles and morpholines and to determine whether sensitivity has changed over the past thirty years. This paper reports the results for morpholine sensitivity.

Variation in *P.striiformis* for virulence for cultivars has been monitored since 1967 by the U.K. Cereal Pathogen Virulence Survey, which maintains a collection of isolates classified on the basis of origin and virulence. This collection was the main source of isolates for fungicide sensitivity tests.

METHODS

Isolates collected between 1961 and 1990

268 isolates of *P.striiformis* , collected between 1961 and 1990 were tested for sensitivity to fenpropimorph using a seedling test. Isolates were stored as freeze-dried uredospores in sealed glass ampoules. Each test included a standard isolate (83/62) for reference. Ten seedlings of the universally susceptible wheat cultivars Sappo or Vuka were grown in 6.5cm pots. Seedlings were sprayed with fungicide when the first leaf had expanded, 7 to 8 days after sowing, using a field application simulator sprayer, delivering 200 $l.ha^{-1}$ at a pressure of 2 bars. The dose rate of fenpropimorph was 187.5 mg AI/l, corresponding to 1/20 field rate.

24 hours after spraying, seedlings were inoculated in a rotary spore inoculator, using fresh uredospores mixed with acid purified talc. After incubation for 48 hrs at 7°C and high relative humidity, seedlings were transferred to a controlled environment growth room with 16 hrs light at 18°C and 8 hrs dark at 11°C.

Four to five days after the appearance of yellow rust pustules on unsprayed seedlings (14 - 15 days after inoculation), the percentage leaf area covered with pustules was assessed on first leaves. An index of infection 'I', comparing the relative infection for the test isolate with the standard isolate, was estimated as follows:

$$I = P_{tf} / P_{to} - P_{sf} / P_{so}$$

where P_t = percentage infection on test isolate
P_s = percentage infection on standard isolate
f = fungicide - treated
o = untreated

Positive values of 'I' indicated higher infection than the standard isolate i.e. lower sensitivity, whilst negative values of 'I' indicated lower infection than the standard i.e. greater sensitivity.

Isolates were classified according to:

1. Year of collection
2. Geographical location, north or south of River Tyne (N or S)
3. Virulence or avirulence for the cultivar Hornet (V or A)
4. Whether or not morpholine fungicide applied to crop prior to sampling (+Mor -M)

Using these classifications, mean 'I' values for contrasting groups of isolates were compared using a t-test, to indicate associations between sensitivity to fenpropimorph and other characteristics.

Comparison of sensitivity to fenpropimorph and fenpropidin

Four isolates were tested for sensitivity to fenpropimorph (187.5 mg AI/l, equivalent to 1/20 field rate) and fenpropidin (375.0 mg AI/l, equivalent to 1/10 field rate). The higher rate of fenpropidin was required to reduce infection to the same degree as fenpropimorph. The isolates comprised two collected from crops in which poor fungicide control had been reported (92/27, from a crop treated triazole and morpholine, and 93/32, from a crop treated with

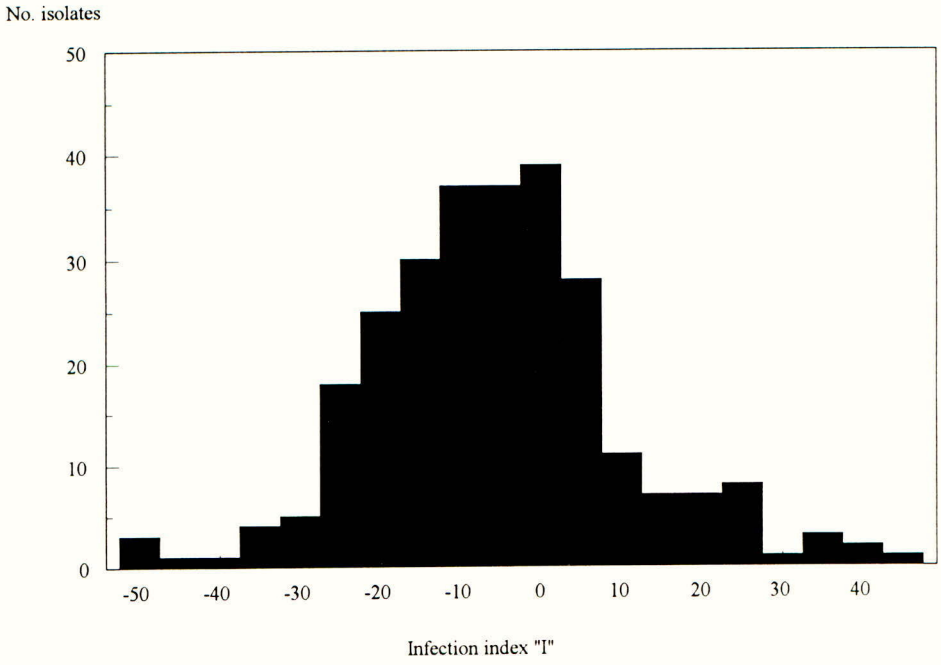
triazoles), one with consistently reduced sensitivity to fenpropimorph (90/20) and the standard isolate (83/62).

RESULTS

Isolates collected between 1961 and 1990

For the 268 isolates tested, values of 'I' ranged from -54 to +41, representing approximately 1% to 65% of the infection level on the nil control (Figure 1).

Figure 1. Infection indices for 268 isolates of *P.striiformis* inoculated onto fenpropimorph - treated seedlings (1/20th field rate)



Mean infection indices for contrasting groups of isolates, and the significance of the differences between them, are given in Tables 1a to 1e.

TABLE 1. Mean infection indices for isolates of *P.striiformis* inoculated onto fenpropimorph - treated seedlings (1/20 field rate).

a) Isolates classified by year

Year	No. isolates	mean 'I'	comparison	significance
pre-1989	95	-7.33	pre-'89 v '89	NS
1989	111	-7.89	'89 v '90	NS
1990	62	-9.45	pre-'89 v '90	NS

b) Isolates classified by geographical location

Location	No. isolates	mean 'I'	significance of difference
N	66	-16.25	P = 0.001
S	188	-5.17	

c) Isolates classified by virulence for cultivar Hornet

Virulence for cv Hornet	No. isolates	mean 'I'	significance of difference
V	102	-12.05	P = 0.01
A	157	-5.11	

d) Isolates classified by location and virulence

Location	virulence for cv Hornet	No. isolates	mean 'I'	Comparison	significance
N	V	49	-14.97	N,V v S,V	P = 0.05
	A	4*			
S	V	53	-9.35	S,V v S,A	P = 0.001
	A	132	-3.31		

* number of isolates too small for valid comparisons

e) Isolates classified by morpholine application prior to sampling.

Morpholine application	No. isolates	mean 'I'	significance of difference
+M	38	-6.82	NS
-M	230	-8.33	

Comparison of sensitivity to fenpropimorph and fenpropidin

Results are given in Table 2.

TABLE 2. Percentage leaf area infected with yellow rust (relative to untreated control) for wheat seedlings treated with fenpropimorph or fenpropidin and inoculated with four isolates of *P.striiformis*

Isolate	Fungicide	
	Fenpropimorph (187.5 mg AI/l)	Fenpropidin (375mg AI/l)
83/62	37.5	41.0
90/20	51.5	53.0
92/27	23.5	39.0
93/32	44.5	45.0

DISCUSSION

Isolates of *P.striiformis* collected between 1961 and 1990 varied widely in their sensitivity to a low dose of fenpropimorph, equivalent to 1/20 field rate. However, there was no evidence that the pathogen has become less sensitive in recent years. The mean sensitivity of isolates collected in 1989 and in 1990 did not differ significantly from that of isolates collected between 1961 and 1988.

There was evidence of an association between geographical location and sensitivity, with isolates from the south being, on average, less sensitive than those from the north. One possible explanation for this is that the use of morpholine fungicides to control yellow rust has been more common in the south than in the north. There was also an association between sensitivity and virulence characteristics, such that isolates lacking virulence for the wheat cultivar Hornet were less sensitive than isolates possessing this virulence. This observation is probably related to the north : south difference, since virulence for Hornet first arose in northern populations (Bayles *et al.*, 1989, Bayles and Stigwood, 1991), which were themselves relatively sensitive to morpholines.

Whether or not a morpholine fungicide had previously been applied to a crop appeared to have no effect on the sensitivity of isolates taken from it. However, the number of samples from morpholine treated crops was small compared with those from untreated crops or crops receiving other fungicides, and this result should therefore be interpreted with caution.

There was no evidence from a limited comparison of the reactions of four isolates to fenpropimorph and fenpropidin that sensitivity to the two morpholine fungicides differed. The ranking of isolates was similar for the two chemicals and isolate 90/20 showed a marked

reduction in sensitivity to both. The isolate taken from a crop in which a morpholine fungicide had reportedly given poor control, (92/27) proved to be the most sensitive of the isolates, underlining the need to exercise caution in attributing poor disease control to insensitivity.

The most important outcome of this investigation was the establishment of a base line sensitivity with reference to isolates with defined sensitivity characteristics. These isolates can be stored for long periods as freeze-dried spores, with negligible risk of genetic change . The use of reference isolates is considered to be vital, since the precise quantity of fungicide delivered to test seedlings can vary significantly depending on the spraying equipment used.

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MONITORING RESULTS OF *MYCOSPHAERELLA FIJIENSIS* TO TRIDEMORPH

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ABSTRACT

Control of black Sigatoka, caused by *Mycosphaerella fijiensis*, presently depends on five fungicide groups, three of which belong to the so called single site inhibitors. Pathogen resistance to benzimidazoles is now general and widespread in most of the banana growing countries of South- and Central-America. The first decline in sensitivity to triazoles has been reported from Belize, Guatemala, Honduras, Costa Rica, Mexico, Panama and Cameroon.

In contrast, monitoring results show that there are no indications of sensitivity changes to tridemorph in any country. The use of tridemorph in fungicide mixtures or alternation programmes is, therefore, an important resistance management strategy to limit further development of resistance to triazoles and benzimidazoles.

INTRODUCTION

TABLE 1.Sequence of products used for the control of Sigatoka and the approximate year of introduction

Year	Product	Application Rate
1934 - 1958	Bordeaux mixture	1000 - 1500 l/ha
1956 -	Mineral oil	7 - 15 l/ha
1958 -	Dithiocarbamates (Mancozeb)	1,5 - 2,5 kg/ha
1970 -	Benzimidazoles	250 - 300 g/ha
1978 -	Chlorothalonil	2 - 3 l/ha
1978 -	Morpholines (tridemorph)	0,6 l/ha
1985 -	Triazoles (propiconazole)	0,4 l/ha

Present day chemical control of *Mycosphaerella fijiensis* depends on five fungicide groups (Table 1), two of which have a multi-site mode of action (mancozeb and chlorothalonil), while benzimidazoles, tridemorph and propiconazole belong to the so called single-site inhibitors. It is widely accepted, that there is little chance of resistance development to the multi-site inhibitors, but they have only protectant activity against fungal infections and, therefore, have to be applied more frequently than the single-site inhibitors, whose limited systemic activity provides a certain degree of curative action.

Benomyl was the first systemic fungicide to be used for Sigatoka control in 1970. Since at that time there was little knowledge of fungicide resistance, benomyl was applied frequently. Reports of Sigatoka resistance to benomyl first occurred in the late 1970's and started the idea of fungicide resistance management in the banana industry.

Tridemorph has now been part of Sigatoka control programmes since 1978 i.e. for up to 16 years in some countries. Based on the experience of benomyl resistance, a periodic monitoring programme was started in 1984 (Cronshaw and Lorenz, 1988) and continued ever since, in order to detect any possible sensitivity changes at an early stage. So far no such changes in sensitivity have been detected.

The triazole fungicide propiconazole was introduced into Sigatoka control programmes in 1985 and is now in its ninth year of use. Monitoring studies were carried out from the beginning and only recently have started to show a reduced sensitivity to propiconazole.

MATERIAL AND METHODS

Materials and methods involved are based on techniques outlined for monitoring the response of *M. fijiensis* to benomyl (Anon. 1983) which were subsequently modified for triazole/morpholine fungicides (Anon. 1988) and are described in some detail by Stover (1992). They are based on the measurement of germ tube growth on water agar amended with various fungicide concentrations.

Generally ascospore-bearing leaf tissue is incubated in plastic bags with moist towelling for 48 hr at room temperature. Leaf tissue for ascospore discharge is removed, cut into pieces of 1 - 2 cm^2, numbered with ink and stapled to filter paper. The filter paper with attached leaf pieces is submerged in tap water for 5 minutes and then placed inside the lid of a Petri dish over 2 % water agar containing a concentration range of tridemorph (0 - 50 ppm a.i.) for 1 hr for ascospore discharge, and then removed. Ascospores are located and identified with the aid of a dissecting microscope. Following ascospore discharge plates are incubated at 26°C for 48 hr. After that time the germ tube length of 50 ascospores per plate is measured. An ascospore is considered to have germinated, if the germ tube is at least 5 µ long. From these data EC_{50}-values can be calculated by using appropriate statistical programmes, or frequency distributions can be drawn using either %

inhibition (based on mean score of the unamended plate) or by graphing the actual germ tube length.

RESULTS

A tridemorph monitoring programme was started in 1984. Since this work was mainly done in the laboratory of Dr. Stover, Honduras, the most consistent data are available from this country. In Table 2 EC_{50}-values are compared from farms where either no tridemorph had ever been applied or where it was part of the commercial spray programmes. Obviously no appreciable differences exist in the sensitivity of ascospores from treated and non-treated areas. When samples are taken at the same date from different locations within the same farm, there is very little variation in sensitivity (Table 2.). On the other hand a certain degree of variation becomes apparent, when samples are taken at the same location but at different sampling dates throughout the year (Table 2), which is probably due to the fact, that ascospores taken during the dry season are presumably less vigourous and consequently more sensitive to fungicides.

TABLE 2.Tridemorph monitoring data from Honduras 1984

Various farms						
	A (nt)*	B (nt)	C	D	E (nt)	F
ED_{50} (ppm a. i.)	2,04	< 1	1,31	1,52	1,03	1,83

Various locations within the same tridemorph treated farm				
	A	B	C	D
ED_{50} (ppm a. i.)	1,35	1,37	1,65	1,79

Various sampling dates from the same non-treated farm					
	Nov.	Dec.	Feb.	Mar.	Aug.
ED_{50} (ppm a. i.)	< 1	< 1	1,4	1,42	3,1

* nt = non-tridemorph treated

TABLE 3.Tridemorph monitoring data from Honduras 1988 - 1992

Santa Catalina Farm (non-tridemorph treated)								
Sample Date	Dec. 88	Apr. 89	Apr. 89	Oct. 89	Feb. 92	Jun. 92	Jun. 92	Oct. 92
EC_{50} (ppm a. i.)	1,98	0,09	1,44	0,11	0,54	0,22	0,23	1,98

Ø = 0,82

Various tridemorph treated farms							
Sample Date	Feb. 88	Feb. 88	Feb. 88	Feb. 88	Oct. 92	Oct. 92	Oct. 92
	Corozal 2	Corozal 16	Santa Rosa 38	Santa Rosa 170	Santa Rosa 40	Corozal 125	Tacamiche
EC_{50} (ppm a. i.)	0,13	0,25	0,05	< 0,01	1,19	1,9	1,09

Ø = 0,66

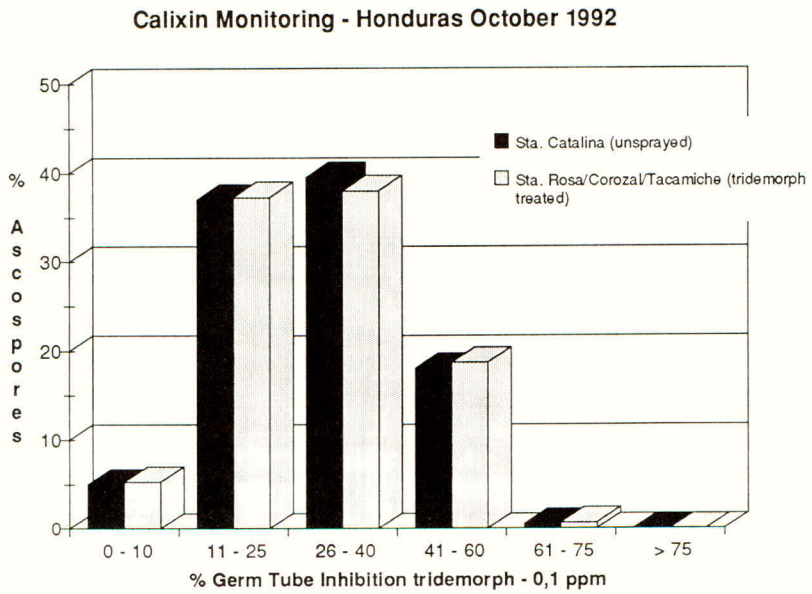
In 1984 further data were obtained from samples from Panama (Ø EC_{50}-value 1,91 ppm) from Costa Rica (Ø EC_{50}-value 1,48 ppm) and from the Philippines (Ø EC_{50}-value 1,0 ppm) which were comparable to those from Honduras.

Table 3 shows data from Honduras for the years 1988 to 1992. Again values from non-treated locations are compared with those from treated areas. The EC_{50}-values show some variation but the average value from the non-treated control farm (Santa Catalina) is very similar to the average from farms that have received tridemorph applications in commercial spray programmes.

The wide range of variation of EC_{50}-values seems to be a general disadvantage of the germ tube elongation test used for *M. fijiensis*. Sampling date, concentration range chosen, storage conditions and differences between laboratories are some of the factors whose influence on the response of ascospores was investigated by Stover (1992). Besides these, at the last FRAC-meeting of the SBI Banana Working Group the major area for improvement was identified as the method of data analysis. It was suggested to focus rather on specific areas of the sensitivity distribution instead of calculating EC_{50}-values.

In Figure 1, therefore data from Honduras are shown where the percentage of germ tube inhibition is presented as a frequency distribution at a given concentration and again this indicates that there is no difference in sensitivity to tridemorph comparing untreated and treated areas.

FIGURE 1. Frequency distribution of % germ tube inhibition at 0,1 ppm tridemorph



CONCLUSIONS

In spite of the fact that tridemorph has now been a part of the Sigatoka control programmes for up to 16 years in some countries, and was used with a wide range of frequencies, the results of the continuing monitoring programme so far indicate that *M. fijiensis* causing black Sigatoka or black leaf streak, does not show any decreased sensitivity to tridemorph.

The same situation is true for the control of cereal powdery mildews in Europe, where after 24 years of use there are still no reports of reduced sensitivity to tridemorph (Clark 1992). The reasons discussed are possible additional effects on processes not related to sterol biosynthesis i.e. an additional mode of action

besides its effects on sterol biosyntheses (James *et al.* 1992). This emphasizes the importance of tridemorph as an alternation or mixture partner to lessen or help prevent further resistance development to benzimidazoles and triazoles. Although no field resistance to tridemorph has occurred, the FRAC recommendations (Table 4) are strongly supported by BASF. The use of tridemorph in a carefully planned resistance management strategy that avoids block treatments and limits the total number of applications per year should be considered as a sensible precaution.

TABLE 4.Recommendations of FRAC, Orlando, January 1993

Fungicides used against Sigatoka	No. of applications / year
triazoles alone	8 (max. 2 consecutive)
triazoles in mixture with other fungicides	10 (max. 2 consecutive)
Calixin alone or in mixture with other fungicides	12 (max. 2 consecutive)
benzimidazoles alone or in mixture with other fungicides	always in alternation
chlorothalonil	no limit
mancozeb	no limit

In addition:

- consecutive applications of triazoles are not recommended
- a 2 - 4 month period free of triazoles is recommended
- in mixtures Calixin 66 % of the normal rate
  triazoles 75 % - 100 % of the normal rate
  benzimidazoles 100 % of the normal rate

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FIELD PERFORMANCE OF MORPHOLINES

Summary of discussion following the papers presenting recent findings on morpholines.

Chairman: Dr James Gilmour, Scottish Agricultural Colleges, Central Office, West Mains Road, Edinburgh, EH9 3JG

A wide ranging and lively discussion followed presentation of papers in the first part of the session. Inevitably, perhaps, delegates concentrated on morpholine action against cereal mildews. Discussion also focussed on fenpropimorph and fenpropidin; little interest was directed towards tridemorph and other morpholines.

In both England and France advisory workers have noticed some fall in the persistence of Corbel and Mistral, but these perceptions were not generally supported by field experimentation in the Netherlands and elsewhere. The environmental impact, especially of high temperatures at the time of spraying, can be considerable, reducing control levels and generating yearly variation in performance. Where newer triazoles, such as tebuconazole and epoxiconazole have replaced triadimenol as standards, the performance of morpholines may be down graded because of improved control achieved by these standards. Greater mildew susceptibility of current wheat cultivars may increase disease pressures and make adequate control more difficult to achieve. With the notable exception of the very mildew susceptible cultivar Apollo, this was probably not the case in the UK. In comparison with the substantial decline in the performance of early triazoles in different parts of N. Europe, similar changes in morpholine performance had not occurred despite their widespread use.

Different assays of fenpropimorph sensitivity, and comparison with standard isolates, have provided experimental evidence, albeit of relatively small changes in both wheat and barley powdery mildew. Choice of these standard isolates is critical, and their relationship to the natural population has not always been made clear. Equally, so called **ring tests** have not always produced consistent results in different laboratories, in marked contrast to similar tests with isolates used as standards in surveys of azole sensitivity. Nevertheless, there was widespread agreement that significant variation exists in natural populations, and that the range of morpholine sensitivity in wild-type populations is less than in selected ones. This does not necessarily lead to a change in population mean, although this can change from year to year. A consensus view emerged from these discussions of the need to re-examine testing methods to obtain more uniform testing protocols to be used amongst workers.

Shifts in sensitivity as measured by bioassay clearly do not correlate with field performance, although this is often a feature of the early phases of the development of practical resistance. The effect of migration is a very real problem in evaluating selection induced changes in wind-dispersed cereal mildews, especially where the fungicides being examined are in widespread use in surrounding farming regions. With present technologies, small plots are generally inadequate, although just what plot sizes are acceptable is not clear. Shifts in sensitivity might well result from directional selection exerted on the whole population, but may equally well reflect the small

population surviving treatment. It should be possible to distinguish between these two possibilities, by examining the overall shape of the population distribution, which should no longer be log normal in the case of the small, surviving population. But morpholine sensitivity assays frequently generate considerable experimental variation making it difficult to detect subtle changes in population distributions.

A consistent feature of laboratory and greenhouse results is the apparent cross resistance between fenpropimorph and fenpropidin, but not with tridemorph. It is perhaps too simplistic to relate this to differences in mode of action, when so many of the biochemical studies have been carried out in yeast, and may not be relevant to plant pathogens. Nevertheless, there was considerable hesitancy at adopting anti-resistance strategies based on mixtures of tridemorph with either fenpropimorph or fenpropidin.

Morpholines are still accepted as low risk fungicides. Attempts to analyse the genetic control of any variation in sensitivity have been inconclusive. Identification of distinct sensitivity classes does not inevitably provide evidence of major gene control, as effects of many genes exerting additive effects within different classes are quite difficult to detect against a background of considerable experimental variation. The picture is not made any clearer by differences in the definition of resistance used by workers, and by references to "**less sensitive**" isolates, and "low level" resistance. However, in a discussion that pulled together the experiences of many practicising pathologists, there was no convincing evidence of practical resistance to morpholines in any target pathogen.

D.W. Hollomon