Fungicide Resistance to Morpholine and Piperidine Fungicides in Barley and Wheat Powdery Mildew *Erysiphe graminis* D.C.

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ABSTRACT

This work was carried out to study the sensitivity of barley and wheat powdery mildew (*Erysiphe graminis* f.sp. *hordei* and *E. graminis* f.sp. *tritici* respectively) to morpholine fungicides. Morpholine fungicides are classified as compounds with a low risk of resistance development. On the other hand, barley and wheat powdery mildews belong to the group of high risk fungi as far as development of fungicide resistance is concerned.

Firstly, mildew isolates collected in the UK were assessed, over the period 1992 to 1995, for their sensitivity to three morpholine fungicides (tridemorph, fenpropimorph and fenpropidin). Barley isolates tended to be more sensitive to the fungicides than wheat isolates. In general, the barley mildew isolates were less sensitive to tridemorph than to fenpropimorph and fenpropidin. Cross resistance was found between fenpropimorph and fenpropidin but there was no significant correlation between barley mildew sensitivity to tridemorph and either fenpropimorph or fenpropidin. Isolates from Scotland were significantly less sensitive to fenpropimorph and fenpropidin than isolates from England. The wheat isolates tended to be more sensitive to fenpropidin than to fenpropimorph. There was no evidence of cross resistance between fenpropimorph and fenpropidin with the wheat isolates tested. A shift towards insensitivity over the period of testing was found for fenpropimorph. Wheat isolates from Scotland were found to be significantly less sensitive to fenpropidin than isolates from England.

Although variation in sensitivity among mildew isolates was recorded, the results of the monitoring work confirmed the findings of field experience that morpholines are continuing to maintain an effective control over barley and wheat mildew. The variation in sensitivity appeared to be as a continuous distribution, probably related at least in part to the use of bulk isolates for most of the sampling. There, was however, no evidence of any part of the population showing a very high level of resistance.

Secondly, it is common practice for farmers to use doses of fungicides below the recommended rate to maximise gross margins, but the effect this has on fungicide sensitivity has not been determined conclusively. To test the selective effect of reduced doses on the fungicide sensitivity of populations of powdery mildew, wheat and barley field experiments were conducted between 1992 and 1995. Isolates recovered from plots receiving reduced and full dose treatments were compared to see whether selection for insensitivity varied with dose rate. No significant differences between the effects of the full commercial rate and reduced doses were found.

Observations from the wheat experiment indicated that relative sensitivities of populations in field plots may change quickly and that account should be taken of the relative fitness of sensitive and less sensitive isolates and of interference from neighbouring population sources in assessing the effects of fungicide treatments.

Finally, preliminary studies on the variation in the early development of isolates of *Erysiphe graminis,* of different sensitivities, in response to exposure to fenpropimorph were carried out. Three different methods of studying the sensitivity of isolates to fenpropimorph were compared, with a view to developing a less time consuming and more accurate method. All three methods showed that exposure to fenpropimorph reduced spore germination. Further fungal development in response to fungicide treatment varied with the method employed. Incidence of spore germination after 24 hours and spore germination, appressorial formation and development of secondary hyphae after 24 and 48 hours, were compared with the standard bioassay used in this work. Results were not consistent and the standard bioassay remained the most consistent and accurate method of measuring sensitivity.

Dedication

This work is dedicated to my late father, my mother and late Aunt Kosita.

Declaration

I, the undersigned, hereby declare that
this thesis has been composed by myself.
All assistance obtained from other sources
has been acknowledged in appropriate places.

Miriam C N Zziwa

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Chapter 1

General Introduction

GENERAL INTRODUCTION

An important development in crop disease control in the 1960s was the introduction of systemic fungicides which exerted their effect from within the plant. They had several advantages over conventional, surface-acting fungicides, including providing some protection over new growth and overcoming certain weathering and distribution problems. Of necessity, the mode of action of systemics requires some measure of specificity to allow control of the fungus without damage to plant tissues exposed to the compound. Unfortunately, associated with their specific mode of action, fungicide resistance problems have tended quickly to follow the introduction of systemic fungicides, with the emergence of strains of the previously controlled target species which are less sensitive or completely insensitive to the compound concerned. There are now on record many instances of the appearance of resistance to systemic fungicides in populations of fungal pathogens of crop plants. The present work is concerned with aspects of fungicide resistance in *Erysiphe graminis* DC. [= *Blumeria graminis* (DC.) Speer], the cereal mildew fungus. Of the classes of systemic fungicides which have been used to control this pathogen, fungicide resistance has developed to two but the third, the morpholines, has shown no clear evidence of a major resistance problem despite the heavy reliance that has been placed on it for over twenty years. The present work focuses on the morpholines and an assessment of the risk of resistance to this group arising in populations of barley and wheat mildew.

Chapter 2

Literature Review

LITERATURE REVIEW

Cereal Powdery Mildew

Powdery mildew, caused by the obligate biotroph *Erysiphe graminis* DC., is a major disease of cereals, especially barley, in the UK (James, 1969; Colhoun, 1971; Gair, Jenkins & Lester, 1978;) and other temperate regions. Surveys of foliar diseases of spring barley in England and Wales in 1967 suggested that powdery mildew was the most important disease of spring barley in England and this probably applied to all major barley-growing areas in Europe (James, 1969). Surveys of winter wheat in England and Wales from 1976 to 1988 also revealed that mildew was the most severe leaf disease in 1970, 1976 and 1977 and that it was important in 1979, 1981 and 1982 (Polley & Thomas, 1991). Losses in individual severely affected crops have been as high as 40 per cent for both wheat and barley (Gair *et al.*, 1978).

The fungus is found only on members of the Gramineae but within the morphological species specialised forms (*formae speciales*) are distinguished on the basis of the principal host genus attacked. For example, that which attacks barley is known as f.sp. *hordei.* According to Marchal (1902) and Jørgensen (1988) specialised forms are recognised on seven different hosts, four on cereals, *hordei*, *tritici*, *avena* and *secalis* Marchal, and three on grasses, *agropyri*, *bromi* and *poae.* Although it is generally accepted that specialised forms are restricted to their particular host so that forms on wheat only affect wheat and not other cereals and forms on these other cereals do not affect wheat, experimental work has shown that this is not strictly correct and that under certain conditions the barley form may attack wheat. According to Moseman & Greeley (1964) and Moseman, Scharen & Greeley (1965), when wheat plants are already infected by f.sp. *tritici*, or are inoculated with f.sp *tritici* simultaneously with f.sp. *hordei*, they are predisposed to infection by f.sp. *hordei* and some spores of the latter can be formed on them. Extensive inoculation experiments in Israel (Wahl *et al.*, 1978) also showed that specialisation was not so strict in *E. graminis* from natural stands of wild grasses, where mildew cultures could be found capable of infecting a wide range of species and genera. Some of the wheat isolates used in the present work were also collected from grasses neighbouring wheat fields. The grouping of powdery mildew into *formae speciales* does not therefore imply a strict parasitic specialisation but rather an adaptation to certain host species (Hirua, 1978). According to Gabriel & Ellingboe (1982) and Torp & Andersen (1982), differences within *formae speciales* are small as disclosed by two-dimensional eletrophoresis of proteins from powdery mildew. Within the *formae speciales* attacking wheat, barley and oats distinctive physiologic races occur. These can be distinguished by the reaction of certain cultivars of the appropriate host. *E. graminis* is heterothallic (Powers & Moseman, 1956) and hybridisation between different *formae* may occur (Hiura, 1962; Moseman & Greeley, 1964). The heterothallism of the pathogen and hybridisation between *formae* result in the continued appearance of new physiological races of the fungus (Colhoun, 1971).

Symptoms

Symptoms may be found on leaves, sheaths, stems and inflorescences (Jones & Clifford, 1983) but leaves are most commonly affected (Parry, 1990). The fungus is conspicuous on the leaves, mainly on the upper surface, but sometimes underneath. The symptoms resulting from an attack of *E. graminis* are very similar on all cereals (Colhoun, 1971) although infection of glumes is more common on wheat (Jones & Clifford, 1983) than on other cereals.

The fungal growth on the surface of the plant, in the form of a superficial mycelium, produces a powdery appearance, particularly in the early stages after infection when spores (conidia) are produced in abundance. The first symptoms of mildew appear as white fluffy pustules: these may remain separate or they may run together to cover substantial areas of the leaf surface. Compared with barley mildew, colonies on wheat tend to remain more discrete (Jones & Clifford, 1983). As the mycelium ages it turns pale brown or grey. The older mycelium forms a thick mat in which are embedded small, black, globose bodies (cleistothecia). When plants are very severely infected the leaves may be deformed and ear development may be wholly or partially checked.

Infection process

Infection begins with the deposition of air-borne conidia, or less frequently ascospores, on the plant surface and involves direct penetration of the cuticle. Germination of conidia on leaves of the host is rapid, reaching a peak within 4-6 hours (Colhoun, 1971). Some workers state that germination occurs at low air humidities but others suggest that at least 85 per cent relative humidity is necessary (Colhoun, 1971). Studies by Manners & Hossain (1963) show that such differences may be due to variations in experimental conditions. They also demonstrate, however, that for ff.sp. *tritici, hordei* and *avenae* the optimum relative humidity is 100 per cent in the absence of free water, but some conidia germinate at 0 per cent relative humidity, the water required for germination then coming from within the spore. These workers reported that 20°C is the optimum temperature for germination at 100 per cent relative humidity, but the optimum temperature may vary according to the air humidity. Conidia can germinate over a wide temperature range (0.5-30°C) and the germ tubes formed can elongate at temperatures between 2 and 30°C (Colhoun, 1971). The best development of mildew occurs between 15 - 22°C Germination is inhibited by the existence of a water film on the leaf surface.

After landing on leaves, conidia germinate by producing from near one end, a short (5 - 10 μm), aseptate, non-appressorial germ tube (Fig. 2.1) in 0.5 - 2 hours (Carver *et al.*, 1995). This is called the primary germ tube. Contact with the host surface is

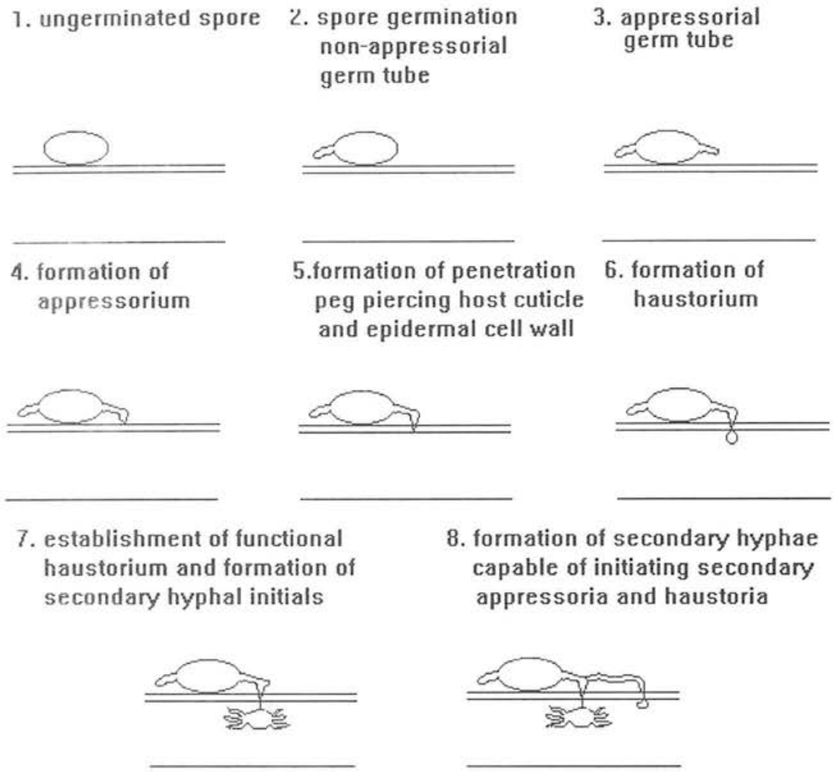


Fig. 2.1. Germination and development of a conidium of *E.graminis*. (After Ellingboe, 1972).

crucial to the continued development of germ tubes and differentiation of infection structures. Although the first germ tube to emerge remains short, it generally makes contact with the host, becoming attached to the epidermal surface and causing localised responses beneath the germ tube contact site. The first function of the primary germ tube is to attach the germling to the host surface and this happens within 1 - 2 hours after inoculation (Carver *et al.*, 1995). Carver and Bushnell (1983) proposed that a second function of the primary germ tube is to gain access to host water. A third function of the primary germ tube is in recognising the contact surface and in consequential intracellular signalling resulting in elongation of the second-formed germ tube, a prerequisite to appressorium formation (Carver *et al.*, 1995). If the first-formed germ tube fails to contact a host epidermal cell surface, it ceases development and presumably becomes redundant. Such tubes are termed subsidiary germ tubes (Kobayashi *et al.*, 1991). Where a subsidiary germ tube is produced, the next-formed germ tube will function as the primary germ tube upon host contact or become an additional subsidiary germ tube if contact fails to be made.

Shortly after a functional primary germ tube is established, a second germ tube which will form the appressorial germ tube appears. This elongates (ultimately 30 - 40 μm), becomes septate, and the tip of the distal cell swells to form the hooked appressorial lobe (by about 9 hours) (Carver *et al.*, 1995). Although germination and formation of appressorial initials are light independent, the maturation of appressoria are light sensitive, both darkness and high light intensity having an inhibitory effect (Masri & Ellingboe, 1966a,b). Penetration is effected by the development of a fine infection process from the appressorium. If this process succeeds in penetrating the host cuticle, host cell wall and defensive barriers laid down by the cell in response to attack, an haustorium is formed in the host epidermal cell within 15 - 20 hours (Carver *et al.*, 1995). Haustoria are characteristically elliptical in shape, with several finger-like appendages protruding from both ends. After the first haustorium is established within the invaded cell, the germ tube continues its apical growth as an ordinary hypha on the surface of the host leaf.

Elongation of the appressorial germ tube is a prerequisite to differentiation of the appressorial lobe from which penetration is effected. The time between emergence of the primary germ tube and of the appressorial germ tube varies between 15 - 60 minutes after the emergence of the primary germ tube (Carver *et al.*, 1995). Often, the primary germ tube and appressorial germ tube emerge from opposite ends of the spore (Fig. 2.1), enhancing the likelihood that they will encounter different host epidermal cells (Carver *et al.*, 1995). This is biologically important as host epidermal cells previously encountered show enhanced resistance to penetration (Carver & Ingerson-Morris, 1989). By 4 - 6 hours after inoculation, most germlings possess an appressorial germ tube that has elongated perceptibly. Extracellular material is secreted beneath this growing tube. A septum forms, and the distal cell starts to swell. There is a tendancy for elongating tubes to locate and follow suture lines between adjacent epidermal cells, which probably accounts for the elongated colonies in the early stages of attack. By 9 - 10 hours the tube reaches its mature length of 30 - 40 μm, and the hook-shaped, smooth-surfaced appressorial lobe is formed. Germlings on host leaves occasionally form a second germ tube that grows away from the leaf and makes no contact with it. Such tubes may grow to considerable length but they remain hypha-like, and an appressorial lobe is not differentiated. Carver & Ingerson-Morris (1987) demonstrated that appressorial lobe differentiation is stimulated by contact between the elongated tube itself and the host surface. Contact between the primary germ tube and host surface, which triggers second tube elongation, is insufficient to stimulate appressorial lobe differentiation. After the appressorial lobe differentiation, about 9 hours after inoculation, for the next few hours the gross morphology of the appressorium remains constant until either the germling dies or attempted infection succeeds and secondary hyphae grow out from branches formed on the appressorial germ tube. If attempted infection from the first appressorial lobe fails, a second lobe may form, proximal to the first and usually on the opposite side to the germ tube. The second appressorial lobe will in turn attempt penetration and if it fails a third lobe may form. Multiple appressorial lobes are commonly found on plants highly resistant to penetration (Carver *et al.*, 1995).

Wart-like bodies appear on the first appressorial lobe surface at about 15 - 18 hours after inoculation (Carver *et al.*, 1995). These bodies increase in number, eventually covering the distal cell of the appressorial germ tube. Wart-like bodies also form on the surfaces of established colonies in other regions, i.e, on hyphal appressoria, at hyphal branches and on conidiophore basal cells. They are similar to the globular bodies seen on conidia (Kunoch *et al.*, 1988) but their function remains to be determined.

After differentiation of the appressorial lobe, a penetration peg emerges from beneath the lobe. The penetration peg is blunt ended and appears to have no cell wall at its tip (McKeen & Rimmer, 1973). Enzymatic attack may be important in cuticle penetration (Pascholati *et al.*, 1992) and it seems probable that the host cell wall is digested enzymatically (Bushnell, 1972; Aist, 1976).

In addition to preformed defences, the host papilla deposited in response to attack must also be penetrated. If penetration is completed, the tip of the penetration peg swells to initiate haustorium formation and the establishment of biotrophic parasitism.

Secondary haustoria are formed in colonies usually within 4 days of inoculation. The mycelium is entirely superficial; only haustoria lie within the host tissue and these are mostly restricted to the epidermal cells. After 5 days, small swellings appear on the surface of hyphae giving rise to conidiophores which may be fully developed 2 days later (Smith & Blair, 1950). Conidiophores arise at right angles from the hyphae at the leaf surface. They are short and consist of a swollen basal cell and a terminal generative cell, which forms a chain of 10-12 conidia with the older terminating the chain. The infection cycle from conidial germination to sporulation is normally 7-10 days (Gjaerum, Tjamos & Viranyi, 1988). As host plants mature, the mycelium darkens in colour and small, dark-coloured, spherical cleistothecia are developed. These are globose at first, but become strongly depressed. In some of the cleistothecia asci containing ascospores are produced. Ascospores are rarely produced on actively growing plants but may be formed after the host tissues have senesced.

Spread of the disease

Mildew survives the winter primarily as a mycelium on living material in the form of volunteer cereals and autumn sown crops (Parry, 1990). The disease can spread significantly by spores from the mycelium during mild weather in the winter, but spread occurs mainly as temperatures rise in spring when the conidia are produced in large numbers and the disease spreads faster; at first to the neighbouring plants and then as spore numbers increase to adjacent crops (Gair *et al.*, 1978). Excessive nitrogen encourages the disease and mildew can be particularly severe in dense crops grown in sheltered, humid places (Parry, 1990). In wheat, the disease is usually most severe on the lower leaves but new leaves are infected as they are produced and these and the ears can be severely affected. Cleistothecia are usually formed after a spell of very warm weather. Pustules with cleistothecia stop producing conidia. Cleistothecia serve two purposes. First, they represent the sexual stage in the life history of the fungus and are therefore the means by which recombination of genetic material can occur and provide the opportunity for the production of new races of the fungus. Second, the cleistothecia are the main means by which the fungus survives the period before and during harvest when there is little or no green plant material available (Gair *et al.*, 1978).

Although *E. graminis* f.sp. *hordei* reproduces asexually during the two annual epidemics in autumn and summer, a sexual cycle occurs in midsummer (Smedegard-Petersen, 1967). Therefore the autumn epidemic is initiated partly by ascospores, the results of sexual reproduction, which infect emerging winter barley seedlings and partly by asexual conidiospores, produced on volunteer plants that germinate from grain that falls from the standing crop or during the harvest (Brown, 1994). It is likely that the relative importance of sexual compared with asexual reproduction is generally low but may vary from place to place and from year to year. Despite the uncertainty about its frequency, sexual reproduction may occur sufficiently often to have a significant effect on population structure.

Epidemics of mildew on cereals are initiated by a large number of spores (O'Hara & Brown, 1996). The epidemic develops quickly as a large number of foci (O'Hara, 1996). During the epidemic the fungus produces asexual conidiospores, which are wind-dispersed, often in large numbers, depending on environmental conditions

(Turner, 1956). Although most of these spores land close to their parental colony, some spores escape the canopy to join the migrant airborne population (Fitt *et al.*, 1987). Wolfe & Schwarzbach (1978) suggested that in spring barley, most of the inoculum which initiates an epidemic comes from outside the crop.

Because of the multifocus nature of mildew epidemics, once such an epidemic is established, almost all of a field becomes infected to some extent (O'Hara & Brown, 1998). The many infections in the field all produce spores. These spores are dispersed away from their point of origin, and the distances over which they disperse is described by their dispersal gradient (Fitt *et al.*, 1987). Once an epidemic is established, spores arrive on a plant from various sources; from other parts of the same plant, from other nearby plants and from the general aerial population of spores.

It has been generally accepted that conidia are short-lived and are unsuitable for long range dispersal (Bruehl, 1967) but doubt has been expressed about this opinion. Wolfe (1967) showed that cereal seedlings isolated in central London from the main inoculum sources by a distance of 12.9 km became infected. Work in Denmark showed that the epidemic in that country owes its annual origin principally to that occurring in Germany. Hermansen *et al.* (1978) showed that viable mildew spores could travel several hundred kilometres between the UK and Denmark. So it appears that conidia play an important role in spread from one locality to another (Colhoun, 1971).

Of environmental factors, the most important influence on disease spread is temperature. Spread occurs more slowly at lower temperatures but spore production is restricted above 25°C (Gair *et al.*, 1978). Severe attacks of mildew may be expected when optimum conditions prevail, i.e. the host is growing rapidly or is responding to nitrogen fertiliser, the weather is warm and there is a plentiful supply of spores. In winter cereals, which grow over a longer period of time than spring crops, a single crop may be subjected to more than one severe attack.

Effect of mildew on the plant and yield

According to Gair *et al.* (1978), mildew affects the plant in various ways. It diverts food materials from the plant to the pathogen and reduces the amount of photosynthetic tissue by causing premature leaf death. It has several adverse effects on the physiology of the plant, including increasing the rates of respiration and transpiration. These changes in turn affect the plant in a number of ways. Severe early attacks of mildew reduce the number of fertile tillers and the size of the ear; they also reduce the size of the leaves which may affect the size of the grain. Later attacks, after the size of the plant has been determined, largely affect the size of the grain though severe attacks may also reduce the number of grains because affected grains do not mature or are so small that they are lost during harvest. A further important effect of mildew, especially associated with attacks in the early growth stages, is the reduction in the size of the root system which in turn can have serious effects on yield especially if crops are subjected to soil moisture stress as commonly occurs in summer. This effect is particularly significant in spring-sown crops when seedlings are affected by mildew. Brooks (1972) observed that early and severe attacks of powdery mildew on spring barley plants greatly reduced vigour and decreased the amount of root, the number of ear-bearing tillers and grain size. Early attack on winter barley retarded development of the root system, increased winter kill and decreased the number of ears reaching maturity. According to O'Rourke (1976), mildewed plants become more sensitive to stress (e.g. drought and low temperature). In the winter crop mildew attack decreases frost hardiness due to the shortage of assimilates that are necessary for hardening.

Grain yield losses are related to the severity and earliness of mildew infection and vary with other factors, especially soil moisture. In relating yield loss to the results of a national disease survey of cereal diseases in the UK, Cook & King (1984) used the formula $y = 2.5\sqrt{x_i}$ for mildew on spring barley and $y = 2.0\sqrt{x_i}$ for mildew on winter wheat, where y = % loss in grain yield and x_i = % disease on leaf 3 at growth stage (GS) 58. Further financial losses can be incurred where mildew causes a reduction in the quality of the harvested grain, by increasing the number of small or shrivelled grains.

Control

The principal methods of controlling powdery mildew are the use of genetic resistance of the host plant, the use of fungicides and through integrated control where variety mixtures are supplemented by fungicides. Control by cultural methods, such as reducing the carry-over of inoculum on stubble, volunteer plants and winter cereals, is also practised. The risk of economic loss due to powdery mildew is reduced by the use of resistant cultivars but the emergence of races in the pathogen population which possess virulence factors to overcome specific resistant factors in the host presents an underlying problem in the exploitation of genetical resistance. Diversification of cultivars has been recommended as a means of restricting the spread of a pathogen race which has specific virulence genes for one cultivar. In spring barley, the *mlo* gene for resistance has provided an important source of resistance to barley powdery mildew over several years, having been deployed in commercial cultivars since 1979 (Jørgensen, 1992; 1994).It has been used with increasing frequency in European spring barley, reaching over 70% of the UK spring barley seed production in 1993 without any erosion in its effectiveness (Newton & Young, 1996). The durability of this monogenic form of resistance, conferred by a series of recessive alleles (Jørgensen, 1992) is unusual. However, Baker *et al.* (1998) recognise that, despite its apparent durability, the dependance on a single gene over a widespread area may pose a serious threat to future spring barley production. An *mlo*-aggressive race of barley mildew from Japan has been reported (Lyngkjaer *et al.*, 1995), while Baker *et al.* (1998) have described the temporary partial breakdown of *mlo*-resistance by the sudden relief of soil water stress, and this could be of importance in speeding up the evolution of aggressive isolates. For winter barley and wheat mildew resistance, there are combinations of minor and major genes in a horizontal, polygenic background (A. Newton, pers. comm.). Although resistance genes have reduced infection levels, changes in the virulence spectrum of the pathogen population continues to present problems to the breeder. Powdery mildew remains important in cereal growing and farmers must rely for control on the application of fungicides. Genetic variation in the mildew population again gives rise to problems, in this case with respect to the level of sensitivity to fungicides.

Up to 1996 three classes of systemic fungicides had been recommended in Britain for control of barley and wheat mildew: hydroxypyrimidines, demethylation inhibitors (DMIs) and morpholines (Table 2.1). However, in common with systemic fungicides in general, problems of fungicide resistance have been encountered. Ethirimol resistance was found among populations of *E. graminis* (Shephard *et al.*, 1975) and the pyrimidine fungicide was soon replaced by triazoles (DMIs). From 1978 onwards these were used exclusively for mildew control, but soon there was a decline in their sensitivity (Fletcher & Wolfe, 1981) and effectiveness. Control of powdery mildew then relied almost exclusively on morpholines until 1997. New products, for example quinoxyfen (Fortress), were consequently developed by the agrochemical companies with novel modes of action to control mildew (Heye *et al.*, 1994; Dutzmann *et al.*, 1996; Hollomon *et al.*, 1996; Longhurst *et al.*, 1996; Ruess *et al.*, 1996).

With the current heavy reliance for control on the morpholine fungicides, selection pressure on the mildew population must be high. Prior to this project, the Scottish

Agricultural College (SAC) at Edinburgh had been monitoring the sensitivity of barley isolates collected mostly from Scotland but also from other parts of Britain, to morpholines since 1988. The results of this survey (1988-1990) showed that over this period the mean sensitivity of isolates tested had changed little, and EC_{50} values for different years fell within the same range. There were, however, slight but significant differences in the mean sensitivity of isolates between seasons, demonstrating that the population was not stable in terms of sensitivity to morpholines (Robertson *et al.*, 1990).

Table 2.1. Systemic fungicides used to control cereal mildew up to 1997.

Hydroxypyrimidines	**DMIs**	**Morpholines**
Ethirimol (barley seed)	Nuarimol (3,6,8)*	Fenpropimorph (1,2,3)
	Prochloraz (3,6,7,8)	Tridemorph
	Propiconazole (1,2,3,6,7,8,11)	Fenpropidin (1,2,4,5,6) (piperidine)
	Triadimenol (spray and in seed treatment mixtures) (2,3,10)	
	Triforine (barley) (8)	

***Other diseases controlled:**

1 Yellow rust
2 Brown rust
3 *Rhynchosporium*
4 Glume blotch
5 Leaf spot
6 *Septoria* spp.
7 Eyespot
8 Net blotch
9 Sharp eyespot
10 Snow rot
11 Sooty moulds

Fungicide Resistance /Insensitivity

When a fungicide controls a fungus effectively at the recommended dose, the fungus is classed as 'sensitive' to the fungicide. However sometimes strains of the fungus may arise, (usually by mutation) which are less sensitive and the pathogen is not adequately controlled. Such a decrease in sensitivity may be caused by genetic or non-genetic changes in the fungal cell. Non-genetic changes are not stable and usually disappear rapidly in the absence of the toxicant (Dekker, 1972). They are therefore of little importance in practice. Genetic changes are more serious. Strains with such changes may be called 'resistant' or 'insensitive'. Fungicide resistance/insensitivity may be defined as the stable, inheritable adjustment by a fungus to a fungicide, resulting in a less than normal sensitivity to that fungicide (Delp & Dekker, 1985). The term resistance will be mainly used in these studies.

Although there are many cases of disease-control failure due to fungicide resistance after application of systemic fungicides (site-specific inhibitors), hardly any such problems have arisen during a century of application of protectant fungicides (multi-site inhibitors) like copper compounds and dithiocarbamates. The few cases of resistance to multi-site inhibitors include the development of resistance to mercury in the oat leaf stripe pathogen, *Pyrenophora avenae* (Noble *et al.*, 1966) and in *P. graminea* which causes a similar disease in barley (Jones *et al*, 1989).

Historical background

Before the 1960s, nearly all fungicides used in agriculture acted at multiple subcellular targets (Henry, 1992). Their biochemical specificity was limited and this restricted their application to external plant therapy. These fungicides (multi-site inhibitors) fall into two distinct groups. The first includes the inorganic Bordeaux mixture and the other copper fungicides and the sulphur compounds. The second group includes the organic compounds such as maneb, dichlone, captan and chlorothalonil. The discovery of fungicides able to be transported within the plant and with specific modes of action brought about the benefit of systemic protection of the plant as well as curative and eradicant disease control, but these were often followed by the problem of resistance.

The mechanism of fungicide resistance is often related to the mechanism of fungicide action (Henry, 1992). It was inevitable that the invention of fungicides with specific biochemical mechanisms would lead to fungicide resistance and resistance mechanisms based on target site changes in the fungus (Henry, 1992).

The first reports of evidence of fungicide resistance date back to the early to mid 1960s. These included resistance to biphenyl fumigation and orthophenylphenate dipping in *Penicillium* spp. causing post-harvest rots in lemons in Carlifonia (Harding, 1962); resistance to hexachlorobenzene seed treatment in *Tilletia foetida* the cause of bunt disease in wheat in Australia (Kuiper, 1965); and resistance to organo-mercurial seed treatment in the oat leaf-stripe pathogen (Noble *et al.,* 1966). These early cases occurred many years after the initial introduction of the non-systemic fungicide treatments concerned. Although they caused considerable difficulty in disease control in some situations, they have proved to be of relatively limited significance in comparison with the more widespread and immediate problems encountered with most of the systemic fungicides which were introduced from the late 1960s onwards.

In following the use of systemics to control cereal mildew, ethirimol was first introduced as a seed treatment ('Milstem') in 1969 (Brooks, 1971), but subsequent monitoring of *E. graminis* populations revealed some decline in sensitivity (Shephard *et al.*, 1975), especially where ethirimol use was frequent. These changes were accompanied by some decline in its effectiveness, although yield benefits were often still obtained. From 1978 onwards, ethirimol was largely replaced, in England at least, by broader spectrum triazoles (DMIs), both as sprays (Bayleton = captafol + tridimefon, Tilt = propiconazole, Radar = propiconazole, Bayfidan = triadimenol) and as a seed treatment (Baytan = fuberidazole + triadimenol). Intensive use of these triazoles was soon followed by some decline both in sensitivity (Fletcher & Wolfe, 1981) and effectiveness, especially on powdery mildew susceptible varieties. Again, however, yield benefits could still be obtained. The decline in effectiveness of triazoles continued and by the mid-1980s the activity of the DMIs had declined to a level where disease control failure was commonly encountered (Clark, 1992).

Reduced sensitivity of *E. graminis* f.sp. *hordei* isolates to tridemorph, introduced in the 1960s (Pommer, Otto & Kradel, 1969), was reported in 1979 by Walmsley-Woodward, Laws & Whittington (1979) and Hollomon (1982) while reduced sensitivity to fenpropimorph has been detected since 1986 (Wolfe, Slater & Minchin, 1987). Robertson *et al.,* (1990) reported variation in responses to *E. graminis*. f sp. *hordei* to the three morpholines in south-east Scotland. Work by Brown & Evans (1992) in Scotland showed that there was substantial variation in the sensitivity of *E. graminis* f.sp. *hordei* to fenpropidin. They reported isolates that were resistant to reduced doses of tridemorph, fenpropimorph and fenpropidin and in addition reported cross-resistance between fenpropimorph and fenpropidin. They indicated, however, that the levels of resistance they found were unlikely to cause a substantial loss of effectiveness of the chemicals immediately after spraying.

Since then, reports have come from France especially Northern France that the effectiveness of fenpropimorph has weakened and that more frequent applications are now needed to achieve a high level of control of wheat powdery mildew (Readshaw & Heaney, 1994). A small decrease in the sensitivity of *E. graminis* f. sp. *tritici* to fenpropimorph has also been found in a number of other European countries; the Netherlands (De Waard, 1992), Germany and Switzerland (Lorenz, Saur &

Schelberger, 1992).

Mechanisms of resistance

Fungi may become insensitive to fungicides by the following mechanisms (Dekker, 1977b; 1984; 1987):

(I) A change at the site of inhibitor action which results in a decreased affinity to the fungicide. There are an increasing number of fungicides, where insensitivity can be attributed to this mechanism, for example, carbendazim, the toxic principle of benomyl and thiophanate-methyl, which binds to tubulin, the major constituent of microtubules, which constitute the spindle. The assembly of microtubules is prevented, and as a consequence mitosis and other cellular processes in which microtubules are involved, are inhibited. Resistance to carbendazim is caused by a single gene mutation, resulting in slightly changed tubulin with a reduced affinity to carbendazim (Davidse, 1982). Insensitivity to multi-site inhibitors cannot arise in this manner as it would require changes at many sites.

(II) Decreased uptake or decreased accumulation of the fungicide in the fungus. The fungal cell may become less sensitive to a fungicide by changes which keep the fungicide from reaching the site of action in sufficient quantity. These changes may hamper entrance by the fungicide through the membrane, or they may lead to increased efflux immediately after entrance, preventing accumulation. Polyoxin antibiotics, which interfere with chitin synthesis in fungi, are used for control of black spot in Japanese pear. Strains of *Alternaria kikuchiana*, resistant to polyoxin B, appeared in orchards treated with the antibiotic, resulting in failure of disease control. Resistance was not due to a change in the target site, chitin synthetase as this enzyme was equally inhibited in cell free systems of resistant and sensitive strains, but it appeared to be caused by a change in the fungal membrane, resulting in reduced uptake (Misato, Kakiki & Hori, 1977). Resistance by this mechanism can affect both conventional and systemic fungicides. The most widely reported and studied mechanisms of azole resistance invole a reduction in the intracellular accumulation of these agents (Hitchcock, Barrett-Bee & Russell, 1986; Goldway *et al.*, 1995)

(III) Detoxification of the fungicide before the site of action has been reached or lack of conversion of a compound into the fungitoxic principle. There may be changes within a cell which increase its capacity to detoxify the fungicide. This may happen by conversion of the fungicide into non-fungitoxic compounds or by binding to other cell constituents before the sites of action have been reached. This has been found to occur rarely. Kitazin-P (5-benzyl 0,0-diisopropyl phosphorothiolate), which among others is used for control of rice blast, interferes with the biosynthesis of phosphatidylcholine. Strains of the causal organism, *Pyricularia oryzae*, were obtained with a moderate, or a high level of resistance to this fungicide. The resistant mechanism of the latter has not been elucidated, but that of the former appears to be due to cleavage of the S-C bond of the molecule by the pathogen, which gives non-fungitoxic derivatives (Uesugi & Sisler, 1978). This mechanism can affect both conventional and systemic fungicides.

(IV) As a counterpart of detoxification the fungicide may convert an inactive compound into a fungicide, i.e. lethal synthesis. This was suggested to occur with *Cladosporium cucumerinum* exposed to 6-azauracil (Dekker, 1972). For antifungal activity 6-azauracil has first to be converted to 6-azauridine-5′-phosphate via 6-azauridine involving the enzymes uridine phosphorylase and uridine kinase. Resistance was associated with the loss of activity of one or the other of these enzymes.

(V) Compensation for the inhibitory effect, e.g. by an increased production of an inhibited enzyme. An example of this phenomenon is strain R II-1 of *Cladosporium cucumerinum*, which is resistant to 6-azauracil. 6-azauracil, after conversion into its nucleotide 6-azauridine monophosphate, inhibits primarily one enzyme in pyrimidine biosynthesis, namely, orotidine monophosphate decarboxylase. The activity of this enzyme was determined in the resistant strain and the wild type fungus, using carboxyl-labelled orotidine monophosphate according to a method described by Dekker (1968). It appeared that the resistant strains produced at least three times as much of the enzyme than the the sensitive fungus (Dekker, 1971). As a consequence the resistant fungus needs more of the fungicide to be inhibited, i.e. it is more insensitive. This mechanism of insensitivity is rarely found.

(VI) Circumvention of the blocked site by the operation of an alternative pathway. If a fungicide blocks a reaction at one site in the fungal metabolism, the fungus may adapt to this situation by shifting its metabolism in such a way that the blocked site is bypassed. Antimycin A acts upon the electron transport in the respiratory chain between cytochromes b and c. Insensitivity of *Ustilago maydis* to this antibiotic could be attributed to a shift in the electron transport at a site preceding cytochrome b to an alternative terminal oxidase (Georgopoulos & Sisler, 1970). This alternate pathway is apparently of considerable value to *U. maydis* for growth in the presence of the antibiotic, which is demonstrated by the fact that a mutant, which lacked this alternative pathway, appeared sensitive to antimycin A. This mechanism of insensitivity is rare.

Genetics of resistance

The emergence of tolerance to fungicides due to non-genetic factors, e.g. adaptive enzyme formation (Partridge & Rich, 1962), seldom reaches a high level and is usually lost after transfer to a fungicide free environment (Dekker, 1972). Of much greater practical importance is resistance which develops due to stable heritable changes following exposure to the antifungal agent (Dekker, 1984). Early work on the genetics of fungicide resistance related to members of aromatic hydrocarbons. Five loci for resistance to aromatic hydrocarbons were identified in *Nectria* *haematococca* by Georgopoulos & Panopoulos (1966) and two in *Aspergillus nidulans* (Threlfall, 1968). Studies on the genetics of fungicide resistance have extended and intensified with the introduction of systemic fungicides and the attendant high risk of the development of resistance problems. Between the systemic fungicide groups, however, there is considerable variation in the risk. The frequency with which fungicide resistant strains will emerge depends in part on the number of mutations required for a certain level of resistance. When a fungicide acts as a multi-site inhibitor more mutations would be expected to be needed for resistance and probably account for the fact that little or no genetical resistance is being found in such non-systemic compounds as the dithiocarbamates and metal toxicants which act at many sites. On the other hand single mutations affecting uptake of toxicants can potentially lead to resistance to multisite inhibitors.

Georgopoulos (1987) distinguished two types of resistance based on the form of genetic control. Resistance to some of the agricultural fungicides develops in one step as a result of mutation of one gene which has a major effect on the phenotype. Such a mutation may achieve the highest level of resistance possible. This does not mean that only one chromosomal locus controls sensitivity to each type of fungicide. If more loci are involved, there is no positive interaction between mutant genes at different loci and a stepwise increase of resistance is not possible. In other words, a mutant allele at one locus is epistatic over wild type allelles at other loci. With this type of genetic control of sensitivity, field populations of sensitive fungi give a discontinuous distribution, i.e. each population consists of at least two distinct subpopulations, one sensitive and one resistant. Because of the lack of overlap between subpopulations, each isolate can be easily classified as unequivocally resistant or sensitive (Georgopoulos 1986).

A stepwise development of resistance is observed with some fungicides which do not belong to the above group. Usually a single mutation may have measurable effects on sensitivity (although not necessarily, particularly under field conditions), but many mutant genes are required to achieve the highest level of resistance possible. The various combinations of resistant genes, and often also modifiers, result in a continuous distribution of sensitivities, so that distinct subpopulations cannot be recognised even after long exposures. And if highly resistant and wild type strains, which differ in many gene pairs, are crossed, a Mendelian ratio of resistant to sensitive progeny cannot easily be recognised. Frequently, the progeny from such a cross will produce a unimodal frequency distribution with no discrete classes with respect to sensitivity. This type of control of fungicide resistance is termed polygenic Georgopoulos (1987).

Brown (1996) has studied the genetics of resistance of *E. graminis* f.sp *hordei* to morpholines, ethirimol and conazoles (triazoles & imadazoles). As far as morpholines are concerned, three types of isolates were collected in a survey in 1988; type S which were sensitive to fenpropidin, fenpropimorph and tridemorph, similar to isolates sampled before morpholine fungicides were introduced; type R which were more fenpropimorph and fenpropidin resistant than any S isolate although they were no more resistant to tridemorph than S-types; and type RM which had greater resistance to fenpropimorph and also showed some resistance to tridemorph. He came up with a number of hypotheses about the genetics of resistance to these chemicals. It was predicted (i) that responses to fenpropimorph and fenpropidin are controlled by the same gene or genes; (ii) that, as the R and S groups of isolates were both genetically diverse, one or very few genes would control resistance; (iii) that the gene or genes in R-type isolates which confer resistance to fenpropimorph and fenpropidin would have little or no effect on tridemorph sensitivity and that either (iv) the fenpropimorph/fenpropidin resistance gene or genes in the RM-type isolates would also confer some resistance to tridemorph or (v) these isolates would have a separate gene conferring reduced tridemorph-sensitivity. The first four predictions were confirmed by genetic analysis.

Of the three main groups of mildew fungicides, Brown (1996) reported that ethirimol sensitivity is under the simplest genetic control in *E. graminis* f.sp *hordei.* A population survey carried out in 1985 detected two groups of isolates. One group was distictly more resistant to etirimol than the other, and there were no intermediate types (Brown & Wolfe, 1990). An RFLP (Restriction Fragment Length Polymorphism) study showed that both the resistant and sensitive groups were genetically diverse (Brown *et al.*, 1990). This led to the hypothesis that ethirimol resistance is controlled by one or very few genes. In crosses of a resistant isolate with each of two sensitive isolates, two distinct groups of progeny segregated. Responses to ethirimol of the sensitive and resistant isolates were similar to the sensitive and the more resistant parents respectively. In both crosses the frequencies of the two classes of progeny fitted 1:1 ratios, indicating that resistance in the more resistant form was was probably conferred by a single allele. No sensitive progeny segregated in a cross of the more resistant isolate with another resistant isolate. It was concluded that one gene controls most or all of the variation in ethirimol responses in these crosses.

Brown (1996) also reported on the genetics of responses to conazoles by *E. graminis* f.sp. *hordei.* Resistance to conazole fungicides is more complex than that to morpholines and ethirimol with several genes involved. In a survey of *E graminis* f.sp. *hordei* in England in 1985, four distinct levels of resistance to triadimenol were detected. There were isolates that were sensitive and those that had a low, medium or high resistance to triadimenol (Brown & Wolfe, 1991). Isolates with a fifth, very high level of resistance were detected in 1988 (Brown *et al.*, 1991b). Distinct levels of resistance to triadimefon were also detected in Germany in 1987 (Wohlleber *et al.*, 1993). Isolates of low, medium and high resistance detected in England in 1985 were genetically diverse as determined by RFLP (Brown *et al.*, 1990). As for ethirimol, it was predicted that each level of resistance would be under fairly simple genetic control. Triadimenol resistance was studied in six crosses (Brown *et al.*, 1992). In one cross of a sensitive isolate with one of medium resistance, all progeny either had medium resistance or were as sensitive as the sensitive parent with a 1:1 ratio segregation. So it was concluded that a single gene controls the triadimenol resistance of the medium resistant isolate. Similarly, another cross between the same sensitive isolate and another isolate with medium resistance resulted in responses indicating control of resistance by a single gene. Three other crosses between a sensitive isolate and one with high resistance have also been studied (Brown *et al.*, 1992; Brown *et al.*, 1996b). In all three cases, the triadimenol responses of the progeny and the segregation ratios were consistent with resistance being controlled by single genes (Brown, 1996). Tests for the existence of minor genes controlling additional variation showed no evidence that any gene affected responses to triadimenol, other than the identified major gene (Brown *et al.*, 1992; Brown, 1996). The linkage of triadimenol response genes to avirulence genes has shown that more than one locus controls triadimenol resistance (Brown, 1996). Thus, there is more than one resistance locus in *E. graminis* f.sp. *hordei* even though only one gene had segregated in each of the studies that had been done by 1996 (Brown, 1996).

Emergence of resistance in the field

The factors that result in the development of resistant strains in the pathogen population and those that determine whether these strains will cause problems in practice, are quite complicated. They can, however, be resolved into four fundamental components; the fungicide, the way it is used, the pathogen and the characteristics of the resistant mutants (Jones, 1981; Wade, 1982).

The fungicide properties govern to a large extent the rate at which resistant strains are likely to develop. The more non-specific a chemical is with respect to its mode of action, the less chance there is of resistant strains occurring (Wade, 1982). Multi-site inhibitors, which characterise the conventional non-systemic fungicides, interfere with a variety of vital metabolic processes. Multiple differences or modifications in the pathogen's genome are necessary to overcome this concerted action. Resistance will occur only after intensive and prolonged use: this is why for example the dithiocarbamates, known to inhibit more than twenty different isolated enzymes, have not suffered serious resistance problems since their introduction (Wade, 1982). However resistance caused by decreased uptake or increased detoxification through metabolism is possible against these compounds (Dekker, 1983; Staub & Sozzi, 1984).

Systemic fungicides, in contrast to non-systemic ones, have a greater degree of biochemical specificity and tend to inhibit only one or two metabolic sites (Wade, 1982). Alterations in only one fungal gene are often sufficient to induce change at the site of action and a resistant strain may emerge. Consequently, resistance to these types of fungicide is more common and can occur sooner after the introduction of the product.

Examples of resistance to systemic fungicides are found with the benzimidazoles and thiophanates, dimethirimol, the antibiotics kasugamycin and polyoxin and metalaxyl (Delp, 1980). According to Wade (1982), these compounds would not have suffered serious practical problems if it had not been for additional influences that led to the dominance of the resistant strains in the pathogen population. In the cases of benzimidazoles, carboximides and phenylamides these influences followed a similar pattern (Russell, 1995). The first major problems developed with the introduction of new areas of fungicide chemistry. The three groups were introduced as single products and were widely used. Resistance to each was under simple genetic control and resistant isolates were easily selected out of natural populations (Russell, 1995).

Of most influence is the 'fitness' of the resistant strains. Fitness refers to the virulence and/or competitive ability of a pathogen (Wade, 1982). It refers to the survival of a pathogen within a large population of organisms and its ability to grow normally and reproduce on its host. For example, some fungicide-resistant forms are not so well adapted for survival and may gradually die out in the absence of the fungicide; this is a result of competition from other, sensitive, forms of the same pathogen and from other organisms (Anon., 1987).

Development of resistance to fungicides is influenced by the fitness of populations to all factors favourable to the production of progeny (Hartl, 1980). Dekker (1995) states that the fitness of fungicide-resistant strains seems to relate to the mechanism by which the pathogen becomes resistant to a fungicide. Some genetic changes conferring resistance are linked to lower fitness in the absence of the fungicide while others are not. If resistance is correlated with reduced fitness, it may slow down resistance developing significantly (Chin, 1987; Milgroom, Levin & Fry, 1989; Shaw, 1989). Most models of the evolution of resistance to fungicides have assumed fitness costs associated with resistance. Although there has been much speculation in the literature with regard to fitness costs associated with fungicide resistance genes, the available evidence is contradictory, e.g. some studies have demonstrated fitness costs associated with resistance (de Waard, Groeneweg & van Nistelrooy, 1982; Miyagi, Hirooka & Araki, 1986; Webber, 1988; Nuninger-Ney, Schwinn & Staub, 1989; de Waard & van Nistelrooy, 1990), while others have not (Smilanick & Eckert, 1986; Crute & Harrison, 1988; Kalamarakis *et al.*, 1989; Lasseron De Falandre, Daboussi & Leroux, 1991). One study found that isolates of *Phytophthora infestans* resistant to metalaxyl had greater fitness than the sensitive isolates, regardless of the presence of the fungicide (Kadish & Cohen, 1988).

Most experiments to measure fitness costs associated with resistance to fungicides have measured fitness in terms of fitness 'components' such as germination frequency, latent period, lesion size, colony forming ability and sporulation, termed as 'predicted' fitness methodology as opposed to 'realised' fitness in which the growth rates of a small number of isolates are compared over several generations (Peever & Milgroom, 1994).

Researchers have studied different component costs of different fungus/fungicide combinations. Engels & de Waard, (1996) studied the fitness of field isolates of *E. graminis* f.sp. *tritici* with reduced sensitivity to fenpropimorph. They studied a broad range of fitness components such as germination, latent period, colony forming ability and production of conidia. In addition they studied the competitive ability and survival in the winter of the isolates. The best method to assess fitness seemed to be competitive ability since results obtained in the various competition experiments were consistent. Using this method they found that the competitive ability of fenpropimorph sensitive (FS) isolates was higher compared to fenpropimorph resistant (FR) isolates in the absence of fenpropimorph, regardless of the composition and complexity of the mixture. This conclusion was based on the observations that the proportion of (FR) isolates in mixtures decreased during the course of the experiments and that at the end EC_{50} values of mixtures were similar to those of wild type isolates. There was a suggestion that in periods without fenpropimorph application and with high mildew infection, the frequency of (FR) isolates may decrease in practice.

Similar results were obtained by Buchenaur & Hellwald, (1985) on work with *E. graminis* on barley and wheat and sterol C-14- demethylation inhibitors and by Al-Mughrabi & Gray, (1995). Al-Mughrabi & Gray tested the competitive ability of triadimefon-resistant and sensitive isolates of *E. graminis* f.sp. *tritici* in mixed inoculations of the fungus using a test tube method. They adapted this method, as measuring fitness in the field is difficult due to the migration of conidia within the field and from outside sources. They studied fitness by growing mixtures of resistant and sensitive isolates for five generations while monitoring the change in sensitivity. They obtained highly significant differences among generation cycles in terms of mean values of log (EC_{50}) for mixtures of resistant and sensitive isolates, indicating that there was a decline in the proportion of resistant isolates as a result of competition with sensitive ones, for five generations in the absence of triadimefon. Results of the LSD test indicated that the mean value of the log (EC_{50}) of all isolate mixtures decreased from 2.39 ± 0.22 μg triadimefon per ml in the first generation to 0.80 ± 0.22 and -0.38 ± 0.22 μg triadimefon per ml after keeping the mixtures of resistant and sensitive isolates for three and five generations respectively, in the absence of triadimefon.

In the studies by Engels & de Waard, (1996), analysis of fitness components of individual isolates demonstrated that the reduced competitive ability could not be attributed to a specific effect on either germination, latent period, colony forming ability or production of conidia. No relationship between single fitness components and sensitivity was observed. Similar results were obtained for field isolates of *E graminis* f.sp. *hordei* resistant to ethirimol and tridemorph by Laws, Walmsley-Woodward & Whittington, (1982), isolates of the same fungus resistant to triadimefon by Butters, Clark & Hollomon, (1984), triadimefon-resistant field isolates of *E. graminis* f.sp. *tritici* by Buchenauer & Hellwald, (1985) and DMI-resistant field isolates of *Pyrenophora teres* by Peever & Milgroom, (1994).

When fungicide resistance is accompanied by reduced fitness, such as in some of the the above mentioned studies, the risk of development of resistance problems in commercial practice may be considered as moderate or low, since low fitness counteracts the build-up of resistance (Al-Mughrabi & Gray, 1995).

Studies with other fungi/fungicide combinations have, however, shown that decreased fitness of resistant isolates is not always the rule (Crute & Harrison, 1988).

Variation in experimental results among studies may be explained by the use of different measures of fitness, fungicides, pathogens or sources of isolates. Another possible explanation is that most of the studies involved few pathogen isolates and fitness differences between resistant and sensitive isolates may have been due to differences in the genetic backgrounds of the isolates rather than fitness costs associated with resistance genes (Peever & Milgroom, 1994).

If the mutation that confers resistance adversely affects fitness, it is unlikely that those strains will dominate the population. For example, on strawberry, strains of *Botrytis* resistant to dicarboximide fungicides can be readily demonstrated in the field and the laboratory (Wade, 1982). They are often inferior to sensitive isolates in terms of viability and pathogenicity and field resistance has not become a problem. But if the mutation that leads to resistance has no serious effect on fitness, for example phenylamide-resistant isolates may be as competitive as sensitive ones (Davidse, 1988), then the fungicide selectively reduces the sensitive wild population (Wade, 1982). The resistant strains will survive and spread at a rate which will depend on the following; (1) the degree of resistance acquired by mutant strains; (2) the number of resistant spores in the pathogen population; (3) the amount of inoculum that survives to the next season; (4) the type of disease and (5) the intensity of fungicide use (Wade, 1982). Fungicide use is crucial and is the criterion over which the grower has most control. Thus an understanding of how it affects the build-up of resistance is of paramount importance.

The intensity of use of a fungicide is a function of five factors: (1) the dose rate, (2) the number of applications per season, (3) the area treated, (4) the efficiency with which the area is covered and (5) the persistence of the chemical (Wade, 1982). Wade (1982) goes on further to say that the higher these parameters, the greater will be the selection pressure exerted on the pathogen population, particularly if only one type of fungicide is being used. Fit resistant strains will be selected for very rapidly and will soon become dominant. Benomyl, for example, was a such an improvement on existing chemicals when first marketed that it was used exclusively and intensively in many situations worldwide (Wade, 1982). However, excellent disease control was in many cases followed by severe resistant problems within two to four seasons. By 1980, there were at least 23 fungal genera resistant to benzimidazoles (Delp, 1980). Intense selection pressures occur more easily in confined areas such as greenhouses and fruit packing stations. Complete and efficient coverage is easily achieved and dilution of resistant strains by untreated sensitive ones does not occur as readily as in the field.

The speed with which a resistant pathogen population will build up in the field, can be influenced by the type of disease or by the nature of the pathogen (Dekker, 1983; 1987). In the presence of the fungicide, resistance will build up more rapidly in an abundantly sporulating pathogen on aerial plant parts than in a pathogen which sporulates sparcely and spreads slowly, such as with certain soilborne root or foot diseases (Dekker, 1987). For example resistance to metalaxyl developed rapidly in *Phytophthora infestans* in potatoes (Davidse *et al.*, 1981) so that control of this disease by the single compound had to be abandoned, but problems with control of various *Phytophthora* root diseases in other crops seem to occur less readily (Dekker, 1987).

The influence which the fungal life cycle may have on the development of resistance was shown in apple orchards treated with benomyl for control of scab and rust. After some time, failure of scab control occurred but there were no problems with control of rust (Dekker, 1987). The explanation may be that *Venturia inaequalis* (scab) does have a repetitive summer cycle on apple which favours build-up of resistance, but *Gymnosporangium juniperi-viriginianae* (rust) does not.

Several methods have been proposed to manage the evolution of resistance to fungicides in pathogen populations. Fungicide mixtures and alternations of fungicides in time and space may delay the evolution of resistance (Koller & Scheinpflug, 1987; Skylakakis, 1987). For these strategies to be effective, resistance to the fungicide used in the mixture and in alternation must not be correlated (no cross resistance). The alternation strategy can delay the evolution of resistance to fungicides if fitness costs are associated with resistance to each fungicide used in the alternation. Apart from use of mixtures and alternation of fungicides, use of the fungicide can be restricted to a limited part of the disease control programme or the fungicide may be withdrawn from the market with a possible reintroduction at a later date (Russell, 1995).

Monitoring for fungicide resistance

Monitoring for fungicide resistance involves testing samples of the target pathogen populations for their sensitivity to one or more fungicides (Brent, 1992). Monitoring for resistance to the systemic fungicides has been carried out world-wide over the past twenty-five years, especially in Europe, the USA and Japan.

For all purposes of monitoring, it is important to obtain base-line data, i.e. data on the initial variation between samples of the target pathogen in their response to a particular fungicide before it has been widely used in the field. It assesses the 'background noise' against which future results of monitoring can be compared, so that any substantial change in response can be detected clearly. Baseline monitoring also serves to test out methods of sampling and bioassy on a sizeable scale. If there is no initial data on variation in response, it can be very difficult to interpret subsequent monitoring results. Some specimen samples should be obtained during base-line monitoring so that any subsequent change made in the bioassay method can be tested on these 'original' isolates.

In the context of resistance research, monitoring may be done for a number of different purposes. Brent (1992) carried out an extensive review of these. He put them into five different groups which include; (1) early warning of potential resistance problems, (2) checking that avoidance strategies are working, (3) complaint monitoring, (4) to advance knowledge of the resistance phenomenon and to validate models and (5) for guidance on fungicide use at local level.

Early warning, often considered to be the main intention of resistance monitoring, is done to give warning of impending danger. It is questionable whether reliable and early indications of a potential resistance problem can in fact be obtained through monitoring, particularly for one step resistance. It is difficult to detect mutants in a field population of the target fungus until relatively high frequencies are obtained (>1%) and failure of disease control is either happening or imminent (Brent, 1992). Brent (1992) goes on further to say that to have a 95% probability of detecting even a 1 in 100 frequency of resistant forms, one would need to test 300 samples. Such a frequency is very much higher than the initial 10^{-7} or 10^{-8} frequency of mutation. Thus the selection and increase in frequency of resistant mutants in successive pathogen generations is likely to occur undetected until the point is reached when the next, or next-but-one, fungicide application will fail to give normal levels of disease control in the field. Thus monitoring generally will not give adequate warning of resistance build-up unless very large and generally impracticable numbers of samples are tested.

However, early warning can play a significant role in some situations, for example if resistant strains exist in high frequency before appreciable loss of disease control is observed as can occur with the DMI fungicides where resistance may be polygenic (Georgopoulos & Skylakakis, 1986). Detection of these resistant strains is worthwhile as a warning of greater, more practically significant resistance especially for other farms, regions or countries where the fungicide was introduced later, where use may have been less intensive or where there were other reasons for build-up occurring at a later time. If pathogen reproduction is slow, as for example in the cereal eyespot (*Pseudocercosporella herpotrichoides*) which sporulates only once or twice a year, some early warning will be useful. If the resistant mutants are less fit, build-up of resistance may be slow or interrupted and useful warning may be obtained. For example the resistance of *Botrytis cinerea* to the dicarboximide fungicides was detected through monitoring before widespread problems occurred (Beever & Byrde, 1982). If strategies to minimise the risk of resistance have been adopted, the rate of build-up of resistance may be brought down to a level at which monitoring can provide a warning so that strategies may be modified or another treament may be used. For example, it was possible to gain early warning of resistance build-up to phenylamide fungicides in *Phytophthora infestans* in potato crops in England that had been treated with formulated mixtures of phenylamides and mancozeb (Carter, Smith & Brent, 1982).

Checking that avoidance strategies are working is a valuable and achievable purpose of monitoring. However, this can be very expensive as it requires extensive monitoring over large regions annually. Monitoring of this nature is required only in special situations of known high risk coupled with major fungicide use. A cheaper and generally adequate alternative approach, is to monitor only for practical performance of the fungicide either in commercial or field experiments by assessing disease levels in crops. Monitoring can be carried out if signs of deterioration are observed, to check if resistance is the cause. Good correlation between the results of performance monitoring and resistance monitoring has often provided convincing evidence of the occurrence of resistance problems. However, sometimes such correlations are not found and alternative reasons have to be found for poor control. For example, Cooke (1992) reported a poor correlation between sensitivity scores and degree of field control by phenylamide-mancozeb mixtures in surveys of phenylamide resistance. In this case, the method of monitoring, which involves multiple spore inocula which fails to distinguish between minority and majority resistance within spore populations, has been cited as an underlying reason for poor correlation.

Complaint monitoring is done at specific sites as a detective exercise to investigate the cause of an apparent lack of performance of a fungicide observed and reported by the grower. Normally this should be done after elimination of other possible reasons such as faulty application technique, wrong dose rate, wrong identification of the pathogen or unusually high disease pressure. If lack of performance is severe then few samples should suffice to determine if there is a resistance problem.

In advancing knowledge of the resistance phenomenon and validating models, sensitivity testing is sometimes done in well-defined field experiments or other situations in which determining factors can be examined for their effect on the rate of build-up. This is difficult and expensive, requiring intensive frequent sampling, large plots, and accurate well-replicated assays for fungicide sensitivity. A long term approach, in which the same plots and treatments are maintained over several years, is often required in order to gain conclusive results (Brent, 1992).

As far as guidance to fungicide use at a local level is concerned, if monitoring methods give good results and if pathogen populations fluctuate in their response to fungicide treatment, then growers could usefully determine on-the-spot which particular fungicides are most likely to be effective at any given time. Du Pont have used this approach for the control of Sigatoka disease in bananas caused by *Mycosphaerella fijiensis* (Anon., 1982). Generally this method is not useful; shifts in fungicide response often occur over lengthy periods and are fairly stable, so there is little point in making frequent checks.

Since the fungicidal properties of morpholines, with large ring N-substituted tetrahydro-1, 4-oxazines, or morpholine derivatives were reported in 1965 (Koenig, Pommer & Sanne, 1965), several have been successfully marketed for the control of plant pathogenic fungi particularly the powdery mildews. The first of these, dodemorph (Kradel & Pommer, 1967), was introduced by BASF as its acetate [trade name Meltatox; chemical name (IUPAC) 4-cyclododecyl-2,6-dimethylmorpholine] into the horticultural market in 1965. This was followed in 1969 by tridemorph [trade names, Calixin (BASF) and Bardew (Schering Agrochemicals - now AgrEvo, Hauxton); chemical name (IUPAC) 2,6-dimethyl-4-tridecylmorpholine], again introduced by BASF (Kradel, Pommer & Effland, 1969) and which was used primarily for the control of powdery mildews on cereals, particularly barley. A decade later, fenpropimorph was described by both BASF (Pommer & Himmele, 1979) and MAAG (Bohnen & Pfiffner, 1979) and marketed in 1980 under trade names, Corbel (BASF) and Mistral (May and Baker) [chemical name (IUPAC) (±)-cis-4-[3-(4-tert-butylphenyl)-2-methylpropyl]-26-dimethylmorpholine], for the control of cereal mildews and rusts. The same year, aldimorph (trade name Falimorph) was introduced by Fahlberg-List for the control of powdery mildew on spring barley.

The extensive investigation of structure-activity relationships that led to the discovery of the commercially successful morpholine fungicides also showed that the piperidine ring version of fenpropimorph, known as fenpropidin [trade names include Patrol (ICI now Zeneca), Tern 750 EC and Mallard 750 EC (Ciba Agriculture.) and chemical name (IUPAC) (RS)-1-[3-(4-tert-butylphenyl)-2-methylpropyl]piperidine] was highly active against cereal powdery mildews and rusts (Bohnen *et al.*, 1986). Because fenpropidin, although not a morpholine, has been shown to have a very similar mode of action to the morpholine fungicides and because of its commercial success, it is included in these studies. Fig. 2.2 shows the chemical structures of morpholine and piperidine fungicides used in these studies.

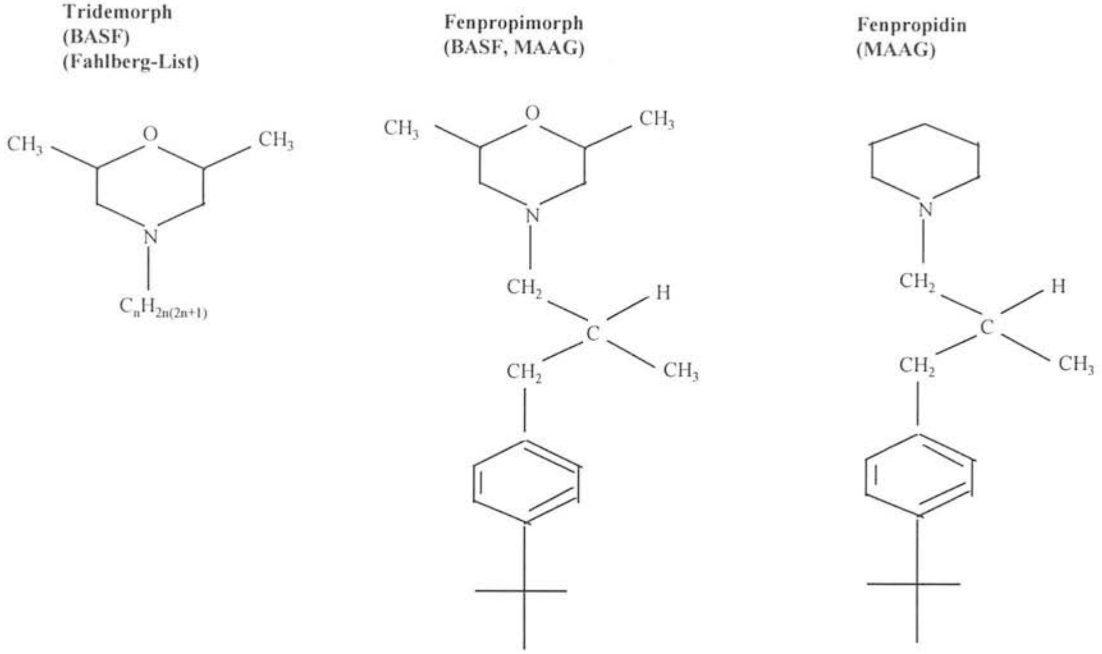


Fig. 2.2. Morpholine and piperidine fungicides (based on Hollomon, 1994).

Mode of action of morpholines

Most investigations on the mode of action of morpholines have been carried out using tridemorph and fenpropimorph in model systems because of the difficulties inherent in working with obligate pathogens. It is now generally accepted that morpholine fungicides act primarily on sterol biosynthesis. Sterols are known to be major structural components of cell membranes where they may contribute to their selective permeability properties and modify membrane fluidity. Inhibition of biosynthesis of sterols is detrimental to fungal growth and development and it is found that interference with sterol biosynthesis results in cell or hyphal membranes becoming disorganised and losing their ability to regulate the movement of substances in and out of the cell or hyphae (Carlisle, 1995). With most fungi, ergosterol is the main membrane sterol. Morpholine fungicides inhibit reduction or rearrangement of C=C double bonds in the ergosterol biosynthesis pathway. Fenpropimorph and fenpropidin both inhibit sterol Δ^{14} reductase strongly and $\Delta^{8} \rightarrow \Delta^{7}$ isomerase weakly, whereas the reverse is the case for tridemorph (Baloch *et al.*, 1984; Girling *et al.*, 1988). Based on this double block in the sterol biosynthesis shunt, the development of resistant pathogens is impeded and a longer life span for representatives of this chemical class could be forecast (Bohen *et al.*, 1986). Ziogas *et al.* (1991) showed that fenpropimorph inhibits an additional enzyme in the sterol pathway in *Nectria haematococca* var. *cucurbitae*: squalene epoxidase.

Uptake and transport in the plant

Tridemorph for the control of cereal mildew (Table 2.1) is taken up readily by plants through their roots, stem and foliage and translocated acropetally, with the transpiration stream, into leaves. Absorption by the leaf surface is assumed to be much slower than by roots and root hairs but after penetration of the leaf, distribution within the tissue occurs relatively quickly. Movement of tridemorph within the plant tissue is predominantly upwards which leads to an accumulation of the active substance in leaf tips and a slight depletion of the active ingredient in the lower leaf parts (Pommer, Otto & Kradel, 1969), particularly in younger and older plants (Deymann, 1981). Uptake of tridemorph is lower in older cereal plants than in younger ones, and degradation occurs more slowly. Basipetal transport does not occur. Tridemorph has protectant and eradicant properties against powdery mildew.

In contrast to barley, in wheat, tridemorph is taken up better by the morphological upper leaf surface than by the lower (Pommer & Kradel, 1971). Since the wheat upper leaf surfaces are considerably more densely covered with stomata than the lower leaf surfaces, the stomata may possibly be regarded as important gates of entry for tridemorph. The fact that leaf surfaces are more densely covered with fine hairs in wheat as opposed to barley, may play a role in the lower uptake of the active compound, since the small air bubbles trapped between hairs may greatly impede the wetting of the epidermal cells by tridemorph. Deyman (1981) observed that wheat plants take up lower quantities of tridemorph than barley plants. After treatment with equal quantities of active compound, the amount of fenpropimorph found in barley and wheat plants is greater than that of tridemorph (Pommer, 1987). Thus the uptake and distribution of active compound may vary according to the plant species, plant age and the fungicide used.

Fenpropimorph, used exclusively as a cereal fungicide, controls powdery mildew and yellow and brown rusts (*Puccinia* spp) and *Rhynchosporium* (Table 2.1). It is systemic and is mainly taken up by the roots but also by the foliage and is translocated acropetally *via* the xylem. Untreated, newly developed leaves are protected to a certain extent. In infected leaves the chemical concentrates around infected areas. A remarkably high vapour pressure supports the activity of the compound, allowing distribution to unsprayed plant surfaces (Rathmell & Skidmore, 1982). It also has eradicant activity.

Fenpropidin is rapidly absorbed by the roots, but only to a minor extent by other parts of the plant. Acropetal transport *via* the xylem has been demostrated but there is no basipetal transport. It has both protective and eradicant properties. Like fenpropimorph, fenpropidin shows a strong secondary dispersion through a vapour phase. Fenpropidin controls several cereal diseases in addition to mildew (Table 2.1).

Resistance to morpholines

Little is known about the mechanisms of resistance to morpholine fungicides. As indicated earlier, for a long time there has been no significant resistance problems to the morpholine SBI (sterol biosynthesis inhibitors) fungicides. This may be related to the ability of these compounds to inhibit multiple steps in the biosynthesis of sterols in fungi (Baloch *et al.*, 1984). Since these compounds may inhibit up to three different enzymes in the sterol biosynthesis pathway, they act as multi-site fungicides rather than single-site fungicides.

Reduced dosage rates of morpholines in relation to resistance

Agrochemical manufacturers spend a lot of money and time evaluating a fungicide before they recommend a dose which will control the target diseases in almost all circumstances (95%) in which the fungicide might be used. This is essential if the manufacturers want to avoid costly claims for poor control. In high disease pressure situations the recommended dose will be essential, but there are many other situations when a lower dose will provide just as effective control (Wale, 1993).

Some growers have been using fungicides below the full dose recommended by manufacturers for many years (Wale, 1994). This practice is economically good for the grower and is good for the environment. However, during the late 1970s and early 1980s when grain prices were high and maximum yield was the target, there was little incentive to reduce the dose. As the grain prices began to diminish, growers reacted by reducing the dose of fungicides in order to maximise gross margins (Wale, 1993). This worked reasonably well in many instances for eradication was still effective with lower doses. However, the persistence of reduced doses was shorter.

Historically, reduced doses were thought to increase the risk of fungicide resistance in pathogen populations (Paveley & Lockley, 1993). However, current expert opinion suggests that potential increases in resistance, associated with a higher proportion of the population surviving the lower fungicide dose, is counterbalanced by a corresponding reduction in the selection pressure exerted on the fungal populations (Paveley & Lockley, 1993). Contrary to this opinion, recent work by Engels & De Waard (1994), Forster *et al.* (1994) and Engels, Mantle & De Waard (1996) has shown that multiple, split applications of the full dose rate over the season reduce the sensitivity of powdery mildew to fenpropimorph compared with a single full recommended dose rate application.

Forster *et al.* (1994) examined the influence of split applications on disease control and on the sensitivity to fenpropimorph in both *E. graminis* f .sp. *tritici* and f.sp. *hordei* in 1991 to 1993 in Northern Germany. Split applications generally resulted in very good powdery mildew control but at the same time showed significant decreases in fenpropimorph sensitivity.

Similarly, Engels & De Waard (1994) tested the effects of Corbel (fenpropimorph) and Tilt Top (375 g fenpropimorph l^{-1} and 125 g propiconazole l^{-1}) split applications on disease control and on sensitivity in *E. graminis* f.sp. *tritici* in field experiments between 1992 and 1993. In 1992 no differences in sensitivity were found between multiple, split and full dose applications. However, in the 1993 experiment isolates collected from plots treated with split applications had, on average, the highest EC_{50} and Q_{50} (degree of resistance) values, suggesting that split applications favour reduction in sensitivity to fenpropimorph.

In a further field experiment conducted in the south of the Netherlands, Engels *et al.* (1996) tested the effect of fenpropimorph-containing fungicides on the sensitivity of *E. graminis* f.sp. *tritici* over a period of three years. The sensitivity to fenpropimorph of the mildew population in plots treated with normal applications of both fungicides, Corbel and Tilt Top, did not change during the growing seasons of the years investigated. In contrast, the sensitivity of the mildew population from plots treated with split applications of Corbel became significantly lower. A significantly lower sensitivity was also found in the mildew population from plots treated with split applications of Tilt Top in 1993 and 1994.

Results from all these experiments indicate that split applications can increase the rate at which *E. graminis* can become less sensitive to fenpropimorph. Because maintenance of sensitivity of the pathogen population is valued highly, Engels *et al.* (1994; 1996) do not recommend the split application strategy of fenpropimorph-containing fungicides for wheat and Forster *et al.* (1994) for wheat and barley mildew control.

Aims of Experimental Studies

While the use of morpholine fungicides remains an important means of controlling cereal mildew, small reductions in sensitivity have been reported from a number of countries and there is a need to have some measure of the occurrence and magnitude of variation in mildew populations. One of the main objectives of the present experimental studies was to monitor isolates of *E. graminis* f.sp. *hordei* and f.sp. *tritici* collected from the field for variation in sensitivity to different morpholine fungicides.

Among the factors that have been suggested to increase the risk of resistance problems is the use of reduced dosage rates and further experiments were carried out to study effects of reduced dosage rates of a morpholine fungicide on sensitivity of barley mildew.

Little work seems to have been done on the effect of morpholines on stages in the mildew infection process although morphologically abnormal sporidia in *Ustilago* *maydis* (Kerkenaar, Barug & Kaars Sijpesteijn, 1979) and abnormal germ tube growth in *Botrytis cinerea* (Kato, Shoami & Kawasi, 1980) are produced by tridemorph. However, the study of fungal development at a microscopic level would seem to offer a method of characterising variation in sensitivity to fungicides as a more sensitive alternative to visual assessments of disease levels. The third section of the present research is concerned with some preliminary studies on variation in development of isolates of *Erysiphe graminis* of different sensitivities in response to exposure to fenpropimorph.

The experimental studies are considered under the following headings:

(a) Sensitivity monitoring of barley and wheat powdery mildew (Chapter 4).

(b) Reduced dosage rates of fungicide in relation to resistance (Chapter 5).

(C) Comparison of four methods of testing for fungicide sensitivity (Chapter 6).

Chapter 3

General Materials and Methods

GENERAL MATERIALS AND METHODS

The Standard Leaf-segment Method

Plant culture

The barley cultivar used throughout the barley mildew experiments was 'Golden Promise' which has no recognised resistance genes against powdery mildew of barley. The wheat cultivar used for isolate maintenance and propagation of wheat mildew was 'Cerco' which similarly carries no known mildew resistance genes against powdery mildew of wheat. The lack of mildew resistance genes in these cultivars, allowed the maintenance and propagation of all isolates of the respective *formae speciales* irrespective of their virulence status. The seed used for producing plants for maintenance and propagation of isolates was untreated with fungicide. The barley seed was obtained from Dodds of Haddington and the wheat seed was obtained from the National Institute of Agricultural Botany (NIAB) in Cambridge. Seed was sown in a general peat compost mix (See Appendix III.i. for composition of compost) in 5″ (12.5 cm) pots. Seedlings, five to a pot, were raised in a glasshouse in Burkhart Isolation Propagators (Fig. 3.1) under conditions of positive pressure to avoid contamination of clean plants by airborne inoculum and cross-contamination between different mildew isolates.

Fungal culture

Powdery mildew isolates were maintained either on detached leaf segments on distilled water agar containing 0.4% Davis agar and 80 mg l^{-1} benzimidazole (incorporated to delay leaf senescence) in a Gallenkamp incubator at 18°C with a 12 hour photoperiod, or on whole plants (two leaves present = Zadok's growth stage (GS) 12) (Stubbs *et al.*, 1986) in Isolation Propagators in a heated greenhouse with

Fig. 3.1. An isolation propagator used to grow disease-free plants and to maintain mildew isolates without cross contamination.

Fig. 3.1.

temperatures fluctuating around 18±4°C. Isolates were subcultured on to fresh leaf segments after two weeks and on to whole plants after three weeks, depending on the method of maintenance, as leaf segments and plants became severely affected by disease and began to senesce. This procedure maintained vigorous colonies for the production of an abundance of viable inoculum for experimentation. Isolates required for experiments were propagated either on leaf segments or on whole plants in the case of barley and on to whole plants in the case of wheat. It was difficult to propagate inoculum of wheat isolates on leaf segments as spores tended to stick on to the leaf segments, making inoculation difficult and inefficient when infected leaf segments were tapped over the treated test segments to dislodge spores.

In sensitivity tests, eight replicate, fungicide-treated, leaf segments were placed on the benzimidazole agar medium in 9 cm (diameter) Petri dishes and then inoculated as uniformly as possible, by tapping heavily infected leaf segments covered with spores over them and distributing the spores with a sterile, fine paint brush. Where whole plants were used for propagating inoculum, inoculation was carried out by dusting the mildew inoculum over the surface of the leaf segment using a sterile fine paint brush. The inoculum used was ten to fourteen days old, as before ten days there was not enough inoculum to work with and after fourteen days the viability of inoculum had declined.

Determination of dose response

Three fungicides [tridemorph (full commercial field spray rate 0.7 l ha^{-1} 'Calixin' in 200 l water ha^{-1}); fenpropimorph (full commercial field spray rate 1.0 l ha^{-1} 'Corbel' in 200 l water ha^{-1}) and fenpropidin (full commercial field spray rate 1.0 l ha^{-1} 'Patrol' in 200 l water ha^{-1})] at different concentrations were used routinely in the fungicide sensitivity screening tests. Table 3.1 details the active ingredients of the different chemicals.

Table 3.1. Chemicals used in the experiments and their active ingredients.

Active ingredient	Product	Active ingredient content (g a.i. l^{-1})
Tridemorph	Calixin	750
Fenpropimorph	Corbel	750
Fenpropidin	Patrol	750

Plants grown to provide leaf material for leaf segments for sensitivity testing were also grown on the isolation propagator but with about ten seedlings per pot rather than the five plants per pot used for maintenance of isolates and production of inoculum. Plants from the isolation propagators were sprayed with fungicide in a perspex spray cabinet using a hand-operated Humbrol sprayer in ascending order of concentration (Fig. 3.2). In all tests each concentration was sprayed twice (using different plants) for replication. Sprayed plants were kept covered for 24 hours to allow sufficient time for fungicide uptake. Barley plants were sprayed for five seconds and wheat plants were sprayed for ten seconds both followed by 15 minutes to allow the fungicide cloud to settle. Spraying wheat plants for ten seconds was a modification of the standard leaf-segment method for barley. The aim was to increase the amount of fungicide that was sprayed so that sprayed leaves approached surface run-off.

After 24 hours, eight 2-2.5 cm leaf segments from each spray replicate were cut from the middle of the second leaves and laid flat with the midrib down, on the agar in the Petri dishes. Leaf segments from each spray replicate were plated in a separate Petri dish.

Fig. 3.2. A perspex spray cabinet with two pots of barley seedlings ready for spraying with fungicide and showing an aerosol sprayer - bottle of fungicide not attached.

Fig. 3.2.

Disease assessment and analysis of results

For all the laboratory sensitivity tests, assessment was carried out on inoculated leaf segments after two weeks incubation in a Gallenkamp incubator at 18°C with a 12 hour photoperiod, which was when untreated control leaf segments showed maximum colony development. Leaf segments were assessed for percentage mildew cover using a hand held magnifier. The data collected for each isolate in each test were recorded as shown overleaf in Table 3.2. This information was then fed into the computer and analysed. A Genstat 5 program was used to calculate the median percentage mildew cover for each treatment level within each replicate and then to fit symmetrical logistic curves to the medians to facilitate the calculation of EC_{50} values. The EC_{50} is estimated from the dose-response curve as the median effective concentration of the pesticide which produces half the maximum effect on the isolate (Brown, 1991). Fig. 3.3. shows a typical dose response curve. The dose response curve is a generalised logistic curve. Appendices III.ii and iii show a copy of the program and an example of the final output from the program illustrating a typical pattern obtained. The graph (Appendix III.iii) illustrates the median values of the percentage leaf cover for the eight leaf segments and the computer fitted values.

The logistic curve fitting procedure used was suitable for all "standard" reactions; for example, isolates that could grow not only on the untreated control but also on the two or three lowest concentrations of the test fungicide. However the program was unable to analyse satisfactorily data from isolates that did not grow on the lowest one or two concentrations of the test fungicide (i.e. very sensitive isolates), or from isolates that grew at a steady rate over several concentrations but were killed at a higher concentration with no steady decline in percentage tissue infected. These were not common responses and when they occurred the test isolate results were noted. In some cases the EC_{50} value of one replicate only was used. For most isolates a geometric mean was taken as the EC_{50} value. This was because a geometric mean does not give much emphasis to extreme numbers. The geometric mean is the nth root of the product of the n data (Zar, 1996). A geometric mean of replicates a and b = √ a x b.

Table 3.2. Example of a disease assessment record sheet.

Test isolate: GM Spray date: 9/5/95 Fungicide: Corbel Assessment date: 24/5/95

Spray Conc.	Rep. Plate	Replicate Leaf Segment							
		1	2	3	4	5	6	7	8
0	1	65	48	40	35	50	70	70	25
0	2	65	70	60	50	45	50	70	55
1/64	1	55	50	45	70	35	50	60	60
1/64	2	50	45	55	60	60	50	55	60
1/32	1	60	40	65	35	50	40	25	25
1/32	2	15	15	42	50	55	35	25	55
1/16	1	15	3	15	5	20	50	25	30
1/16	2	50	20	5	10	20	5	5	5
1/8	1	3	45	0	0	3	25	5	0
1/8	2	0	5	0	5	0	0	0	0
1/4	1	5	0	0	0	3	5	8	0
1/4	2	0	0	0	0	0	0	0	0

Rep. = replicate Conc. = concentration

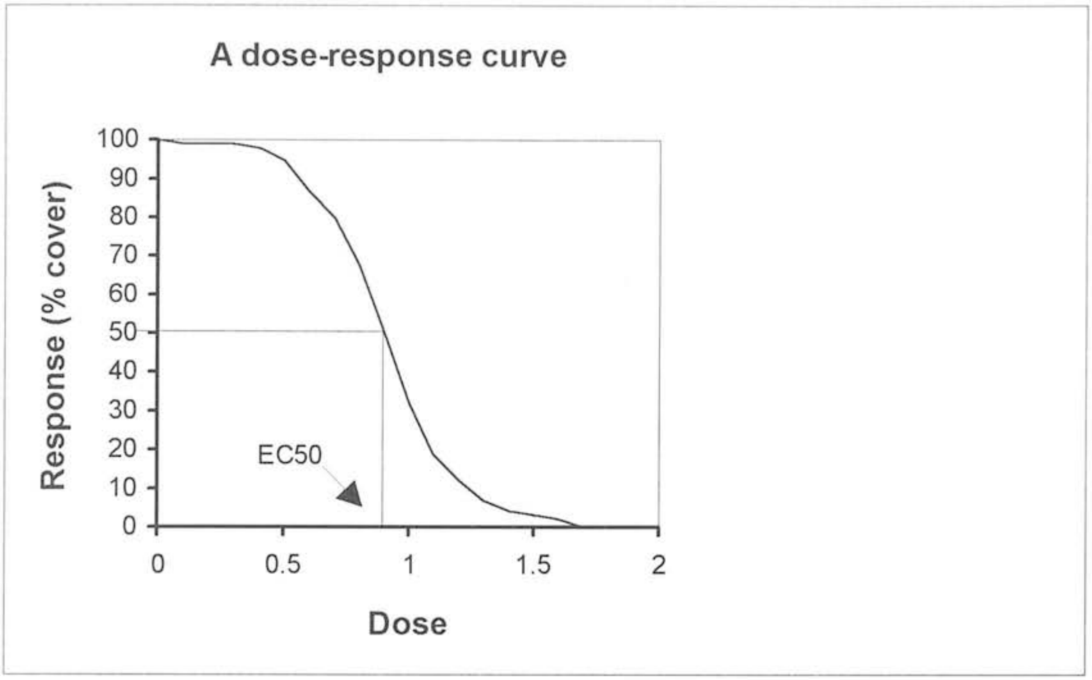


Fig. 3.3. A typical dose-response curve.

The logistic curves in this method are based on the formula:

$$Y = A+C/(1+e^{(-B(X-M))})$$

where A = lower limit of percentage cover

B = growth rate at period of maximum growth of mildew (slope)

C = maximum response of mildew to the fungicide

e = mathematical constant (approximately 2.718)

M = maximum growth period (at point of inflection)

X = fungicide dose

Y = median percentage area infected

Growth anomaly

It has been noted occasionally (Boyle, *et al.*, 1988), with some isolate/fungicide combinations that growth of some isolates was promoted slightly at low fungicide concentrations. To analyse this feature, the program was modified to "unfix" the untreated control median value, enabling the computer to fit a standard symmetrical curve. This feature was also reported by Robertson *et al.* (1996).

Reproducibility of results and stability of isolates

The design of the standard leaf-segment sensitivity test procedure required that the screening of isolates be reproducible and that the sensitivity of individual isolates to particular fungicides remain stable despite repeated subculture of isolates. Normally, repeated subculturing tends to bring about changes in isolates. In the present studies, lack of stability of the subcultured isolates could have been brought about by several factors. First of all, the bulk (as opposed to single colony) isolates used in most of the sensitivity tests may have changed in composition over repeated subculturing, due to competition amongst the spores. Secondly, the fluctuating growth conditions (e.g. temperature) could have affected the resistance of the leaves, and hence development of the isolates, on subculturing. Thirdly, the method of spraying itself could have affected the results as there was no guarantee that all the different leaf positions received equal amounts of fungicide. In these studies about 14 isolates were retested two or more times for replication, although the interval between replicate tests was not longer than a month (Table 3.3). Retesting was done in exactly the same way as the original tests. Although some of the isolates had similar EC_{50} values when they were retested, many isolates had different EC_{50} values. The above-mentioned reasons may account for this lack of similarity. Analysis of variance was carried out to test if the different isolates were genetically different from one another. The ANOVA (Appendix III.iv) showed that the differences between isolates were small compared to differences within isolates and no significant difference in mean EC_{50} was detected between these isolates for either fenpropimorph or fenpropidin. Robertson *et al.* (1996) retested their isolates to check that their sensitivity to the test chemical had remained stable over long periods. Table 3.4 is adapted from Robertson *et al.* (1996). It details some examples of data from the repeated tests they carried out, illustrating the point that in their hands the test procedure (the same as the one used in the present studies) yields reproducible and consistent results. Robertson *et al.* (1996)

used bulk isolates in their sensitivity tests. The EC_{50} values they obtained for each isolate at different times of testing were comparable and significant differences between isolates were detectable for all three morpholines, indicating genetic differences in fungicide sensitivity between the isolates. In contrast to Robertson *et al.* (1996) data, the majority of the isolates presented in Table 3.3 in this study originated from England and were highly sensitive to the morpholines. As a consequence, their dose response curves would have been steep and based on a smaller dose range than those of less sensitive isolates because of complete growth inhibition at higher dose levels. Consequently, the standard error estimates for these EC_{50} values were often quite large and the EC_{50} values therefore only poorly estimated. This may be one reason why differences between isolates were not detected. Alternatively the EC_{50} values of the isolates may in reality have been similar. The method followed by Robertson *et al.* (1996) was not so reproducible in the present experiments.

Table 3.3. Test reproducibility and stability of barley mildew isolates.

Fenpropidin			Fenpropimorph		
Isolate	EC50	SE	Isolate	EC50	SE
IW26	0.011	±0.004	IW227	0.014	±0.003
	0.011	±0.004		0.001	±0.013
	0.005	±0.009	IW2	0.012	±0.001
	0.009	±0.017		0.005	±0.010
IW2	0.006	±0.012		0.014	±0.005
	0.006	0.012	IW225	0.011	±0.002
	0.014	0.002		0.011	±0.005
IW225	0.007	±0.013	22B	0.010	±0.001
	0.012	±0.001		0.006	±0.012
IW43	0.014	±0.006	IW34	0.014	±0.004
	0.014	±0.006		0.012	±0.007
IW34	0.013	±0.003	5G	0.014	±0.004
	0.013	±0.008		0.005	±0.017
IW223	0.012	±0.001	22C	0.014	±0.003
	0.012	±0.001		0.014	±0.005
	0.009	±0.017	736*	0.016	±0.013
5G	0.014	±0.004		0.047	±0.074
	0.014	±0.006	CC139*	0.029	±0.021
	0.012	±0.002		0.030	±0.021
737*	0.017	±0.001			
	0.017	±0.034			
	0.044	±0.030			
736*	0.070	±0.036			
	0.019	±0.038			
	0.013	0.008			

* Isolates originally from Scotland. All other isolates were obtained from Dr D.W. Hollomon and originated from England (Appendix IV.i).

Table 3.4. Test reproducibility and stability of barley mildew isolates. (Adopted from Robertson *et al.*, 1996).

Isolate	Fungicide	Collection date	Test date	EC_{50}	SE
P8	Calixin	25/5/88	31/5/89	0.129	±0.014
			4/1/90	0.125	-
R19	Calixin	14/7/89	11/5/90	0.128	-
			28/8/90	0.124	±0.011
R6	Calixin	14/7/89	7/6/90	0.086	-
			5/7/90	0.093	±0.004
Glf91	Calixin	5/6/91	12/6/91	0.164	±<0.001
			16/7/91	0.161	±0.004
GP91	Calixin	5/6/91	12/6/91	0.108	±0.033
			22/7/91	0.105	-
B21	Mistral	14/7/89	24/7/90	0.030	±<0.001
			17/11/90	0.031	±0.001
R17	Mistral	14/7/89	24/7/89	0.022	-
			14/8/90	0.022	±<0.001
R19	Mistral	14/7/89	24/7/90	0.025	±<0.001
			14/8/90	0.027	±0.003
BSBC	Patrol	18/5/88	4/10/90	0.042	-
			16/11/90	0.038	±0.002
B6	Patrol	14/7/89	4/10/90	0.035	±0.020
			17/5/91	0.034	±<0.001
B2	Patrol	14/7/89	17/10/90	0.035	±0.003
			16/11/90	0.028	±0.001
PSBK	Patrol	1/5/90	7/3/91	0.064	±0.005
			1/5/91	0.066	-
B2	Bayfidan	14/7/89	14/3/90	0.065	±0.004
			17/5/90	0.067	±0.008

- = no standard error calculated

Weaknesses of the test

There was a lot of variation in the test. When spraying, different leaves got different amounts of chemical depending on the angle at which they were held on the plant in relation to the sprayer in the spray cabinet. In testing tridemorph (Pommer, Otto & Kradel, 1969) found that after spraying, the transpiration stream would cause uneven distribution of the fungicide within the leaves such that the bases of leaves were depleted while there was a high concentration of the chemical at the tips. There was no control over the vapour action of the chemicals during the experiment. Both fenpropimorph (Rathmell & Skidmore, 1982) and fenpropidin possess a high vapour pressure. Ideally, Petri dishes with the control leaf segments should either have been hermetically sealed or put in a separate incubator. However, this would have brought in sources of variation due to different incubation conditions.

The age of the different spores within the isolates was not uniform. Older spores are found at the tip of the conidiophores (Jenkyn & Bainbridge, 1978) and this would bring in variation in their response to the chemicals as spores of different ages may take in and respond to the chemical differently. Spores of different ages may differ in viability. This made comparison difficult as the proportion of viable spores was unknown. Although the inoculum was spread over the leaf segments with a fine brush, there was no guarantee that this was uniformly distributed. Ideally, inoculum should have been done in an inoculation tower using a pump so that the spores would have been more evenly distributed. However, mildew spores tend to clump and may be damaged with this technique, which works well for rust spores (Russell, 1978). Lack of even distribution meant that the spores which eventually grew, even within the same replicate, were unequally spread and the percentage mildew cover taken may not have given a true assessment of the potential area of colonisation. This brought in sources of variation other than the fungicide treatment. In addition, the assessment method was also subjective depending on the individual taking the percentage mildew cover. Finally, the experiment lasted four weeks, allowing only a limited number of isolates to be screened per annum. This meant that results should be interpreted with caution as so few isolates may not be representative of the whole mildew population.

Measures taken to counteract the weaknesses

To reduce these sources of variation certain measures were taken. Potted plants to be sprayed with fungicide were evenly placed within the spray cabinet so that they each had equal chances of receiving the fine spray. To reduce the variation brought about by uneven distribution of fungicide within the leaf, all leaf segments used in the tests were cut from the middle of the leaves. To prevent vapour action between different pots from taking place, all pots were covered with propagator tops after spraying. Care was taken to ensure that the inoculum was as evenly distributed as possible over the leaf segments. Before any experiments were conducted, a computer program (Distrain) for training people to estimate disease severity on cereal leaves, written by R. Tomerlin and T. Howell of The Agricultural Research Service of the U.S. Department of Agriculture, Beltsville, Maryland, was used. This reduced the subjectivity of the method of assessment.

Chapter 4

Sensitivity Monitoring of Barley and Wheat Powdery Mildew

SENSITIVITY MONITORING OF BARLEY AND WHEAT POWDERY MILDEW

Introduction

Morpholine fungicides are classified as compounds with a low risk of resistance development (Lorenz *et al.*, 1992; Hollomon, 1994). Work on mode of action identified morpholines as multisite inhibitors of sterol biosynthesis (Hollomon, 1994). Because of this multisite action it is unlikely that resistant mutants will arise rapidly. On the other hand, barley and wheat powdery mildews belong to the group of high risk fungi as far as development of fungicide resistance is concerned (Lorenz *et al.*, 1992), with both pathogens being polycyclic and having the capacity to sporulate abundantly on aerial plant parts, producing many asexual generations in a single season. Therefore, in order to follow closely the sensitivity development of barley and wheat powdery mildews towards morpholines a monitoring project was started at SAC Edinburgh (1988 for barley and 1993 for wheat).

Clearly, knowledge of a change in sensitivity would be very beneficial to growers. Experiments to monitor fungicide resistance were carried out for three main reasons;

(1) as an early warning of potential resistance problems,
(2) in response to complaints of lack of control by fungicides and
(3) for guidance on fungicide use at a local level.

The studies reported here on barley powdery mildew, began in 1991 and were a continuation of the studies started in 1988. Because of reports of lack of control of wheat powdery mildew by fenpropimorph, monitoring for fungicide resistance to wheat powdery mildew was started in 1993.

Materials and Methods

Leaves infected with barley powdery mildew were collected from commercial crops and trial plots from mainly south-east Scotland, but also from other parts of Britain (Fig. 4.1), between 1991 and 1994. On collection, crop details, where available, were recorded with regard to cultivar, area, site, chemical application history and date of collection (Appendix IV.i). Most of the isolates were heterogeneous consisting of inoculum obtained from several pustules on a single leaf collected from the field, but there were also some single colony isolates. The infected leaves were taken to the laboratory where they were cut into 2-2.5 cm leaf segments and incubated for 2-3 days on Davis distilled water agar in an incubator, to allow sporulation to take place, as described in Chapter 3. The different isolates were then subcultured on to fresh leaf segments to increase the inoculum. The isolates were maintained on cv. Golden Promise (see Chapter 3). Every four weeks, four to eight isolates were screened for sensitivity to one of the three fungicides, tridemorph, fenpropimorph and fenpropidin, following the standard leaf-segment method.

Five fungicide concentrations were used for spraying, based on the full commercial field spray rate (C). For tridemorph 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 a.i. l^{-1}. In the first experiments (1991 to part of 1993) fenpropimorph and fenpropidin were applied at 1/256C, 1/128C, 1/64C, 1/32C and 1/16C giving concentrations of 0.015, 0.029, 0.059, 0.117 and 0.234 g a.i. l^{-1}. The concentrations of fenpropimorph and fenpropidin had to be changed in subsequent experiments because most of the isolates were growing equally on all concentrations of the fungicides. The amended concentration range for both chemicals was 1/64C, 1/32C, 1/16C, 1/8C and 1/4C giving concentrations of 0.059, 0.117, 0.234, 0.469 and 0.938 g a.i. l^{-1}. Control plants were sprayed with water. The barley plants were sprayed (see Chapter 3) for

Fig. 4.1. The sources of barley mildew isolates

X = areas from where isolates were collected (figures in brackets refer to number of isolates collected) five seconds followed by 15 minutes to allow the fungicide cloud to settle within the spray cabinet. The sprayed plants were kept covered for 24 hours to allow sufficient time for the fungicide uptake, before the preparation of the leaf segments and their inoculation with isolates of barley powdery mildew. Each concentration was sprayed twice for replication and a Petri dish of eight leaf segments was prepared from each spray replication. The inoculated leaf segments were incubated in a Gallenkamp incubator at 18°C with a 12 hours day and night regime. The leaf segments were assessed (see Chapter 3) for percentage mildew cover two weeks later and EC_{50} values (g a.i l^{-1}) for each isolate were determined.

Seedlings used in the experiments were grown for two weeks to growth stage (GS) 12, when ligules were visible on the second leaf (sometimes referred to as the first true leaf) (see Fig. 4.1a), before spraying. At this growth stage there is enough material to work with on this leaf. Leaf segments (about 2-2.5 cm long) used throughout the experiments were detached from the middle of the leaf to standardise the tissue used in case of uneven distribution within the leaf of the systemic chemicals, which tend to accumulate in the leaf tips and are depleted in the leaf bases. The leaf segments were laid on agar with the midrib down.

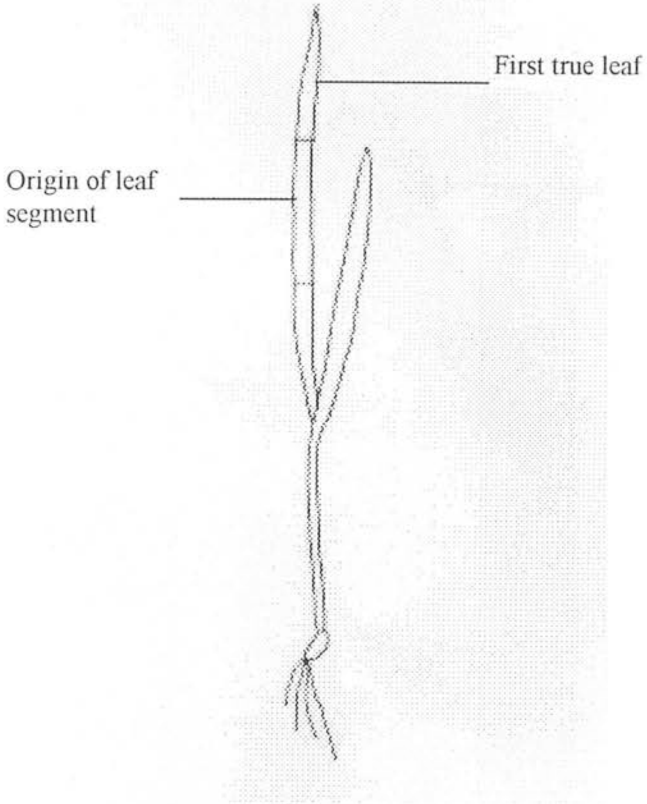


Fig. 4.1a. A barley plant at growth stage (GS) 12

Results

The distribution of isolates under different categories of EC_{50} values (g a.i. l^{-1}) for each fungicide is represented in Fig. 4.2-4.4. Isolates followed a skew distribution for all fungicides with most isolates in the category EC_{50} 0.1-0.2 for tridemorph (Fig. 4.2) and EC_{50} <0.1-0.1 for both fenpropimorph and fenpropidin (Fig. 4.3 & 4.4 respectively). EC_{50} values ranged up to 0.3-0.4 for tridemorph and fenpropidin and up to 0.2-0.3 for fenpropimorph.

The mean EC_{50} values to the different fungicides over years, the ranges between isolates and their standard errors (SE) are summarised in Tables 4.1-4.3.

Generally there was a trend towards an increase in mean EC_{50} values from 1991 to

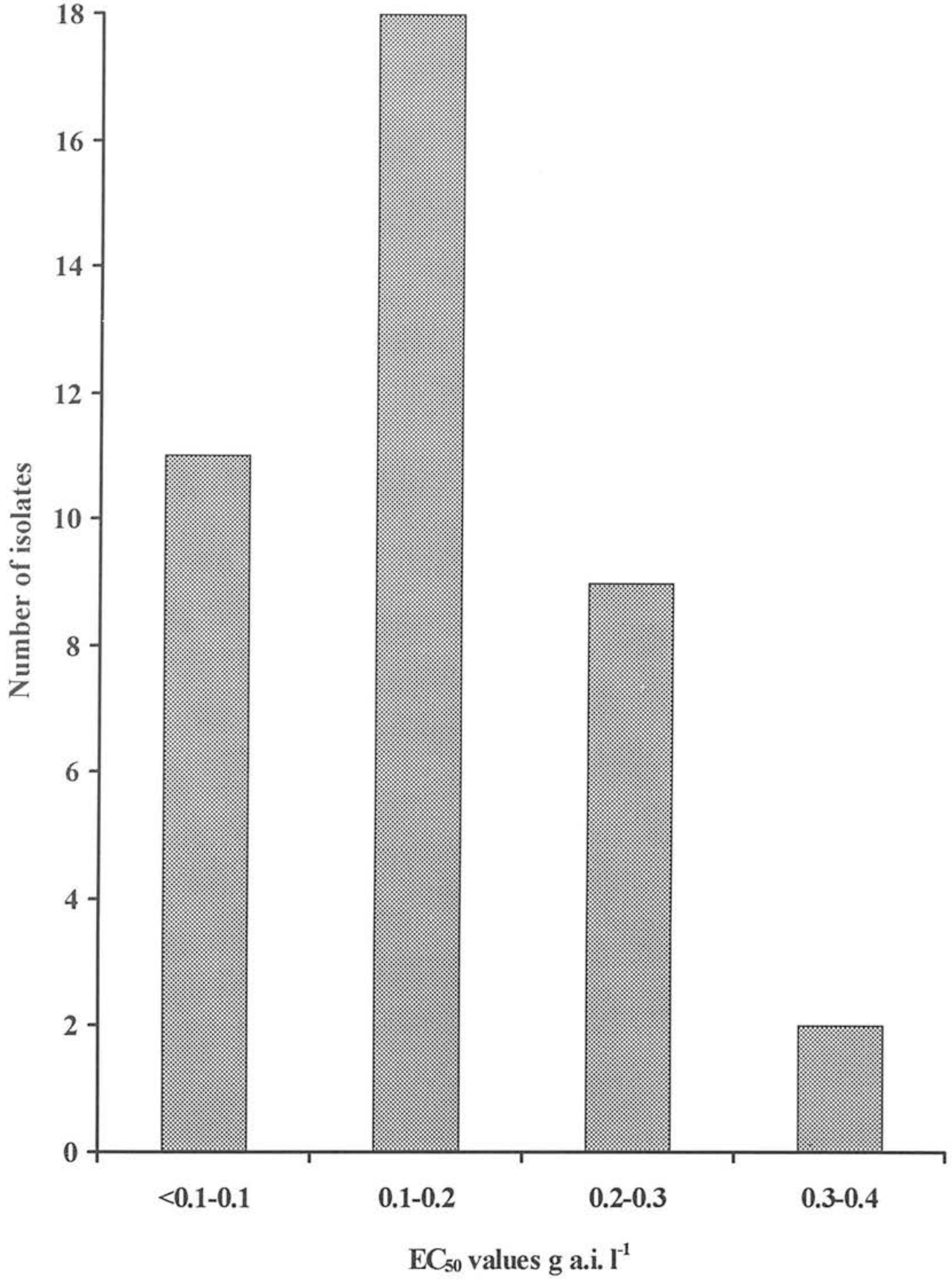


Fig. 4.2. Number of 1991-1993 barley powdery mildew isolates tested against tridemorph under different categories of EC_{50} values.

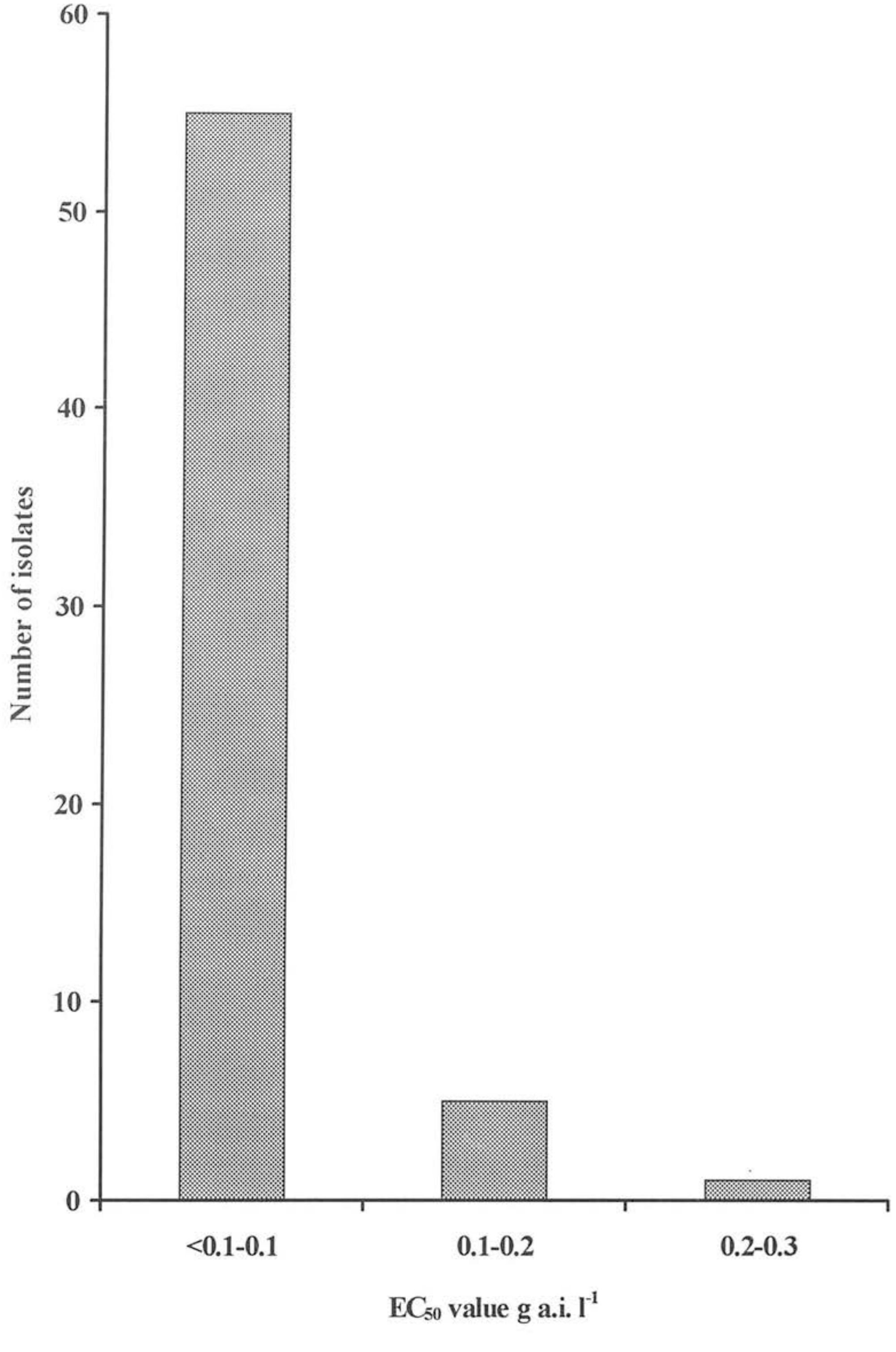


Fig. 4.3 Number of 1991-1994 barley powdery mildew isolates tested against fenpropimorph under different categories of EC_{50} values.

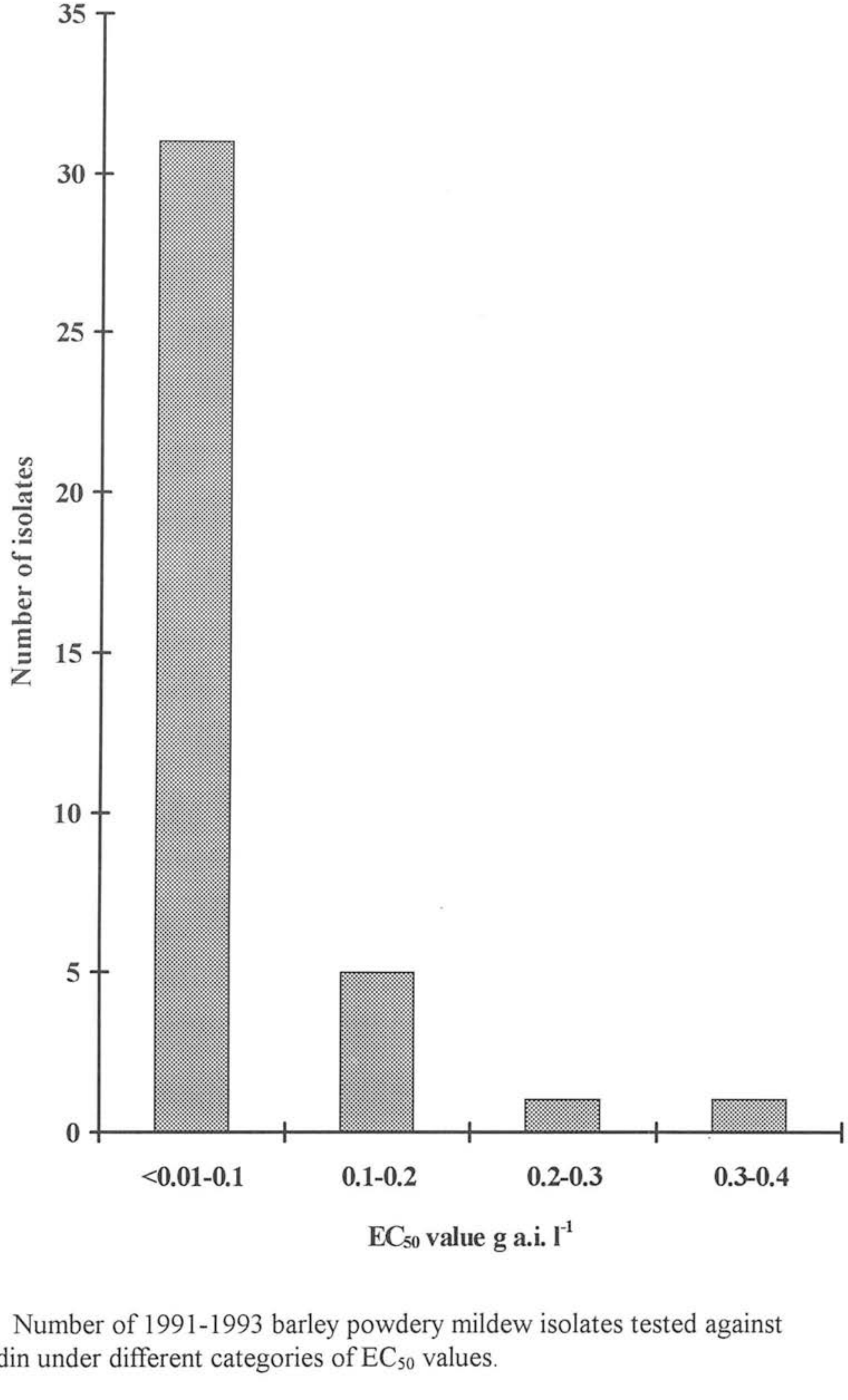


Fig. 4.4. Number of 1991-1993 barley powdery mildew isolates tested against fenpropidin under different categories of EC_{50} values.

Table 4.1. Mean EC_{50} values (g a.i. l^{-1}), range and SE for tridemorph.

Year	No. of isolates	Mean EC_{50}	Range	SE of Mean
1991	9	0.126	0.068-0.213	±0.022
1992	13	0.144	0.009-0.246	±0.018
1993	18	0.182	0.042-0.349	±0.020

Table 4.2. Mean EC_{50} values (g a.i. l^{-1}), range and SE for fenpropimorph.

Year	No. of isolates	Mean EC_{50}	Range	SE of Mean
1991	13	0.038	0.010-0.087	±0.008
1992	16	0.045	0.010-0.119	±0.008
1993	17	0.069	0.010-0.300	±0.019
1994	15	0.062	0.024-0.195	±0.013

Table 4.3. Mean EC_{50} values (g a.i. l^{-1}), range and SE for fenpropidin.

Year	No. of isolates	Mean EC_{50}	Range	SE of Mean
1991	12	0.042	0.009-0.156	±0.014
1992	10	0.039	0.006-0.232	±0.024
1993	16	0.080	0.001-0.351	±0.023

1993 for the three morpholine fungicides. This indicates a slight increase in insensitivity to the fungicides over this period. However, there was a decrease in mean EC_{50} values for fenpropimorph from 1993 to 1994 and for fenpropidin from 1991 to 1992. Apart from fenpropimorph, where the range was smaller in 1994 than in 1993, overall there was an increase in range for all the fungicides from 1991 to 1993.

Fig. 4.5 shows the EC_{50} values of all barley powdery mildew isolates tested against tridemorph between 1991 and 1993. There is variation in sensitivity. The range is continuous from the most sensitive (EC_{50} = 0.009) to the least sensitive (EC_{50} = 0.349) with a 39-fold difference between the two. The mean and median are equal. Fig. 4.6 shows the range of barley powdery mildew isolates tested against fenpropimorph from 1991 to 1994. As for tridemorph, the range between the most sensitive (EC_{50} = 0.010) and the least sensitive (EC_{50} = 0.300) is continuous and there is a 30-fold difference between the two. The mean and the median are far apart because of the much higher EC_{50} values of the six least sensitive isolates. The results for isolates tested against fenpropidin between 1991 and 1993 are shown in Fig 4.7. The range is again continuous with a 351-fold difference between the most sensitive (EC_{50} = 0.001) and the least sensitive (EC_{50} = 0.351) isolate. The mean and the median are far apart with the median falling among the more sensitive isolates and the mean falling among the less sensitive ones.

The fungicide sensitivity of all isolates was compared to that of a 1984 isolate (JB212) tested by Robertson *et al.* (1996) and resistance factors, which are represented graphically in Fig. 4.8-4.10, were calculated basing them on the formula;

Resistance factor of an isolate = EC_{50} of that isolate/EC_{50} of JB212

The 1984 isolate used, JB212, had EC_{50} values for the different fungicides as follows; tridemorph = 0.075 g a.i. l^{-1}, fenpropimorph = 0.024 g a.i. l^{-1} and fenpropidin = 0.011 g a.i. l^{-1}. JB212 was chosen because it was the earliest tested isolate for which relevant data was available. With all three fungicides, JB212 falls among the more sensitive isolates but it is not the most sensitive (Fig. 4.8-4.10).

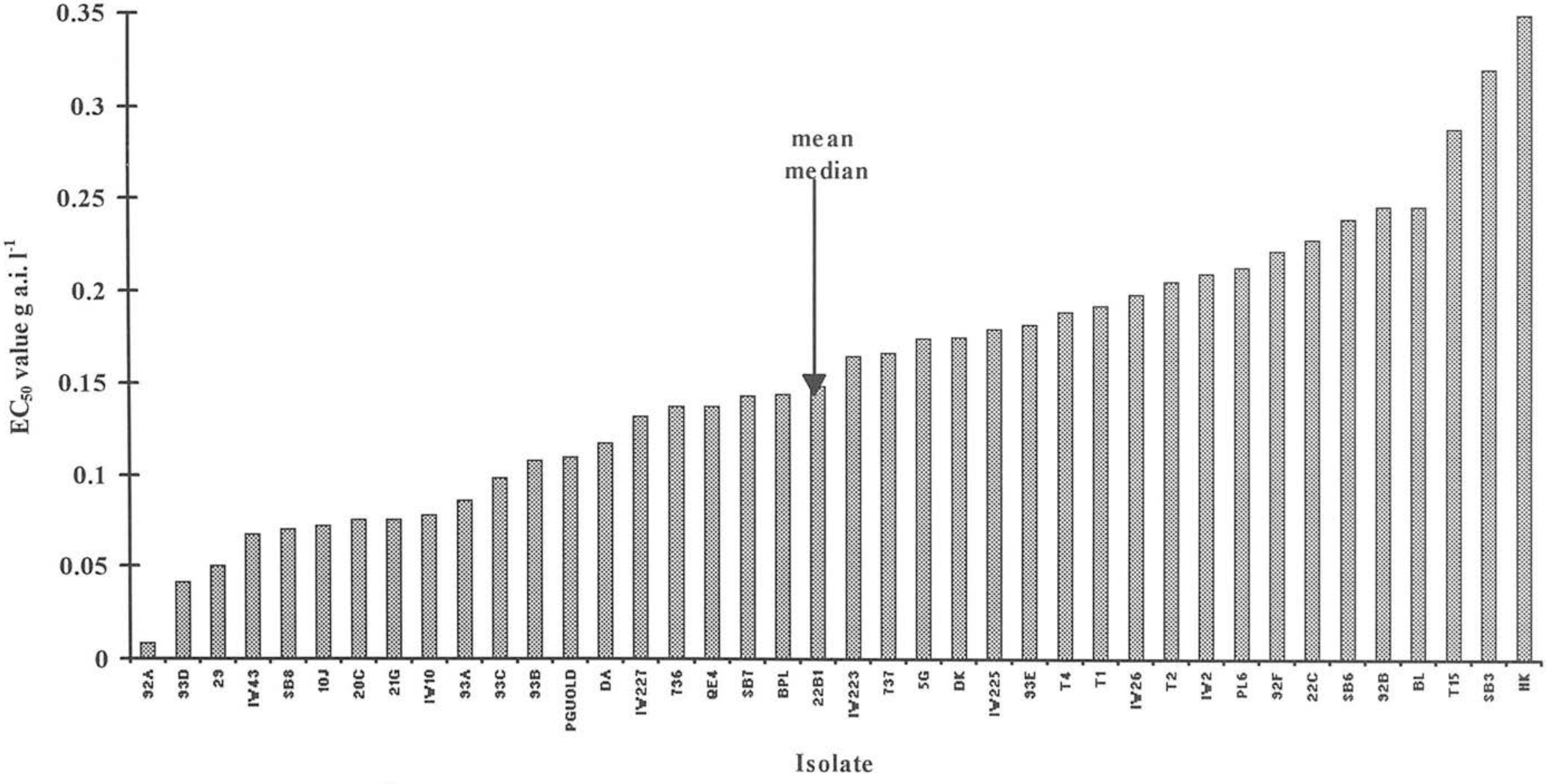


Fig. 4.5. EC_{50} values for barley powdery mildew isolates tested for sensitivity to tridemorph from 1991-1993.

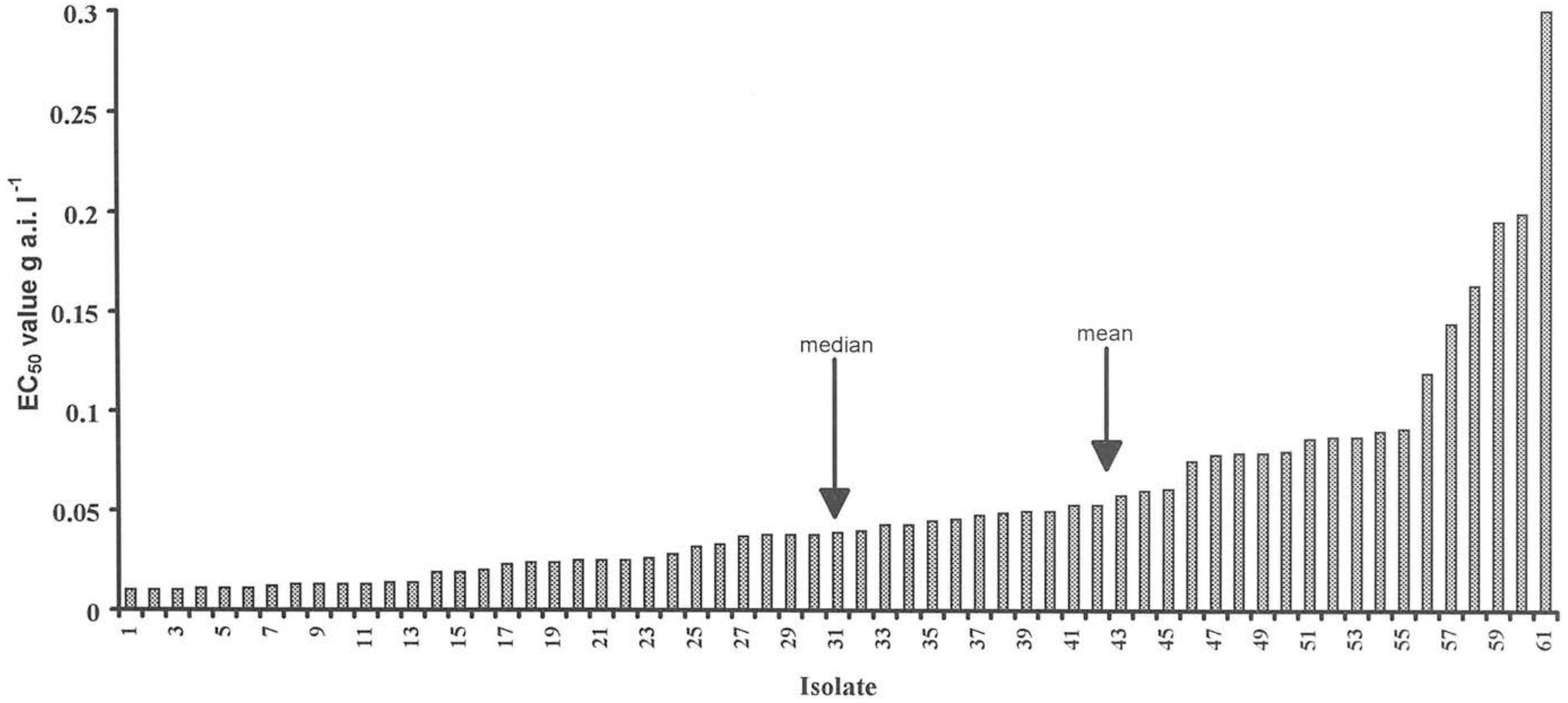


Fig. 4.6. EC_{50} values for barley powdery mildew isolates tested for sensitivity to fenpropimorph from 1991-1994. (Key to isolate numbers overleaf).

Table 4.3a. Key to isolate numbers in Fig. 4.6.

Number	Isolate	Number	Isolate
1	BPL	50	INV
2	22B1	51	SB6
3	SB3	52	21G
4	5G	53	PL11
5	22C	54	PrA
6	IW225	55	T15
7	IW34	56	HR
8	IW2	57	HK
9	IW227	58	GM
10	736	59	LRP
11	DA	60	93B
12	IW223	61	93E
13	SB8		
14	14H		
15	IW43		
16	IW26		
17	93C		
18	T1		
19	BUS94-8		
20	92A		
21	DK		
22	FANS		
23	IW10		
24	LBFF3		
25	20C		
26	PrF		
27	7J		
28	29		
29	93D		
30	Pr2		
31	93A		
32	EH5		
33	BUS94-5		
34	EH3		
35	T4		
36	T2		
37	CoA		
38	EH1		
39	QE4		
40	BUS94-2		
41	92C		
42	92B		
43	2C		
44	737		
45	BUS94-7		
46	SB7		
47	PL6		
48	LD		
49	TY		

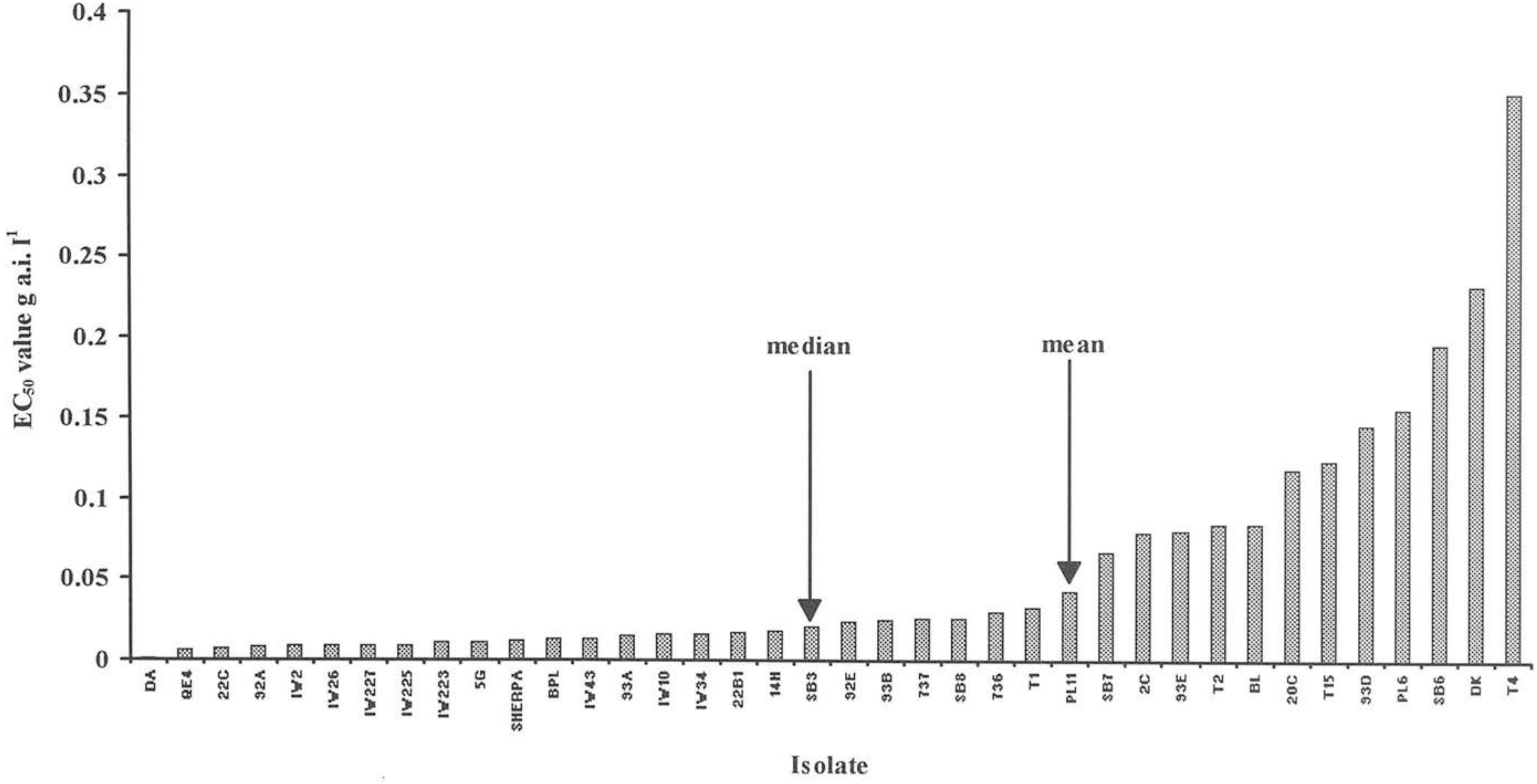


Fig. 4.7. EC_{50} values for barley powdery mildew isolates tested for sensitivity to fenpropidin from 1991-1993.

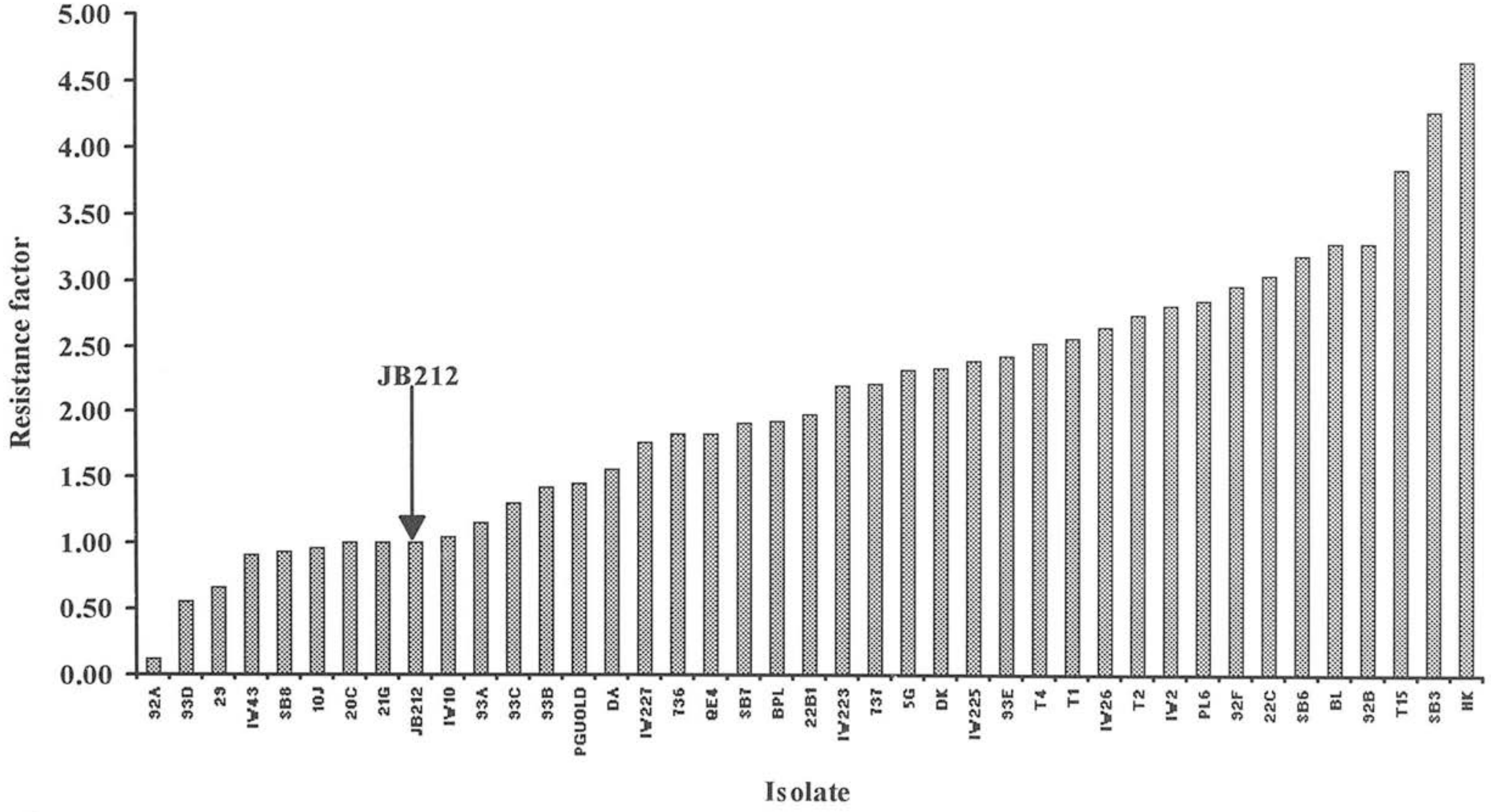


Fig. 4.8. Resistance factors for 1991-1993 barley mildew isolates tested against tridemorph compared with a 1984 isolate (JB212).

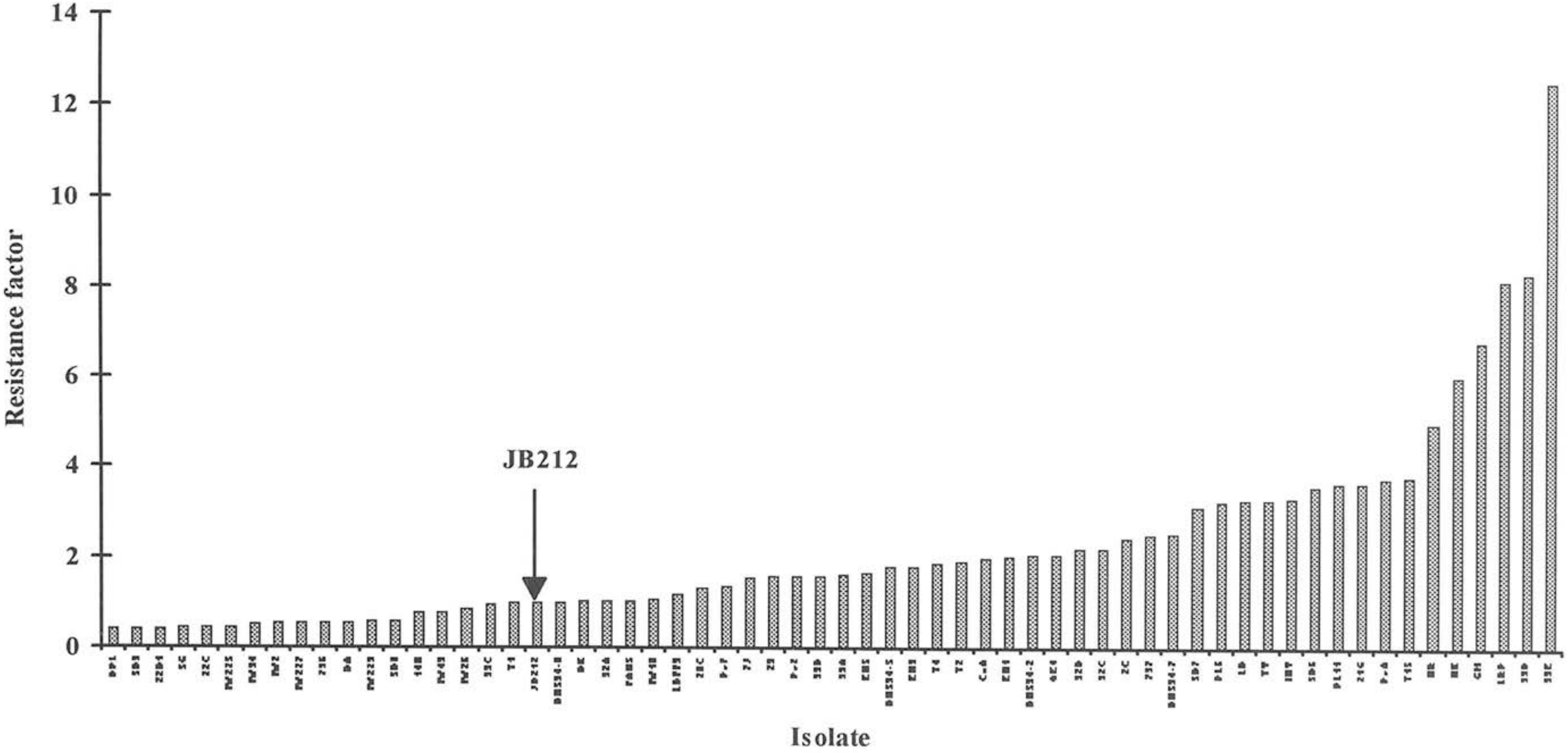


Fig. 4.9. Resistance factors for 1991-1994 barley powdery mildew isolates tested against fenpropimorph compared with a 1984 isolate (JB212).

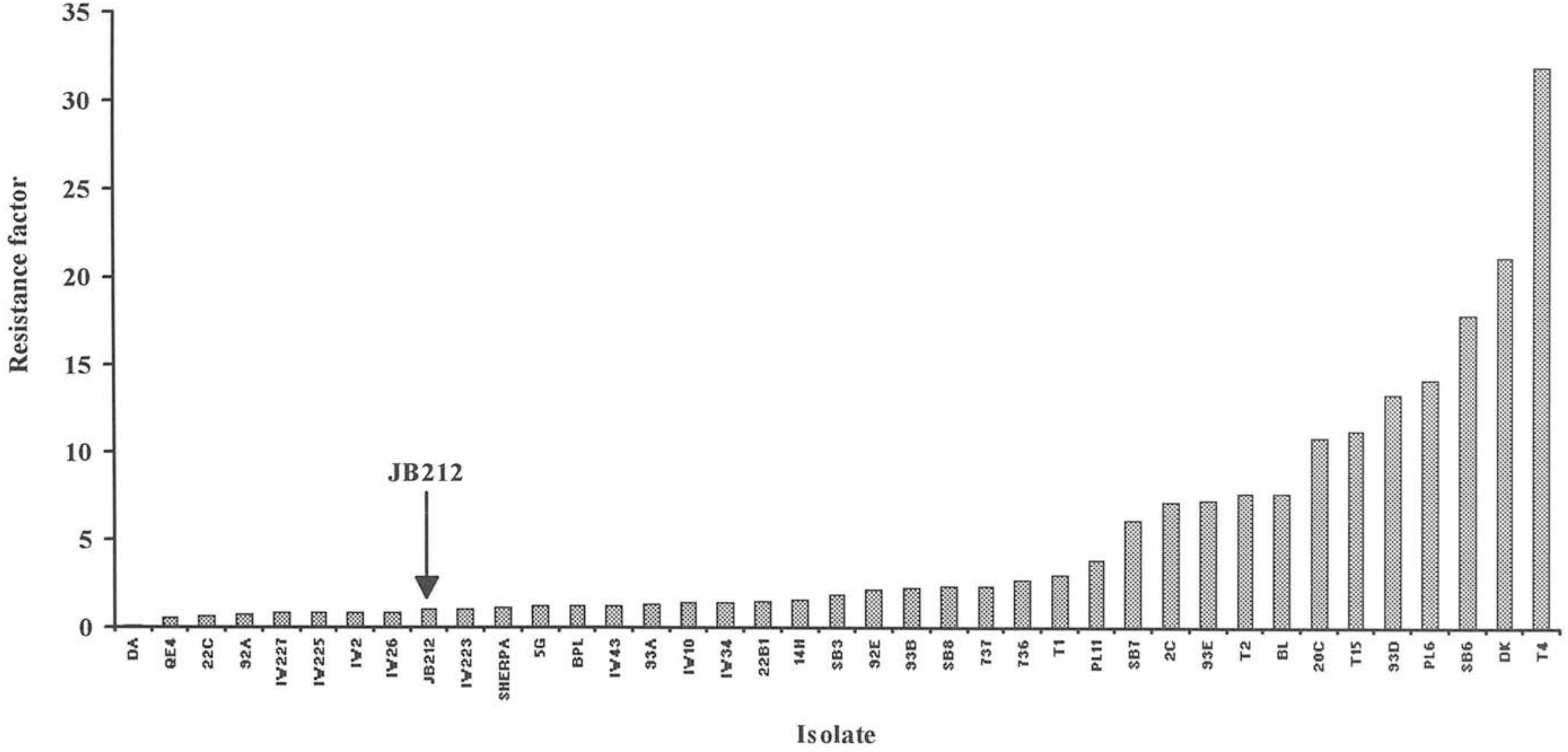


Fig. 4.10. Resistance factors for 1991-1993 barley powdery mildew isolates tested against fenpropidin compared with a 1984 isolate (JB212).

Fig. 4.11-4.13 show correlations of EC_{50} values of barley isolates, collected over the period 1991 to 1993, between tridemorph and fenpropimorph, tridemorph and fenpropidin and fenpropimorph and fenpropidin respectively. Fig. 4.11 shows that there was a slight positive correlation between sensitivity to tridemorph and sensitivity to fenpropimorph but the correlation coefficient $r = 0.073$ with 32 degrees of freedom is not significantly different from zero i.e. there was no relationship between the tridemorph sensitivity and the fenpropimorph sensitivity of these isolates. There was also a slight positive correlation between sensitivity to tridemorph and sensitivity to fenpropidin as shown in Fig. 4.12 but again the correlation coefficient $r = 0.214$ with 30 degrees of freedom is not significantly different from zero. According to the monitoring results, there was therefore no correlation between tridemorph and fenpropimorph and between tridemorph and fenpropidin. However, the positive correlation between sensitivity to fenpropimorph and sensitivity to fenpropidin shown in Fig. 4.13, where $r = 0.426$ with 36 degrees of freedom, is significant ($P = 0.05$).

The data were analysed by the Student *t* test to assess the influence on sensitivity of isolates of their history, i.e. geographic origin of the isolates (whether England or Scotland), year of collection and previous fungicide use on the crop from which samples were taken. Previous fungicide use was analysed according to whether no fungicide had been applied, if there was a history of fenpropimorph application and if other fungicides including other morpholines had been applied. Results from the analyses are summarised in Tables 4.4-4.6. There were no significant differences in any of the fungicide history categories for tridemorph (Table 4.4).

There was a significant difference between isolates collected from Scotland and those collected from England in their sensitivity to fenpropimorph ($P = <0.001$ (d.f. = 21) (Table 4.5). Isolates from Scotland were significantly less sensitive to the fungicide than those from England. Only isolates collected in 1991 and 1992 were analysed for

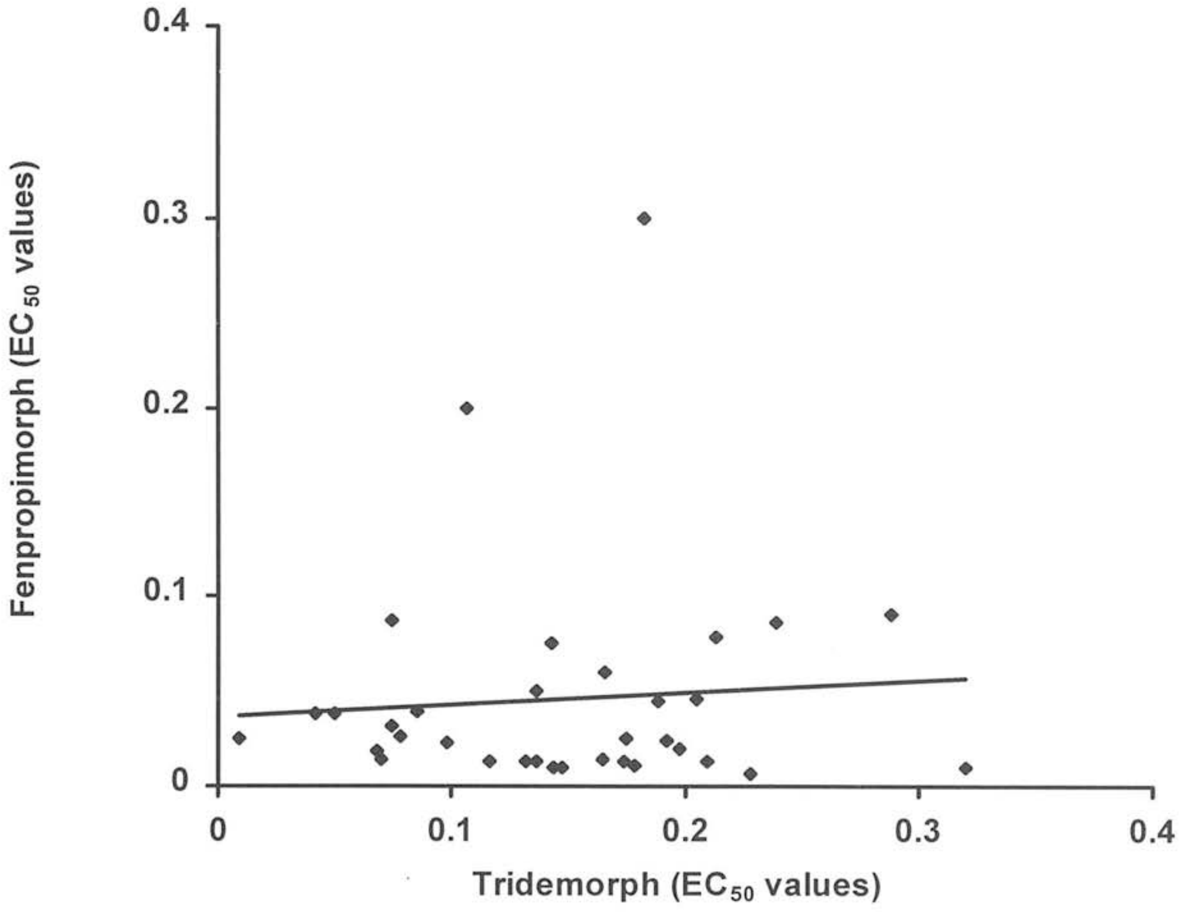


Fig. 4.11. Correlation between tridemorph and fenpropimorph EC_{50} values (g a.i. l^{-1}) for 1991-1993 barley powdery mildew isolates. (r = 0.073, n = 33, n.s)

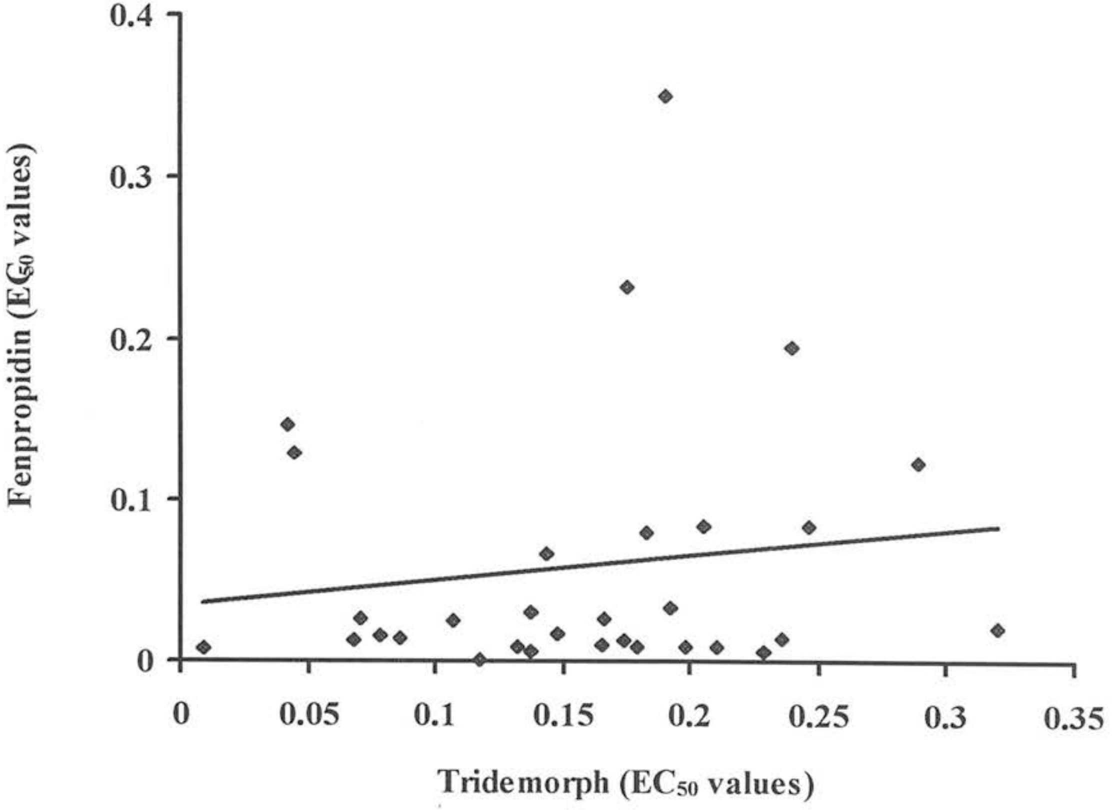


Fig. 4.12. Correlation between tridemorph and fenpropidin EC_{50} values (g a.i. l^{-1}) for 1991-1993 barley powdery mildew isolates. (r = 0.214, n = 31, n.s)

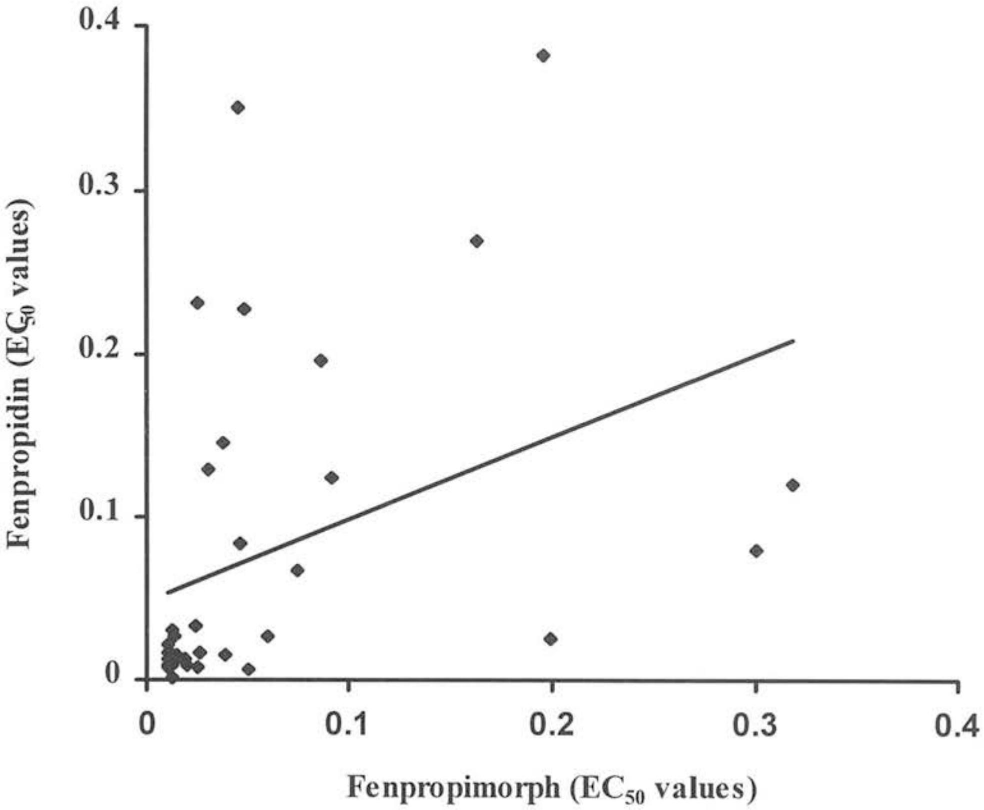


Fig. 4.13. Correlation between fenpropimorph and fenpropidin EC_{50} values (g a.i. l^{-1}) for 1991-1993 barley powdery mildew isolates (r = 0.426, n = 37, *P* = 0.05)

Table 4.4. Average tridemorph EC_{50} values in relation to geographic origin of isolates, year of sampling and previous fungicide treatment of the source crop.

Factor	N	Mean	S.E. of Mean	t-test P-value		
Geographic origin						
Scotland	12	0.139	0.020	-		
England	9	0.136	0.020	0.922(d.f. = 19)	-	
				Scotland	England	
Year (Scotland)						
1991	5	0.116	0.028	-		
1992	7	0.156	0.029	0.341(d.f. = 10)	-	
1993	15	0.175	0.024	0.135(d.f. = 11)	0.619(d.f. = 14)	-
				1991	1992	1993
Year (England)						
1991	4	0.139	0..038	-		
1992	5	0.135	0.023	0.937(d.f. = 5)	-	
				1991	1992	
Fungicide treatment						
No fungicide	9	0.192	0.030	-		
Fenpropimorph	5	0.210	0.029	0.684(d.f. = 11)	-	
Other fungicides	4	0.130	0.033	0.263(d.f. = 8)	0.114(d.f. = 7)	-
				No fungicide	Fenpropimorph	Others

d.f. = degrees of freedom N = number of isolates EC_{50} values = g a.i. l^{-1}

Table 4.5. Average fenpropimorph values in relation to geographic origin of isolates, year of sampling and previous fungicide treatment of the crop source.

Factor	N	Mean	S.E of Mean	t-test *P*-value			
Geographic origin							
Scotland	18	0.054	0.008	-			
England	10	0.018	0.003	<0.001(d.f.= 21)	-		
				Scotland	England		
Year (Scotland)							
1991	8	0.051	0.011	-			
1992	10	0.057	0.011	0.710(d.f. = 16)	-		
1993	15	0.076	0.021	0.292(d.f. = 20)	0.413(d.f. = 20)	-	
1994	13	0.044	0.005	0.523(d.f. = 8)	0.306(d.f. = 13)	0.153(d.f. = 15)	-
				1991	1992	1993	1994
Year England							
1991	5	0.018	0.003	-			
1992	5	0.017	0.005	0.895(d.f. = 6)	-		
				1991	1992		
Fungicide treatment							
No fungicides	13	0.048	0.011	-			
Fenpropimorph	5	0.040	0.011	0.634(d.f. = 12)	-		
Other fungicides	13	0.041	0.007	0.624(d.f. = 19)	0.931(d.f. = 7)	-	
				No fungicides	Fenpropimorph	Others	

d.f. = degrees of freedom N = number of isolates EC_{50} values = g a.i. l^{-1}

Table 4.6. Average fenpropidin values in relation to geographic origin, year of sampling and previous fungicide treatment of the crop source.

Factor	N	Mean	S.E of Mean	t-test P-value		
Geographic origin						
Scotland	13	0.060	0.019	-		
England	8	0.012	0.001	0.033(d.f.= 12)	-	
				Scotland	England	
Year Scotland						
1991	7	0.063	0.022	-		
1992	6	0.054	0.036	0.846(d.f. = 8)	-	
1993	13	0.090	0.027	0.444(d.f.= 18)	0.446(d.f. = 11)	-
				1991	1992	1993
Year England						
1991	5	0.013	0.002	-		
1992	4	0.012	0.002	0.670(d.f. = 6)	-	
				1991	1992	
Fungicide treatment						
No fungicides	7	0.055	0.028	-		
Fenpropimorph	5	0.111	0.061	0.434(d.f. = 6)	-	
Other fungicides	5	0.057	0.044	0.965(d.f. = 7)	0.495(d.f. = 7)	-
				No fungicide	Fenpropimorph	Others

d.f. = degrees of freedom N = number of isolates EC_{50} values = g a.i. l^{-1}

this factor as isolates from England were collected only in those years. There were no significant differences in mean EC_{50} values of isolates between years of collection 1991-1994 or fungicide treatment prior to collection (Table 4.5).

Like fenpropimorph, there was a significant difference in sensitivity to fenpropidin (Table 4.6) between isolates collected from Scotland and those collected from England (P = 0.033 (d.f. = 12)). Isolates from Scotland were significantly less sensitive to fenpropidin than isolates from England. Again like fenpropimorph, there were no significant differences between years of collection or different fungicide treatments.

Discussion

The barley powdery mildew isolates screened in the monitoring work displayed different ranges of sensitivity reactions to the three morpholine fungicides. In general the isolates were less sensitive to tridemorph than to fenpropimorph and fenpropidin (Tables 4.1-4.3). Robertson *et al.* (1996) also found this to be true in monitoring work carried out between 1988 and 1991. They attributed this to the distinct modes of action of the fungicides and to a decrease in sensitivity to tridemorph which has been on the market longer (it was introduced in 1969, Kradel *et al.*, 1969) than the two other morpholines introduced in the 1980s (Robertson *et al.*, 1996). The primary cause of differences in sensitivity to the morpholines would appear to be the mode of action of tridemorph, fenpropimorph and fenpropidin. Fenpropimorph and fenpropidin inhibit sterol Δ^{14} (B1) reductase strongly and $\Delta^{8}\rightarrow\Delta^{7}$ (B2) isomerase weakly, whereas the reverse is the case for tridemorph (Baloch *et al.*, 1984; Girling *et al.*, 1988) (Fig 4.14 (i)). Ziogas *et al.* (1991) also showed that fenpropimorph inhibits an additional enzyme in *Nectria haematococca* var. *cucurbitae*: squalene epoxidase. This difference could also be the reason for the degree of correlations of EC_{50} values for isolates found between the respective fungicides. There was no correlation between isolate EC_{50} values against tridemorph and either fenpropimorph or fenpropidin. However, there was a positive correlation between the fenpropimorph and fenpropidin EC_{50} values of isolates. Therefore, there was cross-resistance between fenpropimorph and fenpropidin, a feature which Brown & Evans (1992) also found. They went on further to hypothesise that cross-resistance between the two chemicals was due to a single mechanism of resistance to both. Brown *et al.* (1991) found only limited cross-resistance between fenpropimorph and fenpropidin on one hand and tridemorph on the other, which again may be attributable to their different modes of action. The overall greater level of toxicity shown by fenpropimorph and fenpropidin compared with tridemorph, as evidenced by their respective average EC_{50} values over the monitoring period, may also be due to their respective modes of action. However, as explained by Robertson *et al.* (1996), the longer period of exposure of mildew populations to tridemorph may also be a factor.

Fig. 4.14 (i). Biosynthetic pathway of ergosterol. A = point of blockage (site of action) of triazoles (demethylation inhibitors = DMI group); B1 & B2 = points of blockage of morpholines (After Sisler & Ragsdale, 1984; Parry, 1990).

squalene → 2,3–oxidosqualene → lanosterol→ eburicol

↓

4,4-dimethylergosterol-trien-ol←A 14α–methylfecosterol←obtusifoliol

↓

4α-methylergosta-trien-ol→ignosterol **B1**→4,4–dimethyl-dien-ol

↓

ergosta-trien-ol←B2 fecosterol← 4α-methylergosta-dien-ol

↓

ergosta-dien-ol→episterol→ ergosterol

As previously noted, the results of genetic analysis by Brown (1996) indicate that the responses to fenpropimorph and fenpropidin are controlled by the same gene or genes, that one or very few genes control resistance, that the gene or genes in the resistant type isolates which confer resistance to fenpropimorph and fenpropidin would have little or no effect on tridemorph sensitivity but that the fenpropimorph/fenpropidin resistance gene or genes in the type of isolates that are more resistant (type RM) than that classified as resistant (type R) would also confer some resistance to tridemorph.

The annual mean sensitivity of screened isolates to tridemorph decreased over the three years (1991-1993) of the study period although the changes were not significant (Table 4.1). The decrease followed a trend shown in previous observations begun in 1988 (Table 4.7). Walmsley-Woodward, Laws & Whittington (1979) also reported a decrease in tridemorph sensitivity over time. They found a widespread occurrence of stability of tolerance to tridemorph in isolates used in their studies. Six of the 51 isolates used were found to be tolerant of high levels of the fungicide and tolerance was stable. Brown & Evans (1992) also reported isolates that showed increased resistance to tridemorph.

The annual mean sensitivity of screened isolates to fenpropimorph decreased from 1991 to 1993 but returned to the 1992 level in 1994 (Table 4.2). From an earlier study (Table 4.7) no clear change in sensitivity with time was found. Several workers have, however, reported reduced sensitivity to fenpropimorph for barley powdery mildew isolates. Wolfe *et al.* (1987) observed the occurrence, in Scotland in 1986 and 1987, of pathogen phenotypes with a combined reduction in sensitivity to the three major fungicide groups. Brown & Evans, (1992) also reported isolates that showed some resistance to fenpropimorph. However, the sensitivities of isolates in the present studies remained within the same range as those tested earlier by Robertson *et al.* (1996) (Table 4.7).

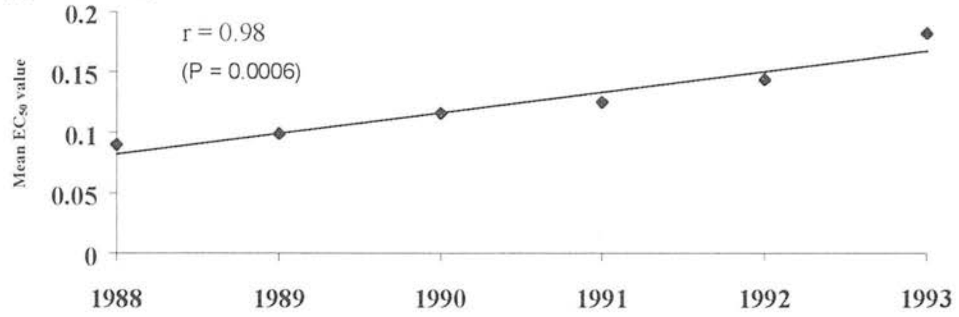
The annual mean sensitivity of isolates to fenpropidin changed from year to year (Table 4.3) although, again, they remained within the same range as those tested by Robertson *et al.* (1996) (Table 4.7). Brown & Evans (1992) referred to isolates that were resistant to fenpropidin but, together with tridemorph and fenpropimorph, they indicated that the levels of resistance they found were unlikely to cause a substantial loss of effectiveness of the chemicals immediately after spraying.

Data sets in Tables 4.1-4.3 and 4.7 have been amalgamated and are presented in Fig. 4.14 (ii). There was a significant positive correlation between mean EC_{50} values and years with the correlation coefficient $r = 0.98$ for tridemorph, indicating that there was a steady decrease in sensitivity of barley powdery mildew to tridemorph. There was however, no significant correlation between mean EC_{50} values and years for fenpropimorph ($r = 0.43$) and fenpropidin ($r = 0.02$).

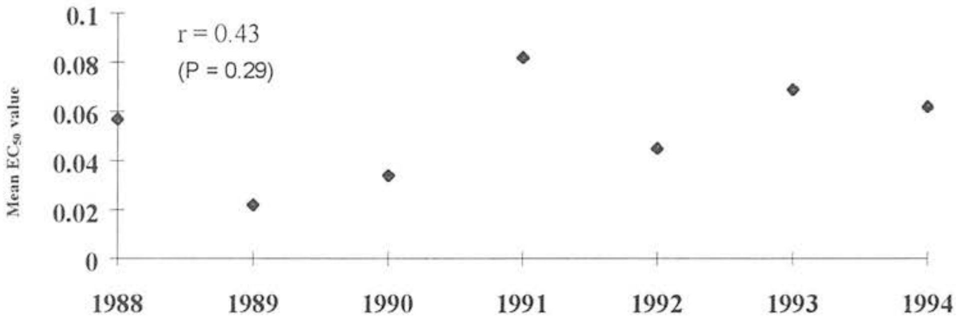
Table 4.7. Annual mean EC_{50} values (g a.i. l^{-1}) (from Robertson *et al.* (1996)).

Year collected	No. of isolates	Mean EC_{50}	Range
Tridemorph			
1984 (JB212)	1	0.075	-
1988	23	0.090	0.031-0.194
1989	30	0.099	0.037-0.165
1990	20	0.116	0.045-0.232
1991	9	0.125	0.081-0.701
Fenpropimorph			
1984 (JB212)	1	0.024	-
1988	13	0.057	0.007-0.118
1989	26	0.022	0.010-0.051
1990	19	0.034	0.008-0.115
1991	3	0.082	0.054-0.118
Fenpropidin			
1984 (JB212)	1	0.011	-
1988	10	0.084	0.003-0.226
1989	25	0.026	0.008-0.050
1990	14	0.079	0.008-0.162
1991	2	0.090	0.057-0.122

(a) Tridemorph



(b Fenpropimorph



(c) Fenpropidin

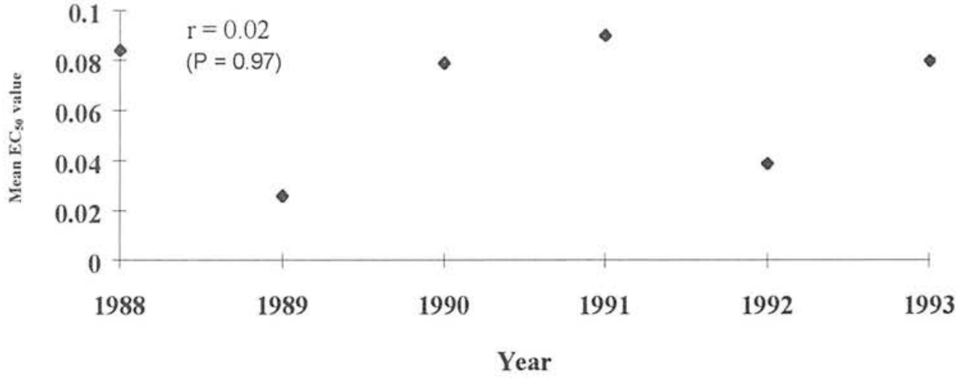


Fig 4.14 (ii). Correlation between mean EC$_{50}$ values from the monitoring work, and years for tridemorph, fenpropimorph and fenpropidin (Data from Robertson *et al.* (1996) and present study combined).

Isolates showed a continuous range in variation in sensitivity for all three fungicides. Robertson *et al.* (1990) also found a continuous distribution of EC_{50} values for the three chemicals. This contrasts with Brown, Slater & See (1991) who found distinct classes of sensitive and resistant isolates. Brown *et al.* (1991) used single colony isolates, while in the studies of Robertson *et al.* (1990) and in the present work mostly bulk (heterogeneous) isolates were used and these may have consisted of several clones of *E. graminis* f.sp. *hordei* with varying degrees of sensitivity. As Brown *et al.* (1991) indicated, varying proportions of resistant and sensitive isolates in a bulk (heterogeneous) isolate would give rise to the appearance of a continuous distribution of EC_{50} values and they considered that their results were not at variance with those of Robertson *et al.* (1990). The same observations would apply in relation to the present work and the findings of Brown *et al.* (1991).

It is interesting to note that when isolates from the work of Brown *et al.* (1991) classified as sensitive (CC1) or at two levels of resistance (CC139 and CC151) to fenpropimorph and fenpropidin were tested as part of the present study using the standard leaf-segment method at SAC, the resistant isolates CC139 and CC151 fell within the range of our more resistant isolates but that isolate CC1 showed a greater sensitivity than any of the most sensitive isolates in the present work. This again may be explained by the use of single colony isolates as opposed to bulk isolates. Had selection in the present work been made within our more sensitive isolates a sensitivity equivalent to that of Brown *et al.* (1991) may have been identified.

The variation among isolates in their sensitivity to the three fungicides may be related in part to the history of fungicide use and of cultivar use in different localities. Both Brown *et al.* (1991) and the present work showed that sensitivity to fenpropimorph and fenpropidin was more widespread in isolates, including CC1, from England than in isolates from Scotland. Brown & Evans (1992) classified all the isolates they collected from England as sensitive and those from Scotland as resistant. This frequency of resistance may be explained by the extensive cultivation of the mildew susceptible cv. Golden Promise in Scotland in the 1980s and the need to protect this cultivar by the extensive use of morpholine fungicides (Gilmour, 1984; Robertson *et al.*, 1990). As a consequence, selection would be against the more sensitive isolates.

In England greater use was made of mildew resistant cultivars allowing less use of fungicides and thus less selection against sensitivity. Differences in the cultivars used may also give rise to genetical variation in mildew populations involving linkages between sensitivity factors and other inheritance characteristics. Another explanation would be the greater area in cereals in England, and higher mildew levels creating a larger population and a larger potential reservoir of sensitive isolates.

Comparison of the sensitivity of isolates included in this study with sensitivity data published for a 1984 isolate (JB212) from England (Robertson *et al.*, 1996) revealed that JB212 fell among the more sensitive isolates for all three fungicides. Most of the isolates in the present study had EC_{50} values which were higher than those for JB212 for all fungicides. This might be an indication of a reduction in sensitivity to the fungicides since 1984. However, since JB212 was not the most sensitive isolate when compared to our isolates this means that there are still some sensitive isolates within the populations. Most of the isolates which were more sensitive than JB212 came from England (Fig. 4.8-4.10, Appendix IV.i). However, sensitive isolates still persisted in a smaller proportion in Scotland.

Despite the suggestion of a link between the extent of previous fungicide use and sensitivity, no relationship was found between fungicide contact in the immediate history of the crop and sensitivity (Tables 4.4-4.6), in keeping with the results of Robertson *et al.* (1990).

The lack of variation in sensitivity between years might suggest that a residue of sensitive isolates persist and become re-established in the population when fungicides are not being applied and resistant isolates have no selective advantage. Circumstantial evidence might indicate that more sensitive isolates are more fit. This is because of (a) failure to find extremes of resistance in the bulk isolates when compared with those of Brown & Evans (1992), (b) the persistence of sensitive isolates in the population over many years and (c) which result in possibly the annual re-establishment of sensitive isolates in the field when fungicide use diminishes. Under crowded infection conditions, Engels & de Waard (1996) found that isolates of *E. graminis* f.sp. *tritici* with reduced sensitivity to fenpropimorph displayed a lower competitive ability than wild type isolates.

Although there is evidence of resistance in the work of Brown *et al.* (1992) and in the present work, advisory experience would suggest that morpholines still give effective control of barley powdery mildew (S. Oxley, pers. comm.). As already indicated, a reason to account for this would be the mode of action of the morpholines which involves more than one site (Fig. 4.4). Fungicides acting at a single site, e.g. the MBCs, have proved more prone to resistance problems.

Wheat powdery mildew

Materials and Methods

Leaves infected with wheat powdery mildew were collected from commercial crops and trial plots from the east of Scotland between 1993 and 1995 and from a 1994 trial at Morley Research Centre (Norwich) (Fig.4.15). Wherever possible, crop details were recorded on collection of isolates (Appendix IV.ii). Many of the isolates were heterogeneous consisting of inoculum obtained from several pustules on a single leaf collected from the field, although there were some single colony isolates. Of the 33 isolates tested against fenpropimorph 48% were single pustule isolates and of the 23 isolates tested against fenpropidin 65% were single pustule isolates. The infected leaves were taken to the greenhouse where they were cut into leaf segments and incubated in Petri dishes for 2-3 days, to allow sporulation to take place as described in Chapter 3. The different isolates were then subcultured on to fresh leaves of whole plants of cv. Cerco which had been raised in isolation propagators (Chapter 3). Isolates were subcultured several times to increase the amount of inoculum before they were tested for sensitivity. Isolates were tested for sensitivity to fenpropimorph and fenpropidin following the variations for wheat as stated in the General Materials and Methods (Chapter 3).

Wheat plants to be sprayed were grown, ten to a 12.5 cm pot, to growth stage 12 (GS 12). Five fungicide concentrations of fenpropimorph and fenpropidin, based on the full commercial field spray rate C were used. The concentration range for both fungicides was 1/64C, 1/32C, 1/16C, 1/8C, and 1/4C giving concentrations of 0.059, 0.117, 0.234, 0.469 and 0.938 g a.i. l^{-1}. The wheat plants were sprayed with the test fungicide in ascending order of concentration (see Chapter 3), for 10 seconds followed by 15 minutes to allow the fungicide cloud to settle within the spray cabinet. Control plants were sprayed with water. Two replicates were sprayed at each concentration. As for barley, the sprayed plants were kept apart for 24 hours to allow sufficient time for the fungicide uptake, after which eight leaf segments were

Fig. 4.15. The sources of wheat mildew isolates.

X = areas from where isolates were collected (figures in brackets refer to number of isolates collected) prepared from the middle of the second leaves of the sprayed plants and placed on benzimidazole agar. The leaf segments were inoculated with the different isolates using a sterile fine paint brush and were then incubated at 18°C with a 12 hour photoperiod. The leaf segments were assessed for percentage mildew cover two weeks later and EC_{50} values (g a.i. l^{-1}) were determined for each isolate as described in Chapter 3. In order to screen as many wheat isolates as were tested, a decision was made to concentrate on fenpropimorph and fenpropidin (chemicals that are commonly used on wheat) and leave out tridemorph.

Results

The distribution of isolates under different categories of EC_{50} values is represented in Fig. 4.16 and 4.17. For fenpropimorph, isolates follow a normal distribution, with EC_{50} values of 0.2-0.3 for most isolates. For fenpropidin, the distribution of isolates is skewed with most isolates having EC_{50} values within the range <0.1-0.1. EC_{50} values ranged up to 0.5-0.6 for fenpropimorph and up to 0.2-0.3 for fenpropidin.

The mean of EC_{50} values of isolates tested against fenpropimorph and fenpropidin for different years, their range and their standard error (SE) are summarised in Table 4.8. There was an increase in mean, minimum and maximum EC_{50} values from 1993 to 1995 for fenpropimorph. The increases in means between 1993 and 1995 and between 1994 and 1995 were significant (Table 4.9). However, there were no clear differences in EC_{50} values for fenpropidin in 1993 and 1994. Compared with barley isolates (Tables 4.2 & 4.3) the mean EC_{50} values for wheat isolates are higher in all the years of testing.

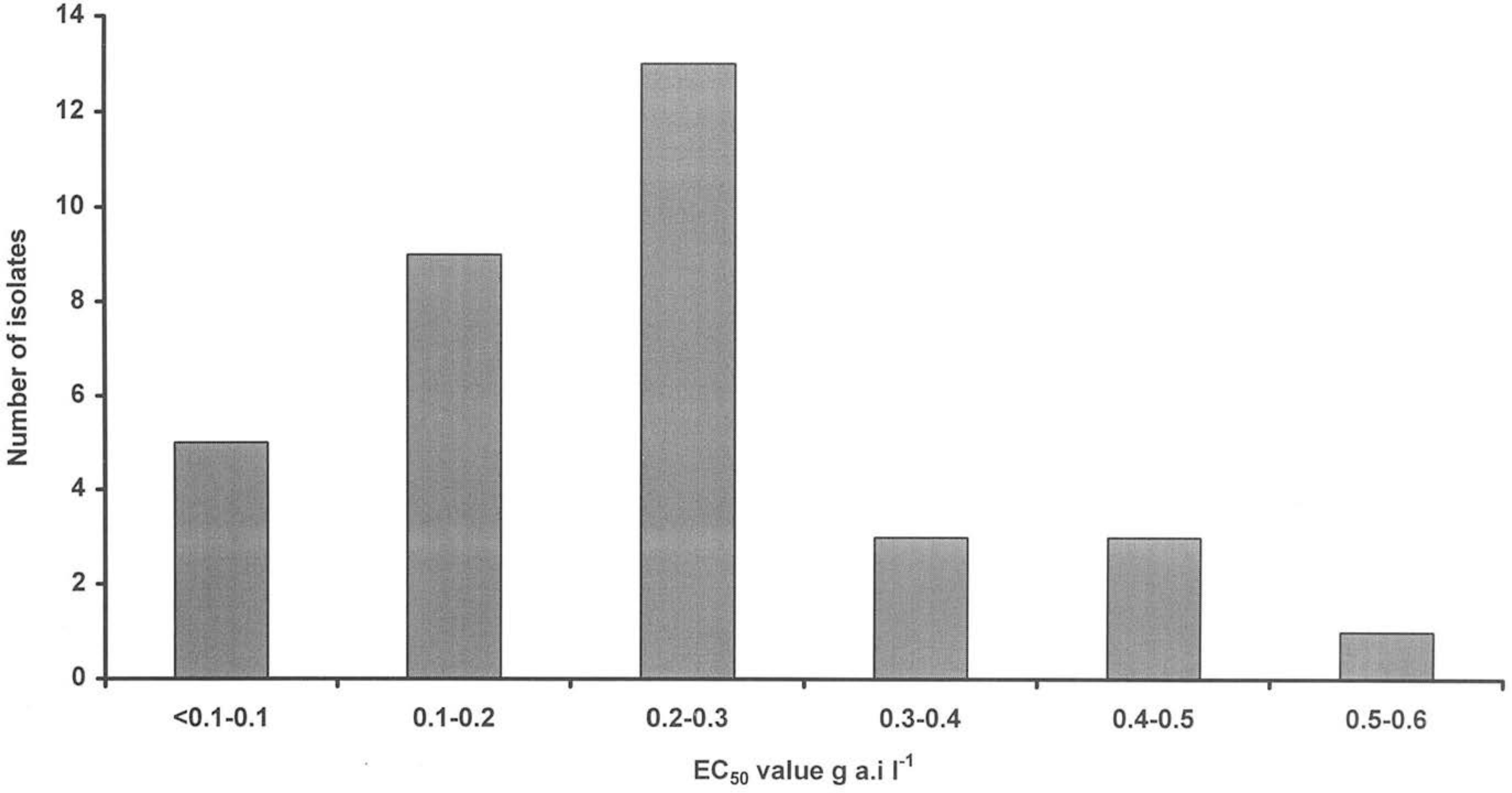


Fig. 4.16. Number of 1993-1995 wheat powdery mildew isolates tested for sensitivity to fenpropimorph under different categories of EC_{50} values.

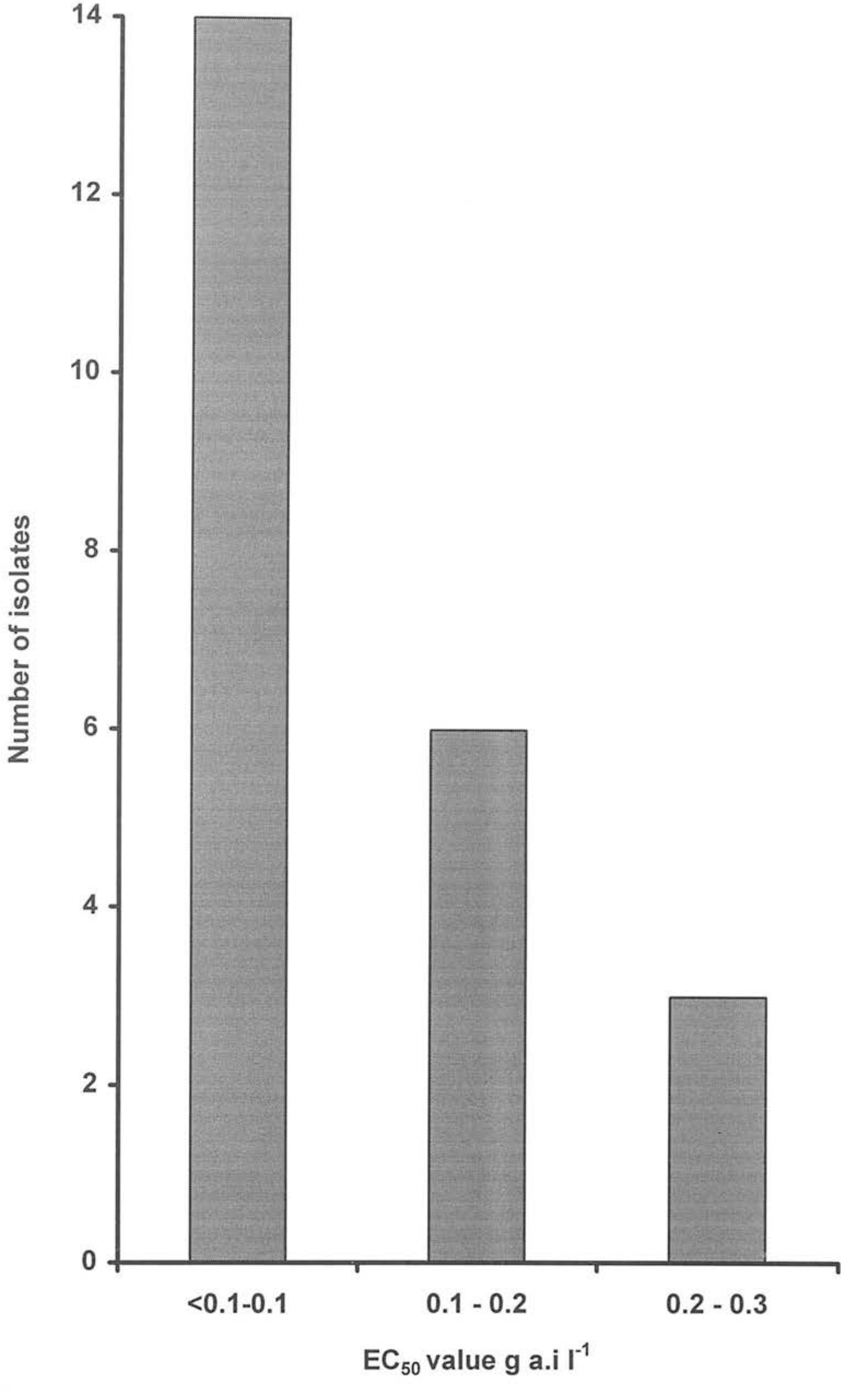


Fig. 4.17. Number of 1993-1994 wheat powdery mildew isolates tested for sensitivity to fenpropidin under different categories of EC$_{50}$ values.

Table 4.8. Mean EC_{50} values (g a.i l^{-1}), range and SE from the wheat monitoring work, for fenpropimorph and fenpropidin.

Year	No. of isolates	Mean EC_{50}	Range	SE of Mean
Fenpropimorph				
1993	9	0.167	0.012-0.244	±0.027
1994	13	0.196	0.062-0.324	±0.022
1995	12	0.327	0.163-0.548	±0.036
Fenpropidin				
1993	11	0.113	0.014-0.255	±0.022
1994	12	0.080	0.017-0.207	±0.018

Fig. 4.18 shows the EC_{50} values of all wheat isolates tested for sensitivity to fenpropimorph between 1993 and 1995 indicating the variation in sensitivity. The range is continuous from the most sensitive isolate (EC_{50} = 0.012) to the least sensitive one (EC_{50} = 0.548) with a 46-fold difference between the two. The mean and the median fall on either side of isolate 51CAMP.

Fig. 4.19 shows the EC_{50} values of wheat mildew isolates tested for sensitivity to fenpropidin in 1993 and 1994. The variation was again continuous from the most sensitive isolate (EC_{50} = 0.014) to the least sensitive one (EC_{50} = 0.255) with a 18-fold difference between the two. The mean and the median fall within two isolates of one another with the median lower than the mean.

Fig. 4.20 shows the relationship between EC_{50} values for fenpropimorph and fenpropidin of wheat mildew isolates collected in 1993 and 1994. The figure suggests a negative correlation between the two, but the correlation coefficient $r = -0.265$ with 17 degrees of freedom is not significantly different from zero.

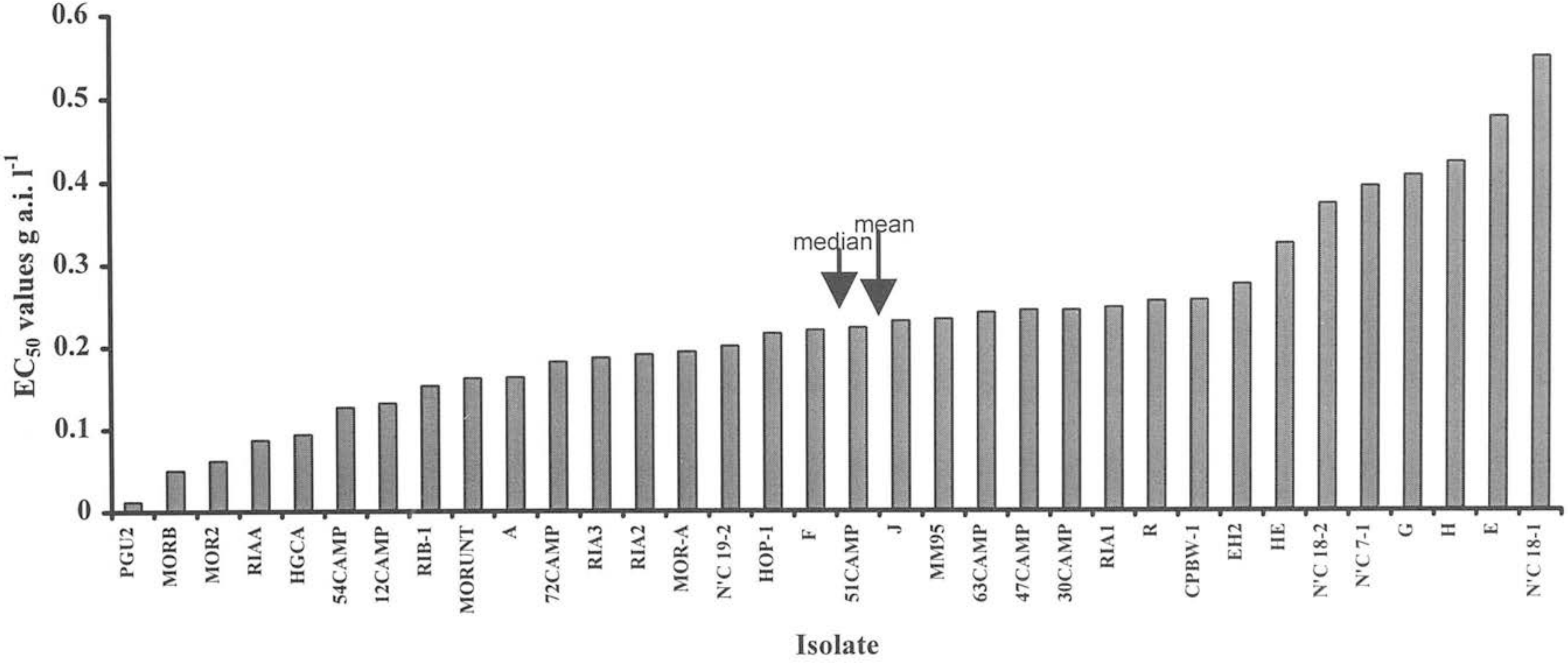


Fig. 4.18. EC_{50} values for wheat powdery mildew isolates tested for sensitivity to fenpropimorph from 1993-1995.

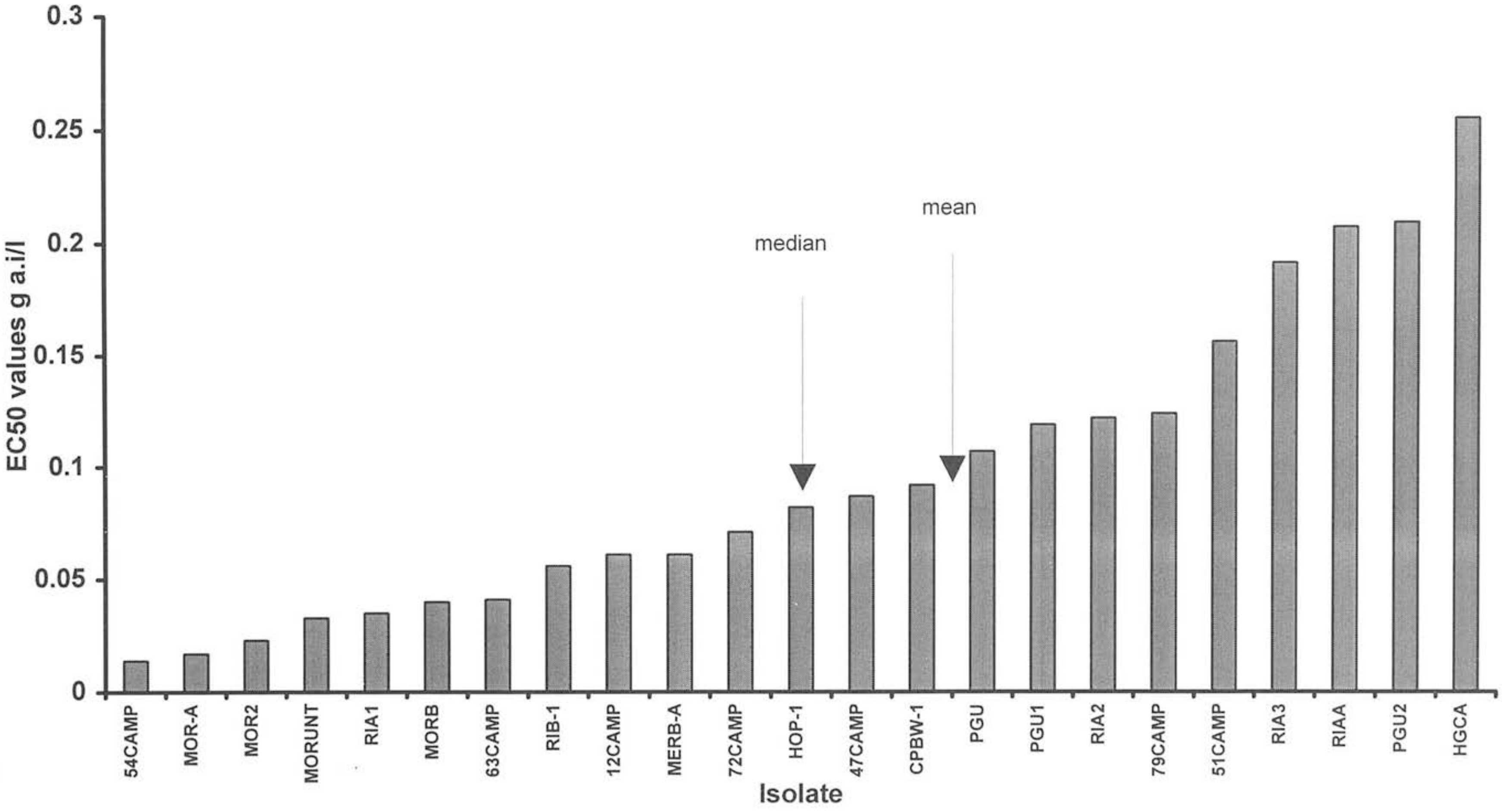


Fig.4.19. EC_{50} values for wheat powdery mildew isolates tested for sensitivity to fenpropidin from 1993-1994.

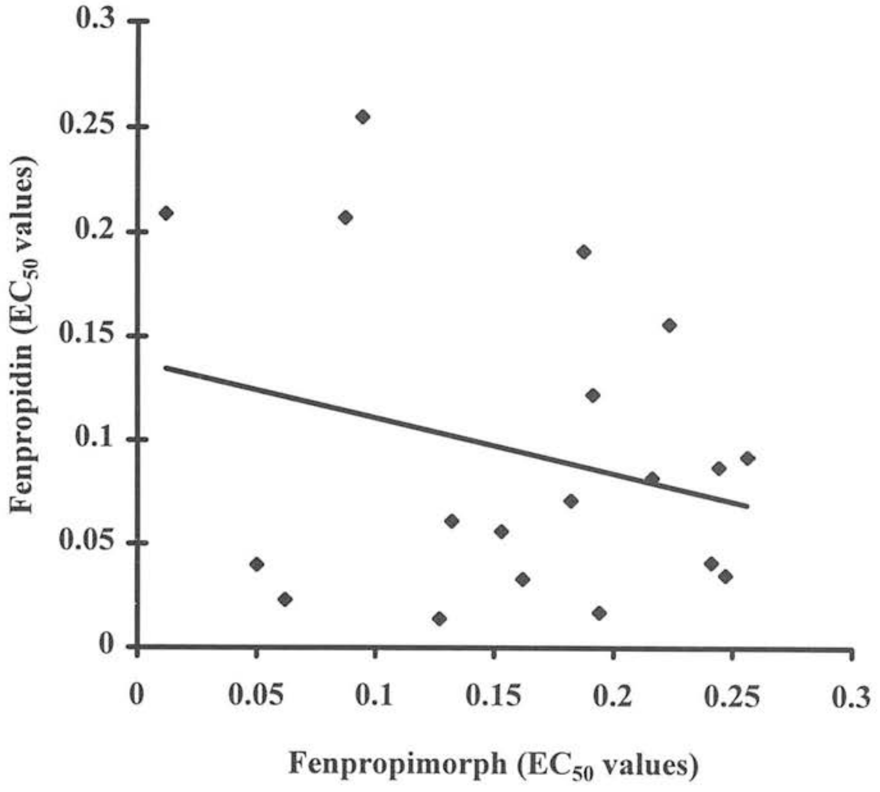


Fig. 4.20. Correlation between fenpropimorph and fenpropidin EC_{50} values (g a.i. l^{-1}) for 1993 and 1994 wheat powdery mildew isolates. (r = -0.265, n = 19, n.s.)

The data were analysed using the t-test to assess any influence on fungicide sensitivity of the immediate history of the crops from which samples were obtained; i.e. geographic origin and years of collection. Sampling from England was done in one year only so data for this latter factor was not analysed for isolates from England. Almost all the samples for which information was available came from untreated crops so data could not be analysed for previous fungicide history. The results of the analyses are summarised in Tables 4.9 and 4.10.

The mean EC_{50} value to fenpropimorph of isolates from Scotland was not significantly different from that of those from England (Table 4.9). With this fungicide there was no significant difference in the mean EC_{50} values of isolates collected in 1993 and 1994. However, there were significant differences in mean EC_{50} values between 1994 and 1995 ($P = 0.018$) and between 1993 and 1995 ($P = 0.002$) (Table 4.9), values for 1995 being greater than for the previous years.

Unlike the results for fenpropimorph, there was a significant difference in mean EC_{50} values of isolates obtained from Scotland and those obtained from England in their sensitivity to fenpropidin (Table 4.10). Isolates from Scotland were significantly less sensitive to fenpropidin than isolates from England ($P = 0.01$). The difference in mean EC50 values between years was not significant for fenpropidin.

Table 4.9. Average fenpropimorph EC_{50} values in relation to geographic origin and year of sampling.

Factor	N	Mean	SE of Mean	t-test P-value		
Geographic origin						
Scotland	9	0.215	0.024	-		
England	4	0.117	0.036	0.062 (d.f. = 6)	-	
				Scotland	England	
Year Scotland						
1993	9	0.167	0.027	-		
1994	9	0.215	0.024	0.195(d.f.= 16)	-	
1995	12	0.327	0.036	0.002(d.f. = 19)	0.018(d.f.= 18)	-
				1993	1994	1995

d.f. = degrees of freedom N = number of isolates EC_{50} values = g a.i. l^{-1}

Table 4.10. Average fenpropidin EC_{50} values in relation to geographic origin and year of sampling.

Factor	N	Mean	SE of Mean	t-test P-value	
Geographic origin					
Scotland	8	0.106	0.022	-	
England	4	0.028	0.005	0.010 (d.f. = 8)	-
				Scotland	England
Year Scotland					
1993	11	0.113	0.022	-	
1994	12	0.080	0.018	0.272 (d.f. = 20)	-
				1993	1994

d.f.= degrees of freedom N = number of isolates EC_{50} values = g a.i. l^{-1}

Discussion

The wheat mildew isolates tested tended to be more sensitive to fenpropidin than to fenpropimorph confirming advisory experience that fenpropimorph has shown less effective control of wheat powdery mildew than fenpropidin (Anon, 1996; S. Oxley, pers. comm.). Fenpropidin was the principal fungicide used on winter wheat in 1996 in Scotland, (no data for 1995 is available) accounting for 13% of the total spray area of fungicide active ingredients (Snowden & McCreath, 1996). Fig. 4.16. shows an approximately normal distribution of isolates tested against fenpropimorph whereas Fig. 4.17. shows a skew distribution towards the sensitive isolates for isolates tested against fenpropidin.

There was a shift towards reduced sensitivity to fenpropimorph in 1995 compared with the two previous years. No assessments were made on the sensitivity of isolates to fenpropidin in 1995 but the 1993 and 1994 data show no evidence of a decline in sensitivity. This is unlike the case of barley powdery mildew, where fenpropimorph is more effective and is the more commonly recommended of the two. During this period fenpropimorph was the main fungicide used in Scotland on both winter and spring barley, accounting for 21% and 23% respectively of the total spray areas of fungicide active ingredients (Snowden & McCreath, 1996). Furthermore, unlike the results for barley mildew isolates, where there was a significant positive correlation between EC_{50} values for fenpropimorph and fenpropidin, indicating cross-resistance between the two chemicals, for the wheat mildew isolates there was no evidence of any positive correlation. Cross-resistance between the two fungicides for wheat mildew has however, been reported in preliminary studies (Anon, 1996) and, further work is needed (a) to explain the variation of wheat mildew in response to fenpropimorph and fenpropidin when the mode of action of the two fungicides is apparently similar (Fig. 4.14) and (b) whether cross-resistance would have been identified by using a larger number of isolates, particularly if isolates had been tested against fenpropidin as well as fenpropimorph, in 1995. Lack of cross resistance could be due to minor differences in the mode of action of the two chemicals. As already mentioned, Ziogas *et al.* (1991) showed that fenpropimorph inhibits an additional enzyme in the sterol pathway in *Nectria haematococca* var. *cucurbitae*: squalene epoxidase. The slight negative correlation with regard to behaviour of isolates to the two fungicides appears to relate to three isolates with high EC_{50} values to fenpropidin and low EC_{50} values for fenpropimorph (Fig. 4.20). Had these three isolates been excluded, there is suggestion of a positive correlation. Results for fenpropidin are consistent with the fact that there were no reports of reduced sensitivity of wheat powdery mildew to it since its introduction in 1986.

There have been a number of reports where wheat powdery mildew isolates have been found to have reduced sensitivity to fenpropimorph. Lorenz, Saur & Schelberger (1992) carried out long term monitoring of wheat powdery mildew sensitivity towards fenpropimorph in Switzerland and Germany. From 1982 to 1988, there were only insignificant changes in sensitivity of wheat powdery mildew populations towards fenpropimorph in both countries. The first signs of a sensitivity shift became apparent in 1989 in both countries, and continued into 1990 but not beyond 1991. Field performance of fenpropimorph when applied according to label recommendations remained unaffected in their studies.

The sensitivity of wheat powdery mildew to morpholine fungicides has been monitored by the UK Cereal Pathogen Virulence survey since 1987. Bayles *et al.* (1997) found that most isolates they tested proved less sensitive to fenpropimorph than the sensitive control isolates collected during the 1970s although there was little evidence of any shift in sensitivity between 1987 and 1995. When they tested isolates from trials in England in 1996, however, they found few isolates which were as sensitive as the control isolates collected in the 1970s. They also found that 32% of 1996 isolates were less sensitive than the insensitive control isolate from 1994 indicating a shift towards insensitivity in 1996 compared with 1994.

Readshaw & Heaney (1994) also made a survey of fenpropimorph sensitivity in *E. graminis* f.sp. *tritici* in Northern France between 1991 and 1993. Compared with 1991/1992, the 1993 data showed a shift towards decreased fenpropimorph sensitivity in the wheat powdery mildew in all regions of Northern France. However, they made no attempt to relate their results to field performance of fenpropimorph which remained good (Russell, 1993).

Isolates from Scotland were found to be significantly less sensitive to fenpropidin than isolates from England (Table 4.10). This was not true for fenpropimorph where there were no significant differences between the two countries (Table 4.9).

Overall, barley isolates tended to be more sensitive to the morpholines than wheat isolates. Evidence would suggest that differences in history of use of the fungicides on the two crops is not involved as morpholines have been used over a long period and presumably more extensively on barley compared with wheat and this would give an expected greater level of resistance to emerge in barley mildew. The more likely explanation would seem to relate to differences in pathogen or host factors of physiological, morphological or biochemical nature which account for the greater vulnerability of barley mildew to the action of the chemical.

Chapter 5

Reduced Dosage Rates of Fungicide in Relation to Resistance

REDUCED DOSAGE RATES OF FUNGICIDE IN RELATION TO RESISTANCE

Introduction

Many farmers in Scotland use reduced doses of morpholines, usually in mixtures, as standard practice to control mildew infections in cereals. Because of the possibility that such practices might influence the selection pressure for insensitive isolates in the mildew population, field experiments were carried out to determine if the use of reduced doses was likely to influence the sensitivity of powdery mildew to fenpropimorph, the most commonly used morpholine (Bowen *et al.*, 1992).

This chapter reports the results of four field experiments carried out over the period 1992 to 1995 with spring barley and one in 1994/95 with winter wheat, to establish if any shift in sensitivity could be measured following a repeat application of reduced doses as applied in standard practice.

Sensitivity of barley powdery mildew to fenpropimorph in relation to reduced dosage rates of the fungicide

Materials and methods

In 1992 a large spring barley field experiment (Field experiment 1) was laid out at Boghall farm in the Midlothian Region of Scotland. The barley cultivar used was 'Golden Promise'. Fertiliser, herbicide and any micro-nutrient treatments were uniform across all plots, and accorded with local practice, whereby the seedbed was treated with $N:P_20_5:K_20$ in the ratio of 60:60:60 kg ha^{-1} and the nitrogen top dressing was 60 kg ha^{-1}. The herbicide Swipe P (bromoxynil + ioxynil + mecoprop-P) was applied twice at three l ha^{-1}. Certified seed for the trial was obtained from a seed merchant and had been treated with organomercury disinfectant to control foot rot and leaf stripe (*Pyrenophora* sp.).

Foliar fungicide treatments were applied as two spray programmes of either fenpropimorph (Corbel) alone or in a mixture with propiconazole (Tilt 250 EC) at a range of rates. The first fungicide application was made when mildew first developed on the plants and the second spray was applied three weeks later. There were eight treatments as shown in Table 5.1, with three replicates of each laid out in blocks. Three treatments (A, B & C) involved fenpropimorph used alone at different doses while in a further four treatments (D, E, F & G) fenpropimorph was sprayed in a mixture with the triazole fungicide propiconazole. An unsprayed control treatment (U) was included and the final treatment used involved a single 1/4 rate of both fungicides applied only at the second spray timing. Plot sizes were 24 m by 16 m. To facilitate spraying, plots within blocks were not completely randomised (Fig. 5.1). Because of the large plots, it was necessary to spray with a tractor rather than a knapsack sprayer so plots were set out to allow tractor access for spraying and to minimise spray drift between the different dose plots. All fungicides were applied in 200 l ha^{-1} of water at a pressure of 0.2 MPa, using a tractor-mounted Allman hydraulic sprayer with standard flat nozzles.

In the spring of 1993 and 1995 three similar large field experiments were laid out at Boghall farm; Field experiment 2 and 3 in 1993 and Field experiment 4 in 1995. The barley cultivar used was 'Golden Promise' bought as certified seed, ready treated with Panoctine Plus seed treatment containing guazatine and imazalil for the control of brown foot rot, *Fusarium* foot rot, seedling blight in barley, foot rot, leaf stripe and net blotch in barley. Plot sizes were 24 m by 16 m.

Fungicide treatments for 1993 and 1995 were fenpropimorph at full rate and at three reduced doses, applied as two spray programmes. As in 1992 the first fungicide application was made when mildew first developed on the plants and the second spray was applied three weeks later. There were four treatments in 1993 and 1995 as shown in Table 5.2 with three replicates of each, laid out in blocks. To reduce uncontrolled variation brought about by high levels of freely mobile inoculum in untreated plots, there were no unsprayed plots in 1993 and 1995 (Figs.5.2-5.4).

Table 5.1. Fungicide spray programme used in Field experiment 1 (1992) with fenpropimorph and propiconazole applied as commercial products Corbel and Tilt 250 EC respectively.

Treatment	First application (g a.i. ha^{-1})	Second application (g a.i. ha^{-1})	Dose rate of full rate
U	nil	nil	nil
A	fenpropimorph (750)	fenpropimorph (750)	1
B	fenpropimorph (375)	fenpropimorph (375)	1/2
C	fenpropimorph (187.5)	fenpropimorph (187.5)	1/4
D	fenpropimorph (750) propiconazole (125)	fenpropimorph (750) propiconazole (125)	1 1
E	fenpropimorph (375) propiconazole (62.5)	fenpropimorph (375) propiconazole (62.5)	1/2 1/2
F	fenpropimorph (187.5) propiconazole (31.25)	fenpropimorph (187.5) propiconazole (31.25)	1/4 1/4
G	nil	fenpropimorph (187.5) propiconazole (31.25)	1/4 1/4

	Application date	Growth stage
First application	22 May 1992	15-23
Second application	12 June 1992	37-39

Fig. 5.1. Field layout of the reduced dose Field experiment 1 (1992). Letters A-G & U correspond to treatments in Table 5.1.

C	G	U	D	C	G
B	F	A	E	B	F
A	E	B	F	A	E
U	D	C	G	U	D

Table 5.2. Fungicide spray programme used in Field experiments 2, 3 (1993) and 4 (1995) with fenpropimorph applied as the commercial product Corbel.

Fenpropimorph dose	First application (g a.i. ha^{-1})	Second application (g a.i. ha^{-1})
Full dose *	750	750
¾ dose	562.5	562.5
½ dose	375	375
¼ dose	187.5	187.5

* Dose rate as a proportion of full commercial dose

Experiment	First application date	Second application date	Growth stage
2 & 3	8 June 1993		35
2 & 3		23 June 1993	45-49
4	15 June 1995		40-45
4		28 June 1995	60

Fig. 5.2. Field layout of Field experiment 2 (1993). Letters A-D correspond to treatments; full, 3/4, 1/2 and a 1/4 dose respectively.

C	D	B	A	C	D
A	B	C	D	B	A

Fig. 5.3. Field layout of Field experiment 3 (1993). Letters A-D correspond to full, 3/4, 1/2 and a 1/4 dose repectively.

A	B	D	C	D	A
C	D	B	A	C	B

For the purposes of mildew sampling, about 15 infected leaves were collected from the middle of plots at three times in the season, just prior to spraying and three weeks after both the first and second sprays. Infected leaves were collected from random leaf layers. All leaves from within the same plot were put in one bag and taken to the laboratory where they were cut into 2-2.5 cm leaf segments. These were placed on distilled Davis water agar containing 80 mg l^{-1} benzimidazole, in 9 cm (diameter) Petri dishes and incubated in a Gallenkamp incubator at 18°C for 2-3 days to allow sporulation of the pustules to take place. The inoculum was then transferred, with a fine brush, to fresh leaf segments on agar for increasing the amount of inoculum (as described in Chapter 3). After two weeks the increased bulk of inoculum from each plot was tested for sensitivity to fenpropimorph in the laboratory following the standard leaf-segment method used in the monitoring work.

Fig.5.4. Field layout of Field experiment 4 (1995). Letters A-D correspond to full, 3/4, 1/2 and a 1/4 dose respectively.

D	A	D
C	B	C
B	C	B
A	D	A

Results

From the records of the Adopt-a-crop monitoring scheme, carried out by SAC for the Scottish Office, Agriculture, Environment and Fisheries Department (SOAEFD) for the 1992 growing season, the barley powdery mildew levels in the surrounding areas (Perth, Fife, Angus, Borders and Lothian) varied. Around the 12th of May when the first samples were collected from the experimental plots, there were trace-1% amounts of mildew in 15% of the spring barley crops and 5-10% mildew in 10% of the crops. By the 2nd of June around the time when the second samples were taken, 30% of the crops had trace-1% amounts of mildew and another 30% of the crops were covered by 5-10% or more mildew. On the 7th of July, around the time when the third samples were taken, 40% of the crops had trace-1% amounts of mildew while 20% of the crops had 5-10% mildew. The high disease incidence was reflected in the high disease pressure within the experimental plots. This was enhanced by the large amounts of inoculum from the untreated plots. Because there were high levels of mildew in both the treated and the untreated plots it was easy to obtain samples before and after the first spray. However, after the second spray it was extremely difficult to obtain samples because the disease cycle was far advanced. Plants were senescing due to severe disease pressure earlier on. Glasshouse grown barley plants of the same cultivar, grown in pots, had to be put in the plots and mildew was recovered from these and bulked on leaf segments over a period of two weeks. Many growers applied fungicides to their crops in this season. One of the most common fungicides applied was fenpropimorph, accounting for 41% of the total spray area of active ingredients (Bowen *et al.*, 1992).

According to Adopt-a-crop records, the powdery mildew levels in the surrounding areas in 1993 were lower than in 1992. On the 10th of June, around the time when the first samples were taken from the experimental plots, 10% of the spring barley crops had trace amounts of mildew while another 10% had 0.1-5% mildew. On the 24th of June, around the time when the second samples were taken, 11% of the crops had trace-1% amounts of mildew while 22% of the crops had 0.1-5% amounts of mildew. On 15th of July before the third samples were taken, 23% of the crops had trace-1% amounts of mildew while 8% of the crops had 0.1-1% mildew cover. The disease levels in the surrounding areas in 1995, were higher than in 1993. On the 15th of June, around the time when the first samples were taken from the experimental plots, 28% of the spring barley crops had 0.1-1% amounts of mildew while 12% of the crops had 2-5% mildew cover. On the 29th of June, around the time when the second samples were taken 21% of the spring barley crops had 0.1-1% amounts of mildew and 16% of the crops had 2-5% or more of mildew. On the

13th of July, before the third samples were collected, 42% of the spring barley crops had 0.1-1% mildew while 25% of the crops had 2-5% or more of mildew. In contrast to 1992, the powdery mildew levels in the experimental plots were very low in 1993 and 1995 and it was difficult to obtain samples at the sampling times. The Pesticide Usage in Scotland records are taken every two years so while there were records for 1992, there were none for 1993 and 1995. However, the disease levels in the crops in the areas surrounding the experimental plots were high enough to warrant pesticide usage and fenpropimorph was the most popular fungicide again.

The results of sensitivity assessment of isolates from different treatments for the field experiments are summarised in Tables 5.3-5.6. An analysis of variance (ANOVA) of the data was conducted using a Genstat program which calculates SED values for direct comparison of means. Although the sampled isolates varied in their sensitivity to fenpropimorph, there were no significant differences between mean EC_{50} values either in response to fungicide treatment or to dose in all experiments.

The EC_{50} values averaged over all treatments at the different sampling times in 1992 were 0.096 before spraying, 0.074 after the first spray and 0.069 after the second spray giving no evidence of an overall reduction in sensitivity over the experimental period. At the first sampling, in Field experiment 1, leaves from replicate plots were bulked together and so the results were not included in the statistical analysis, while for the samples after the first and second spray the SED (d.f. = 29) was 0.058 which meant that differences were not significant with any treatment comparisons (Table 5.3).

For samples assessed after the second spray application in Field experiment 1 (Table 5.3) the untreated isolates had an EC_{50} value of 0.053 g a.i. l^{-1} while those exposed to fenpropimorph alone showed comparable values of 0.032 for the highest dose and 0.025 for the lowest dose. Where fenpropimorph was combined with propiconazole the equivalent values were 0.026 and 0.136. Apart from the isolates which received treatment C (187.5 g a.i. l^{-1} fenpropimorph) equivalent of a 1/4 dose and E (375 g a.i. l^{-1} fenpropimorph and 62.5 g a.i. l^{-1} propiconazole) equivalent of a 1/2 dose, all the other isolates had lower mean EC_{50} values after both sprays than those of the same plots before any spray had been applied. Before any sprays had been applied, there was a wide range of variation in the mean EC_{50} values. These ranged from 0.001 to 0.244 (a 244 fold difference). The equivalent values after one spray are 0.029 to 0.139 (5-fold) and after two sprays are 0.025 to 0.136 (5-fold) (Table 5.3).

The EC_{50} values in 1993 averaged over all treatments before spraying, after one spray and after two sprays were respectively 0.068, 0.117 and 0.080 (SED (d.f = 18) = 0.035, n.s.), in Field experiment 2 and 0.087, 0.054 and 0.025 (SED (d.f. = 20) = 0.029) n.s.) in Field experiment 3. When fenpropimorph was applied at full or reduced doses to all plots in the two experiments (Tables 5.4 & 5.5), the range between the mean EC_{50} values before any sprays had been applied was from 0.023 to 0.111 (a 5-fold variation) in Field experiment 2 and from 0.027 to 0.188 (a 7-fold variation) in Field experiment 3. After the first spray the range in experiment 2 was 0.029 to 0.181 (6-fold). The equivalent values for experiment 3 were 0.012 and 0.115 (10-fold). The equivalent values for experiments 2 and 3 after the second sprays were 0.030 to 0.120 (4-fold) and 0.011 to 0.038 (3-fold) respectively. The plots that had started off with the most sensitive isolates at the beginning of the season did not consistently contain the most sensitive isolates after the second spray.

In 1995 (Field experiment 4) isolates collected after the first spray died out during propagation, before their fenpropimorph sensitivity could be determined. The difference in average EC_{50} values for all samples before spraying (0.148) and after two sprays (0.229) was not significant (SED (d.f. = 13) = 0.040). Before any sprays had been applied the mean EC_{50} values of isolates from the different plots ranged from 0.111 for the plots receiving the 3/4 dose of fenpropimorph to 0.174 from plots receiving the lowest dose. The EC_{50} values after the second spray application ranged from 0.174 from plots receiving the highest dose to 0.342 for those receiving half the full dose (Table 5.6). Taken over all treatments, the mean EC_{50} values obtained in 1995 (mean = 0.198±0.022) were significantly higher ($P < 0.001$) than those obtained from treatment plots planted in 1992 (mean = 0.056±0.022) and 1993 (mean = 0.066±0.013).

Table 5.3. Sensitivity of isolates from Field experiment 1 (1992) to fenpropimorph based on mean EC_{50} values in g a.i. l^{-1}.

Sampling time		Fungicide treatments g a.i. ha^{-1}								
	fenpropimorph	Nil	750	375	187.5	750	375	187.5	187.5*	Mean
	propiconazole	Nil	Nil	Nil	Nil	125	62.5	31.25	31.25*	
Before spray+		0.092	0.05	0.111	0.001	0.128	0.048	0.244	0.096	0.096
After 1 spray		0.034	0.035	0.102	0.055	0.118	0.029	0.139	0.077	0.074
After 2 sprays		0.053	0.032	0.086	0.025	0.026	0.110	0.136	0.086	0.069
		SED = 0.058, n.s. (d.f.=29)								SED= 0.021 n.s. d.f.=29
Mean		0.044	0.034	0.094	0.040	0.072	0.070	0.137	0.081	
		SED =0.041, n.s. (d.f.=29)								

+ Samples from replicate plots bulked and not included in statistical analysis

*Only second spray application n.s. = not significant

Table 5.4. Sensitivity of isolates from Field experiment 2 (1993) to fenpropimorph based on mean EC_{50} values in g a.i. l^{-1}.

Site	Sampling time	Fenpropimorph treatments g a.i. ha^{-1}				
		750	562.5	375	187.5	Mean
1	Before spray	0.068	0.023	0.111	0.070	0.068
	After 1 spray	0.029	0.181	0.102	0.156	0.117
	After 2 sprays	0.120	0.081	0.087	0.030	0.080
		SED = 0.070, n.s. (d.f. = 18)				SED= 0.035n.s. d.f.=18)
Mean		0.072	0.095	0.100	0.086	
		SED =0.040, n.s (d.f. =18).				

Table 5.5. Sensitivity of isolates from Field experiment 3 (1993) to fenpropimorph based on mean EC$_{50}$ values in g a.i. l^{-1}.

Site	Sampling time	Fenpropimorph treatments g a.i. ha^{-1}				
		750	562.5	375	187.5	Mean
2	Before spray	0.027	0.102	0.029	0.188	0.087
	After 1 spray	0.059	0.115	0.031	0.012	0.054
	After 2 sprays	0.015	0.038	0.036	0.011	0.025
		SED = 0.057, n.s.				SED =
		(d.f. = 20)				0.029n.s
						(d.f.=20)
Mean		0.034	0.085	0.032	0.070	
		SED=0.033, n.s.				
		(d.f. = 20)				

Table 5.6. Sensitivity of isolates from Field experiment 4 (1995) to fenpropimorph based on mean EC50 values in g a.i. l^{-1}

Sampling time	Fenpropimorph treatments g a.i. ha^{-1}				
	750	562.5	375	187.5	Mean
Before spray	0.147	0.111	0.158	0.174	0.148
After 2 sprays	0.174	0.207	0.342	0.191	0.229
	SED = 0.080, n.s.				SED =
	(d.f = 13)				0.040
					n.s.
Mean	0.161	0.159	0.250	0.183	(d.f.=13)
	SED =0.056, n.s.				
	(d.f. =13)				

Discussion

There was no evidence from the 1992, 1993 and 1995 field experiments that exposure to fenpropimorph or variation in doses of fenpropimorph affected the level of sensitivity of isolates of barley powdery mildew to the fungicide. However, Brown & Evans (1992) reported that at one of the localities in which they carried out their experiments increasing doses of fenpropimorph, up to one-quarter of the commercial rate, selected greater resistance. They also reported an increased frequency of insensitivity after exposure to fenpropimorph sprays. They concluded, however that the reductions in sensitivity that they observed were unlikely to result in any reduction in field performance.

Hollomon (1978) studied the effect of ethirimol resistance on the development of barley powdery mildew. A field experiment was carried out in which barley was grown from seed treated at three ethirimol levels or left untreated. Plots were inoculated with an ethirimol-sensitive strain at seedling emergence. An additional control plot (no ethirimol) was not inoculated but was left to be naturally infected. Ten infected leaves were collected and conidia from them were tested for ethirimol sensitivity at growth stages 3, 6 and 10 using an *in vitro* germ-tube growth assay (Hollomon, 1977). Less mildew was present on ethirimol-treated plots than on the untreated plot where the natural population was the source of infection. On all untreated plots ethirimol-sensitivity was the same regardless of how the epidemic was established. Where ethirimol was used, sensitivity decreased although not in relation to the selection pressure applied i.e. dose rate. Although mildew had increased on all plots by growth stage 6, effects of ethirimol on ethirimol sensitivity were still apparent. Changes in sensitivity in the mildew population were largely confined to untreated plots. Little if any of the sensitive isolate that was used in inoculation now remained and ethirimol sensitivity was now fairly uniform throughout the experiment. Higher ethirimol rates had certainly not selected for greater insensitivity. Hollomon (1977) states that the *in vitro* biossay used in these experiments was the most variable of three bioassays he developed. Yet he went ahead and used it in this particular experiment. Doubt may be cast on the validity of his results although they are similar to the results of the present studies of the reduced doses.

The lack of evidence of any change in sensitivity may be explained in terms of selection. In evolution populations are constantly being sifted for superior types. In this sifting the primary force is selection in which individuals with certain characteristics are favoured in reproduction. The contribution of offspring to the next generation is called the fitness of the individual. If the differences of fitness are in any way associated with the presence or absence of a particular gene in the individual's genotype then selection operates on that gene (Falconer, 1981). When an allele is subject to selection its frequency in the offspring is not the same as in the parental population since parents of different genotypes will reproduce with different levels of success depending on their fitness. In this way selection causes a change in gene frequency and consequently also of genotype frequency and heredity. Selection can act effectively only on heritable differences. It cannot create variability but acts only on that already in existence.

In theory, application of reduced doses could lower the intensity of selection. Selection pressure is likely to be small or even absent if inoculum enters the crop from an outside source. This is due to the diluting effect of the inoculum coming from outside which may not have been subjected to any selection pressure before it entered the plots. In the reduced dosage rate experiments there was variability in the experimental population on which selection could act. This is shown in Tables 5.3 to 5.6 where the mean sensitivities of the isolates from the plots before any sprays had been applied are shown to be variable, especially those of the 1992 experiment where the variation was 244-fold. It is also possible that there may have been some individuals within the population which were not sensitive to the different dose rates.

Failure to detect these however, may indicate that they were less fit (Wade, 1982; Anon., 1987; Engels & de Waard, 1996) and so were unable to respond to selection by forming the bulk of subsequent generations. The selection pressure in the form of fungicide resistance was not sufficiently strong to bring about a change in the population. Moreover, inoculum in the population could not be controlled so that contamination from outside the crop or from untreated plots in the 1992 experiment could mask effects of any selection pressure. To ensure that most of the inoculum came from the experimental plots, large plots were used and samples were obtained from the middle of the plots. However, this could not guarantee that all the inoculum came from the experiment, as spores can be wind borne over huge distances. Failure to detect any resistant individuals could also have been due to the fact that the period of three weeks in which samples were taken may not have been long enough for the resistant individuals to multiply to sufficient numbers that could be detected. Within the three weeks between sprays there could have been only two and a half conidial generations, given a length of one generation as eight days (Kilian & Kranz, 1991).

Hypotheses have been put forward regarding the effect of reduced dosage rates on fungicide sensitivity (Shaw & Pijls, 1994). There are three differing potential hypotheses as to how reduction of the rate of a single fungicide application might affect the rate at which resistance evolves. The reduced dose might increase the rate of resistance evolution because partly resistant forms could survive and subsequently give rise to more resistant individuals by crossing or mutating; the reduced dose might do nothing over the range within which fungal growth was affected or the reduced dose might reduce the rate of evolution because more sensitive forms survive to reproduce and breed in the future. As far as the current work is concerned, the second hypothesis seems to have applied as there was no evidence from the experiments that reduced doses had any effect on sensitivity.

When a crop is sprayed with a fungicide, there is bound to be variation in the amount of fungicide each individual pustule or lesion encounters in terms of space and time. A reduced dose may kill many of the sensitive strains but a high proportion of the population may remain. This will include the less sensitive strains. Each time a reduced dose is repeated, the less sensitive strains will remain and be allowed to multiply. With many repeated doses it is assumed that the proportion of the less sensitive strains in the population will be so high that there will be loss of control. This refers to the first theory cited above. Current expert opinion contradicts this theory. Suggestions have been made that potential increases in resistance associated with a higher proportion of the population surviving a lower dose is counterbalanced by a corresponding reduction in selection pressure exerted on the fungal population (Paveley & Lockley, 1993). When a full dose is applied, most of the population is eradicated. It is assumed that any mildew remaining did not just escape but was probably not sensitive to the chemical. If the surviving fungal population is made up of a majority of these strains, they stand a better chance of contributing genes to the next generation. Some of these individuals may have existed in the original population or they may represent spontaneous mutants. If lack of control of these strains is associated with a particular allele, then the frequency of that allele would increase in the next generation, as most of the individuals which survived would have possessed it. Here selection is exerting a high pressure for that allele.

However, in the case of reduced doses, a higher proportion of the more sensitive strains will remain with the less sensitive ones. Unless there are barriers in mating, which seems unlikely, there will be mating between the more sensitive strains and the less sensitive ones. What will determine the frequency of alleles associated with resistance in the offspring will be the fitness of the offspring. Mutated individuals which are resistant to fungicides have often been found to be less fit than the sensitive strains (Wade, 1982; Anon, 1987). If selection was to act against that allele for resistance, then there would not necessarily be more resistant individuals in the next generation even if reduced doses were repeated. Mating was not involved in the 1992, 1993 and 1995 experiments as powdery mildew is largely clonal and the conditions were not conducive for mating. In this case any resistant mutants or survivors depended on the rate of asexual reproduction.

A paper entitled "The Effect of Reduced Doses on the Sensitivity of Powdery Mildew to Fenpropimorph in Barley Field Trials" in relation to this work has been published and a copy is attached in the Appendices.

Sensitivity of wheat powdery mildew to fenpropimorph in relation to reduced dosage rates of the fungicide.

Materials and methods

A winter wheat experiment was superimposed in a winter crop in the autumn of 1993 to investigate the effect of reduced doses of fenpropimorph on fungicide sensitivity of wheat powdery mildew. However, this experiment had to be abandoned as no mildew infected the plots, throughout the growing season. Another winter wheat experiment to investigate the effect of reduced doses of fenpropimorph on fungicide sensitivity of wheat powdery mildew was established in a similar way to the barley experiments in the autumn of 1994 at Boghall farm in the Midlothian region of Scotland (Fig. 5.5). Plot sizes were 24 m by 16 m. Fertiliser, herbicide and any micro-nutrients were uniform across all plots and accorded with local practice whereby the seedbed was treated with P_20_5:K_20 in the ratio of 60:60 kg ha^{-1}. The cultivar used was 'Mercia' which has a powdery mildew resistance score of 5, which is considered to be medium on a 1-9 scale where 9 is considered as high resistance (Anon, 1997). The certified seed used, was treated with Panoctine which contains guazatine and is used to control bunt (*Tilletia* sp.), *Septoria* spp., seedling blight in wheat and reduces *Fusarium* foot rot. It does not control any foliar diseases.

The fungicide programme followed was the same as that used for barley in 1993 and 1995 as shown in Table 5.2 with a full dose treatment and three reduced dose rates, equal to 3/4, 1/2 and a 1/4 the full commercial recommended rate of fenpropimorph. There were three replicates laid out in blocks as shown in Fig 5.5. The first fungicide application was made when mildew first developed on the plants (2 June 1995) at GS 39-43 and the second spray was applied three weeks later (28 June 1995) at GS 66. To reduce uncontrolled variation in mildew isolates as a result of large amounts of mobile inoculum from untreated plots, there were no unsprayed plots. The field used for the experiment was surrounded on three sides by uncultivated upland and woods and the site was therefore isolated from neighbouring wheat fields. All fungicides were applied, in 200 l ha^{-1} of water at a pressure of 0.2 MPa, using a tractor mounted Allman hydraulic sprayer with standard flat fan nozzles.

About fifteen infected leaves were sampled from each plot on two occasions; just before the first (1 June 1995) and second (28 June 1995) sprays when, on each sampling occasion, all leaves from the same plot were put in the same bag. These were taken to the laboratory where they were cut into 2-2.5 cm leaf segments and incubated in a Gallenkamp incubator at 18°C, with a 12 hour photoperiod, on distilled Davis water agar containing 80 mg l^{-1} benzimidazole in 9 cm (diameter) Petri dishes. This was to allow sporulation to take place. After three days the heterogeneous inoculum was subcultured on to whole plants of cv. Cerco which has no known resistant genes against powdery mildew of wheat. On the third sampling occasion (24 July 1995), whole plants were uprooted from the middle of the plots three weeks after the second spray as there was little active mildew on the leaves. These plants were taken to the greenhouse where they were incubated on isolation propagators for three days, to allow the mildew present to sporulate, after which inoculum from different plots was subcultured and propagated on to fresh plants of cv. Cerco. Inoculum from different leaves was not mixed. Using the heterogeneous inoculum, sensitivity tests were carried out in the laboratory following the standard leaf-segment method adapted for wheat powdery mildew (Chapter 3). The levels of mildew in the plots were recorded at the time of sampling, by assessing the percentage surface area infected with mildew on each of the top three leaves on a sample of ten plants taken from the central area of plots.

Fig. 5.5. Field layout of the 1995 winter wheat, reduced dose experiment. Letters A-D correspond to full, 3/4, 1/2 and a 1/4 the recommended commercial dose respectively.

D	C	B	A
A	B	C	D
D	C	B	A

Results

From Adopt-a-crop records for 1995, disease levels of wheat powdery mildew in the areas surrounding the experimental plots were on average low. On the 1st of June on the day when the first samples were collected from the experimental plots, 62% of the winter wheat crops had 0.1-1% mildew while 12% of the crops had 2-5% mildew. On the 29th of June around the time when the second samples were collected, 70% of the winter wheat crops had 0.1-1% mildew. No records are available for the mildew cover of the winter crops on the 24th of July, the day of the third sampling of the experimental plots. In the experimental plots disease development was slow over the winter period and did not reach a very high level. However, assessment of the levels of mildew present in the plots at each assessment timing (Table 5.7) revealed that there was an influx of mildew into the plots after the second spray, which may have re-introduced more sensitive 'wild type' isolates from the general mildew population into the experimental plot population. The leaves near the base of the stems were mostly affected and there was hardly any disease development on the younger leaves early in the season as is normal with mildew infection (Table 5.7). Apart from the samples obtained after the second spray, when the disease was advanced and colonies were less actively sporulating, the other samples were obtained with ease.

The results of sensitivity assessments for the 1995 wheat experiment are summarised in Tables 5.8-5.9. Although the sampled isolates varied in their sensitivity to fenpropimorph, there were no significant differences between mean EC_{50} values for isolates in relation to the concentration of fungicide to which they had been exposed and sampling time (Table 5.8). Isolates collected from plots treated with a 3/4 (562.5 g a.i. l^{-1}) dose seemed the most sensitive throughout the experiment. There was a significant (P = 0.049) decrease in the mean sensitivity of isolates, averaged for fungicide dose rates collected after the first sprays had been applied (Table 5.9). However, the mean sensitivity to fenpropimorph for isolates collected after the second spray application, was not significantly different from the mean values at the beginning, prior to any sprays being applied, although the range of sensitivities at this last timing was greater than at the other two sampling times. Thus, isolates with significantly reduced sensitivity remained within this sample group (Table 5.9).

Table 5.7. Mean percentage wheat mildew cover on top three leaves in relation to sampling time, leaf position and fenpropimorph treatment.

	Before spray (1/6/95) GS 39-43			After 1 spray (28/6/95) GS 66			After 2 sprays (24/7/95) GS 73		
Dose	Leaf 1	Leaf 2	Leaf 3	Leaf 1	Leaf 2	Leaf 3	Leaf 1	Leaf 2	Leaf 3
Full	0	0	1.1	0	0	1.3	0.1	8.8	7.9
3/4	0	0	0.6	0	0.1	0.9	0.2	3.8	10.8
1/2	0	0	2.2	0	0	2.2	2.5	7.0	9.3
1/4	0	0	0.1	0	0	1.5	0.1	6.3	14.0
SED(d.f.=6)	-	-	0.95	-	0.56	0.60	0.92	3.02	4.29
Significance			n.s.		n.s.	n.s.	n.s.	n.s.	n.s.

n.s. = not significant

Table 5.8. Sensitivity of isolates (mean EC_{50} values in g a.i. l^{-1}) from the 1995 wheat experiment to fenpropimorph in relation to sampling time and fungicide dose rate.

	Fenpropimorph treatments g a.i. ha^{-1}			
Sampling time	750	562.5	375	187.5
Before spray	0.263	0.131	0.209	0.193
After 1 spray	0.348	0.263	0.274	0.328
After 2 sprays	0.253	0.136	0.173	0.197
	SED = 0.0934, n.s. (d.f. = 16)			
Mean	0.288	0.177	0.219	0.239
	SED = 0.054, n.s. (d.f. =16)			

Table 5.9. Sensitivity of isolates (mean EC_{50} values in g a.i. l^{-1}) from the 1995 wheat experiment to fenpropimorph in relation to sampling time, averaged for fungicide treatments.

Sampling time	Range	Mean
Before spray	0.027-0.310	0.199
After 1 spray	0.161-0.441	0.303
After 2 sprays	0.021-0.448	0.190
	SED = 0.0467, P = <0.05 (d.f. = 16)	

Discussion

A decline in mean sensitivity of mildew isolates to fenpropimorph was detected in the field experiment after fenpropimorph had been applied. However this shift in sensitivity was short-lived, as after the second sprays had been applied the mean sensitivity reverted back to near the value it had been before any sprays had been applied. Nevertheless, after two sprays of fenpropimorph when more 'wild type' mildew inoculum invaded the plots (Table 5.7), less sensitive isolates, found after the first spray, could still be detected in the present field experiment mildew population. This shift in sensitivity was not related to dose rate applied and there was no evidence that reduced doses increased or decreased the sensitivity of the mildew isolates assayed. With work on barley powdery mildew, Brown & Evans (1992) reported that they could find no correlation between dose rate and frequency of insensitivity, but they reported an increased frequency of insensitivity after exposure to fenpropimorph sprays. A small decrease in sensitivity of *E. graminis* f.sp. *tritici* to fenpropimorph has been found in a number of West European countries (De Waard *et al.*, 1992; Lorenz *et al.*, 1992; Felsenstein, 1994; Readshaw & Heaney, 1994).

Although the findings presented indicate that reduced doses do not cause an increased risk of morpholine resistance in *E. graminis* f.sp. *tritici*, multiple split applications of fenpropimorph, alone or in a mixture with propiconazole, have been shown to cause larger reductions in sensitivity than fewer full rate applications (Forster *et al.*, 1994; Engels *et al.*, 1996). This reduction in sensitivity has been shown where the total amount of fenpropimorph applied was equal in both regimes (Schulz, 1994). The degree of mildew control has been shown to be critical in determining the size of the shift in sensitivity probably as a result of the selection pressure exerted by improved mildew control (Schulz, 1994). Several other authors of papers where shifts are reported in response to split applications, also comment that disease control was superior in these treatments (Forster *et al.*, 1994; Engels *et al.*, 1996) so that although there are reductions in sensitivity, the control was effective. In the experiment reported here the levels of mildew assessed in the plots show that disease control was as good in the reduced dose plots as in the full dose plots, so that this selection pressure, if it exists, was similar in all treatments.

Multiple split applications of fenpropimorph can cause a larger reduction in sensitivity than a lower number of full rate sprays (Forster *et al.*, 1994; Schulz, 1994; Engels *et al.*, 1996), and the work reported by these researchers would lead to the conclusion that it may be the multiple and continuous nature of the applications, and therefore the selection pressure, rather than the dose that was critical. Standard practice in the UK is to reduce the dose from the full commercial dose when controlling mildew, to reduce input costs (Paveley & Lockley, 1993), rather than to increase the number of applications made to the crop. In addition, the maximum number of applications for many formulated products containing fenpropimorph is restricted in the UK to two or three depending on the formulated product used (Whitehead, 1997), so that more (over three) multiple applications of reduced rates are seldom applied. Some formulated products containing either fenpropimorph, fenpropidin or tridemorph do permit a maximum total dose, rather than a maximum application number, to be applied which would allow multiple applications of reduced doses to be made.

If the degree of mildew control determines the size of the shift in sensitivity to fenpropimorph and if control is sometimes inferior with reduced doses where the length of persistence of the fungicide can be shorter (Hardwick *et al.*, 1994; Jørgensen, 1994) then, in theory, the selection pressure from reduced doses may actually be less than for highly effective full dose sprays.

Reduced rates of fungicide clearly still provide effective control of mildew in the field situation (Wale *et al.*, 1993). The results of the five experiments, where reduced doses of morpholine were applied twice in the season as is common agricultural practice, confirm the observations of advisers (S. Oxley, pers. comm.) that reduced doses have not significantly affected the sensitivity of the pathogen to fenpropimorph or the effectiveness of control.

In conclusion, there was no evidence from the experiments described here that reduced doses of fenpropimorph, applied following normal UK agricultural practices, are likely to reduce the sensitivities of barley or wheat mildew isolates in treated plots. Shaw & Pijls (1994), with work on *Septoria* and a reduced dose of flutriafol, suggested that resistance was no more likely to be selected by reduced rates than by full rates but as with the present study, there is no report of populations of *Septoria* with high levels of resistance to flutriafol. There was evidence from the wheat experiment in this present work that application of fenpropimorph in itself could reduce the sensitivity of powdery mildew to the fungicide.

In qualifying the results of this work, it should be recognised that lack of evidence of reduction in sensitivity may be attributed to the method followed in the experiment. First of all, while it would have been ideal to isolate single colonies, heterogeneous inoculum in form of bulk isolates were sampled throughout the experiment. This was for practical reasons. If single colony isolates had been sampled the numbers involved would have been too great to be manageable. To get single colony isolates representative of the plot populations, would have required sampling many isolates. To propagate these single colony isolates would have required many propagators and the isolates would have had to go through many generations to get enough inoculum, to be used in the sensitivity tests. In any case, by the time sampling was done, the pustules had gone beyond the single colony phase. The heterogeneity of the inoculum was confirmed by a preliminary experiment carried out on differential hosts. Some of the field samples were grown on differential hosts to characterise the inoculum. The differential cultivars used were as follows;

Differential cv.	European code	Resistance genes	WMR group
Cerco	none	none	0
Galahad	Pm2	*Pm2*	2
Armada	Pm4b	*Pm4b*	4
Aquila	Pm5	*Pm5*	5
Brimstone	Pm2, Pm6	*Pm2, Pm6*	2,6
Ambassador	Pm8	*Pm8*	8

All the samples tested grew on all the differential hosts, indicating that the isolates were of heterogeneous inoculum. But with heterogeneous samples, a wider genetic base was sampled and there were increased chances of detecting resistant isolates.

A further limitation of the test method used was that there was no control over the dilution effect of cross-contamination from outside the experimental plots. Any inoculum coming from outside would have been selected depending on the fungicide treatment it would have been exposed to. Even though there could have been resistant isolates, their effect could have been masked by large amounts of inoculum coming from outwith the trial area especially with the barley experiment in 1992 where mildew levels were very high in the surrounding areas.

Thirdly, the germination rate of the sampled spores is known to be low after two weeks (Cartner, 1996). This could have further diminished the chances of picking up resistant isolates. Lastly, if colonies able to grow at the higher dosage rates in the dilution series had been selected and tested further, they may have shown populations with different response curves and higher EC_{50} values.

A paper entitled "Effect of Application Rate on the Sensitivity of *Erysiphe graminis* f.sp. *tritici* to Fenpropimorph" in relation to this work has been published and a copy is attached in the Appendices.

Chapter 6

Comparison of Four Methods of Testing for Fungicide Sensitivity

COMPARISON OF FOUR METHODS OF TESTING FOR FUNGICIDE SENSITIVITY

Introduction

The standard leaf-segment method of testing the sensitivity of powdery mildew isolates to morpholines followed in this project involves the calculation of EC_{50} values, after visual assessments of mildew development on leaves from fungicide treated plants. This method is slow and demanding on materials and space, resulting in testing only a small number of isolates each year. Monitoring is therefore restricted, so that reacting quickly to a reduced effectiveness of morpholines would prove difficult. In addition, the number of samples tested is often too low to be fully representative of the field population.

Two alternative methods of testing for fungicide sensitivity, based on microscopic observations on the early development of the fungus exposed to fungicide introduced into agar, were investigated in these studies. These methods are referred to as (a) cellophane/agar and (b) leaf/agar. The main objective was to compare these two alternative methods with the standard leaf-segment method to evaluate their suitability. The cellophane/agar method involved assessments of germination of spores on cellophane over fungicide treated agar and the leaf/agar method involved assessments of germination and further development of spores on leaf segments over fungicide-treated agar. Adding the fungicide to agar in the alternative methods was a simpler operation than introducing it through the plant and the microscopic observations enabled fungal development to be assessed at 24 or 48 hours after inoculation. The standard leaf-segment method is similar to the way the fungicide is applied in the field. It does, however, require treating a series of plants at different dosage rates and a delay of 14 days between inoculating plants and visually assessing mildew cover. A modified leaf-segment method was also tested in this study, with leaves being examined microscopically from 24 hours to six days after inoculation. The development of the alternative methods was aimed at providing methods which were not only more rapid and more economical with materials than the standard leaf-segment method but, in the case of the leaf/agar method, would also give a possible fuller understanding of fungal behaviour in relation to fungicide resistance, for example how it might relate to inoculum potential or sporulation capacity.

The concentrations of fungicide required to inhibit fungal development when incorporated into the agar in the alternative test methods were considerably lower than those used in the standard leaf-segment method. For this reason, and to avoid using very small numbers in this results chapter, the fungicide concentrations and EC_{50} values are given in μg a.i. ml^{-1} rather than g a.i. l^{-1}. To convert between the two the μg a.i. ml^{-1} data should be divided by 1000.

Preliminary experiments had been carried out using three barley isolates in a supervised Honours student project which showed that, in cellophane/agar and leaf/agar tests, spore germination was progressively reduced with increasing dosage rate (Cartner, 1996). This student project was, however, too limited in scope to establish any relationship between resistance of isolates as assessed by the standard leaf-segment method and spore germination response to fungicide exposure. The present work covered a more comprehensive study including early fungal colony development as well as spore germination.

Materials and methods

The barley powdery mildew isolates used in these studies were single spore isolates obtained from the Cereals Research Department, John Innes Centre at Norwich. They were DH14 which had been found fenpropimorph/fenpropidin sensitive, CC148 which was categorised resistant to fenpropimorph and fenpropidin, CC152 having a resistance to fenpropidin similar to CC148 but a somewhat lower resistance to fenpropimorph and CC52 which had been found to have a low sensitivity to fenpropidin (J. Brown, pers. comm.). Two sets of wheat powdery mildew experiments were conducted. The wheat powdery mildew isolates tested in the first set of experiments (wheat 1) were chosen randomly from the isolates that were being monitored (Chapter 4). They were RIA2, MORUNT, MOR2, CPBW-1, RA2 and 63CAMP. Those tested in the second set of wheat experiments (wheat 2) were isolates that had shown a range of sensitivity in the conventional leaf segment methodology and were used as standard in SAC monitoring work. These were termed "sensitive" (S) (isolate 96a), "moderate" (M) (isolate 96b) and "resistant" (R) (isolate 96c).

Standard leaf-segment method

The standard leaf-segment method was followed as described in Chapter 3. The concentration range of fenpropimorph was 1/64, 1/32, 1/16, 1/8 and 1/4 of the recommended field rate, giving concentrations of 59, 117, 234, 469 and 938 μg a.i. ml^{-1} respectively. Fourteen days-old seedlings were sprayed with the range of concentrations of fenpropimorph. These were covered with propagator tops and left overnight. The following day, 2-2.5 cm long leaf segments were cut from the middle of the second leaf. Eight leaf segments were placed on agar (Oxoid No. 3 at 4 g l^{-1}) incorporated with 80 mg l^{-1} of benzimidazole in each of two replicate Petri dishes. The inoculum used in these experiments was propagated on whole plants of either cv. Golden Promise (barley) or on cv. Cerco (wheat). Inoculum from 10 days-old colonies or from 15 days-old colonies, shaken to remove older spores at 12 days-old, was shaken onto autoclaved coloured art paper. Sterile fine brushes were used to transfer the inoculum to leaf segments as evenly as possible. The Petri dishes with the inoculated leaf segments were incubated in a Gallenkamp incubator at 18°C with a 12 hour night and day regime. Percentage mildew cover was assessed 14 days after inoculation and the data were analysed by Genstat to give median EC_{50} values as described in Chapter 3. The EC_{50} is the dose required to reduce the percentage germination to half that of the control. The Genstat analysis generated two independent estimates based on the two replicate plates for each dose level. Each series of plates had eight replicate leaf segments. The program gave the logarithms (logs) of the two EC_{50} estimates for each isolate and the log EC_{50} data were analysed by ANOVA for differences between the mean log EC_{50} values. A geometric mean EC_{50} value was calculated from the replicate log EC_{50}s after back transformation.

In addition, a modified version of the standard leaf-segment method using the same range of fungicide concentrations was set up to study fungal development after 24 and 48 hours and 6 days in the barley and second set of wheat experiments. Instead of eight, two leaf segments were put in each Petri dish and there were three replicate plates instead of two. Percentage germination, appressorial formation, development of secondary hyphae and conidiophores were assessed microscopically under ultra-violet light with a Leitz Orthoplan II epifluorescence microscope (UV microscope). This had a 100W mercury vapour lamp, a dichroic mirror and Biener filters. Calcofluor (fluorescent dye which stains fungal structures on the surface easily) at 0.1% (w/v) in aqueous solution was used to stain the spores. Fifty spores were systematically counted across each field of view, counting each spore that was encountered. Photographs were taken at x400 magnification. The data were analysed by logit transformation to give EC_{50} values.

Logit analysis analyses quantal responses (all or nothing responses: the spores were either germinated or not) to give quantitative results, as described by Hewlett & Plackett (1979). The most important quantitative result is the EC_{50} value for the isolates.

First, the logit l (a transformed value) of the proportion that germinated relative to the control is calculated:

$$l = \log_e \{P/(1-P)\}$$

where P = proportion relative to the control

There is assumed to be a linear relationship between the logit of this proportion and the log of fungicide dose. Logits are plotted against log-doses and can be fitted to the points, whose equation is:

$$l = \theta + \Phi \log z$$

where l is the logit of the response at log-dose z

(θ = intercept, Φ = slope)

The EC_{50} is related to the slope and the intercept, log-EC_{50} being equal to $-\theta/\Phi$, enabling the EC_{50} to be simply calculated by inverse of the log function (antilog). The logit technique was adapted for use on a Microsoft Excel spreadsheet, where a matrix can produce graphs and generate EC_{50} values from the inverse log of intercept/slope.

Cellophane/agar method

Oxoid agar No.3 (4 g l^{-1}) with a range of concentrations of fenpropimorph was prepared in 9 cm Petri dishes. The dose range used was 1/16384, 1/2048, 1/1024, 1/512, 1/256 and 1/128 of the recommended field rate, giving concentrations of 0.23, 1.8, 3.7, 7.3, 14.6 and 29.3 μg a.i. ml^{-1} respectively. The control Petri dishes had no fungicide. There were three replicates of each concentration and control. The isolates to be tested were propagated on whole plants. Cellophane circles which had been sterilised by autoclaving twice at 121°C for 15 minutes, were placed on the agar media and inoculated with the spores from 10 days-old colonies or from 15 days-old colonies shaken to remove older spores at 12 days so that no spores were more than three days old. The inoculum was collected by shaking whole plants onto autoclaved coloured art paper. The cellophane circles were inoculated with a sterile fine brush. The Petri dishes with the inoculated cellophane circles were incubated in a Gallenkamp incubator at 18°C with a twelve hour day and night regime. Germination was assessed quantitatively by use of a light Microlux II microscope to count the proportion of spores with germ-tubes 24 hours later. According to Manners (1949) and Manners & Hossain (1963), a spore is germinated when the germ-tube is as long as it is broad. This definition was followed when assessing germination. One hundred spores were counted under each field of view five times to give a total of 500 spores for each of the three replicates. The data were analysed by logit transformation in which EC_{50} values were calculated.

Leaf /agar method

Segments (2-2.5 cm long) were cut from the second leaves of plants which had been grown to the two leaf stage (Zadok's GS 12) on an isolation propagator. These were placed on 8 g l^{-1} (0.8%) Oxoid agar No. 3 with different doses of fenpropimorph incorporated with antibiotics (20μg ml^{-1} of streptomycin and chloramphenicol) to prevent bacterial contamination on the leaf segments and 80 mg l^{-1} of benzimidazole to delay leaf senescence. The fenpropimorph dose range was 1/512, 1/256, 1/128, 1/64 and 1/32 of the field rate, giving concentrations of 7.3, 14.6, 29.3, 58.6 and 117.2 μg a.i ml^{-1} respectively. The control Petri dishes had no fungicide. Two leaf segments were placed in each dish and there were three replicate plates. Spores from 10 days-old colonies or from 15 days-old colonies shaken to remove older spores at 12 days were collected from whole plants by shaking the spores on to autoclaved coloured art paper. The leaf segments were lightly brushed with spores, using a sterile fine brush. The Petri dishes were incubated in a Gallenkamp incubator at 18°C with a 12 hour day and night regime. Germination and growth were assessed with a (UV) microscope after 24 hours, 48 hours and 6 days. Photographs were taken at x400 magnification. Calcofluor (fluorescent dye) was used to stain the spores, the hyphae and the conidiophores. Fifty spores were systematically counted across the field of view, counting each spore that was encountered. The counted spores were sorted into categories of germination i.e. whether germ tubes, appressoria, secondary hyphae (Ellingboe, 1972) or conidiophores were present. Logit transformation was used in the analysis of total germination and these data were plotted against log dose so that EC_{50} values were calculated. Percentages of germination, germ tubes, appressoria and secondary hyphae were calculated for the experiments, assessed 48 hours after inoculation. Looking at the different categories was more informative 48 hours after inoculation than after 24 hours as more structures had developed.

Three experiments were carried out in these studies.

(1). Response of four barley mildew isolates to fenpropimorph using different methods of assessment.

Four isolates, DH14, CC148, CC152 and CC52 were assessed based on the following methods and observations:-

	germination		appressoria		secondary hyphae	conidiophores	leaf area (visual)
	24 h	48 h	24 h	48 h	48 h	6 d	14 d
cellophane /agar	x	-	-	-	-	-	-
leaf/agar	x	x	x	x	x	x	-
leaf-segment	-	-	-	-	-	-	x
leaf-segment (modified)	x	x	x	x	x	x	-

(2). Response of six wheat mildew isolates to fenpropimorph using different methods of assessment (wheat 1 experiment).

Six isolates, RIA2, MORUNT, MOR2, CPBW-1, RA2 and 63CAMP were assessed based on the following methods and observations:-

	germination		appressoria		secondary hyphae	conidiophores	leaf area (visual)
	24 h	48 h	24 h	48 h	48 h	6 d	14 d
cellophane /agar	x	-	-	-	-	-	-
leaf/agar	-	x	-	x	x	x	-
leaf-segment	-	-	-	-	-	-	x

(3). Response of three wheat mildew isolates to fenpropimorph using different methods of assessment (wheat 2 experiment).

Three isolates, S, M and R were assessed based on the following methods and observations:-

	germination		appressoria		secondary hyphae	conidiophores	leaf area (visual)
	24 h	48 h	24 h	48 h	48 h	6 d	14 d
cellophane /agar	x	-	-	-	-	-	-
leaf/agar	x	x	x	x	x	x	-
leaf-segment	-	-	-	-	-	-	x
leaf-segment (modified)	x	x	x	x	x	x	-

Results

Visual assessments from the standard leaf-segment method.

The relative resistance, based on EC_{50} values of isolates in all three experiments, determined by the standard leaf-segment method of assessment, is represented in Table 6.1 and Figures 6.1 (i-iii). The figures show fitted symmetrical logistic curves obtained for the two replicates from the Genstat analysis of the standard leaf-segment method data. Log EC_{50} values were obtained for each replicate and analysed by ANOVA for all isolates in the three experiments. The analysis revealed significant differences between the mean log EC_{50} values of the isolates. Student t tests were used to identify which means were significantly different (A.E. Hunter, pers. comm.).

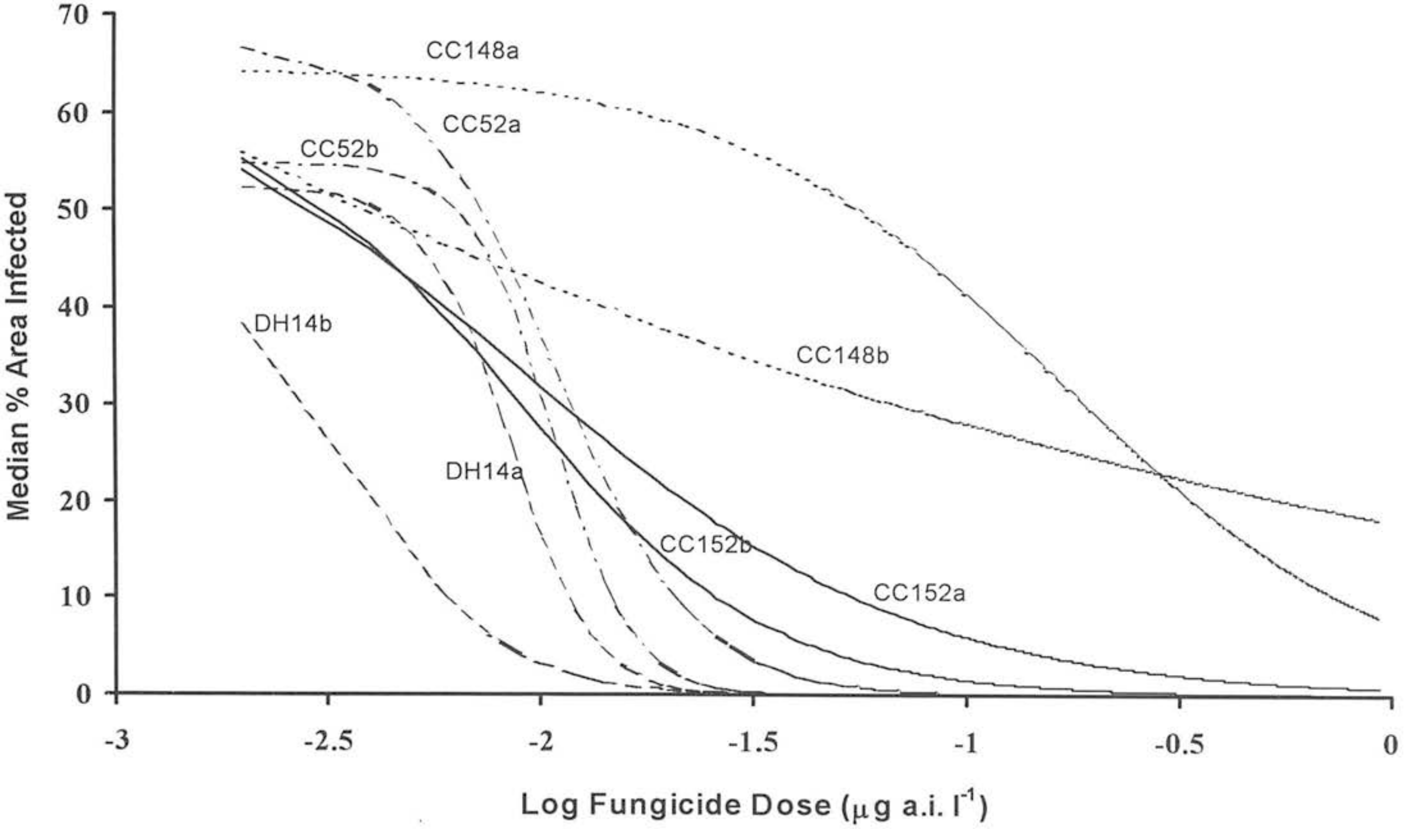


Fig. 6.1.(i) Median % area infected vs log fenpropimorph dose for the barley mildew isolates.

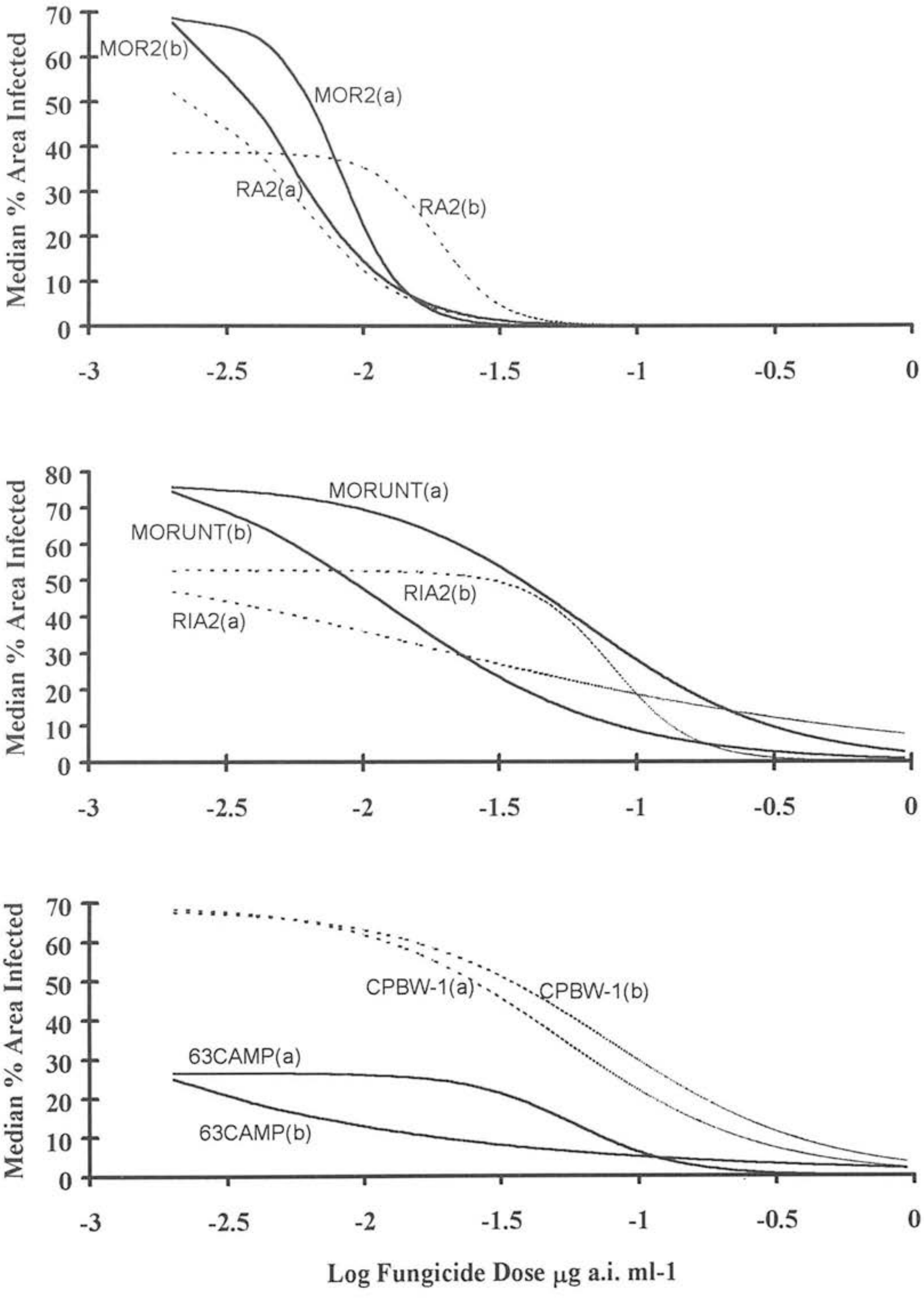


Fig. 6.1(ii). Median % area infected vs log fenpropimorph dose for the wheat 1 powdery mildew isolates.

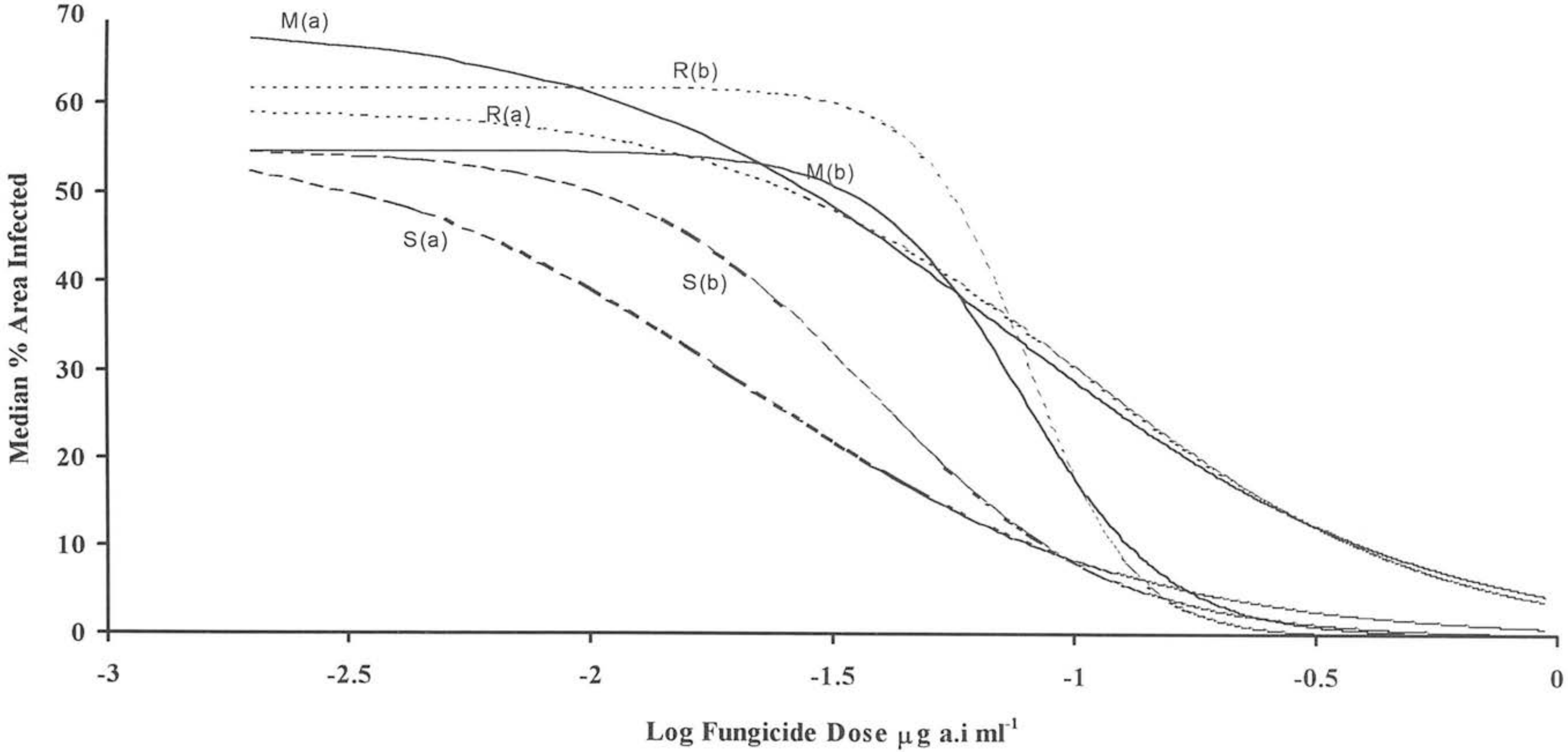


Fig. 6.1(iii). Median % area infected vs log fenpropimorph dose for the wheat 2 powdery mildew isolates.

Table 6.1 shows that, of the barley isolates DH14 was the most sensitive with a geometric mean EC_{50} value of 54 $\mu g\ ml^{-1}$ and CC148 was the least sensitive with a geometric mean EC_{50} value of 408 μg ml. The difference between the mean log of CC148 and the means of the other three isolates was very highly significant $P = 0.001$ (Table 6.1 and Fig. 6.1(i)). However, there were no significant differences between the mean log EC_{50} values of the other three isolates.

Wheat 1 data are the same as that presented in the monitoring work in Chapter 4. MOR2 was found to be the most sensitive with a geometric mean EC_{50} value of 62 μg a.i. ml^{-1} while CPBW-1 was the least sensitive with a geometric mean EC_{50} value of 256 $\mu g\ ml^{-1}$. The mean for MOR2 was significantly less than that for MORUNT, RIA2 ($P < 0.05$) and 63CAMP and CPBW-1 ($P < 0.01$). RA2 which was also relatively sensitive showed a mean which was significantly less than that for 63CAMP and CPBW-1 ($P < 0.05$) (Table 6.1 and Fig. 6.1(ii)).

In the second wheat experiment the isolates S (sensitive), M (moderate) and R (resistant) fell into the expected order of ranking, the geometric mean EC_{50} values ranging from 162 to 302 μg a.i. ml^{-1}. However, the differences between the means of the log EC_{50} values of the different isolates were not significant (Table 6.1 and Fig. 6.1(iii)).

Table 6.1. Relative resistance of isolates to fenpropimorph based on visual assessments after 14 days in the standard leaf-segment test method.

Barley experiment	Isolate	Mean $\log_e EC_{50}$ * 50 + μg a.i.ml^{-1} (Fig.6.1)	Geometric mean μg a.i. ml^{-1}
	DH14	4.647 a **	54
	CC152	4.855 a	78
	CC52	4.932 a	89
	CC148	6.127 b	408
Wheat1 experiment	MOR2	4.723 m	62
	RA2	4.922 m, n	87
	MORUNT	5.356 n, o	162
	RIA2	5.486 n, o	191
	63CAMP	5.675 o	241
	CPBW-1	5.722 o	256
Wheat2 experiment	S	5.357 x	162
	M	5.652 x	235
	R	5.865 x	302

*SED = ±0.268 (d.f. = 11)

** different letters denote significant differences between means within each experiment

Microscopic observations of fungal development

During the experiments, photographs were taken to show the different stages of fungal development from germination to the growth of secondary hyphae. Fig. 6.2-6.5 illustrate germinating spores and germ tubes with appressoria and secondary hyphae of barley mildew as used in the assessment in the cellophane/agar, leaf/agar and the modified leaf-segment methods. In the cellophane/agar method (Fig. 6.2 & 6.3), spores were able to germinate on cellophane and they produced either a single primary germ tube (Fig. 6.2) or multiple germ tubes (Fig. 6.3) 24 hours after inoculation. None of the germinating spores produced appressoria in the cellophane/agar method. In the leaf/agar method, spores produced either a primary germ tube alone or a primary germ tube and an appressorial germ tube (Fig. 6.4) 24 hours after inoculation. Secondary hyphae could be seen 48 hours after inoculation (Fig. 6.5).

Fig. 6.2. Spores germinating on cellophane 24 hours after inoculation showing abnormal behaviour and a failure to form appressoria. (bar represents 10 μm).

Fig 6.3. A spore on cellophane germinating to produce multiple germ tubes (bar represents 10 μm).

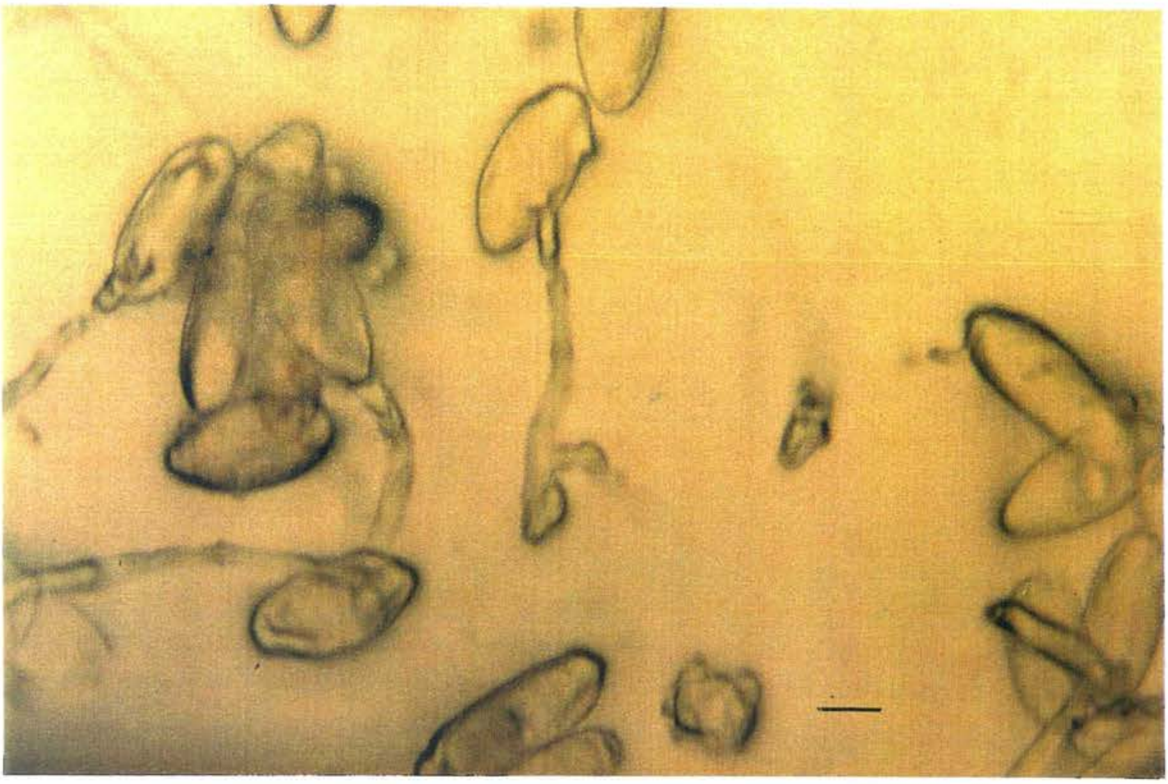


Fig. 6.2

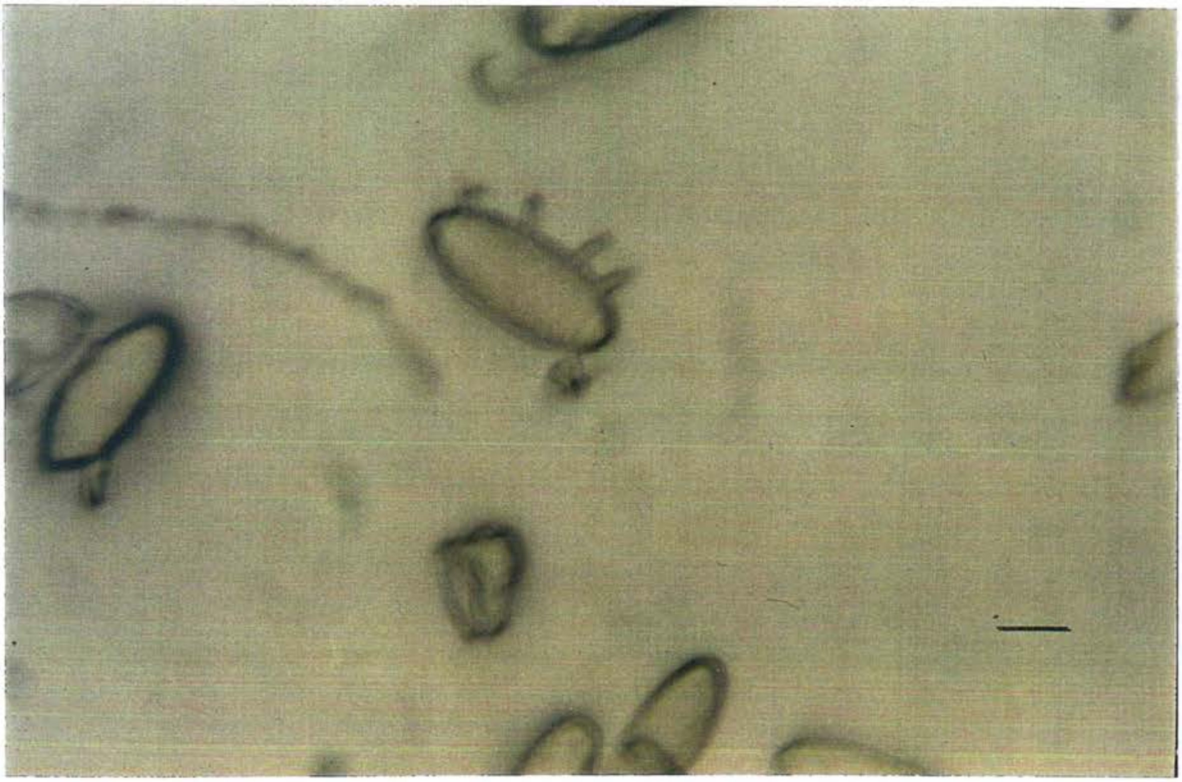


Fig. 6.3.

Fig 6.4. Two spores germinating on leaf surfaces forming both primary germ tubes for attachment and appressorial germ tubes for penetration (bar represents 10 μm).

Fig. 6.5. Secondary hyphae growing on the surface of the leaf showing the original spore. This represents a later stage in development once penetration and haustorial formation have occurred (bar represents 10 μm).

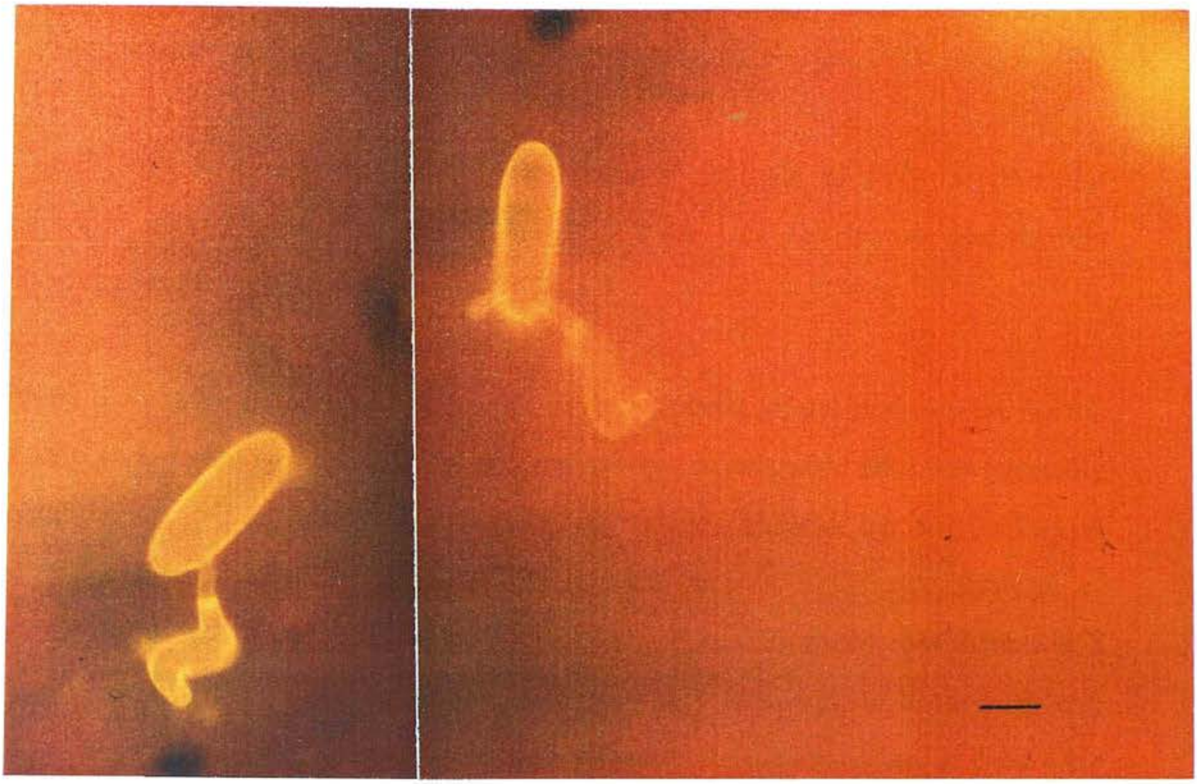


Fig. 6.4.

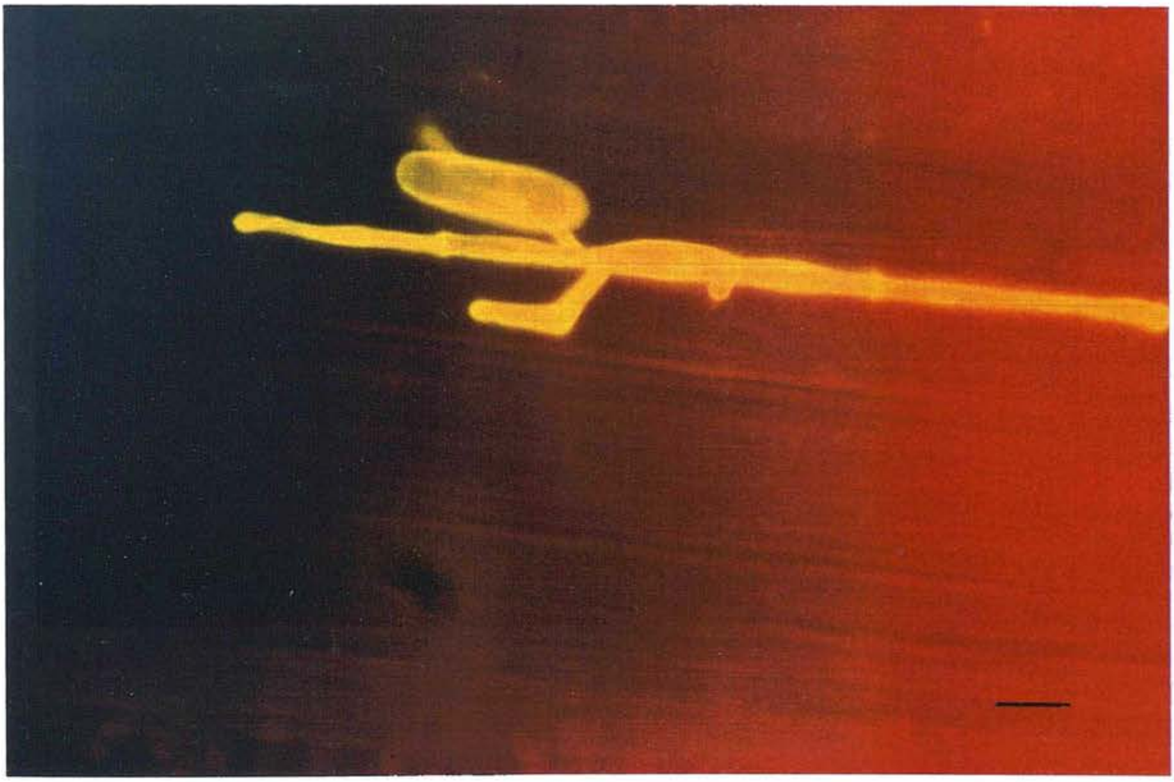


Fig. 6.5.

Microscopic assessments of fungal development by different methods

Cellophane/agar method - spore germination rates

Figures 6.6-6.8 show germination percentages of isolates at different doses of fenpropimorph in the three cellophane/agar tests. On the whole, germination percentages were low for the control.

Figure 6.6 shows the response by barley mildew isolates. Germination rates for the control varied with isolate but in all cases an increase in fungicide dose was generally accompanied by a reduction in germination percentage. Three of the isolates had no germination at the highest dose of 29.3 μg a.i. ml^{-1}. A chi-square analysis of germination rates of the different barley isolates (Table 6.2) shows that there was a significant difference between isolates in the way they responded to different doses. Closer examination of the contribution of each isolate to the chi-square at each dose revealed that isolate CC148 produced more germinated spores at dose 3.7 μg a.i. ml^{-1} than expected but showed only half the expected germination at the14.6 μg a.i. ml^{-1} concentration. DH14 had fewer germinated spores at the control and more at the 14.6μg a.i. ml^{-1} dose than expected. Isolate CC152 produced more germinated spores at the control and the highest dose than expected. Isolate CC52 produced more germinated spores at the control and fewer at the 14.6 μg a.i. ml^{-1} dose than expected.

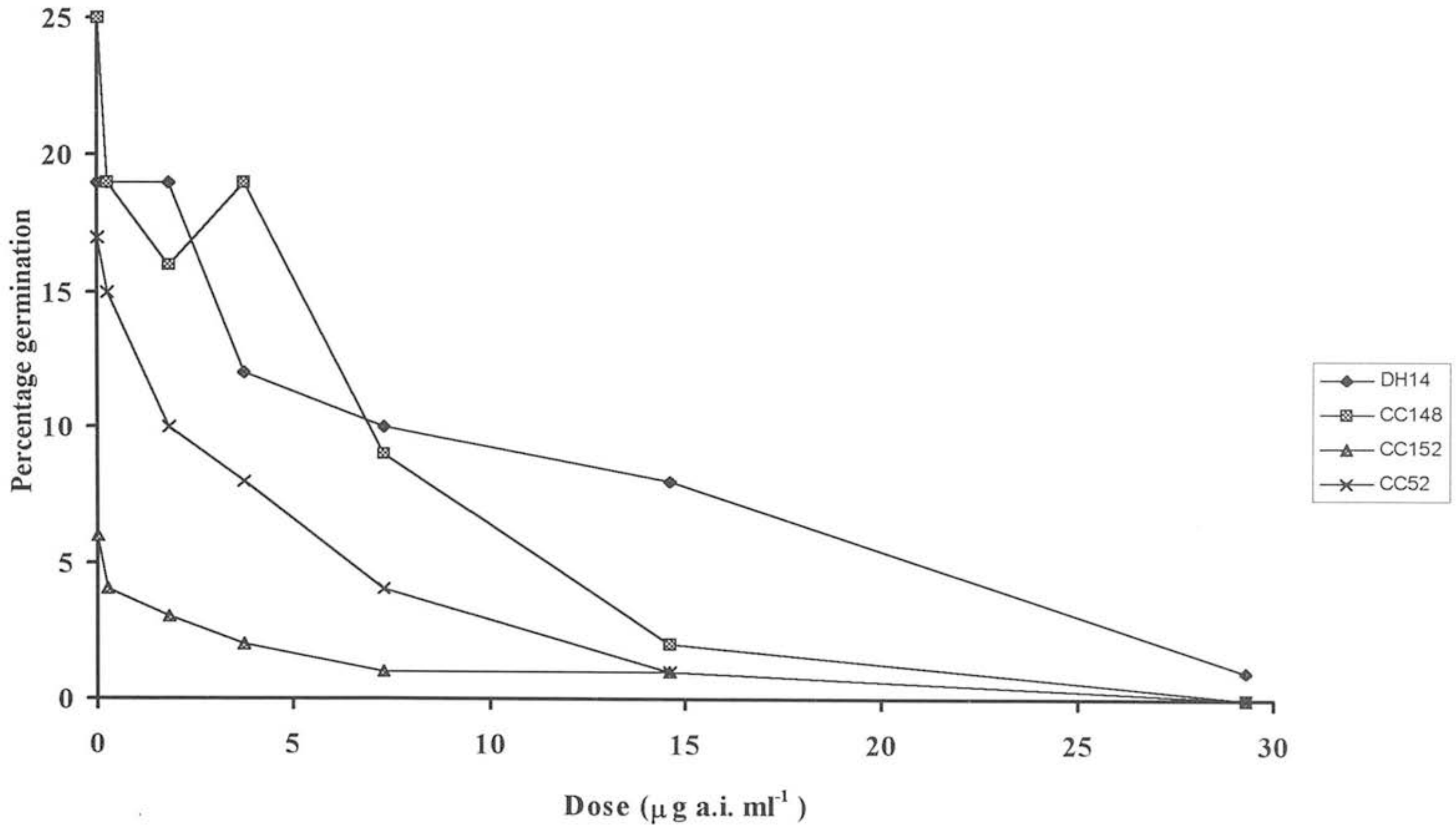


Fig. 6.6. Spore germination rates of four barley powdery mildew isolates in relation to fenpropimorph dose in the cellophane/agar method.

Table 6.2. Chi-square analysis of the germination rates of the barley isolates in the cellophane/agar method with different fenpropimorph dose rates (observed germinated spores out of 1500 in each case).

	Observed number of germinated spores (Expected number of germinated spores)				
Dose µg a.i. ml^{-1}	CC148	DH14	CC152	CC52	*P* value
0	374 (370)	283 (357)	96 (67)	260 (218)	
0.23	291 (312)	284 (301)	58 (57)	220 (184)	
1.8	236 (256)	278 (247)	40 (47))	147 (151)	
3.7	281 (215)	182 (207)	24 (39)	100 (126)	
7.3	141 (134)	150 (129)	17 (24)	58 (79)	
14.6	34 (68)	126 (66)	8 (12)	18 (40)	
29.3	6 (8)	11 (8)	5 (1)	0 (5)	$P < 0.001$

Fig. 6.7 shows the germination response by wheat 1 mildew isolates in the cellophane/agar method. Isolate MORUNT had the highest percentage germination at the control. Response of isolates to fungicide was uneven, especially at the lower doses, but increase in dose resulted generally in a reduction in germination percentage. Four of the isolates had no germination at the highest concentration of 29.3 μg ml^{-1}. Some isolates such as RIA2 showed a percentage germination at the lower doses which was higher than the control. Table 6.3 shows the chi-square analysis of the germination rates for the wheat 1 isolates. The isolates differed significantly in their response to different doses of fenpropimorph $P < 0.001$ but the response of most isolates to increasing fungicide dose tended to be erratic.

Fig. 6.8 illustrates the germination response to fungicide by wheat 2 mildew isolates in the cellophane/agar test. The percentage germination at the control was again low for all isolates. Increase in dose resulted in a reduction in germination with isolates S and M having no germination at the highest dose of 29.3 μg a.i. ml^{-1}. Table 6.4 shows the chi-square analysis of germination rates for the wheat 2 isolates. There was a significant difference in the way the three isolates responded to the different doses of fenpropimorph $P < 0.001$. Isolate S gave higher germination levels than expected at the lower doses whereas isolate M gave higher germination levels at the higher doses than expected. The response was mixed for isolate R.

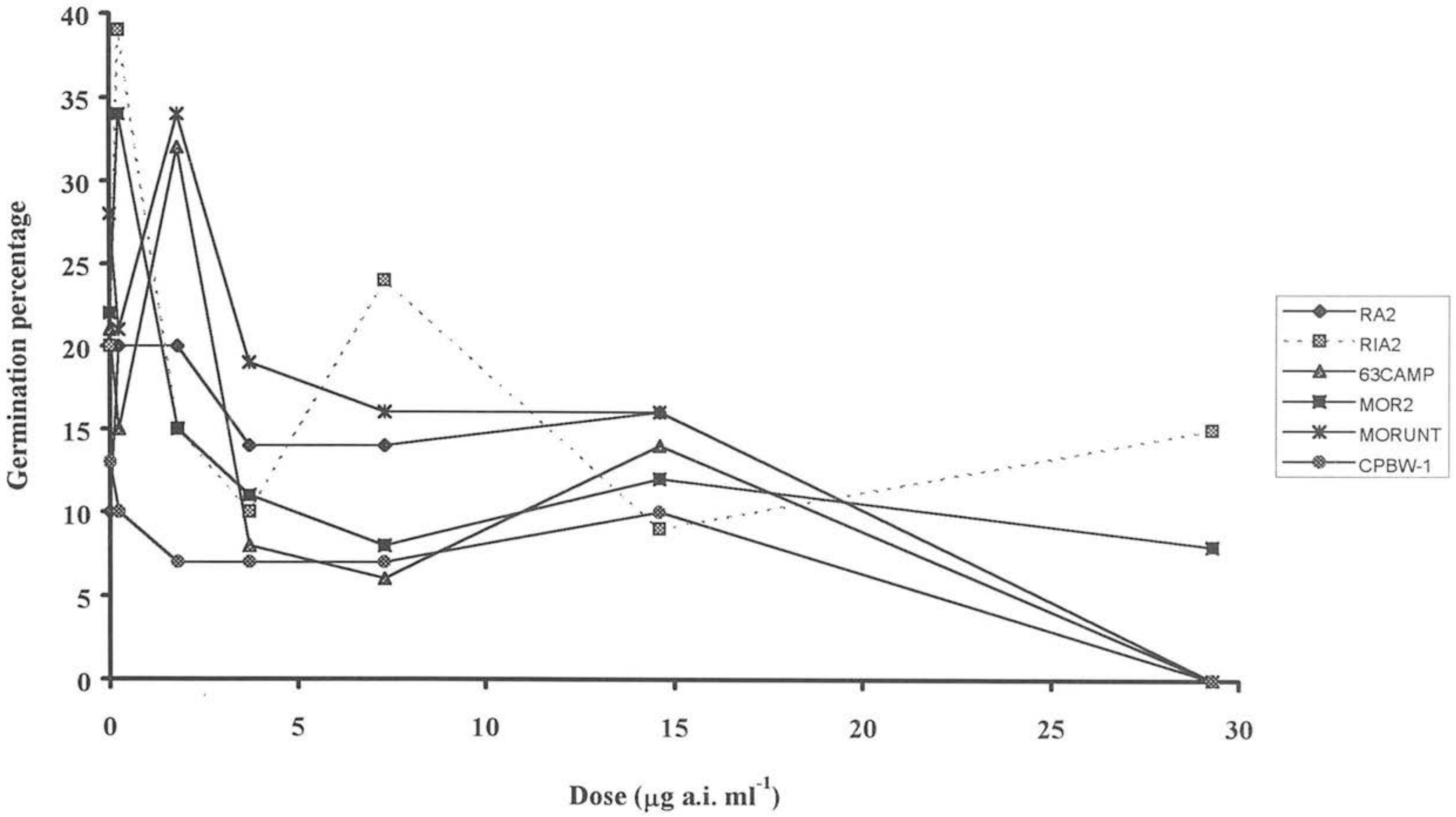


Fig. 6.7. Spore germination rates of six wheat 1 powdery mildew isolates in relation to fenpropimorph dose in the cellophane/agar method.

Table 6.3. Chi-square analysis of germination rates of the wheat 1 isolates in the cellophane/agar method with different fenpropimorph dose rates (observed germinated spores out of 1500 in each case).

	Observed number of germinated spores (Expected number of germinated spores)						
Dose μg a.i. ml^{-1}	MOR2	RA2	MORUNT	RIA2	63CAMP	CPBW-1	*P* value
0	335 (293)	148 (259)	424 (388)	298 (336)	310 (275)	190 (154)	
0.23	504 (359)	306 (316)	322 (474)	588 (411)	220 (336)	146 (189)	
1.8	227 (317)	304 (280)	510 (419)	221 (363)	474 (297)	108 (167)	
3.7	161 (176)	204 (155)	278 (233)	150 (202)	123 (165)	108 (93)	
7.3	114 (176)	124 (155)	237 (233)	353 (202)	89 (165)	108 (93)	
14.6	187 (206)	261 (181)	247 (272)	140 (236)	216 (193)	144 (108)	$P < 0.001$

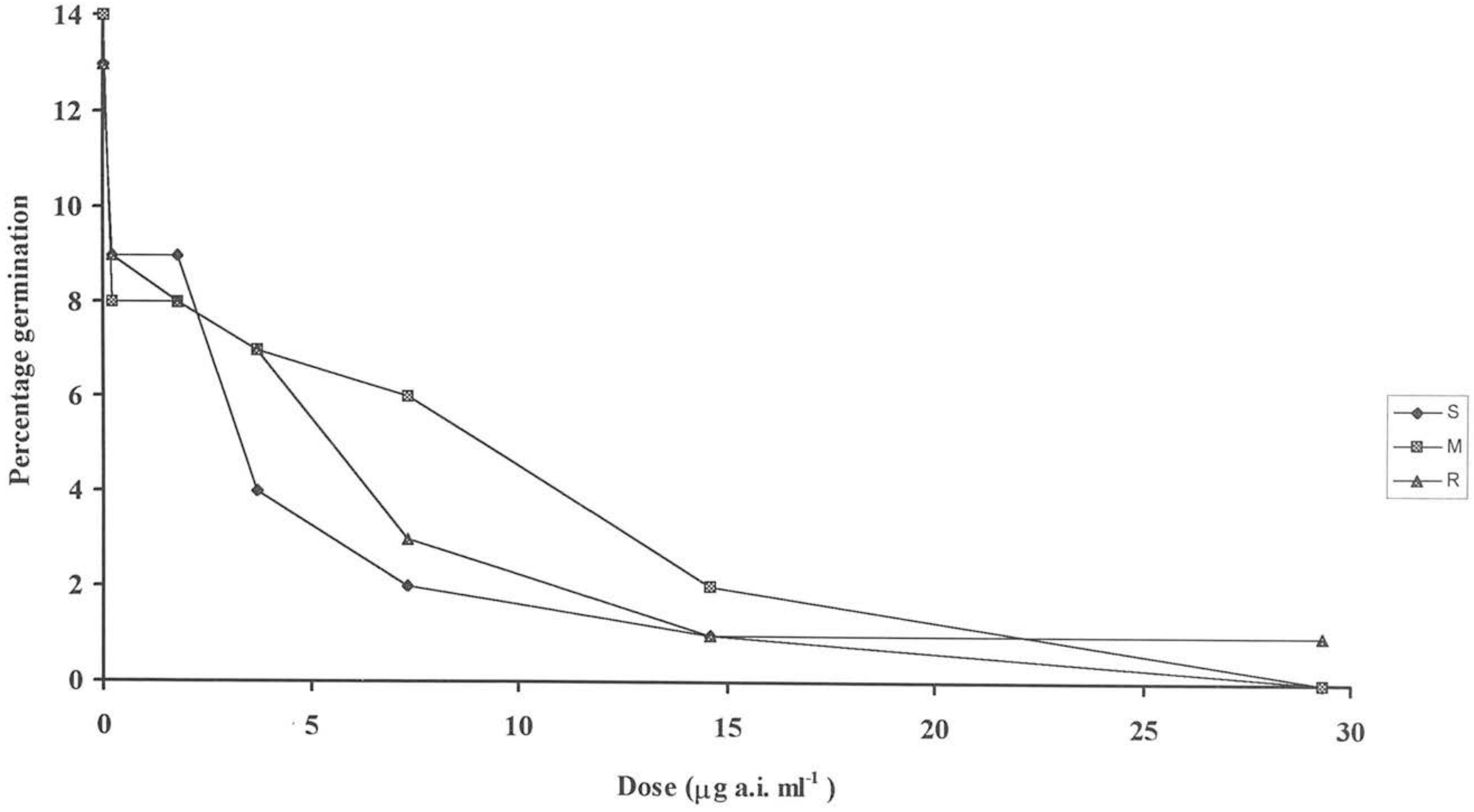


Fig. 6.8. Spore germination rates of three wheat 2 powdery mildew isolates in relation to fenpropimorph dose in the cellophane/agar method.

Table 6.4. Chi-square analysis of germination rates for the wheat 2 mildew isolates in the cellophane/agar method with different fenpropimorph dose rates (observed germinated spores out of 1500 in each case).

Observed number of germinated spores (Expected number of germinated spores)				
Dose μg a.i. ml^{-1}	S	M	R	*P* value
0	192 (178)	216 (222)	188 (196)	
0.23	133 (115)	114 (144)	139 (127)	
1.8	130 (113)	126 (140)	121 (124)	
3.7	57 (79)	107 (98)	100 (87)	
7.3	25 (47)	88 (58)	44 (52)	
14.6	12 (18)	33 (22)	14 (19)	$P < 0.001$

The leaf/agar and the modified leaf-segment methods - rates of spore germination and production of appressoria and secondary hyphae

Fig. 6.9 shows the results for the four barley mildew isolates in the leaf/agar test method. There was a very sharp decline in the percentage of germinated spores with increasing dose. There was negligible germination at 29.3 μg a.i. ml^{-1}, with no germination for three isolates at the maximum dose. There was a more gradual decline in percentage of germinated spores with increasing dose for the modified leaf-segment method, and isolates still showed 5-25% germination at the maximum dose (Fig. 6.10). The actual percentage of spores producing appressoria or appressoria and secondary hyphae tended to follow the trend of percentage germination in both methods (Fig. 6.9 & 6.10).

Tables 6.5 and 6.6 show chi-square analyses of the total germinated spores in the leaf/agar and modified leaf-segment test methods respectively. For the leaf/agar test method, only two doses and the control were analysed as the germination rates for the other doses were too low to be included in the chi-square analysis. Table 6.5 shows that the response of the different isolates to the doses and control was similar as the differences were not significant ($P = 0.2$).

For the modified leaf-segment test method the differences in response of the isolates to the fungicide doses were highly significant ($P < 0.01$) (Table 6.6). Examination of the chi-square contribution by each isolate at the different doses to the differences showed that isolates CC148 had a lower germination and CC52 a higher rate at the highest dose than what was expected. Isolate CC52 also had a lower germination than expected at dose 117 μg a.i. ml^{-1}. The chi-square analysis of the secondary hyphae produced by all isolates was, however not significant at the 5% level (Table 6.7). Thus isolates did not differ in the proportion of secondary hyphae they produced from germinated spores at the different doses.

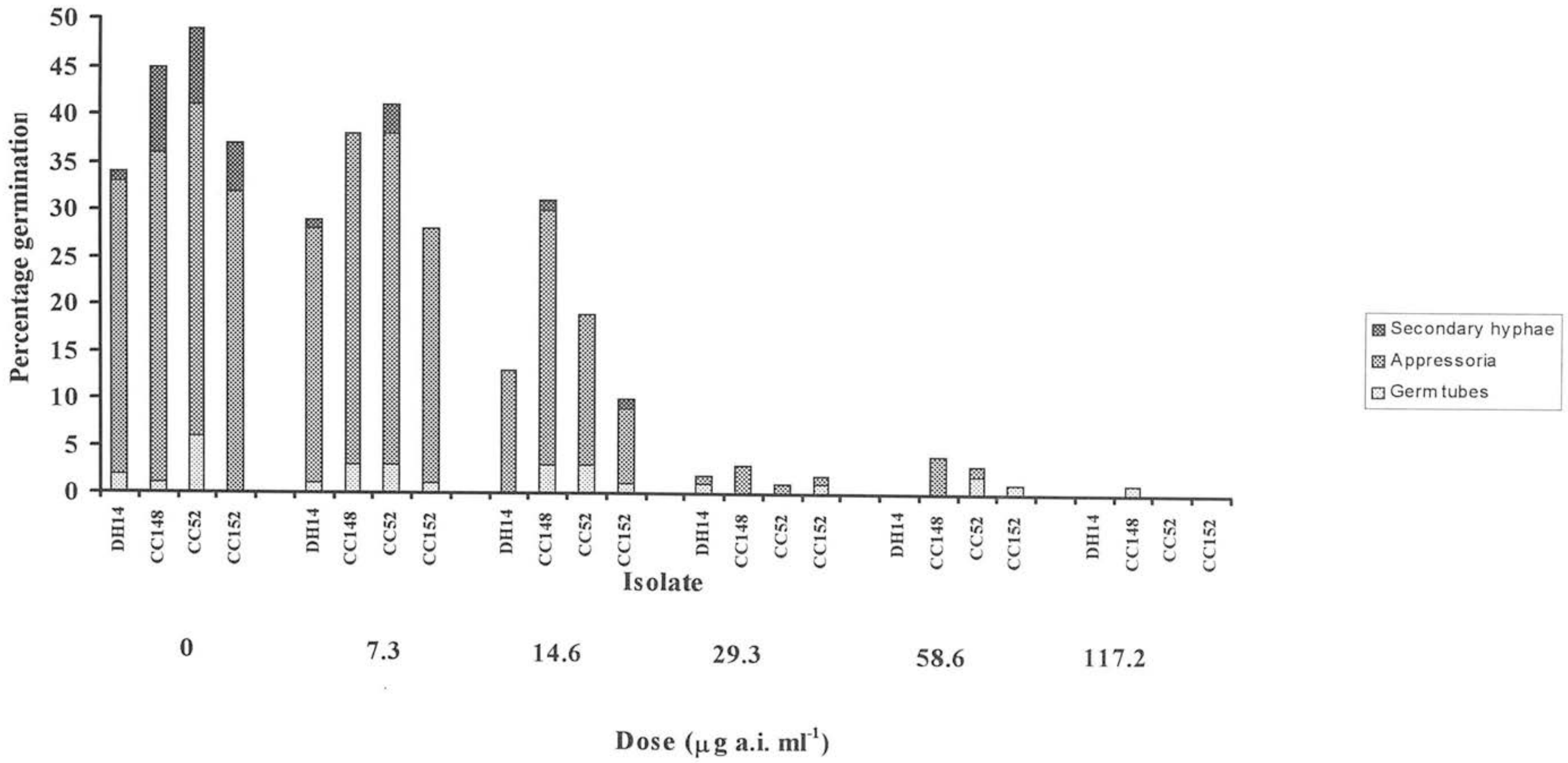


Fig. 6.9. Spore germination rate and percentage of spores with germ tubes only, appressoria or appressoria plus secondary hyphae for four barley powdery mildew isolates 48 hours after inoculation in the leaf/agar method with different fenpropimorph dose rates.

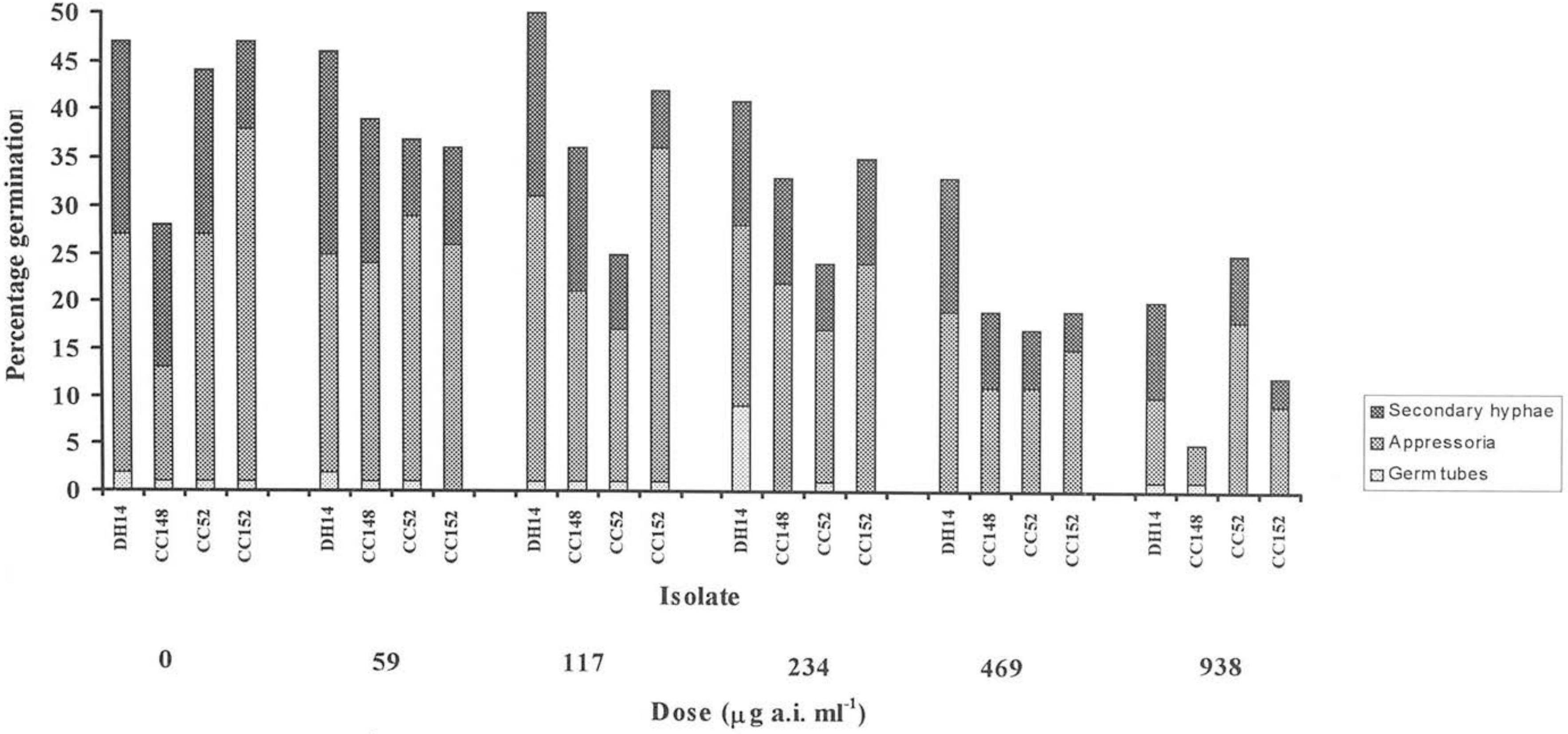


Fig. 6.10. Spore germination rates and percentage of spores with germ tubes only, appressoria or appressoria with secondary hyphae for four barley powdery mildew isolates 48 hours after inoculation in the modified leaf-segment method with different fenpropimorph dose rates.

Table 6.5. Chi-square analysis of total germination of barley isolates in the leaf/agar test method with different fenpropimorph dose rates (observed germinated spores out of 150 spores in each case).

	Observed number of germinated spores (Expected number of germinated spores)				
Dose µg a.i. ml^{-1}	CC148	DH14	CC152	CC52	*P* value
0	73 (79)	53 (51)	56 (50)	68 (70)	
7.3	58 (64)	43 (42)	41 (41)	62 (57)	
14.6	47 (35)	20 (23)	16 (22)	29 (31)	$P = 0.2$

Table 6.6. Chi-square analysis of total germination of barley isolates in the modified leaf-segment test method with different fenpropimorph dose rates (observed germinated spores out of 150 spores in each case).

	Observed number of germinated spores (Expected number of germinated spores)				
Dose µg a.i. ml^{-1}	CC148	DH14	CC152	CC52	*P* value
0	62 (61)	71 (82)	70 (66)	65 (59)	
59	59 (55)	70 (73)	54 (59)	56 (52)	
117	55 (53)	75 (71)	62 (57)	39 (51)	
234	50 (45)	60 (61)	53 (49)	36 (44)	
469	32 (31)	50 (42)	29 (34)	25 (30)	
938	8 (21)	31 (28)	19 (23)	34 (20)	$P < 0.01$

Table 6.7. Chi-square analysis of secondary hyphae produced by all barley isolates across fenpropimorph doses in the modified leaf-segment test method (observed germinated spores out of 150 spores in each case).

	Observed secondary hyphae (Expected secondary hyphae				
Dose μg a.i. ml^{-1}	CC148	DH14	CC152	CC52	*P* value
0	23 (24)	30 (35)	14 (16)	25 (18)	
59	23 (21	32 (31)	15 (14)	12 (16)	
117	23 (19)	28 (28)	9 (13)	14 (14)	
234	17 (17)	19 (24)	17 (11)	10 (12)	
469	12 (12)	21 (17)	6 (8)	6 (9)	
938	0 (7)	15 (10)	5 (5)	7 (5)	$P = 0.07$

In the case of the leaf/agar method (Fig. 6.9), where germination was only appreciable at the control and two lowest doses, a higher percentage of germinated spores tended to show no further development beyond the appressorial stage, even with no fungicide compared, with the modified leaf-segment method (Fig. 6.10), where further development to produce secondary hyphae was appreciable at all doses.

The percentages of spores which germinated and the percentages of spores producing appressoria and secondary hyphae, averaged for all the barley mildew isolates, in the leaf/agar and the modified leaf-segment tests are represented in Figs. 6.11 & 6.12. Fig. 6.11 shows that there was a very sharp decline in percentage of germinated spores with increasing dose in the leaf/agar test. There was negligible germination from 29.3 μg a.i. ml^{-1}. Just like Fig. 6.10, Fig 6.12 shows that there was a more gradual decline in percentage of germinated spores with increasing dose for the modified leaf-segment test. The percentage of spores producing appressoria or appressoria and secondary hyphae tended to follow the trend of percentage germination in both methods.

In the leaf/agar method using the combined data for the four barley isolates, chi-square analysis showed that the different germination classes differed significantly ($P < 0.001$) in their response to fenpropimorph at the lowest dose levels compared to the control (Table 6.8). This was related to the secondary hyphae production. An appreciable number of control (no fungicide) appressoria went on to produce secondary hyphae but few secondary hyphae were produced by appressoria at the two fungicide doses. Low germination at the highest doses precluded their inclusion in this analysis. This was in contrast to the modified leaf-segment test method where apart from isolate CC148 at the highest fungicide dose of 938 μg a.i. ml^{-1} (Fig. 6.10), some appressoria went on to produce secondary hyphae (Table 6.9) at all dose rates. Although there was less germination at higher doses, still the same proportion of spores of isolates developed into appressoria and secondary hyphae.

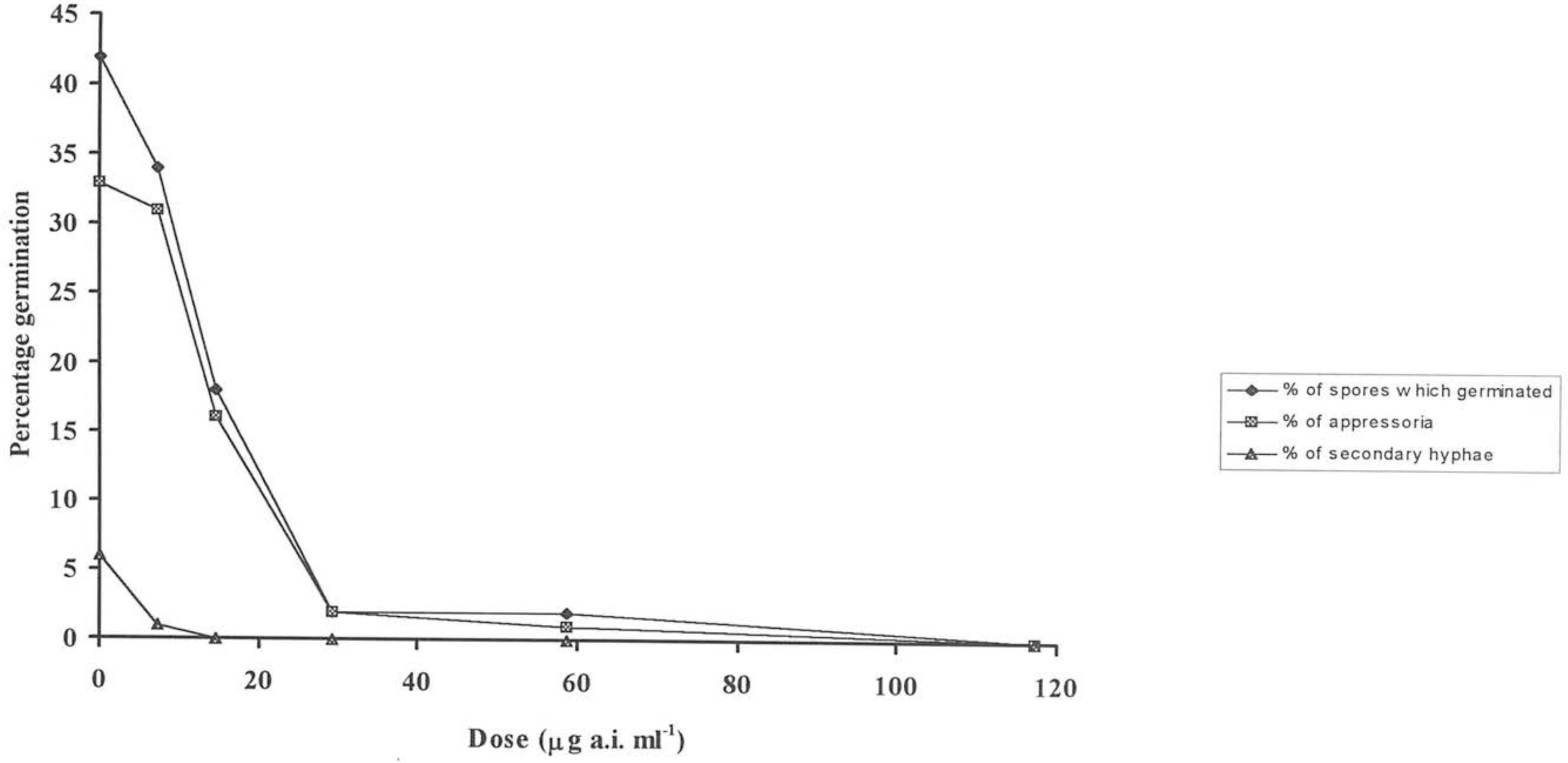


Fig. 6.11. Percentage of spores which germinated and percentage of spores producing appressoria and secondary hyphae averaged for all barley isolates in the leaf/agar method with different fenpropimorph dose rates 48 hours after inoculation.

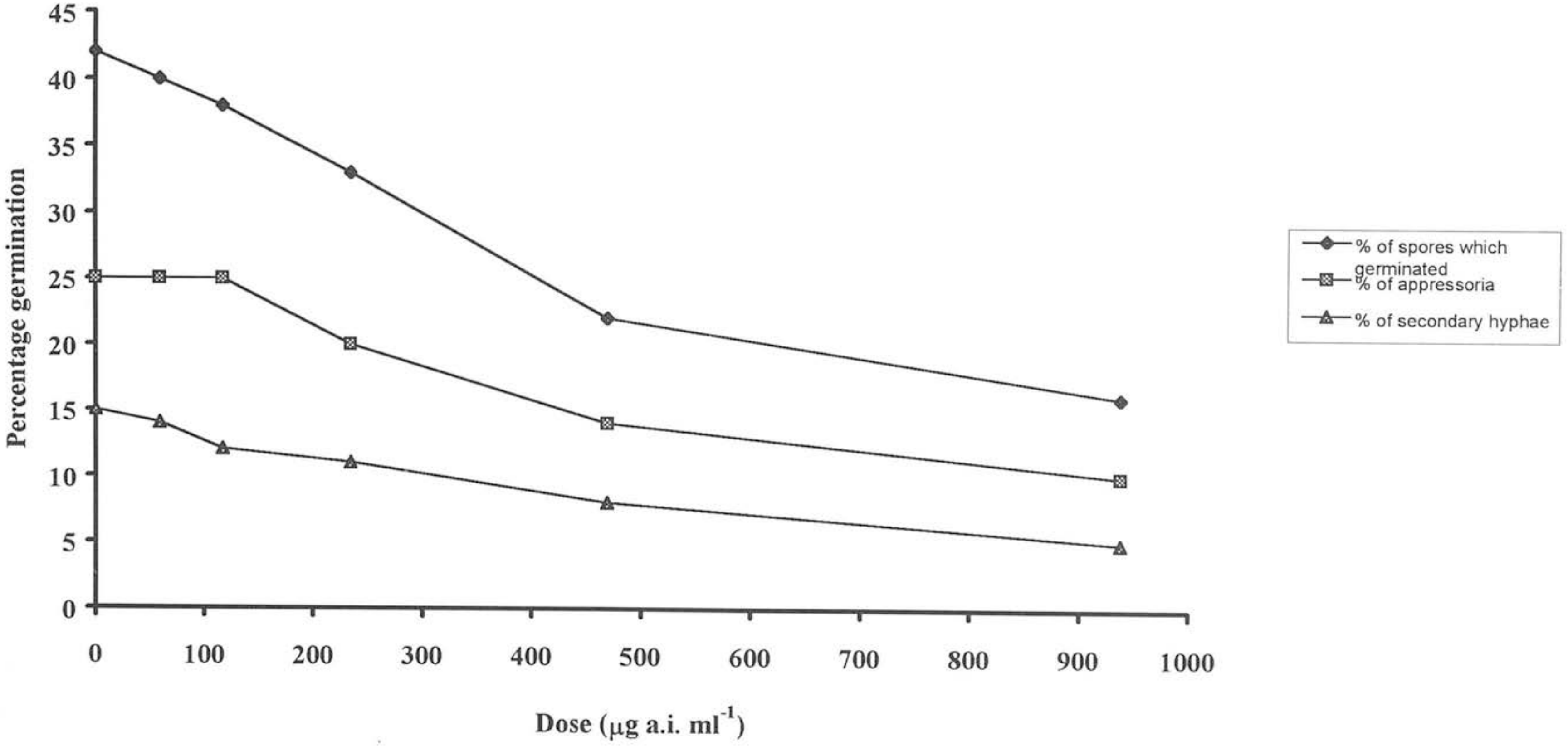


Fig. 6.12. Percentage of spores which germinated and percentage of spores producing appressoria and secondary hyphae averaged for all barley powdery mildew isolates in the modified leaf-segment method with different fenpropimorph dose rates 48 hours after inoculation.

Table 6.9 shows that the difference between the germination classes was not significant at the 5% level.

Table 6.8. Chi-square analysis of germination classes across three fenpropimorph doses for barley mildew isolates in the leaf/agar method (observed germinated spores out of 450 spores in each case).

	Observed numbers (Expected numbers)			
Dose μg a.i. ml^{-1}	Germ tubes	Appressoria	Hyphae	*P* value
0	8 (14)	200 (213)	42 (23)	
7.3	11 (11)	187 (174)	6 (19)	
14.6	12 (6)	96 (95)	4 (10)	$P < 0.001$

Table 6.9. Chi-square analysis of germination classes across fenpropimorph dosage rates for barley mildew isolates in the modified leaf-segment method (observed number of germinated spores out of 450 spores in each case).

	Observed numbers (Expected numbers)			
Dose μg a.i. ml^{-1}	Germ tubes	Appressoria	Hyphae	*P* value
0	7 (9)	170 (171)	92 (89)	
59	8 (8)	151 (153)	82 (80)	
117	6 (8)	151 (147)	74 (76)	
234	15 (7)	121 (126)	63 (66)	
469	0 (5)	88 (86)	48 (45)	
938	4 (3)	61 (58)	27 (30)	$P > 0.05$ n.s.

The abundance of hyphal growth and conidiophores found on leaf segments in the modified leaf-segment test method, 6 days after inoculation in the barley experiments is represented in Table 6.10. For all isolates there was some hyphal growth at all doses although it was only abundant at the control and lower doses. For all isolates there were no conidiophores on leaf segments treated with the last two highest doses.

Most of the isolates had abundant coniodiophores only on untreated leaf segments or those treated with the lowest doses.

Table 6.10. Abundance of hyphae and conidiophores found on leaf segments of the modified leaf-segment method with different fenpropimorph dose rates 6 days after inoculation in the barley experiment.

Isolate	Dose μg a.i. ml^{-1}	Hyphae	Conidiophores
DH14	0	2	2
	59	2	2
	117	1	1
	234	1	1
	469	1	0
	938	1	0
CC/148	0	2	2
	59	2	1
	117	2	1
	234	2	1
	469	1	0
	938	1	0
CC/52	0	2	2
	59	2	2
	117	1	0
	234	1	0
	469	1	0
	938	1	0
CC/152	0	2	2
	59	2	2
	117	1	1
	234	1	1
	469	1	0
	938	1	0

Key

2 = Abundant 1 = Sparse 0 = None

Fig. 6.13 shows the results for the six wheat 1 mildew isolates in the leaf/agar test method. The isolates were not tested in the modified leaf-segment test method. Although the decline in germination was sharp, it was not as sharp as it was for the barley isolates in the leaf/agar test method. Four of the isolates did not have any germination at the highest dose and one at the second highest dose. All isolates produced secondary hyphae at the control but with fenpropimorph, only two of the isolates produced secondary hyphae and at only one fungicide dose.

Table 6.11 shows the chi-square analysis of wheat 1 isolates at three doses and the control in the leaf/agar test method. The last two doses were not analysed because the numbers were very low. The differences between the isolates in their response to the fungicide were highly significant $P < 0.001$. Examination of the contribution of each isolate to the differences at each dose revealed that the significance was brought about by isolate RA2 having more germination and RIA2 less at dose 7.3 μg a.i. ml^{-1} than expected, isolate MORUNT germinating less than expected at dose 14.6 μg a.i. ml^{-1} and isolate 63CAMP having less germination than expected at the control.

Fig 6.14 illustrates the percentage of spores which germinated, the percentage with appressoria and the percentage with secondary hyphae averaged for all wheat 1 isolates in the leaf/agar test method 48 hours after inoculation.. There was a sharp decline in the percentage of all categories from the control to 29.3 μg a.i. ml^{-1} of fungicide. There was negligible germination after then. The average percentage of germinated spores at the control was higher for wheat 1 than for the barley isolates. There was also more hyphal production at the control. The actual percentage of spores producing appressoria and secondary hyphae tended to follow the trend of percentage germination (Fig. 6.14).

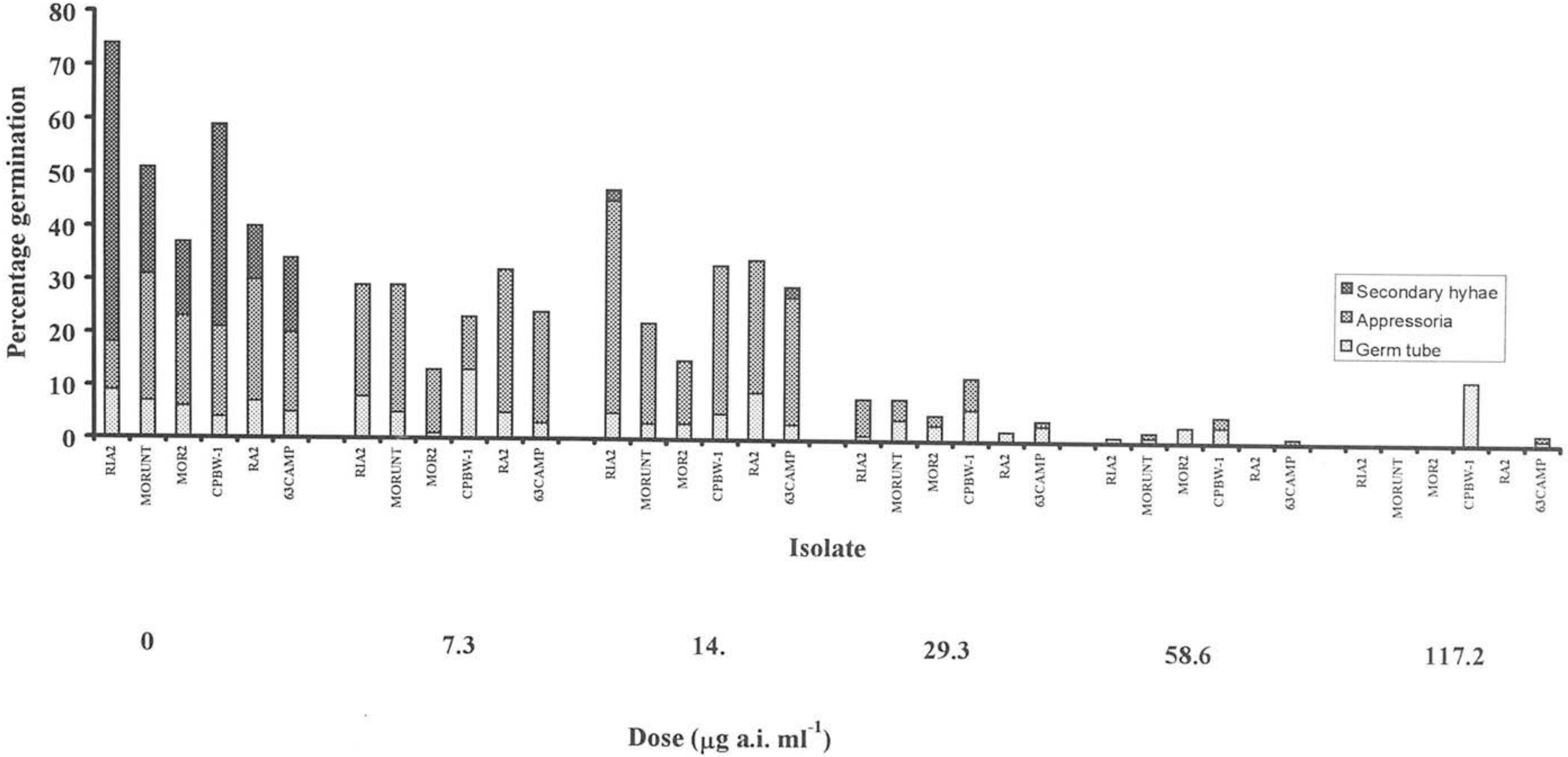


Fig. 6.13. Spore germination rate and percentage of spores with germ tubes only, appressoria or appressoria plus secondary hyphae for six wheat powdery mildew isolates, 48 hours after inoculation in the wheat 1 leaf/agar test method with different dose rates of fenpropimorph.

Table 6.11. Chi-square analysis of total germination rates of the wheat 1 isolates in the leaf/agar test method with different fenpropimorph dose rates (observed germinated spores out of 155 spores in each case).

	Observed number of germinated spores (Expected number of germinated spores)						
Dose μg a.i.ml^{-1}	MOR2	RA2	MORUNT	RIA2	63CAMP	CPBW-1	*P* value
0	55 (46)	52 (65)	61(65)	111 (103)	46 (59)	93 (82)	
7.3	20 (24)	47 (34)	44 (34)	40 (53)	35 (30)	32 (42)	
14.6	23 (29)	47 (41)	30 (41)	70 (65)	45 (37)	51 (52)	
29.3	6 (5)	1 (7)	12 (7)	12 (12)	7 (7)	9 (9)	$P < 0.001$

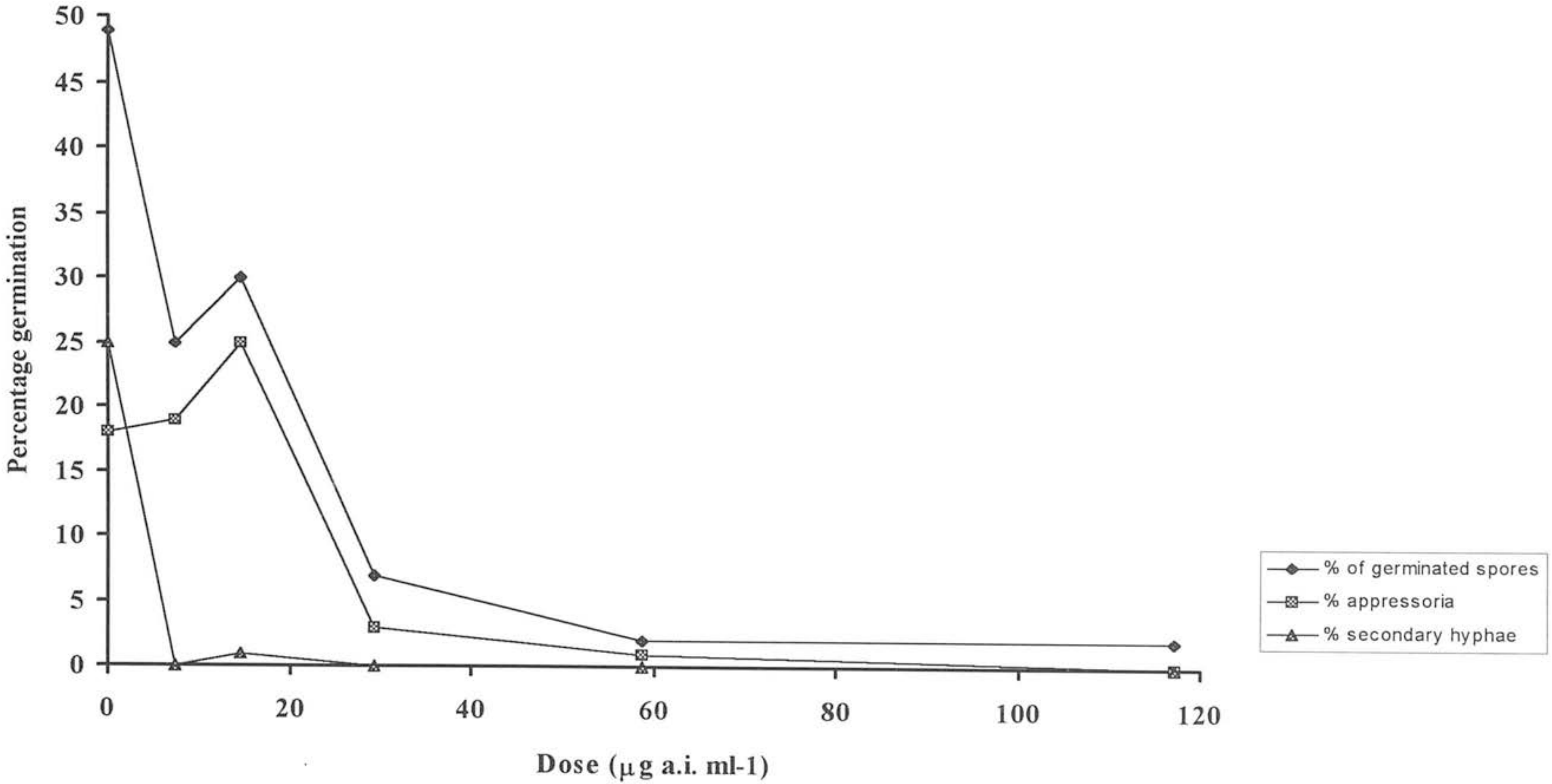


Fig. 6.14. Percentage of spores which germinated and percentage of spores producing appressoria and secondary hyphae averaged for all six powdery mildew isolates in the wheat 1 leaf/agar test method with different fenpropimorph dose rates, 48 hours after inoculation.

Table 6.12 shows the chi-square analysis of different germination classes i.e. germ tubes, appressoria and secondary hyphae. Only two doses together with the control are tabulated here as the numbers at the higher doses were too low for chi-square analysis. The differences between the way the different germination classes responded to fungicide were very highly significant $P < 0.001$. The very high significance was brought about by the fact that a good proportion of the appressoria at the control proceeded to develop into secondary hyphae whereas very few appressoria developed into secondary hyphae in the presence of fungicide.

Table 6.12. Chi-square analysis of germination classes across three doses for the wheat 1 mildew isolates in the leaf/agar test method with different fenpropimorph dose rates (observed germinated spores out of 930 spores in each case).

	Observed numbers (Expected numbers)			
Dose μg a.i. ml^{-1}	Germ tubes	Appressoria	Hyphae	*P* value
0	52 (65)	159 (254)	191 (89)	
7.3	49 (36)	177 (141)	0 (49)	
14.6	43 (44)	227 (168)	6 (59)	$P < 0.001$

Fig. 6.15 illustrates the results for the three wheat 2 mildew isolates in the leaf/agar test method. As for the barley and wheat 1 mildew isolates there was a sharp decline in the percentage of germinated spores with increasing fenpropimorph dose. Germination from 29.3 μg a.i. ml^{-1} onwards was low. There was no germination for isolate M at the highest dose. No germ tubes only were recorded in this method and only occasional secondary hyphae at the lower doses only. There was a more gradual decline in percentage of germinated spores with increasing dose in the modified leaf-segment test method and isolates still showed 25 to over 30 percent germination at the maximum dose (Fig. 6.16). A substantial proportion of germinated spores produced secondary hyphae at all doses.

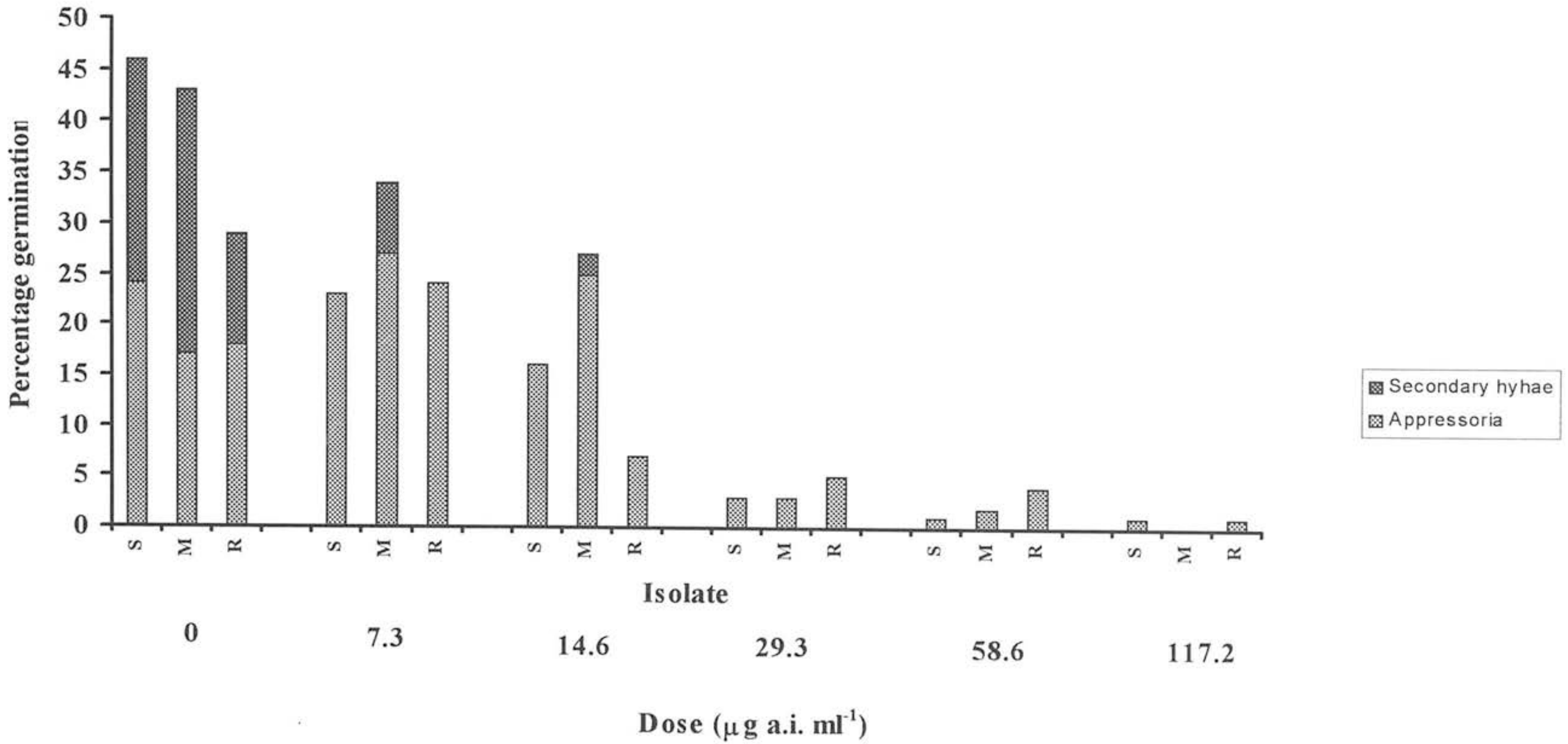


Fig. 6.15. Spore germination rate and percentage of spores producing appressoria or appressoria plus secondary hyphae for three wheat powdery mildew isolates 48 hours after inoculation in the wheat 2 leaf/agar method with different fenpropimorph dose rates.

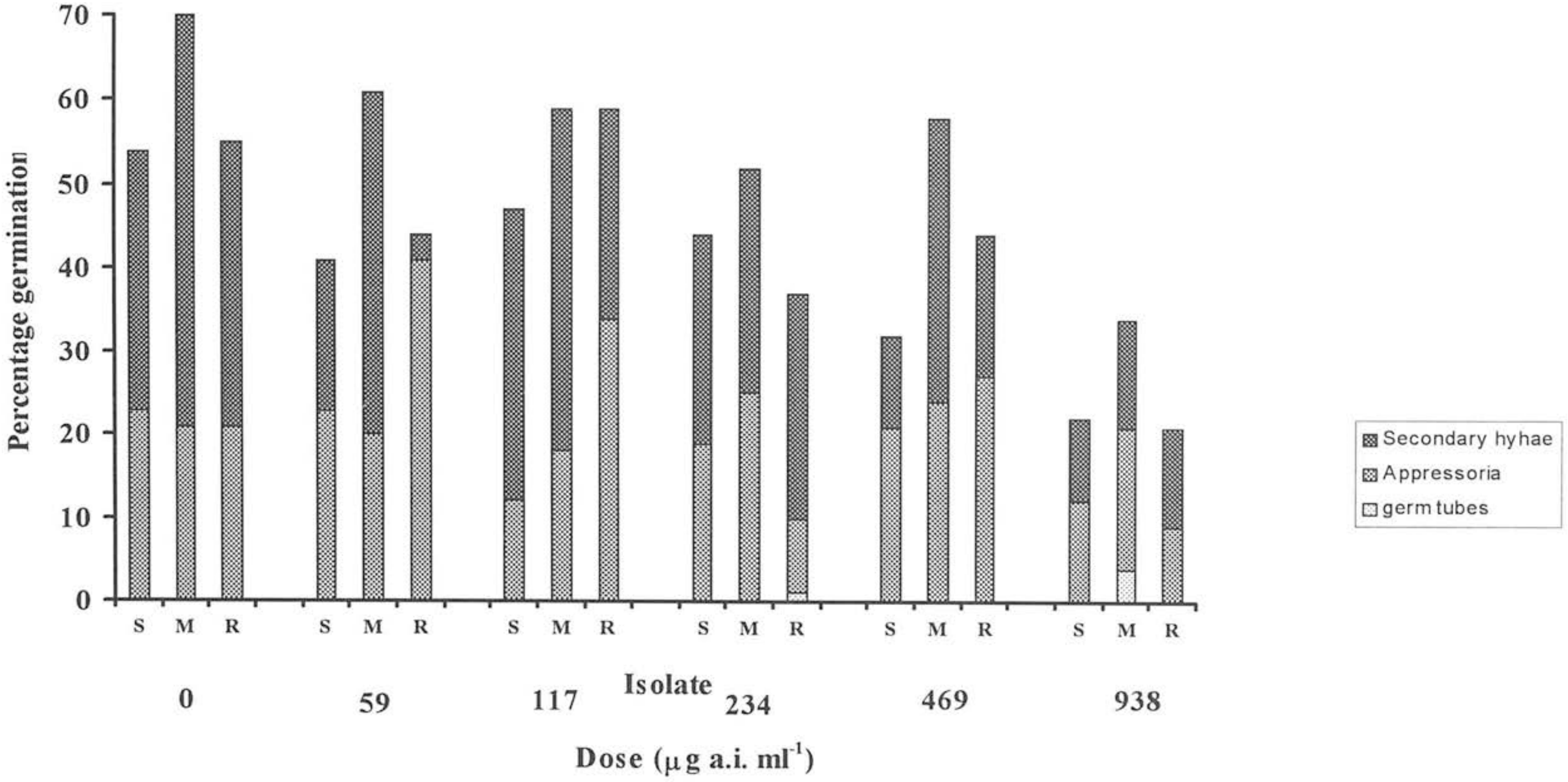


Fig. 6.16. Spore germination rates and percentage of spores producing germ tubes only, appressoria or appressoria plus secondary hyphae for three wheat powdery mildew isolates 48 hours after inoculation in the wheat 2 modified leaf-segment method with different fenpropimorph dose rates.

Table 6.13. Chi-square analysis of total germination rates of the wheat 2 mildew isolates in the leaf/agar test method with different fenpropimorph dose rates (observed germinated spores out of 300 spores in each case).

Observed number of germinated spores (Expected number of germinated spores)				
Dose μg a.i. ml^{-1}	S	M	R	*P* value
0	141 (118)	85 (114)	102 (96)	
7.3	70 (84)	90 (80)	72 (68)	
14.6	48 (52)	76 (50)	21 (42)	
29.3	12 (13)	9 (12)	15 (11)	
58.6	3 (7)	5 (7)	11 (6)	
117.2	2 (1)	0 (1)	2 (1)	$P < 0.001$

Table 6.13 shows the results of the chi-square analysis of germination rates of the wheat 2 mildew isolates in the leaf/agar method. There were high significant differences in the way the isolates responded to the fungicides $P < 0.001$. Examination of the contribution of each isolate to the differences at each dose revealed that the significant difference was brought about by isolate S having a higher germination at the control than expected, isolate M having a lower than expected germination at the control and a higher than expected germination at the second dose (14.6 μg ml^{-1}) and isolate R having a lower than expected germination at the second dose and a higher than expected germination at the second highest dose.

Table 6.14. Chi-square analysis of total germination rates of the wheat 2 mildew isolates in the modified leaf-segment test method with different fenpropimorph dose rates (observed germinated spores out of 150 spores in each case).

Observed number of germinated spores (Expected number of germinated spores)				
Dose μg a.i. ml^{-1}	S	M	R	*P* value
0	81 (76)	105 (107)	82 (84)	
59	64 (64)	91 (89)	68 (70)	
117	71 (70)	88 (99)	88 (78)	
234	61 (56)	79 (78)	56 (62)	
469	48 (57)	87 (80)	66 (63)	
938	33 (34)	52 (48)	36 (38)	$P = 0.7$

Table 6.14 shows the results of the chi-square analysis of germination rates of the wheat 2 mildew isolates in the modified leaf-segment method. The isolates responded similarly to the fungicide as the differences were not significant $P = 0.7$.

Just like with the barley and wheat 1 mildew isolates, germination was only appreciable at the control and two lower doses in the wheat 2 leaf/agar test method. Germinated spores at the other doses did not develop beyond the appressorial stage. Unlike the leaf/agar test method, the appressoria in the modified leaf-segment method went on to develop into secondary hyphae.

Table 6.15. Chi-square analysis of secondary hyphae produced by all wheat 2 isolates in the modified leaf-segment test method with different dose rates of fenpropimorph (observed germinated spores out of 150 spores in each case).

	Observed secondary hyphae (Expected secondary hyphae)			
Dose μg a.i. ml^{-1}	S	M	R	*P* value
0	47 (51)	74 (75)	51 (46)	
59	29 (23)	42 (34)	37 (20)	
117	53 (45)	61 (66)	37 (40)	
234	38 (35)	39 (51)	40 (31)	
469	16 (28)	51 (41)	26 (25)	
938	15 (17)	26 (26)	18 (16)	$P < 0.001$

Table 6.15 shows a highly significant difference between secondary hyphae produced by the isolates at different doses in the modified leaf-segment test. Examination of contribution to these differences by the different isolates revealed that isolate S produced fewer hyphae at the second highest dose than expected, isolate R produced more hyphae at the lowest dose (59 μg ml^{-1}) than expected and isolate M gave less hyphae at dose 234 μg a.i. ml^{-1} and more hyphae at the second highest dose than expected.

The percentages of spores which germinated and the percentages producing appressoria and secondary hyphae averaged for the wheat 2 mildew isolates in the leaf/agar and the modified leaf-segment test methods are represented in Fig. 6.17 and 6.18 respectively. There was a very sharp decline in percentage of germinated spores with increasing dose in the leaf/agar test method and a less pronounced and rather erratic decline in germination of spores with increasing dose in the modified leaf-segment test method. The percentage of spores producing appressoria and appressoria and secondary hyphae tended to follow the same trend as percentage germination in both methods.

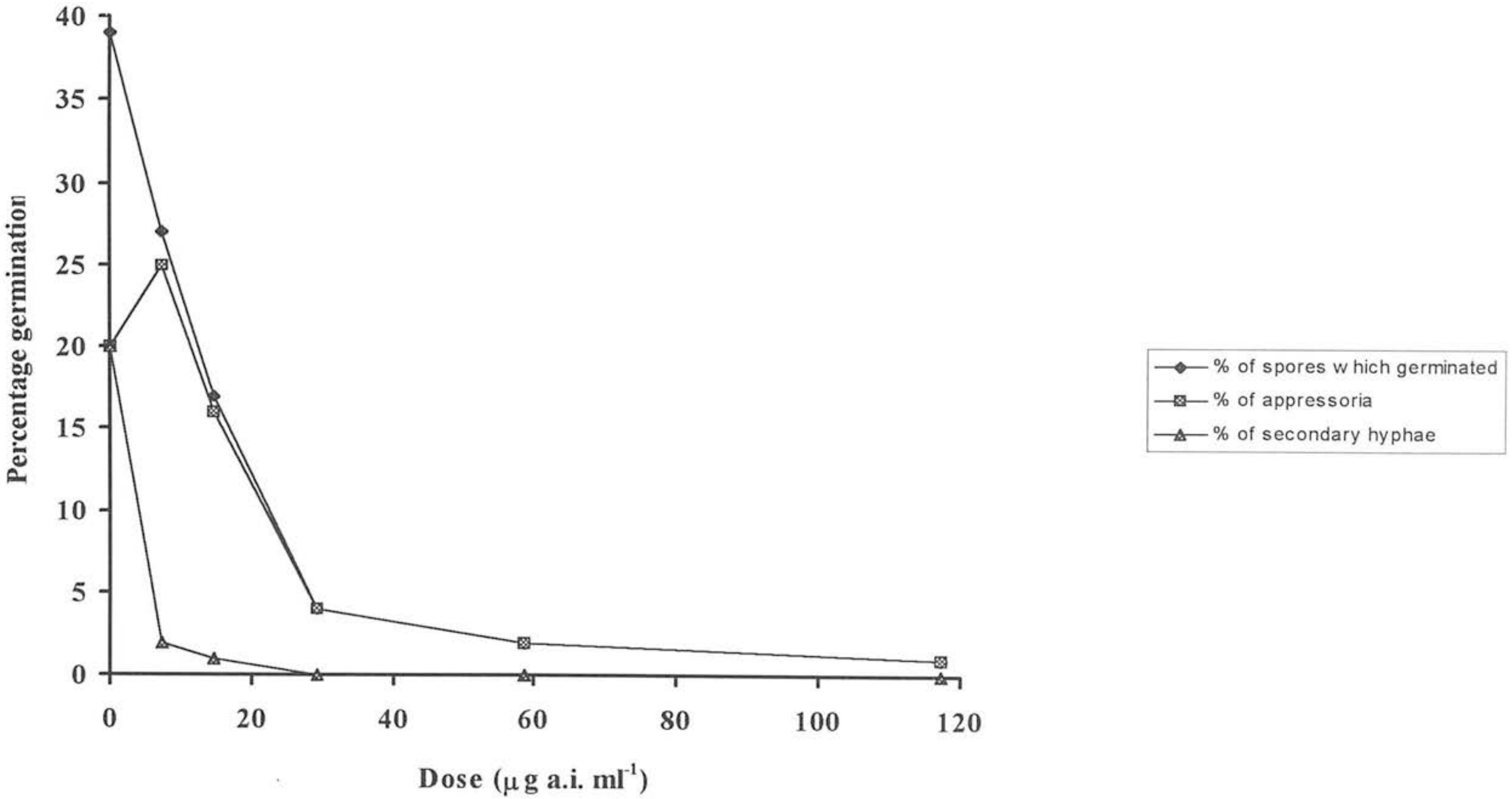


Fig. 6.17. Percentage of spores which germinated and percentage of spores producing appressoria and secondary hyphae averaged for three wheat powdery mildew isolates in the wheat 2 leaf/agar method with different fenpropimorph dose rates, 48 hours after inoculation.

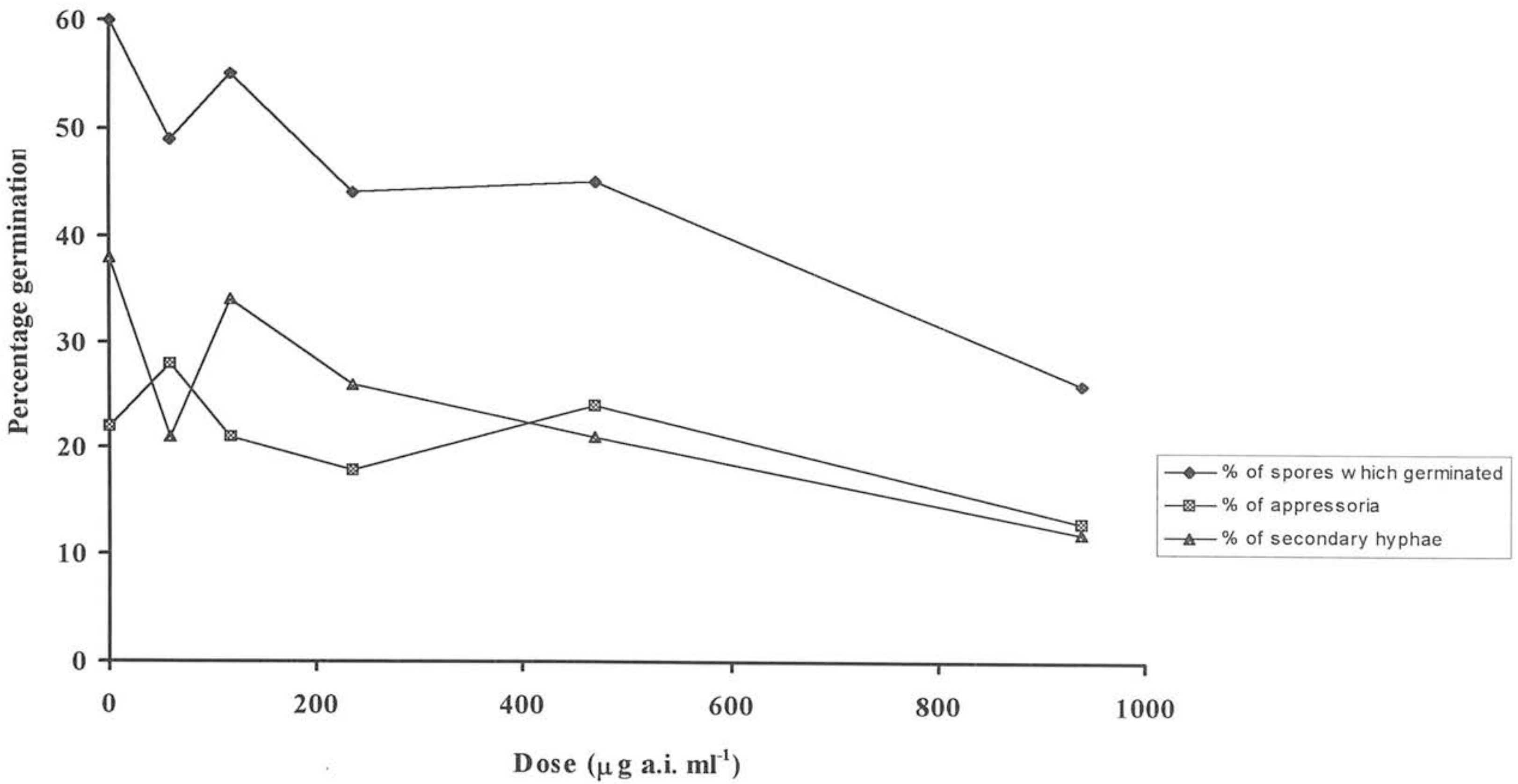


Fig. 6.18. Spore germination percentage and percentage of appressoria and secondary hyphae in the wheat 2 modified leaf-segment method with different fenpropimorph dose rates, 48 hours after inoculation.

Tables 6.16 and 6.17 show the results of chi-square analyses of the different germination classes in the wheat 2 leaf/agar and modified leaf-segment tests respectively, combined for all isolates. For the leaf/agar test method, only the control and first dose data are analysed as beyond the first dose the numbers were too low for chi-square analysis. The differences in the germination classes were very highly significant. Just like barley and wheat 1 mildew isolates the wheat 2 isolates produced abundant secondary hyphae at the control but produced very few secondary hyphae at the lowest dose.

Table 6.16. Chi squared analysis of germination classes across two fenpropimorph dosage rates for wheat 2 mildew isolates in the leaf/agar method (observed germinated spores out of 750 spores in each case).

	Observed numbers (Expected numbers)		
Dose μg a.i.ml^{-1}	Appressoria	Hyphae	*P* value
0	143 (205)	151 (89)	
7.3	222 (160)	7 (64)	$P < 0.001$

Table 6.17 shows a highly significant difference between the different classes in the modified leaf-segment method probably brought about by the unusual behaviour of isolate R which produced very few secondary hyphae at the lowest dose rate (Fig. 6.16).

Table 6.17. Chi-square analysis of germination classes across fenpropimorph dosage rates for wheat 2 mildew isolates in the modified leaf-segment method (observed germinated spores out of 450 spores in each case).

	Observed numbers (Expected numbers)		
Dose μg a.i. ml^{-1}	Appressoria	Hyphae	*P* value
0	96 (118)	163 (141)	
59	117 (88)	75 (104)	
117	96 (113)	151 (134)	
234	80 (91)	119 (108)	
469	108 (92)	93 (109)	
938	56 (52)	59 (63)	$P < 0.001$

The abundance of hyphae and conidiophores in the wheat 2 modified leaf/segment test method six days after inoculation is represented in Table 6.18. For all isolates in the wheat 2 experiment there was some hyphae growing at all doses although it was only abundant at the control. For all three isolates there were no conidiophores on leaf segments treated with the last two doses. All isolates had abundant conidiophores only at the control.

Table 6.18 Abundance of hyphae and conidiophores found on leaf segments of the modified leaf/segment test method with different dose rates of fenpropimorph 6 days after inoculation in the wheat 2 experiment.

Isolate	Dose µg a.i ml^{-1}	Hyphae	Conidiophores
S	0	2	2
	59	1	1
	117	1	0
	234	1	0
	469	1	0
	938	1	0
M	0	2	2
	59	1	1
	117	1	1
	234	1	1
	469	1	0
	938	1	0
R	0	2	1
	59	1	1
	117	1	1
	234	1	0
	469	1	0
	938	1	0

Key

2 = Abundant 1 = Sparse 0 = None

Relationships between sensitivity test methods

The EC_{50} values from all test methods are given in Table 6.19 for barley mildew isolates, in Table 6.20 for wheat 1 isolates and in Table 6.21 for wheat 2 isolates. The EC_{50} values were calculated by a Genstat 5 program which fitted symmetrical curves and calculated EC_{50} values, in the case of the standard leaf-segment test method, and by logit analysis for all the other methods.

A striking feature of the logit analysis was the consistency in the type of data generated by the three methods. The cellophane /agar data gave data points that were clearly curved; the highest and lowest doses had to be excluded from the analysis in order to accommodate the fitted straight line. The leaf/agar test method gave data that conformed well to the fitted line and generally fell close to the fitted line. In contrast the modified leaf-segment method data points were linear but they were much more scattered and this was more pronounced at low doses. Characteristic graphs for each assessment method are shown in Fig. 6.19-6.21 and are typical examples of the relationships obtained for the respective assessment methods.

As Tables 6.19-6.21 clearly show, the actual values obtained from the other methods are different from those obtained from the standard method. The relative sensitivities of the isolates in the cellophane/agar and leaf/agar tests tended to be lower than those of the standard method and its modified form. An attempt was made at ranking the different assay methods. There was very little consistency between the different methods in terms of ranking. This was further demonstrated when correlation analysis was applied to the data (Fig. 6.22).

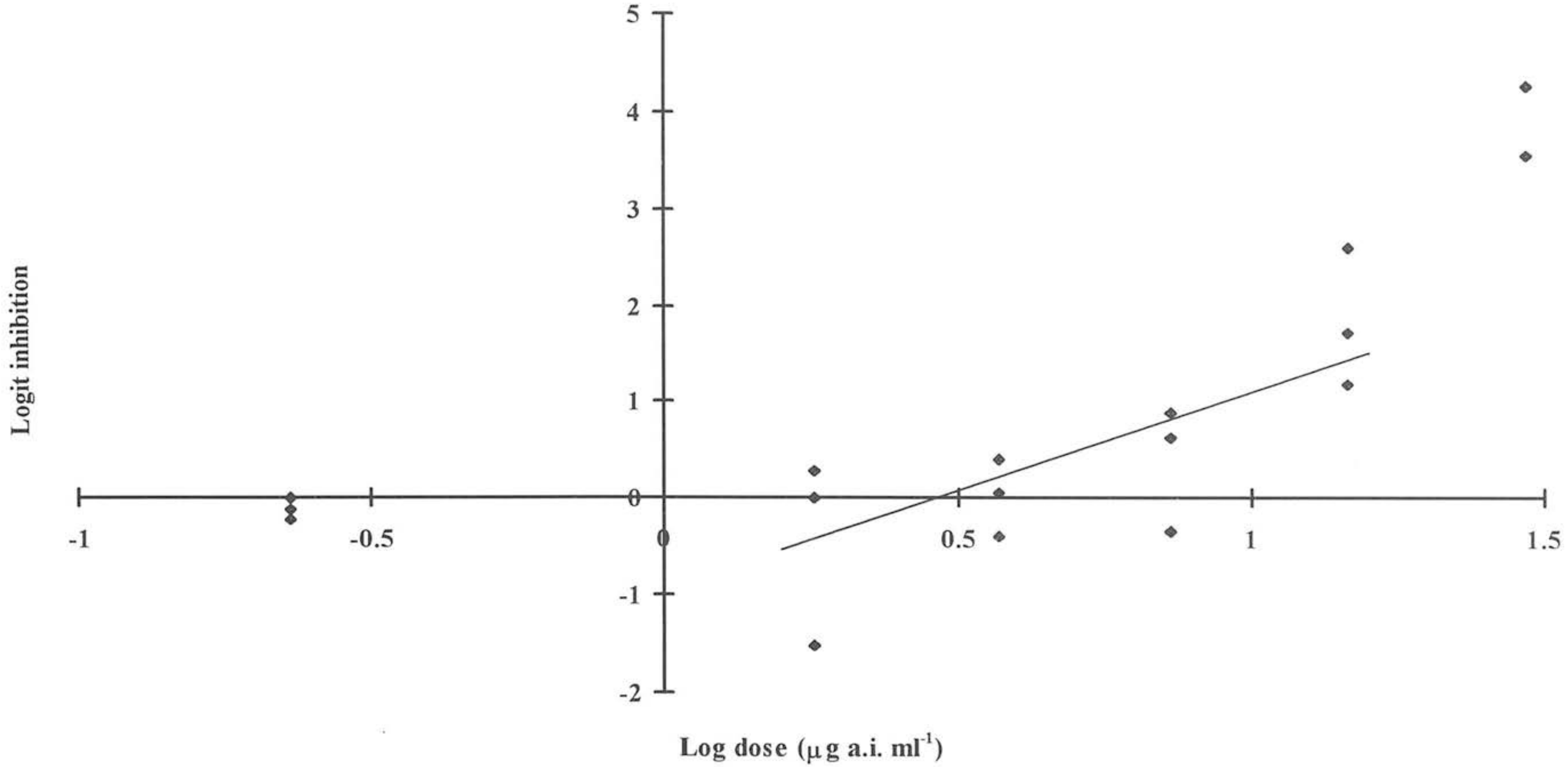


Fig. 6.19. Logit inhibition (germination) vs log dose of fenpropimorph of wheat mildew isolate M, 24 hours after inoculation in the wheat 2 cellophane/agar test method.

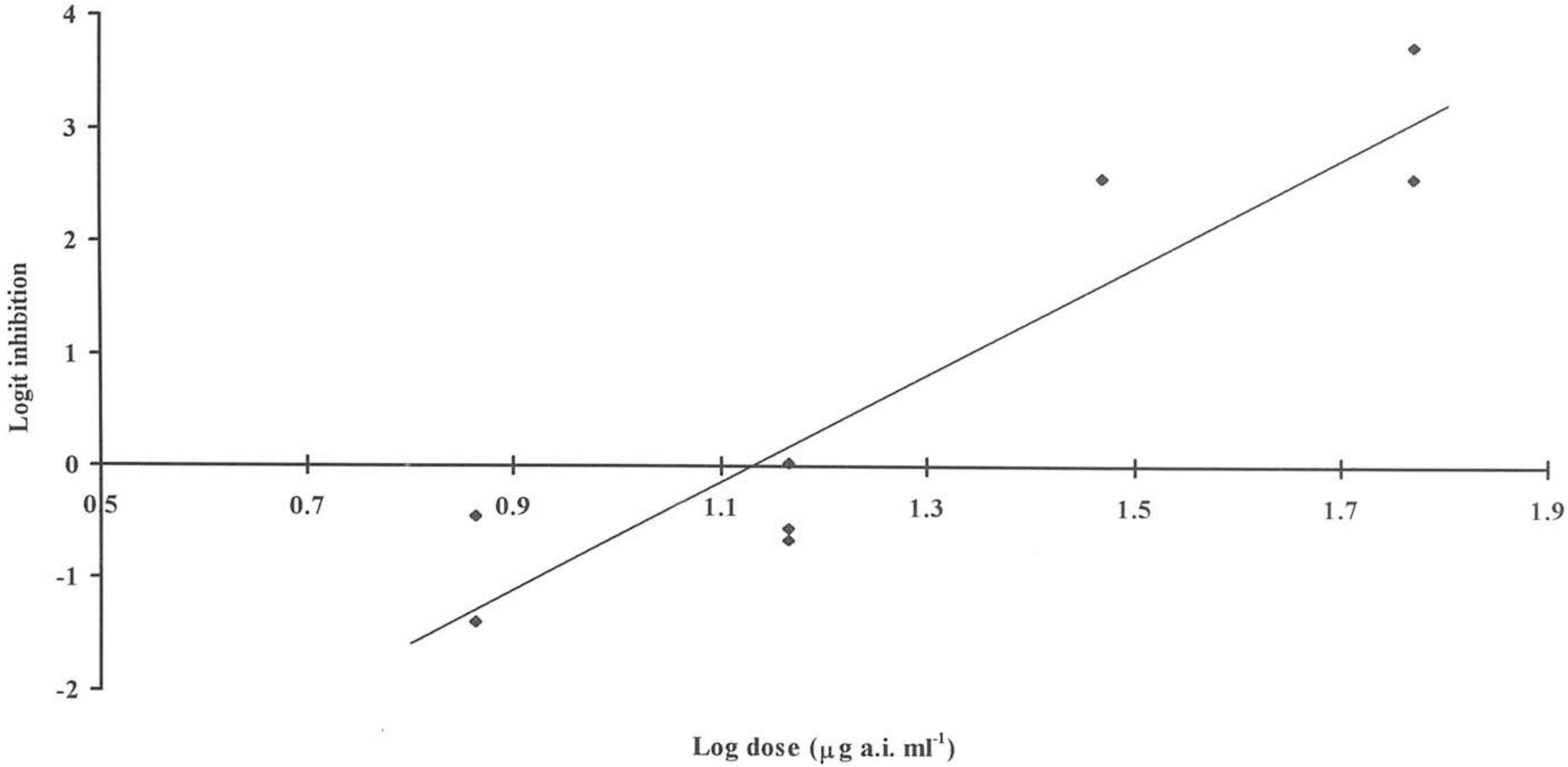


Fig. 6.20. Logit inhibition (germination) vs log dose of fenpropimorph of wheat mildew isolate M, 48 hours after inoculation in the wheat 2 leaf/agar test method.

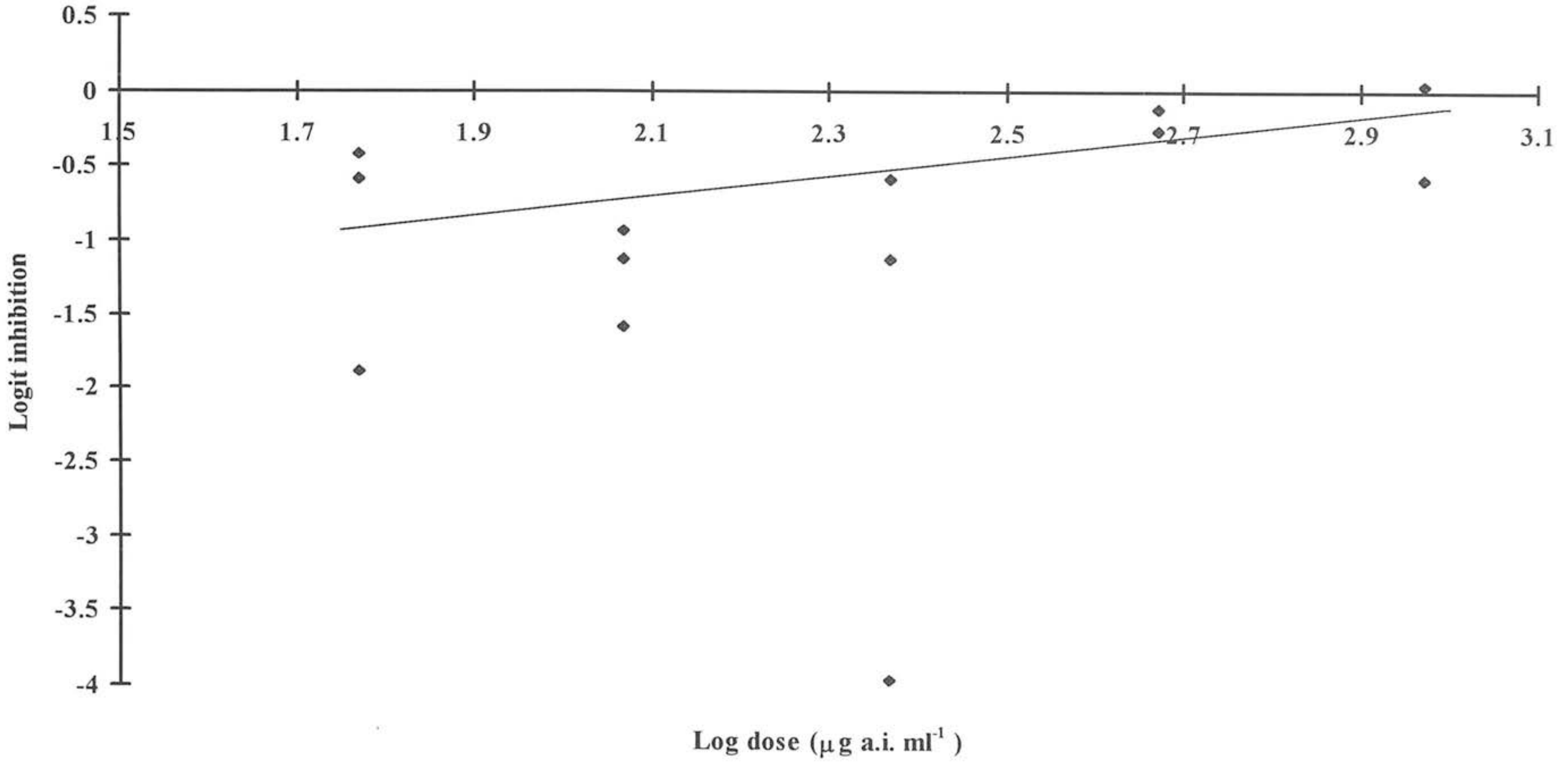


Fig. 6.21. Logit inhibition (germination) vs log dose of fenpropimorph of wheat mildew isolate M, 24 hours after inoculation in the wheat modified leaf-segment test method.

Table 6.19. EC_{50} values (fenpropimorph μg a.i. ml^{-1}) from all different test methods for the barley experiments.

	Method of testing										
Isolate	Leaf-seg. mildew 14 d	Leaf-seg. germin. 24 h	Leaf-seg. germin. 48 h	Leaf-seg. appress. 24 h	Leaf-seg. appress. 48 h	Leaf-seg. Hyphae 48 h	Cel./agar germin. 24 h	Leaf/agar germin. 24 h	Leaf/agar germin. 48 h	Leaf/agar appress. 24 h	Leaf/agar appress. 48 h
DH14	54	228	ND	222	293	770	8.44	8.45	10.09	ND	11.12
CC152	78	267	261	241	168	176	0.50	14.59	10.14	15.18	12.10
CC52	89	126	348	123	406	26	0.80	12.27	13.05	13.26	12.78
CC148	408	188	343	191	298	332	3.87	12.31	11.85	11.79	ND

seg. = segment germin. = germination appress. = appressoria cel./agar = cellophane agar ND = not determined

Table 6.20. EC_{50} values (fenpropimorph μg a.i. ml^{-1}) from all different test methods for the wheat 1 experiments.

	Method of testing			
Isolate	Leaf-segment mildew 14 d	Cellophane/ agar germin. 24 h	Leaf/agar germin. 48 h	Leaf/agar appress. 48 h
MOR2	62	3.26	5.31	11.35
RA2	87	22.27	23.40	10.45
MORUNT	162	1.65	12.49	10.93
RIA2	191	4.90	6.56	9.68
63CAMP	241	0.11	6.86	8.07
CPBW-1	256	2.62	5.87	3.75

germin. = germination   appress. = appressoria

For the correlation analysis the EC_{50} values obtained from all experimental methods (Tables 6.19-6.21) were combined and used to get a matrix of scatter plots to show relationships between columns of the tables, using a program called SPSS (A. Hunter. pers. comm.). SPSS stands for Statistics Package for Social Sciences which can do correlations. The correlations assume a linear relationship and are only meaningful if there are many data points. Because of this, data from all three experimental test methods were combined. Different columns/rows represent different methods of testing after different time regimes. Fig 6.22 illustrates correlations between results of the different methods. A more pronounced border has been put along the edges of those whose correlations are either positively or negatively significantly correlated. Many of the results of the different methods are not significantly correlated. However, the positive correlation coefficients for the following are significant at the 5% level: standard leaf-segment method after 14 days and modified leaf-segment method after 24 hours (germination); modified leaf-segment method after 24 hours (germination) and modified leaf-segment method appressoria after 24 hours; leaf/agar method after 24 hours (germination) and

Table 6.21. EC_{50} values (fenpropimorph µg a.i. ml^{-1}) from all different test methods for the wheat 2 experiments.

	Method of testing									
Isolate	Leaf-seg. mildew 14 d	Leaf-seg. germin. 24 h	Leaf-seg. germin. 48 h	Leaf-seg. appress. 48 h	Leaf-seg. Hyphae 48 h	Cel./agar germin. 24 h	Leaf/agar germin. 24 h	Leaf/agar germin. 48 h	Leaf/agar appress. 24 h	Leaf/agar appress. 48 h
S	162	542	651	31	113	2.25	12.56	7.96	13.45	16.52
M	235	1449	1350	164	122	2.87	13.94	13.48	14.17	13.86
R	302	911	799	208	70	3.18	11.73	9.83	11.94	10.25

seg. = segment germin. = germination appress. = appressoria cel./agar = cellophane agar ND = not determined leaf/agar method appressoria after 24 hours. Correlation coefficients are significant at the 1% level for the modified leaf-segment method secondary hyphae and cellophane/agar method (germination) and for cellophane/agar method (germination) and leaf/agar method (germination) after 48 hours. The correlation coefficient for the modified leaf-segment method after 24 hours and after 48 hours (germination) was very highly significant.

The methods which were significantly negatively correlated at the 5% level included; the modified leaf-segment method after 24 hours (germination) and the leaf/agar method after 48 hours (germination); the modified leaf-segment method secondary hyphae and the leaf/agar method after 24 hours (germination); and the cellophane agar method and the leaf/agar method after 24 hours (germination).

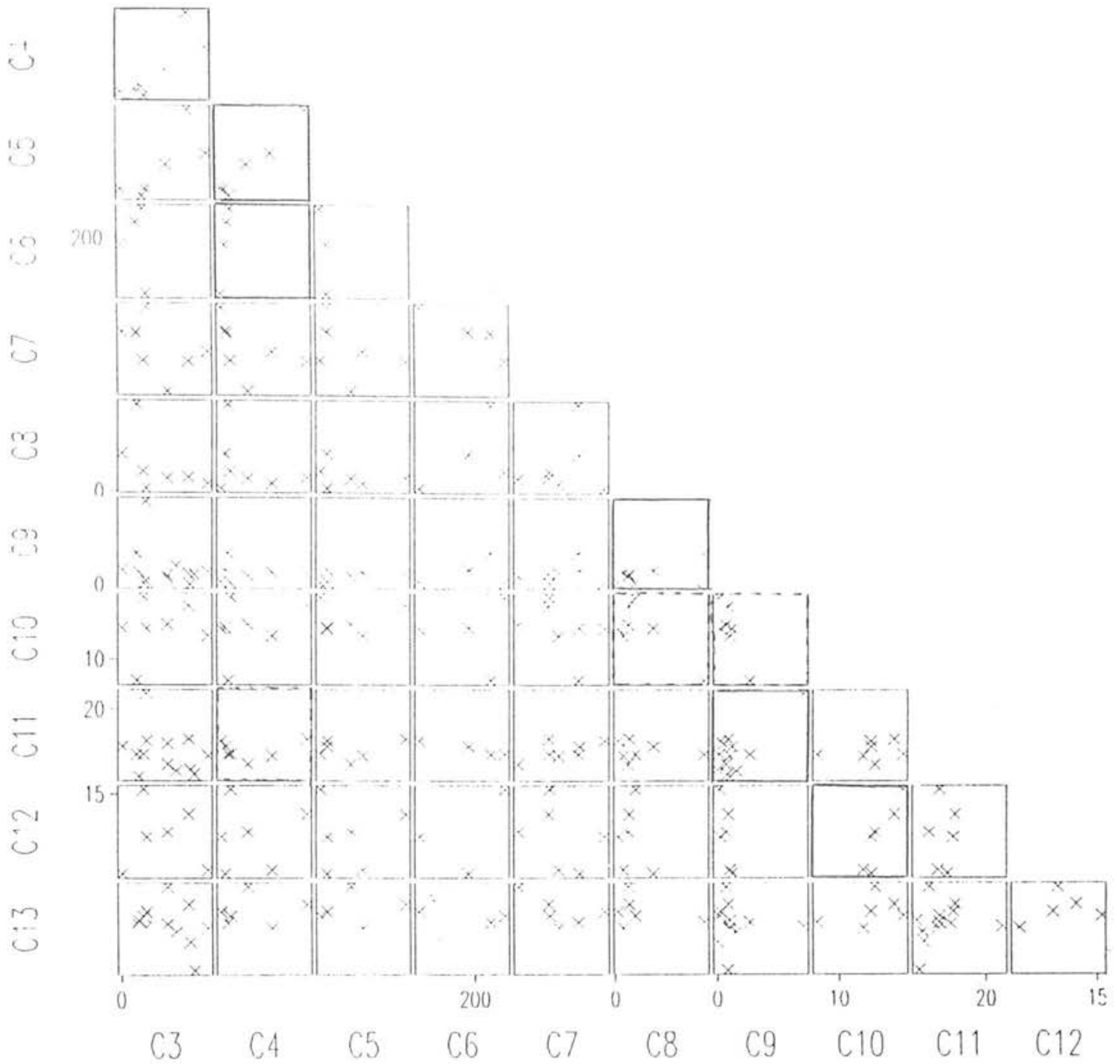


Fig. 6.22. Scatter plots of each pair of variables (all methods combined).

KEY
C3 = Leaf-segment 14 days (percentage mildew cover of leaf area)
C4 = Leaf-segment 24 hours (germination)
C5 = Leaf-segment 48 hours (germination)
C6 = Leaf-segment appressoria 24 hours
C7 = Leaf-segment appressoria 48 hours
C8 = Leaf-segment hyphae 48 hours
C9 = Cellophane/agar (germination)
C10 = Leaf/agar 24 hours (germination)
C11 = Leaf/agar 48 hours (germination)
C12 = Leaf/agar appressoria 24 hours
C13 = Leaf/agar appressoria 48 hours
—— significant positive correlation
- - - - significant negative correlation

Discussion

The process of primary infection by powdery mildew of barley and wheat consists of a number of morphologically identifiable stages (Fig. 2.1); spore germination; formation of appressorial initials (swelling of germ tubes); maturation of appressoria; formation of a penetration peg which penetrates the host cuticle and epidermal cell wall; formation of a haustorium in the epidermal cell of the host; and the formation of secondary hyphae which elongate and are capable of initiating secondary appressoria and haustoria (Ellingboe, 1972). The formation of elongating secondary hyphae capable of initiating secondary infections is taken as evidence that the host and parasite have established compatible, functional relations (Ellingboe, 1972). The stages assessed in these studies after 24 and 48 hours, 6 and 14 days were, germination, appressorial formation, formation of secondary hyphae and conidiophores. In the cellophane/agar method, only primary germ tubes were produced by the spores and sometimes these were multiple as shown in Fig 6.2. Appearance of multiple germ tubes has been reported in earlier studies (Hitoshi *et al.*, 1977; Cartner, 1996). Hitoshi *et al.* (1977) observed multiple germ tubes on conidia on barley leaves and Cartner (1996) on both barley leaves and cellophane. Only one of the germ tubes grows extensively and forms a typical appressorium as shown in Fig. 6.3 obtained from the leaf/agar method. In the cellophane/agar method, only one stage in the infection process was assessed as beyond 24 hours after inoculation no further development of the germ tubes seemed to take place, possibly because of a lack of nutrients from the cellophane circles. In a preliminary experiment (Cartner, 1996) where assessment times of 24 hours and 48 hours after inoculation were tested, there was no significant difference between the two different times which is why the former was used in the present studies.

The ranking of barley mildew isolates by their EC_{50} values in the standard leaf-segment method revealed that DH14 was the most sensitive followed by CC152 and CC52. CC148 was the least sensitive. These isolates fell into the expected order of ranking. From the information already known about these isolates, DH14 is known to be fenpropimorph sensitive and CC148 is known to be fenpropimorph resistant. CC152 has a lower resistance to fenpropimorph than CC148. The sensitivities of these isolates were categorised by Brown (pers. comm.) who followed the standard leaf-segment method, although in his assessments he counted colony numbers instead of taking percentage mildew cover as was done in the present studies. While no significant differences were found between DH14, CC152 and CC52, CC148 was very highly significantly different from the rest. There was no expectation for the ranking of isolates in the wheat 1 experiment but significant differences were found between some of the isolates. However, although wheat 2 isolates, which had been categorised 'sensitive', 'moderate' and 'resistant'in a conventional method of testing using whole plants at SAC, were ranked in that order from the standard method after 14 days the differences between them were not significant (Table 6.1).

From microscopic observations, germination percentages for controls were rather low in all methods. Cartner (1996) improved germination percentages by using inoculum collected from 10 day-old colonies instead of 12 day-old colonies. However, after 10 days there is very little inoculum to work with. Inoculum collected from 15 day-old colonies shaken at 12 days old meant that there was more inoculum to work with. Other workers have used inoculum collected one day after shaking the plants and have found an improvement in germination (Zaracovitis, 1966). Some isolates had a higher germination percentage at the lower doses than at the control in the cellophane/agar method. Low doses seemed to stimulate germination. Such stimulation has been observed previously in sensitivity tests on barley powdery mildew (Williamson, 1983; Robertson *et al.*, 1990) and cereal brown rust fungi (Boyle *et al.*, 1988).

Whereas there was a sharp decline in the percentage of germinated spores with increasing doses in the leaf/agar method, this decline was more gradual in the cellophane/agar and the modified leaf-segment methods. It seems the fungicide is better able to reach the germinating spores in the leaf/agar than in the other two methods. While for the genstat curve fitting program it is better to calculate EC_{50}

values from a curve with a gradual decline than from one with a sharp decline, of the data generated by the three alternative methods those from the leaf/agar test suited the logit analysis best.

The chi-square analyses of the different germination and further development classes revealed differences between the leaf/agar method and the modified leaf-segment method in response to the morpholine fungicide in the barley and wheat 2 experiments (Tables 6.8 & 6.9 and 6.15 & 6.16). The control appressoria proceeded to produce secondary hyphae but very few appressoria developed into secondary hyphae at the various fungicide doses in the leaf/agar method. Therefore, there was generally no functional relationship between the mildew and the leaf segments at the fungicide doses, as formation of secondary hyphae capable of initiating secondary infections is taken as evidence that the parasite has succeeded in the initial infection stage (Ellingboe, 1972). Unlike the leaf/agar method, an average of about half of the germinated spores with appressoria of all the isolates in the modified leaf-segment method also developed secondary hyphae. It seems that the fungicide had little effect on at least the early development of secondary hyphae as this occurred at all doses of fungicide. According to Mercer (1988), fenpropimorph does not suppress the germination of fungal spores to any great extent but is highly effective in inhibiting mycelial growth. Lack of suppression of mycelial growth in this case may be attributed to there being a too low fenpropimorph concentration or to a delay in the expression of an inhibitory effect. The suppression of germination by morpholines in the present work is, however, more difficult to explain: where fungicide was introduced into agar there may have been some direct contact with the fungus rather than a totally systemic action. However, this would not account for reduced germination with the modified leaf-segment method. Cartner (1996) also demostrated germination suppression in the presence of morpholines with cellophane/agar tests.

In comparing the order of ranking of isolates based on results from the different tests, there was very little consistency. The significant positive correlations found in the results tended to be mostly between the different variations of the leaf-segment method. There was also a positive correlation between two variations of the leaf/agar test method. The only two positive correlations found between different methods were, that between the modified leaf-segment secondary hyphae and the cellophane/agar test methods and that between the cellophane/agar and the leaf/agar after 48 hours. Since these correlations are based on single experiments more work will have to be done to confirm that these methods actually correlate.

It is recorded in the literature that considerable variation is a component of all assay methods (Hollomon, 1994). When he tested a group of isolates using a vapour test and a detached leaf test he found different rankings of isolates all of which were tested in both methods. The vapour test involved using fungicide-treated agar in wells in a Petri dish with five different rows. Only one row of wells was fungicide-treated. Approximately 2.5 cm leaf segments were placed diagonally on the agar surface in each well and were inoculated with mildew. The test was designed to exploit the vapour active property of the fungicide. Vapour from the fungicide-treated agar in the first row of the Petri dish diffused across the dish, inhibiting development of sporulating disease on the leaf segments at a distance from the vapour source dependent on the fungicide sensitivity of the test isolate.

Hollomon (1994) suggested that to allow comparisons between test occasions, standard strains of known sensitivity should be included in all assays. While standard strains of known sensitivity to DMIs have given similar rankings in "ring" tests carried out in different laboratories, the same is apparently not the case for standards used in morpholine tests (Hollomon, 1994). "Ring" tests are tests carried out in different laboratories using different methods to see if they come up with similar results. Apart from the isolates used in the wheat 1 experiments whose sensitivities were not known, the isolates used in the present studies were standard isolates of known sensitivity. The present studies seem to agree with the findings of Hollomon (1994) as far as correlations of different assay methods are concerned.

The logit analysis worked well for the leaf/agar method and less well for the modified leaf-segment method because the data points were scattered. For the cellophane/agar method it was less useful because data curved and some data points had to be left out to accommodate the linear relationship. A possible explanation for the variability of the modified leaf-segment method could be due to the application of the formulated pesticide to the plant. The pesticide was diluted more at the lower doses. This dilution could have affected the adjuvants and spreaders and could have made the fungicide more patchy. This effect was not found in the other two methods because the fungicide was incorporated in the agar and would have been more uniform in distribution.

Compared with the standard leaf-segment method, the alternative two methods allowed assessments to be done after a short period but the actual assessments involving microscopic observations took longer so that there was little saving of the time involved. Results from these two methods from visual and microscopic observations did not show consistent relationships. The standard leaf-segment method is established as a routine method of assessment of sensitivity to systemic fungicides and the results of assessment by alternative methods involving microscopic observations of early fungal development, suggested that such methods would not give comparable readings. It should be perhaps noted that the range of variation in sensitivity of isolates of cereal mildews to morpholines is relatively small and if fungi/fungicide combinations had been used where there were more clear-cut qualitative distinctions between resistance and sensitivity then the alternative methods used in this study might have given a better assessment. This view is supported by the findings of Hollomon (1994) who reported that standard strains of known sensitivity to DMIs have given similar rankings in “ring” tests. With DMIs differences in sensitivity do tend to be qualitative rather than giving the continuous distribution of variation which was found in responses to the morpholines in the present work.

Chapter 7

Concluding Discussion

CONCLUDING DISCUSSION

The results presented in this thesis confirmed the findings of field experience that morpholines have continued to maintain an effective level of control of barley and wheat powdery mildew. In the many monitoring tests that were carried out lethal dose rates invariably proved to be less than the equivalents of dosage rates which were recommended for use in practice. For fenpropimorph and fenpropidin the lethal dose rate was, at most, 1/4 the recommended dose rate and for tridemorph it was 1/2, or less, the recommended dose rate. Moreover, mildew susceptible barley and wheat cultivars were used in the standard leaf-segment tests, and field performance of the morpholines would probably be enhanced where mildew resistant cultivars were grown. Compared with other systemic fungicides used against powdery mildew, for example the triazoles, morpholines continued to show a remarkable durability as a control agent.

However, variations in sensitivity relating to both fungicide factors and the host/pathogen combination were observed. There was also an interaction between the two factors as there was a differential response of the various isolates to the three morpholine fungicides. Barley mildew isolates were more sensitive to fenpropimorph and fenpropidin than to tridemorph (EC_{50} g a.i. l^{-1} range = 0.009 - 0.349, median = 0.148). They were also slightly more sensitive to fenpropidin (range = 0.001 - 0.351, median = 0.023) than to fenpropimorph (range = 0.010 - 0.30, median = 0.039). Wheat mildew isolates were not tested against tridemorph but, like barley mildew, they were more sensitive to fenpropidin (range = 0.014 - 0.255, median = 0.082) than to fenpropimorph (range = 0.12 - 0.548, median = 0.222). Although it is difficult to explain why sensitivity to fenpropimorph and fenpropidin differed when they both have similar modes of action, the results confirmed the field experience with wheat mildew that fenpropidin is more effective for controlling the pathogen on wheat than fenpropimorph. Whether it is the biological attributes of barley and wheat plants or of the respective mildew *formae speciales,* in relation to the nature of the fungicides, that brings about these differences needs to be looked into further. Overall, barley mildew isolates tended to be more sensitive to the morpholines than wheat mildew isolates.

In comparison with fenpropidin and fenpropimorph, tridemorph has been used over a longer period. Tridemorph has been used since 1969 (Kradel *et al*., 1969), whereas fenpropimorph has been used only since 1980 and fenpropidin since 1986 (Robertson *et al.*, 1990). Tridemorph has a different mode of action in as much it targets similar enzymes to fenpropimorph and fenpropidin but to a different extent. These factors can account for differences in sensitivities, more particularly towards tridemorph and the other two chemicals. As far as barley mildew was concerned, fenpropimorph was found to be cross-resistant with fenpropidin. This was expected as both chemicals have similar modes of action. However, there was no cross-resistance between either fenpropimorph or fenpropidin with tridemorph. These findings confirm the findings of Brown & Evans (1992).

There was variation in sensitivity of isolates collected from different geographical areas, which may be associated, at least in part, with the history of fungicide use. Generally, barley mildew isolates collected from England tended to be more sensitive to the morpholines than isolates from Scotland. This feature was attributed to the previous intensive use in Scotland of morpholines during the period when the mildew -susceptible cv Golden Promise was grown by many farmers by the virtue of the cultivar's good malting qualities and good agronomic characteristic features, e.g. ability to remain standing under Scottish conditions. 'Golden Promise' was intensively grown from 1968 to 1988 and the morpholines were much used on it in the 1980s (D. Cranston, pers. comm.) This intensive use of morpholines on barley resulted in the loss in Scotland of the more sensitive individuals in the mildew population, which appear to have been retained in England. However, it does not seem to have led to the appearance in Scotland of isolates with a qualitatively higher level of resistance. The population in Scotland has been monitored since 1988 and the range of sensitivities has remained more or less the same despite the continuing use of morpholines. Fenpropimorph and fenpropidin were reported, for example, to be still used widely on barley and wheat respectively, in Scotland in 1996 (Snowden & McCreath, 1996).

The results of monitoring studies provided little evidence that morpholine fungicide application to a crop influenced the sensitivity of isolates collected from it. This was also reported by Robertson *et al.* (1990) on work with barley powdery mildew and morpholines and by Barnard (1992) on work with wheat and barley yellow rust and triadimenol and fenpropimorph. These observations are in contrast with other pathosystems where the appearence of fungicide resistance followed the intensive use of the fungicide within the crop sample, e.g. benzimidazole resistance in *Pseudocercosporella herpotrichoides* the cause of eyespot disease of cereals (Griffin *et al.*, 1982).

Although variation was encountered in the field experiments with reduced fungicide dosage rates, the sensitivities of isolates remained within the range of Robertson *et al.* (1996). These experiments illustrated how population changes can occur quickly, e.g. with wheat mildew (Table 5.9) which showed a significant decrease in sensitivity of isolates collected after the first fenpropimorph spray had been applied. The population quickly reverted back however; the mean sensitivity to fenpropimorph for isolates collected after the second spray was not significantly different from mean values at the beginning, prior to any sprays being applied. It seems there was an ingress of inoculum that had not been subjected to the treatment in the plots which invaded the plots after the second spray. The less sensitive individuals after the first spray which could have been less fit could also have been outcompeted by the more sensitive ones. The observations on field populations of mildew in relation to fungicide treatment emphasise the importance of taking into account a) the relative fitness of sensitive and less sensitive isolates and b) interference from outside population sources.

Both barley and wheat mildew isolates collected in the monitoring work showed a continuous variation in the way they responded to morpholines. Mostly bulk isolates were used in the tests and this would account, at least to some extent, for the type of variation seen. The single colony isolates that were used, were chosen as isolates with very contrasting sensitivities and identified the extremes of range of variation, for example the very sensitive CC1 and the more resistant CC151. The use of bulk isolates would tend to moderate the range of variation but there was no suggestion from all the monitoring results that the existence of very resistant isolates was overlooked. In future work, however, it would be of interest to assess the range of variation in bulk isolates, particularly more resistant ones, from single colony isolates.

The results of the present work (e.g. Fig. 4.5-4.7) showing continuous variation in sensitivity to morpholines in cereal mildew, suggest that variation is quantitative. However, while using single colony isolates of barley mildew, Brown *et al.* (1991) and Brown (1998) distinguished distinct levels of sensitivity to fenpropimorph and fenpropidin. Brown (1998) suggests that samples from pathogen populations may be collected either as pure genotypes such as single colony isolates of powdery mildew or as bulk isolates which consist of many individuals, possibly of many genotypes, and are usually intended to be representative of the pathogen population on a plant, in a field or in some larger area. He goes on further to say that more precise information can be obtained from single colony isolates than from bulk isolates, but if it is more important to collect information from many sampling sites than to have precise data about the population at any individual site, it may be more cost-effective to use bulk isolates. However if a bulk isolate has to be maintained for several generations before being tested, there is danger that some genotypes will outcompete others, with the result that the bulk isolate, when it is tested, is not actually representative of the population from which it was sampled. The choice to use bulk isolates in most of the present monitoring work was made for practical reasons. The aim was to cover as broad a spectrum of the pathogen population as possible and the use of bulk isolates rather than single colony isolates was considered more efficient in terms of time and use of facilities for this purpose. From the results it is suggested that bulk isolates not only gave a good indication of the field situation but also behaved consistently in terms of ranking, in tests at different times over the experimental period.

According to Brown (1998), how the responses of a pathogen population to a fungicide should be summarised depends on the nature of resistance to the chemical, the kind of sample tested, the quantity estimated and the use to which the data will be put, for example whether resistance is quantitative or qualitative, whether bulk or single isolates are being used, whether the quantity estimated is for example MIC or EC_{50} and whether the data used for observation is for advisory services or is for more detailed scientific studies. Brown (1998) distinguished three kinds of distribution of responses to fungicides: the 'all-or-nothing type, several distinct levels of response and a continuous distribution of responses. The use of bulk isolates is not as straightforward as single colony isolates for resistance that exists as two or more discrete levels. Although resistance to the morpholines may appear as discrete levels when single colony isolates are used, in the present work it appeared as a continuous distribution presumably because bulk isolates were mostly used.

Throughout this work the leaf-segment method of testing, based on visual assessments of mildew from fungicide-treated leaves, has been used as a standard. Although drawbacks have been recognised in this method, it simulates treatments in practice and has given consistent results in the assessment of isolates. None of the alternative methods tested proved sufficiently reliable nor gave evidence of significant advantages over the standard method. It is suggested, however, that microscopic studies at early infection stages might be pursued further. The reduced germination of spores on fungicide treated leaves was not expected: further studies on this and the biological characteristics of isolates varying in sensitivity would be of interest.

Comparatively few isolates of mildew were tested by the standard method. When looking for rare isolates, one must test more isolates than the standard method can do. One may have to use more crude, less sensitive methods, for example an adaptation of the standard method where plants treated at the recommended rate and untreated plants are put in fields and the amount of mildew that grows on them is assessed. Further tests can be made on treated plants which have mildew growing. Thus, rather than using a series of concentrations, a single dose may be used to pick out extremes and EC_{50} values of only the insensitive ones would be measured. Using sprayed plants rather than relying on those sprayed in the field would ensure that there was a standard dosage rate. It would also provide evidence of any geographical and time (within or between seasons) variation.

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Appendices

APPENDIX III.I

Compost composition

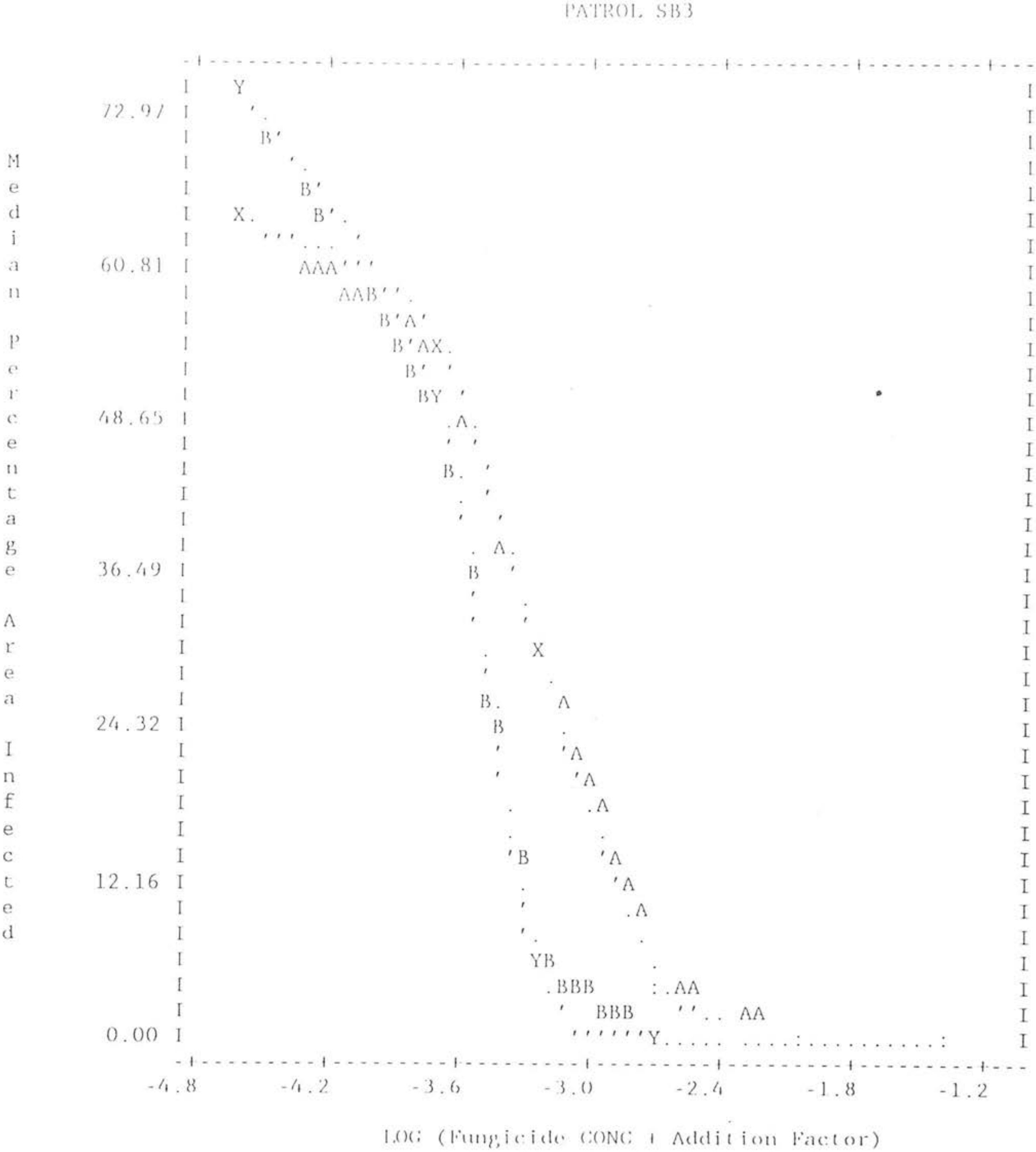
310 l bulrush sphagum moss peat (Irish) medium grade
120 g ammonium nitrate
120 g potassium nitrate
120 fritted trace elements
450 g superphosphate
675 g magnesium limestone
675 g dolomotic limestone

Appendix III.ii.

```
"HGCA Barley Mildew Morpholine Fungicide Project: Program to calculate EC5(
output [width=80] 1
"Replicates summarised as MEDIANS not MEANS, to reduce effect of extremes"
"Model   original to EXCLUDE Pa0 from vector of fitted values 18.9.88"
"MODIFIED FOR SUSAN'S BARLEY MILDEW DATA  27.5.90  JG"
"MODIFIED to analyse Spray Cabs A & B COMPLETELY separately   5.7.90   JG"
"MODIFIED to reduce weighting of Zero values in regression   5.7.90  JG"
"MOD JG 27.8.90 FITCURVE substituted for FITNONLIN and UTcontrol in model"
"To improve fit, ADDITION FACTOR should be set separately for each fungici

TEXT [NV=1] TFile, Title,TscA
TEXT [NV=1] TscA,TscB; VALUES=!T('Spray Cabinet A1'),!T('Spray Cabinet B2'
SCALAR Ntests, Addf, MedWtPa
VARIATE [NV=12] V[1...10]
VARIATE [NV=6] Conc,MedPaA,MedPaB
READ [CH=2] TFile, Ntests : PRINT TFile
PRINT [WIDTH=130] TFile
READ [CH=2] Conc  "6 Concs of fungicide, least first"
READ [CH=2] Addf  "Addition factor to allow for LOG of 0.0 Conc"
PRINT [ORIENT=ACROSS; RLWIDTH=4] Conc; FIELDW=8
PRINT Addf

FOR I=1...Ntests
PAGE
READ [CH=2] Title "of individual test"
PRINT Title
READ [CH=2] V[1...10]
PRINT V[1...10]; FIELDW=6; DEC=0



CALC  MedPa=VMED(V[!(3...10)])
CALC MnPa=VMEAN(V[!(3...10)])
CALC VarPa=VVAR(V[!(3...10)])
CALC LConc=LOG(Conc)
CALC LConcA=LOG(Conc + Addf)
CALC WtPa=1/VarPa    "calculate Weight for Non-Zero values only"
CALC MedWtPa=MED(WtPa)    "NB  based on Weights of Non-Zero Variances"

PRINT [ORIENT=ACROSS] MedWtPa; FIELDW=8; DEC=4
CALC WtPa=1/(VarPa+1.0)    "Recalculate Weights: Zero Variance values = 1"
CALC WtPa=WtPa*(WtPa.LE.3*MedWtPa) + (3*MedWtPa*(WtPa.GT.3*MedW(Pa))
   "substitutes Median Weight for any Weight more than 3 * Median value"

PRINT [ORIENT=ACROSS] Conc,LConc,LConcA; FIELDW=9
  :  &   MnPa,MedPa,VarPa,WtPa; FIELDW=9; DEC=3(2),4

SCALAR LConcA0
SCALAR MedPa0A, MedPa0B       "for Zero Fungicide Concs"
VARIATE [NVAL=6] MedPa6,LConcA6,WtPa6,FPaA,FPaB   "for Non-Zero Fungicide C
VARIATE [NVAL=12] LConcA12     "for ALL Funcicide Concs.   NB 12 values"
  "extract Untreated Control Pa values for A & B"
EQUATE [OLDF=!(1,-11)] MedPa; MedPa0A      "Spray Cab A"
EQUATE [OLDF=!(-1,1,-10)] MedPa; MedPa0B     "Spray Cab B"
EQUATE [OLDF=!((1,-1)6)] MedPa; MedPaA   "Spray Cab A values"
EQUATE [OLDF=!((-1,1)6)] MedPa; MedPaB   "Spray Cab B values"


VARIATE FormatA,FormatB; VALUES !(((1,-12),-1)6), !(-1,((1,-12),-1)6)
FOR Tspraycab=TscA,TscB; EqFormat=FormatA,FormatB; FPa=FPaA,FPaB;  \
    UPa=MedPa0A,MedPa0B; EC50=EC50A,EC50B; LEC50=LEC50A,LEC50B;  \
    SELEC50=SELEC50A,SELEC50B; %SE=%SEA,%SEB
```

```
PRINT Tspraycab

EQUATE [OLDF=EqFormat] MedPa,WtPa; MedPa6,WtPa6  "for Spray Cab A or B"

PRINT MedPa6,LConcA,WtPa6; FIELDW=8; DEC=4



"EC50s estimated by FITCURVE  symmetrical logistic   ALL 6 concs in model"
MODEL [WEIGHTS=WtPa6] MedPa6; FITTED=FPa
FITCURVE [CURVE=LOGISTIC; CONSTANT=OMIT;  \
          PRINT=MODEL,ESTIMATES,FITTED] LConcA

RKEEP MedPa6; ESTIMATES=Estml; SE=Seml
PRINT Estml,Seml
VARI [NVAL=1] EC50,LEC50,SELEC50; DEC=3
EQUATE [OLDF=!(-1,1)] NEWS=LEC50,SELEC50; OLDS=Estml,Seml
PRINT LEC50,SELEC50
CALC EC50=EXP(LEC50) - Addf    "to remove Addition factor"
PRINT EC50
CALC %SE=100*SELEC50/ABS(LEC50)

PRINT Title,Tspraycab,UPa,EC50,LEC50,SELEC50,%SE; FIELDW=35,20,8,8,8,8,8

ENDFOR


SCALAR Yu,AVEC50,GMEC50
"Average EC50   and Geometric Mean value of EC50s"
CALC AVEC50=(EC50A+EC50B)/2  :  & GMEC50=EXP((LEC50A+LEC50B)/2) - Addf
CALC Yu=MAX(MedPa)

GRAPH [TI=Title; YL=0; YU=Yu;   \
       XT='LOG (Fungicide CONC + Addition Factor)';  \
       YT='Median Percentage Area Infected']  \
       MedPaA,MedPaB,FPaA,FPaB,MedPaA,MedPaB;   \
       LConcA,LConcA,LConcA,LConcA,LConcA,LConcA;   \
       Method=L,L,C,C,P,P; SYMBOL='A','B',*,*,'X','Y'

VARIATE [NVAL=6] %PaA, %PaB
CALC %PaA,%PaB=100*(MedPaA,MedPaB)/(MedPa0A,MedPa0B)
PRINT [ORIENT=ACROSS] Conc,LConcA,FPaA,MedPaA,%PaA,FPaB,MedPaB,%PaB;  \
 FIELDW=7; DEC=2,7(1)
PRINT EC50A,LEC50A,SELEC50A,%SEA, EC50B,LEC50B,SELEC50B,%SEB, AVEC50,GMEC50; \
            FIELDW=8,9,9,6, 15,9,9,6, 15,9; DEC=(3,3,3,1)2,3,3

ENDFOR

STOP
```

Appendix III.iii.



Median percentage area infected by isolate SB3 vs log fungicide concentration of fenpropidin (Patrol). A = results from spray cabinet A, B = results from spray cabinet B, X = fitted line for A and Y = fitted line for B. Line fitting involved a Genstat 5 program which fitted logistic curves to the median percentage area infected data.

Appendix III. iv. Analysis of variance for fenpropimorph and fenpropidin monitoring data presented in Table 3.3.

Fenpropidin

SUMMARY

Groups	Count	Sum	Average	Variance
736	3	0.102	0.034	9.81 x 10^{-4}
IW26	4	0.036	0.009	8.00 x 10^{-6}
IW2	3	0.026	0.009	2.13 x 10^{-5}
IW225	2	0.019	0.010	1.25 x 10^{-5}
IW43	2	0.028	0.014	0
IW34	2	0.026	0.013	0
IW223	3	0.033	0.011	3.00 x 10^{-6}
5G	3	0.040	0.013	1.33 x 10^{-6}
737	3	0.078	0.026	2.43 x 10^{-4}

ANOVA

Source of variation	SS	df	MS	F	*P*-value	F crit
Between groups	0.00183	8	0.00023	1.44363	0.25271	2.59109
Within groups	0.00254	16	0.00016			
Total	0.00437	24				

Fenpropimorph

SUMMARY

Groups	Count	Sum	Average	Variance
IW227	2	0.015	0.008	8.45 x 10^{-5}
IW2	3	0.031	0.010	2.23 x 10^{-5}
IW225	2	0.022	0.011	0
22B	2	0.016	0.008	8.00 x 10^{-5}
IW34	2	0.026	0.013	2.00 x 10^{-6}
5G	2	0.019	0.010	4.05 x 10^{-5}
22C	2	0.028	0.014	0
736	2	0.063	0.032	4.81 x 10^{-4}
CC139	2	0.059	0.030	5 x 10^{-7}

ANOVA

Source of variation	SS	df	MS	F	*P*-value	F crit
Between groups	0.00134	8	0.00017	2.53804	0.08456	3.07166
Within groups	0.00066	10	6 x 10^{-5}			
Total	0.02000	18				

Appendix IV. i. History of barley powdery mildew isolates.

Isolate	Collection date	Cultivar	Site of collection	Treatment
20C	1991	-	-	-
14H	1991	-	-	-
21G	1991	-	-	-
SHERPA	1991	Sherpa	-	-
2C	1991	-	-	-
PL11	1991	-	-	-
PL6	1991	-	-	-
7J	1991	-	-	-
10J	1991	-	-	-
IW2*	April 1991	Pastoral	Cirencester	None
IW10*	April 1991	Golden Promise	Askam Bryan	Devel. fungicide
IW26*	April 1991	Golden Promise	Askham Bryan	Devel. fungicide
IW34*	April 1991	Pastoral	Newport	Devel. fungicide
IW43*	May 1991	Pastoral	Riseholm	Devel. fungicide
IW227*	April 1992	Golden Promise	Long Ashton	None
IW225*	April 1992	Golden Promise	Long Ashton	None
22B1*	1992	-	-	-
IW223*	April 1992	Golden Promise	Long Ashton	None
737	1992	-	-	-
736	1992	-	-	-
QE4	1992	-	-	-
92A	1992	Clarine	-	-
92B	June 1992	Tyne	East Lothian	Calixin, Corbel, Tilt
92C	June 1992	Vodka	East Lothian	" " "
92D	June 1992	Bianka	East Lothian	" " "
92E	June 1992	Brigend	East Lothian	" " "
DK	June 1992	Derkado	East Lothian	" " "
HR	June 1992	Corniche	Hawick	
LD	June 1992	Tyne	Kelso	Fenpropidin
TY	1992	Tyne	-	-
INV	1992	Golden Promise	Inverness	None
29*	1992	-	-	-
93A	1993	Clarine	-	-
93B	1993	-	Elgin	-
93C	1993	Golden Promise	Midlothian	None
93D	1993	-	East Lothian	-
93E	1993	-	Aberdeen	-
DA	1993	-	Dalkeith	-
HK	1993	Golden Promise	Midlothian	None
T15	July 1993	Blenheim	Aberdeenshire	None
SB8	July 1993	Blenheim	"	None
T1	July 1993	Blenheim	"	Calixin & Corbel
T2	July 1993	Blenheim	"	" "
T4	July 1993	Blenheim	"	" "
SB3	July 1993	Blenheim	"	" "
SB6	July 1993	Blenheim	"	None
SB7	July 1993	Blenheim	"	None
BL	1993	Blenheim	-	-
CoC*	1994	Corniche	-	-
PrA*	1994	Prisma	St. Boswells	
EH1*	1994	Golden Promise	Midlothian	None
EH3*	1994	Golden Promise	"	None

Appendix IV.i. contd

Isolate	Collection date	Cultivar	Site of collection	Treatment
EH5*	1994	Golden Promise	"	None
BUS94-5*	1994	Golden Promise	Midlothian	Tilt Turbo
PrF*	1994	Prisma	St. Boswells	-
BUS 94-2*	1994	Golden Promise	Midlothian	Tilt Turbo
BUS 94-7*	1994	Golden Promise	-	-
Fans	1994	-	-	-
Pr2*	1994	Prisma	-	-
LBFF	1994	-	-	-
CC1*	1972	Sultan	-	Ethirimol
CC139*	1986	-	Scotland	-
CC151*	1988	-	Moray	-

* single spore isolates
\- information not available

Appendix IV.ii. History of wheat powdery mildew isolates.

Isolate	Collection date	Cultivar	Site of collection	Treatment
51CAMP	1993	Apollo	Edinburgh	None
47CAMP	1993	Apollo	"	Indar, Tilt
54CAMP	1993	Apollo	"	None
30CAMP	1993	Apollo	"	"
72CAMP	1993	Apollo	"	"
63CAMP	1993	Apollo	"	"
12CAMP	1993	Apollo	"	"
HGCA	1993	-	-	-
PGU*	October 1993	Cerco	Edinburgh	None
PGU1*	October 1993	Cerco	"	None
PGU2*	October 1993	Cerco	"	None
RIAA*	July 1994	Rialto	-	-
RIA1*	July 1994	Rialto	-	-
RIA2*	July 1994	Rialto	-	-
RIA3*	July 1994	Rialto	-	-
MORUNT*	1994	Apollo	Morley R. Centre (Norwich)	None
MOR-B	1994	Apollo	" " "	None
MORB-A	1994	Apollo	" " "	None
CPBW-1*	July 1994	Breeders no.	Borders	None
RIB1 *	June 1994	Riband	East Lothian	Folicur, Bravo
MERB-A	July 1994	Mercia	-	-
EH2*	1994	Volunteer	Midlothian	None
HE	1994	Hereward	Aberdeenshire	-
HOP-1*	1994	-	E. Lothian	-
A	June 1995	Hunter	"	Baytan
E	June 1995	Lynx	"	Panoctine
F	June 1995	Lynx	"	Cerevax
G	June 1995	Hunter	"	Beret
H	June 1995	Hereward	"	Panoctine,
ImpactExcel				
N'C7-1*	1995	Jubilatka	Midlothian	None
N'C18-1*	1995	NFC 403-4-2	"	None
N'C18-2*	1995	"	"	None
N'C19-2*	1995	SH 1825-91	"	None
R	1995	-	-	-

* single spore isolates
\- information not available

THE EFFECT OF REDUCED DOSES ON THE SENSITIVITY OF POWDERY MILDEW TO FENPROPIMORPH IN BARLEY FIELD TRIALS

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ABSTRACT

Three field experiments were carried out in 1992 and 1993 to study the effect of reduced doses of fenpropimorph alone, or mixed with propiconazole, on the sensitivity of barley powdery mildew *Erysiphe graminis* f.sp. *hordei*. There were no significant differences between the full commercial dose and reduced doses in their effects on the sensitivity of powdery mildew to fenpropimorph. The sensitivity of the experimental isolates fell within the same range of EC_{50} values as that found in previous sensitivity tests with fenpropimorph. It is is concluded that fenpropimorph is still effective in controlling barley powdery mildew.

INTRODUCTION

Considerable efforts have been directed towards breeding barley for resistance to powdery mildew caused by *Erysiphe graminis* f.sp. *hordei*. Although the use of resistant cultivars has reduced infection levels, changes in the virulence spectrum of the pathogen population present problems to the breeder. The disease remains important in barley growing and farmers must continue to rely for control on the application of fungicides. Genetic variation in the mildew population again gives rise to problems, in this case with respect to the level of sensitivity to fungicides. After the development of resistance to some of the early systemic fungicides used for barley mildew control, the demethylation inhibitors (DMI, azoles) and aminopyrimidine (ethirimol) (Fletcher & Wolfe, 1981; Wolfe, 1985; Heaney, Martin & Smith,1988), control of powdery mildew has relied almost exclusively on one class of fungicides. Commonly referred to as the morpholines, the group consists of two morpholines, fenpropimorph and tridemorph, and a piperidine - fenpropidin. These fungicides are marketed commercially as the individual active ingredients, as well as in mixtures with each other or other compounds.

With such heavy reliance for control on one group of fungicides, selection pressure on the mildew population must be significant. Brown and Evans (1992) described isolates that were resistant to reduced doses of tridemorph, fenpropidin and fenpropimorph, and in addition reported cross resistance between fenpropimorph and fenpropidin. They indicated, however, that the levels of resistance they found were unlikely to cause a substantial loss of effectiveness of the chemicals immediately after spraying.

The Scottish Agricultural College at Edinburgh has been monitoring the sensitivity to fenpropimorph of isolates of barley powdery mildew, collected mostly from Eastern Scotland but also from other parts of Britain, since 1988. The results of this survey show that over this period the mean sensitivity of isolates tested to fenpropimorph has changed little, and EC_{50} values for different years fall within the same range as shown in Table 1. There were however significant differences in the mean sensitivity of isolates between seasons, demonstrating that the population is not stable in terms of sensitivity to morpholines.

Table 1. Sensitivity to fenpropimorph in isolates of powdery mildew collected from 1988 to 1992

	Sensitivity to Fenpropimorph mean EC_{50} value in g/l		
Year	Mean	Range	SED
1988	0.057	0.007 - 0.119	$\pm$0.0118
1989	0.021	0.010 - 0.051	$\pm$0.0023
1990	0.033	0.008 - 0.115	$\pm$0.0061
1991	0.082	0.010 - 0.119	$\pm$0.0189
1992	0.029	0.010 - 0.108	$\pm$0.0140

Many farmers use reduced doses of morpholines, usually in mixtures, as standard practice to control mildew infections. Because of the possibility that such practices might influence the selection pressure for insensitive isolates in the mildew population, field experiments were carried out to determine if the use of reduced doses was likely to influence the sensitivity of powdery mildew to fenpropimorph, the most commonly used morpholine.

This paper reports the results of three field experiments carried out in 1992 and 1993 to establish if any shift in sensitivity could be measured following a repeat application of reduced doses as applied in standard practice.

MATERIALS AND METHODS

In the spring of 1992, a large field experiment was laid out at Boghall Farm, at Bush Estate in the Lothian Region of Scotland. The barley cultivar used was Golden Promise. Plot sizes were 24m by 16m. Fertiliser, herbicide and any micro-nutrient treatments were uniform across all plots, and accorded with local practice. Seed for the trial was treated with a single purpose seed treatment only (mercury).

Fungicide treatments consisted of two spray programmes of fenpropimorph alone or in a mixture with propiconazole. The first fungicide application was made when mildew first developed on the plants and the second spray was applied three weeks later. There were eight treatments, shown in Table 2, and three replicates of each laid out in blocks. To facilitate spraying plots within blocks were not completely randomised.

Table 2. Fungicide programmes evaluated in 1992 field experiment

Treatment	First application	Second application
U	nil	nil
A	fenpropimorph 1.0*	fenpropimorph 1.0
B	fenpropimorph 0.5	fenpropimorph 0.5
C	fenpropimorph 0.25	fenpropimorph 0.25
D	fenpropimorph 1.0 +propiconazole 0.5	fenpropimorph 1.0 +propiconazole 0.5
E	fenpropimorph 0.5 +propiconazole 0.25	fenpropimorph 0.5 +propiconazole 0.25
F	fenpropimorph 0.25 +propiconazole 0.125	fenpropimorph 0.25 +propiconazole 0.125
G	nil	fenpropimorph 0.25 +propiconazole 0.125

* dose rates as a proportion of the full commercial dose of the products used: full commercial doses for the products used were as follows:

Active ingredient	Product	g AI / ha
fenpropimorph	Corbel	750
propiconazole	Tilt 250 EC	125

All fungicides were applied using a tractor mounted Allman hydraulic sprayer with standard flat fan nozzles in 270 l / ha of water at a pressure of 2 bars

Infected leaves were sampled from the middle of plots at three times during the season; before spraying and three weeks after both the first and second sprays. Isolates from leaves from each plot were tested for sensitivity to fenpropimorph in the laboratory following the method reported in detail by Robertson *et al.*, (1990). Isolates were cultured on detached leaf segments of Golden Promise and maintained on Davis minimal medium containing 80 mg/l benzimidazole. To determine the sensitivity of isolates in tests, seedlings of Golden Promise were grown to the two leaf stage and then fenpropimorph solutions applied at concentrations of 0.015, 0.029, 0.058, 0.117 and 0.234 g AI / l in a spray cabinet using a Humbrol spray gun for five seconds. Control plants were sprayed with water. Each spray treatment was repeated in the same cabinet for replication. Segments of the treated leaves were then plated on the minimal medium and inoculated with the experimental isolates. The mildew cover after 14 days incubation at 18 °C was analysed using a Genstat 5 programme which allowed EC_{50} values to be calculated.

Following the field methodology described, two further experiments were laid out at separate sites at Bush Estate in the spring of 1993 but only fenpropimorph was sprayed at full and at three reduced doses as shown in Table 3. To reduce uncontrolled variation brought about by freely mobile inoculum in untreated plots, there were no unsprayed plots. There were three replicates of each of the four treatments, laid out as before. Plot sizes were 24m by 17m. The seed was treated with guazatine plus imazalil.

Table 3. Fungicide programmes evaluated at two sites in 1993

Treatment	First application	Second application
A	Fenpropimorph 1.0*	Fenpropimorph 1.0
B	Fenpropimorph 0.75	Fenpropimorph 0.75
C	Fenpropimorph 0.5	Fenpropimorph 0.5
D	Fenpropimorph 0.25	Fenpropimorph 0.25

* dose rate as a proportion of the full commercial dose of fenpropimorph as follows:

Active ingredient	Product	g AI / ha
fenpropimorph	Corbel	750

Sampling of infected leaves for tests for sensitivity to fenpropimorph in the laboratory were carried out as in 1992.

RESULTS

The results for the three field experiments are summarised in Tables 4 and 5. Although the sampled isolates varied in their sensitivity to fenpropimorph there were no significant differences between mean EC_{50} values for isolates in relation to the concentration of fungicide to which they had been exposed.

For samples assessed after the second spray application in 1992 (Table 4), the untreated isolates showed an EC_{50} value of 0.056 g/l while those exposed to fenpropimorph alone showed comparable values of 0.032 for the highest dosage rate and 0.025 for the lowest. Where fenpropimorph was combined with propiconazole the equivalent values were 0.023 and 0.086.

Table 4. Sensitivity of isolates from 1992 experiment to fenpropimorph based on mean EC_{50} values in g/l

Sampling time	Fungicide treatments								
	U	A	B	C	D	E	F	G	SED
Before spray	0.092	0.05	0.111	0.001	0.128	0.048	0.244	0.096	-
After 1 spray	0.034	0.035	0.102	0.055	0.118	0.029	0.139	0.077	±0.0470
After 2 sprays	0.056	0.032	0.086	0.025	0.023	0.073	0.136	0.086	±0.0636

In 1993, when fenpropimorph was applied alone at full or reduced rates to all plots at two sites (Table 5), the EC_{50} values at site 1 after the second spray application ranged from 0.120 for the isolates from plots receiving the highest dosage rate to 0.030 for those receiving the lowest. At site 2 the equivalent values were 0.015 and 0.011.

Table 5 Sensitivity of isolates from 1993 experiments to fenpropimorph based on mean EC_{50} values in g/l

Site	Sampling time	Fenpropimorph treatments				
		A	B	C	D	SED
1	Before spray	0.068	0.023	0.111	0.070	
	After 1 spray	0.029	0.181	0.102	0.156	
	After 2 spray	0.120	0.081	0.087	0.030	±0.0695
2	Before spray	0.027	0.102	0.029	0.188	
	After 1 spray	0.059	0.115	0.031	0.012	
	After 2 sprays	0.015	0.038	0.036	0.011	±0.0574

In considering the variation in sensitivity between times of sampling, there were no significant differences between the mean EC_{50} values for isolates sampled before any spray application, after one application of fenpropimorph or after two applications, in both seasons. In 1992, the mean EC_{50} of all the isolates collected before any sprays were applied was 0.096. After the first spray the mean was 0.064 for all treatment plots that had received fenpropimorph alone and 0.095 for those that received fenpropimorph plus propiconazole. After the second spray the comparable values were 0.049 and 0.077. In 1993 the mean EC_{50} for all isolates collected from treatment plots before spray application at site one was 0.068 and at site two was 0.086. After one spray the mean values were 0.117 (site 1) and 0.054 (site 2) and after the second spray 0.080 and 0.025 respectively.

DISCUSSION

There was no evidence from the field experiments conducted in 1992 and 1993 that variation in dose rates of fenpropimorph affected the level of sensitivity of isolates of barley powdery mildew exposed to this fungicide within two growing seasons. There was also no significant difference between the sensitivity of untreated plots in 1992 and those which had received fenpropimorph sprays. The range of sensitivities of the isolates tested in all three trials fell within the range found during routine monitoring from 1988 onwards. Brown and Evans (1992) also reported that they could find no correlation between dose rate and frequency of insensitivity, although they did report an increased frequency of resistance after exposure to fenpropimorph sprays. They concluded that the reductions in sensitivity that they observed were unlikely to result in any reduction in field performance.

Reduced rates of morpholine clearly still provide effective control of mildew in the field situation (Wale et al., 1993). The results of the three trials reported here, where reduced doses of morpholine were applied twice in the season as is common agricultural practice confirm the observations of farmers and advisers that reduced doses have not significantly affected the performance of fenpropimorph against the pathogen. Further trials over several years, however would be necessary to establish the long term effects of reduced dose rates on fenpropimorph sensitivity in fungal populations.

In conclusion there was no evidence that reduced doses of fenpropimorph, applied following normal agricultural practices, are likely to reduce the sensitivities of mildew isolates in treated plots. In keeping with previous observations, fenpropimorph was found to retain its effectiveness as a fungicide for the control of barley powdery mildew in the field.

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Effect of Application Rate on the Sensitivity of *Erysiphe graminis* f.sp. *tritici* to Fenpropimorph*

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Abstract: Two sprays of fenpropimorph per season were applied to a winter wheat field trial, at a range of rates, which included the full commercial rate, in order to test the effect of fenpropimorph sprays on the sensitivity of *Erysiphe graminis* f.sp. *tritici*. While a reduction in the sensitivity of the mildew isolates was detected after fungicide application, this was not dependent on the rate of fungicide applied. Reduced rates were not found to induce a larger shift towards insensitivity than the full commercial rate. Powdery mildew isolates were collected from 1993 to 1996 and their sensitivity to fenpropimorph determined in order to monitor sensitivity changes in the population. While a decline in sensitivity was noted from season to season, there was no correlation between a lower sensitivity and the rate of fenpropimorph previously used. Isolates collected in Scotland were found to be significantly less sensitive than those sampled in the south of England.

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Key words: reduced doses, fungicide sensitivity, fenpropimorph, *Erysiphe graminis* f.sp. *tritici*

1 INTRODUCTION

After the development of resistance to some of the early systemic fungicides used for the control of wheat and barley powdery mildew caused by *Erysiphe graminis* DC, such as the demethylation inhibitors and aminopyrimidines,[1–4] control of powdery mildew in cereals has relied almost exclusively on one class of fungicide, the most commercially important members of which are the two morpholines, fenpropimorph and tridemorph, and one piperidine, fenpropidin. The group is commonly referred to as the morpholines and they act by inhibiting ergosterol synthesis from lanosterol to differing degrees at three or more sites.

With reliance for wheat mildew control resting on a single group of fungicides, the risk of insensitivity in the target population causes concern despite the multi-site action of the morpholines, and monitoring programmes have been carried out since the introduction of these chemicals.[7] Sensitivity in the mildew population to the morpholines has remained high,[8] but in recent seasons several surveys have reported a decline in the sensitivity of isolates in Europe to fenpropimorph,[9,10] although at the present time field control remains good.

Morpholines are usually applied in mixtures, which is a recommended part of an anti-resistance strategy.[11] Another standard practice, however, is to reduce the rate applied. Reduced rates can give good mildew control and reduce the input costs to the crop,[12] but there is the possibility that such reduced rates could influence the likelihood of resistant isolates arising in the mildew population. Larger reductions in sensitivity

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following multiple split applications rather than following fewer full-rate applications have been reported,[13–15] but the influence of reduced rates *per se* is not clear.

This paper reports the results of a field experiment carried out in 1995 as well as data gathered from a survey of mildew isolates collected in the United Kingdom over a period of several years. Isolates from both investigations were tested for sensitivity to fenpropimorph to establish whether any shift in sensitivity could be measured following a repeat application of reduced rates of fenpropimorph, as applied in standard practice.

2 MATERIALS AND METHODS

A winter wheat (*Triticum aestivum* L.) trial was planted in the autumn of 1994 at Boghall Farm on the Bush Estate in the Midlothian region of Scotland. The cultivar used was Mercia. Plot sizes were 24 × 16 m. Fertiliser, herbicide and any micro-nutrient treatments applied were uniform across all plots, and accorded with local practice. The seed was treated with guazatine.

Fungicide treatment consisted of two spray applications of fenpropimorph at one of three reduced rates or the full commercial rate (Table 1). The first fungicide application was made when mildew (*E. graminis* f.sp. *tritici* Marchal) first developed on the plants and the second spray was applied three weeks later. To reduce uncontrolled variation in mildew isolates as a result of freely mobile inoculum from untreated plots, there were no unsprayed plots. The field used for the trial was surrounded on three sides by uncultivated upland and woods, and the site was therefore isolated from neighbouring wheat fields. All fungicides were applied in 270 litre ha^{-1} water using a tractor-mounted Allman hydraulic sprayer with standard flat fan nozzles at a pressure of two bars.

Infected leaves were sampled from the middle of plots at three times during the season; just prior to spraying and three weeks after both the first and second sprays. Leaves were sampled from as low down in the crop canopy as possible on the premise that these leaves would have received the lowest doses of fungicide. The levels of mildew in the plots were recorded at the time of sampling, by assessing the percentage surface area on each of the top three leaves infected with mildew on a sample of ten plants taken from the central area of the plots.

TABLE 1
Fungicide Programmes Evaluated

Treatment Reference[a]	*First application 2 June 1995 GS 39-43*	*Second application 28 June 1995 GS 66*
1·0	Fenpropimorph 1·0	Fenpropimorph 1·0
0·75	Fenpropimorph 0·75	Fenpropimorph 0·75
0·5	Fenpropimorph 0·5	Fenpropimorph 0·5
0·25	Fenpropimorph 0·25	Fenpropimorph 0·25

[a] Fenpropimorph was applied as 'Corbel' 750 g litre^{-1} EC; the treatment number represents the proportion of the full commercial rate (750 g AI ha^{-1}) used.

Bulk isolates collected from leaves from each plot were tested for sensitivity to fenpropimorph in the laboratory using a method adapted from one used to assess the sensitivity of *E. graminis* f.sp. *hordei* Marchal.[16] Mildew pustules from 10–15 leaves were bulked on whole plants of the variety Cerco, which carries no known mildew resistance genes, and maintained in an isolation propagator to prevent cross-contamination. To determine the sensitivity of isolates in tests, seedlings of Cerco were grown to the second true leaf stage and fenpropimorph solutions were then applied at doses of 0·058, 0·117, 0·234, 0·469 and 0·938 g litre^{-1} in a spray cabinet using a Humbrol spray gun for 10 s. Control plants were sprayed with water. Each spray treatment was replicated using the same spray cabinet. Treated sets of plants were kept apart for 24 h before the preparation of leaf segments. Eight segments (2 cm long) were cut from the second true leaf of the treated plants from each concentration and spray cabinet combination and then plated on Davis minimal medium containing benzimidazole (80 mg litre^{-1}) and inoculated with the experimental isolates. Inoculation was carried out by dusting the mildew inoculum from the heavily infected plants evenly over the surface of the leaf segment using a fine, sterile paint brush. The percentage mildew cover after incubation for 14 days at 18°C and 24 h light per day was assessed visually and the data analysed using a Genstat 5 programme which fitted symmetrical logistic curves and allowed median efficacy (EC_{50}) values to be calculated.

Additional mildew isolates collected from crops throughout the east of Scotland and East Anglia, England, were assayed for sensitivity to fenpropimorph. Survey isolates were taken from within commercial crops or from field trials, and the fungicide history of the crop, variety, area and date of collection were recorded. These isolates were bulked from single pustules and tested for fenpropimorph sensitivity as described above. The sensitivity of these isolates was then correlated with their known history.

3 RESULTS

3.1 Field trial results

The results for the field experiment are summarised in Tables 2, 3 and 4. There was a significant ($P = 0·049$) decrease in the sensitivity of isolates collected after the first sprays had been applied. Although the sampled iso-

TABLE 2
Sensitivity of Isolates from 1995 Field Experiment to Fenpropimorph

	EC_{50} values (g litre^{-1}) *Fenpropimorph treatments*			
Sampling time	*1·0*	*0·75*	*0·5*	*0·25*
Before spray	0·263	0·131	0·209	0·193
After one spray	0·348	0·263	0·274	0·328
After two sprays	0·253	0·136	0·173	0·197
SED				0·0934 $P = 0{\cdot}049$

lates varied in their sensitivity to fenpropimorph, there were no significant differences between mean EC_{50} values for isolates in relation to the concentration of fungicide to which they had been exposed (Table 2). The mean sensitivity to fenpropimorph for isolates collected after the second spray application was not significantly different from the mean values at the beginning, prior to any sprays being applied; however the range of sensitivities at this last timing was greater than at the other two sampling times, so that isolates with significantly reduced sensitivity remained within this sample group (Table 3). Assessment of the levels of mildew present in the plots at each assessment timing (Table 4) revealed that there was a large influx of mildew into the plots after the second spray, which may have introduced more sensitive 'wild type' isolates from the general mildew population into the trial plot population.

TABLE 3
Sensitivity of Isolates from 1995 Field Experiment to Fenpropimorph

	EC_{50} (g litre^{-1})	
Sampling time	*Range*	*Mean*
Before spray	0·027–0·310	0·199
After one spray	0·161–0·441	0·303
After two sprays	0·021–0·448	0·190
SED		0·0467 $P = 0{\cdot}049$

3.2 Survey results

Analysis of the fenpropimorph sensitivity of mildew isolates collected between 1993 and 1996 as part of a survey for fungicide sensitivity in Scotland are shown in Table 5. The sensitivity of the isolates to fenpropimorph was compared to the known fungicide history of the isolates. There was no indication that previous treatment with fenpropimorph had significantly influenced the sensitivity of the isolates collected ($P = 0{\cdot}794$), or that the rate applied had influenced the sensitivity of the isolates collected ($P = 0{\cdot}624$).

The survey data gathered between 1993 and 1996 show a decline in the mean sensitivity to fenpropimorph from season to season (Table 6) with a significant decrease ($P < 0{\cdot}001$) in sensitivity between 1994 and 1995. This shift in the mean sensitivity of isolates was continued into the following year (1996) though no further significant decrease was detected.

Significant differences in sensitivity were detected in the survey isolates when the geographical source of the isolates was considered (Table 7). The analysis of EC_{50}

TABLE 5
Sensitivity of Survey Isolates to Fenpropimorph

Fenpropimorph treatment	*EC_{50}(g litre^{-1})($\pm SD$)*
Full dose	0·249 (±0·133)
Half dose	0·226 (±0·101)
Quarter dose	0·221 (±0·135)
Nil	0·342 (±0·142)
Pooled SD	(±0·133)

TABLE 4
Mean Percentage Mildew Cover on Top Three Leaves

	Before spray *GS 39–43* *Leaf*			*After one spray* *GS 66* *Leaf*			*After two sprays* *GS 73* *Leaf*		
Treatment	*1*	*2*	*3*	*1*	*2*	*3*	*1*	*2*	*3*
1·0	0	0	1·1	0	0	1·3	0·1	8·8	7·9
0·75	0	0	0·6	0	0·1	0·9	0·2	3·8	10·8
0·5	0	0	2·2	0	0	2·2	2·5	7·0	9·3
0·25	0	0	0·1	0	0	1·5	0·1	6·3	14·0
SED	0 n.s.	0 n.s.	0·95 n.s.	0 n.s.	0·56 n.s.	0·60 n.s.	0·92 n.s.	3·02 n.s.	4·29 n.s.

TABLE 6
Sensitivity to Fenpropimorph in Isolates of Powdery Mildew Collected from 1993 to 1996

Year	*Mean*	EC_{50} (*g litre*$^{-1}$)	
		Range	*SD*
1993	0·167	0·012–0·244	±0·081
1994	0·185	0·062–0·324	±0·083
1995	0·327	0·163–0·548	±0·124
1996	0·341	0·185–0·546	±0·128
Pooled SD			±0·107

data revealed that isolates collected in Scotland were significantly less sensitive to fenpropimorph than isolates collected in East Anglia ($P = 0{\cdot}006$).

There were no significant differences between the mean EC_{50} values of isolates derived from the following varieties:- Apollo, Mercia, Rialto, Riband, Lynx, Hunter, Hereward and Beaver ($P = 0{\cdot}263$). The month in the year in which the isolates was sampled did not significantly influence the sensitivity ($P = 0{\cdot}186$).

4 DISCUSSION

4.1 Reduced rates

A decline in the sensitivity of mildew isolates to fenpropimorph was detected in the field experiment after fenpropimorph had been applied; however this shift in sensitivity was not related to the rate applied and there was no evidence that reduced rates increased or decreased the sensitivity of the mildew isolates assayed. Even after two sprays of fenpropimorph, when more 'wild type' mildew inoculum invaded the plots, the less-sensitive isolates could still be detected in the field trial mildew population. A shift following the use of fenpropimorph could not be detected in the survey data, probably as a result of the high degree of variation in the population. These findings, that reduced rates of fenpropimorph did not pose an increased risk of insensitivity arising in *E. graminis* f.sp. *tritici*, were also found for barley mildew, *E. graminis* f.sp. *hordei*, where no correlation between rate of fenpropimorph and sensitivity has been found in field trials,[17] but exposure to fenpropimorph, regardless of rate, did cause an increase in the frequency of 'resistance' in the barley mildew isolates sampled.[18]

TABLE 7
Sensitivity of Isolates to Fenpropimorph Related to Sampling Area

Isolate source	EC_{50} (*g litre*$^{-1}$) (±*SD*)
Scotland	0·271 (±0·128)
East Anglia	0·165 (±0·087)
Pooled SD	(±0·119)

Although the findings presented in this paper indicate that reduced rates do not cause an increased risk of resistance, multiple split applications of fenpropimorph, alone or in a mixture with propiconazole, have been shown to cause larger reductions in sensitivity than fewer full-rate applications.[13,14] This has been shown even where the total amount of fenpropimorph applied was equal in both regimes.[15] The degree of mildew control has been shown to be critical in determining the size of the shift in sensitivity, probably as a result of the selection pressure exerted by improved control.[15] Several other authors of papers where shifts are reported in response to split applications also comment that disease control was superior in these treatments.[13,14] In the trial reported here the levels of mildew assessed in the plots show that disease control was as good in the reduced-rate plots as in the full-rate plots, so that this selection pressure, if it exists, was equal in all treatments.

Multiple split applications of fenpropimorph can cause a larger reduction in sensitivity than a lower number of full-rate sprays,[13–15] and the work would lead to the conclusion that it may have been the multiple and continuous nature of the applications, and, therefore, the selection pressure, rather than the dose, that was critical. Standard practice in the United Kingdom is to reduce the rate from the full commercial rate when controlling mildew, to reduce input costs, rather than to increase the number of applications made to the crop. In addition, the maximum number of applications for many formulated products containing fenpropimorph is restricted in the United Kingdom to two or three depending on the formulated product used,[19] so that multiple applications of reduced rates are seldom applied. Some formulated products containing either fenpropimorph, fenpropidin or tridemorph do permit a maximum total dose, rather than a maximum application number, to be applied, which would allow multiple applications to be made.

If the degree of mildew control determines the size of the shift in sensitivity to fenpropimorph and if control is sometimes inferior with reduced rates where the persistence of the fungicide can be shorter,[20,21] then, in theory, the selection pressure from reduced rates may actually be less than for highly effective full-rate sprays.

Survey data indicated that geographical location was much more influential on the sensitivity of isolates tested. English isolates assayed as part of the annual survey at SAC were significantly more sensitive that Scottish isolates. This could be due to differences in cultural practices between the two areas. The variety Riband is the predominant variety in Scotland and, as it yields well in response to fungicides, inputs therefore tend to be high.

4.2 Cross-resistance to other fungicides

Cross-resistance exists in barley powdery mildew, *E. graminis* f.sp. *hordei*, between fenpropimorph and fenpropidin, whereas sensitivity to tridemorph is considered to be negatively associated with these two.[18,22] Preliminary studies with *E. graminis* f.sp. *tritici* on a limited number of isolates have indicated that fenpropimorph could be moderately cross-resistant to fenpropidin with a low level or no cross-resistance to tridemorph.[9,23] Cross-resistance would hasten the decline in sensitivity to the other morpholines within the group, but is not in keeping with survey data that show no corresponding decline in sensitivity to fenpropidin relative to that for fenpropimorph (F. J. Burnett—unpublished data). The genetics of cross-resistance to the morpholines in *E. graminis* f.sp. *tritici* are not understood.

4.3 Fitness and stability of isolates with reduced sensitivity

In the absence of fungicide the proportion of isolates with reduced sensitivity falls,[23,24] which would imply that they are less fit. However, they could still be detected, though at low numbers, with the premise that, once insensitive isolates arise, they may persist at low number in the population even where there is no selective advantage.

5 CONCLUSION

In conclusion, sprays of fenpropimorph did cause a significant reduction in the sensitivity of mildew isolates assayed in a field trial, but this reduction was not influenced by the rate of fenpropimorph applied. Reduced doses can therefore be used where applicable without increasing the risk of insensitivity to fenpropimorph. Research has shown, however, that the number of applications should be kept to a minimum and multiple application should probably be avoided wherever possible. In keeping with previous observations, fenpropimorph was found to retain its effectiveness as a fungicide for the control of wheat powdery mildew in the field.

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