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FUNGICIDE INSENSITIVITY IN CEREAL BROWN RUST FUNGI

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by

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ABSTRACT

This project was aimed at monitoring a range of barley brown rust (*Puccinia hordei*) and wheat brown rust (*Puccinia recondita* f.sp. *tritici*) isolates for insensitivity to different fungicides applicable to cereal rust control and to relate the responses of isolates to their source, history and virulence characteristics. Brown rust isolates collected mainly from southern England during 1987-1989 whose virulence characteristics had been determined at Aberystwyth were screened against fenpropimorph, fenpropidin, propiconazole, triadimenol, flutriafol and tridimefon using leaf segments on benzimidazole agar. EC50 values or ratios of fungal growth on sprayed leaf segments to that on controls were determined. Both barley and wheat brown rust isolates varied in their sensitivity to the fungicides with greater variation being shown by wheat brown rust. There was a greater risk of insensitivity to triazoles, particularly triadimenol than to morpholines especially with barley brown rust. No pattern was found in relationships between virulence characteristics, source and insensitivity.

i. OBJECTIVES

The main objectives of these studies have been to develop screening methods to monitor a comprehensive range of isolates of brown rust of barley (*Puccinia hordei* Otth.) and wheat (*Puccinia recondita* Rob. & Desm.f.sp. *tritici* Eriks. & Henn.= *P. triticina*) for insensitivity to different groups of fungicides applicable to cereal rust control, and to relate the responses of isolates to their source, history and virulence characteristics.

ii. INTRODUCTION

Agricultural crops are under constant attack by harmful organisms. For centuries traditional crop rotations and other cultural practices were employed to combat this problem but around the 19th century chemical control became more important and nowadays chemicals play a major role in plant protection.

Although control of diseases with chemicals is very beneficial, it is increasingly faced with the problem of the capacity of the target pathogens to develop resistance to the toxicant (Dekker, 1977).

The first chemicals to be used in the control of fungal diseases were eradicant and protectant fungicides and these rarely failed to give effective control of the pathogens they were designed to combat. For decades, apart from few odd exceptions, such fungicides have remained as effective as when they were first used.

In the late 1960's the first systemic fungicides were introduced. These offered a more efficient and cost-effective way of combating fungal diseases. Very quickly resistance to these new fungicides began to develop and for some it has become common (Dickinson & Lucas, 1982).

When a fungicide controls a fungus effectively, the fungus is said to be 'sensitive' to the fungicide. However, sometimes forms of the fungus may occur 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. They are therefore of little significant importance in practice. Genetic changes are more serious. Forms with such changes may be called 'resistant' or 'tolerant' or 'insensitive'. The term 'insensitive' will be used in this report. Dekker (1984) described the mechanisms by which fungi may become less sensitive to fungicides as follows:

(i) a change at the site of inhibitor action which results in a decreased affinity to the fungicide;

(ii) decreased uptake or decreased accumulation of the fungicide in the fungus;

(iii) detoxification of the fungicide before the site of action has been reached or lack of conversion of a compound into the fungitoxic principle;
(iv) compensation for the inhibitory effect, e.g. by an increased production of an inhibited enzyme;
(v) circumvention of the blocked site by the operation of an alternative pathway.

Effective systemic fungicides exert their effect from within the plant and are generally characterised by a highly selective mode of action with regard to host plant and parasite. They tend to be site-specific inhibitors, i.e. they act by interfering with the metabolism of the fungal cell at one particular site. This specific mode of action, if it involves only one or a few genes, is more likely to be circumvented and give rise to populations of insensitive strains than multi-site inhibition which characterises the mode of action of most conventional protectants' activity at the plant surface.

Since the widespread use of site-specific fungicides, pathologists have become increasingly aware of the possibility of insensitive strains of pathogens occurring both to these and other fungicides. A considerable international effort has been put into the examination of fungal isolates for reduced sensitivity, mechanisms of insensitivity, population studies and fungicide management.

Brown rust is an important disease of winter and spring barley and winter wheat in the UK, causing significant losses of yield in some seasons, especially in southern England (Cook, Jenkins & King 1981). Some currently popular cultivars are susceptible and control is highly dependent on systemic triazole and morpholine fungicides.

Repeated use of site-specific fungicides can increase the likelihood of insensitivity developing, such as the appearance of triazole insensitivity in cereal mildew populations leading to loss of control in the field (Fletcher & Wolfe, 1981; Wolfe, 1985). There have been few studies on the sensitivity of rust fungi to fungicides, but insensitivity to oxycarboxin was recorded in *Puccinia horiana* where the fungicide failed to control chrysanthemum rust (Abiko, Kishi & Yoshioka, 1977).

General characteristics of cereal brown rusts

Brown (leaf) rust fungi are obligate parasites, mostly found on leaf laminae though they may also infect sheaths and ears. The brown pustules containing uredospores have a scattered distribution and this together with their colour enables brown rust to be distinguished from yellow rust. The orange brown colour deepens with age. Black pustules containing teleutospores are formed late in the season. The sexual stage is rare and insignificant epidemiologically, brown rust overwintering largely within living host tissues following infection by uredospores.

Barley brown rust

Barley brown rust (*Puccinia hordei* Otth.) is widely distributed wherever the crop is grown, particularly in the cool temperate regions of barley cultivation (Clifford, 1988a). In the past, this rust was considered economically unimportant in Britain. However, in 1970 it was the most important leaf disease of spring barley and was also at high levels in 1971. These epidemics were associated with highly susceptible spring barley varieties that were being grown at the time (King, 1972, 1977; Priestley, 1978). There were also high levels of this rust in England and Wales in 1989, 1990 and 1991 (Polley & Slough, 1992).

Barley brown rust can affect susceptible varieties of spring and winter barley throughout the country although it is most severe in spring barley grown in southern England. It is often present on volunteer plants in the autumn. In spring and early summer, the disease can occur during tillering and stem extension stages of growth but usually remains slight until after flag leaf emergence. If weather conditions are favourable, it may then increase and very rapid development may occur after ear emergence. This late season development is characteristic and may be related to temperature and the time taken for inoculum to build up (Melville, 1979). Plants can be and have been heavily infected in recent years in winter (Clifford, pers. comm.).

Free moisture, usually satisfied by night-time dew or light rain, is essential for germination and penetration. Germination occurs over the temperature range 5-25°C and is high at 10-20°C. Under optimum conditions, sporulation begins 6-8 days after infection but can take up to 60 days at 5°C (Clifford, 1988a).

Effects on the host depend on the duration and severity of infection. Spring barley, especially if late-sown, is particularly at risk. Early severe infections can result in reduced root and shoot growth, with stunting and a reduction in numbers of fertile tillers and grains per ear (Lim & Grant, 1981; Udeogalanya & Clifford, 1982). Epidemics tend to occur late and consequently the most common effect is on grain size and quality (Newton *et al.*, 1945; Teng & Close, 1977). Yield reductions have been estimated at 0.5-1% for each 1% increment of rust assessed at the milky ripe stage of growth (Clifford, 1988a).

The principal methods of control are through the use of genetically resistant cultivars or fungicides (Jones and Clifford, 1983).

Wheat brown rust

Wheat brown rust (*Puccinia recondita* Rob. & Desm. f.sp. *tritici* Eriks. & Henn.) is arguably the most important single disease of wheat on a world-wide basis (Clifford, 1988b). It is also the most damaging cereal rust world-wide (Samborski, 1985). It is important on both spring and autumn-sown cultivars . It is damaging in Britain (Clifford, 1988b). Surveys in the late seventies and early eighties indicated an increase in its prevalence although previously it had been considered unimportant.

Spore germination and penetration occur over a temperature range of 5-25°C although the optimum is in the range 10-20°C. The generation time is approximately 7-10 days under conditions of warm, moist weather (Jones & Clifford, 1983).

Control is principally through the use of genetically resistant cultivars and there is a long history of breeding for resistance in N. America, Europe and Australia (Jones & Clifford, 1983). Breeding has relied largely on the use of major genes that confer resistance to specific races of the pathogen but which have been overcome by others. The search for more durable forms of resistance has resulted in the identification of partially expressed resistances that show reduced infection frequency and longer latent period, resulting in 'slow rusting' in the field (Clifford, 1988b). Cultural practices aimed at balanced host nutrition can be beneficial.

Chemical control of cereal rusts

Progress in the development of effective chemicals for the control of cereal rusts was slow at the beginning and the use of available fungicides appeared not to be economically feasible.

Sulphur is generally regarded as the first fungicide. Since it possesses low mammalian toxicity and it is easily available, its effectiveness against cereal rusts was examined in the late 19th century (Rowell, 1968). Because of the inadequate knowledge of physical characteristics and application techniques, sulphur and its compounds rendered insufficient control of cereal rust diseases.

The unsatisfactory efficacy of sulphur and other inorganic fungicides (e.g. copper compounds) led to great efforts being put into breeding rust-resistant varieties (Rowell, 1968; Buchenaur, 1982). However, physiological races of various rust species emerged that limited the duration of resistant varieties. After an interruption of several decades, intensified studies on the control of cereal rusts by chemical means were resumed. Various preparations of sulphur were tested (Bailey & Greaney, 1925) and, although results showed that applications of sulphur dusts alleviated the severity of various cereal rusts and yield losses, their effectiveness was not sufficient. Applications had to be initiated as soon as the first symptoms appeared and regularly repeated treatments with increased dosages were required to

obtain satisfactory disease control. Usually under humid-warm conditions, these frequent treatments resulted in severe phytoxicity problems (Gassner & Straib, 1936).

Sulphur caused lysis of germ tubes and inhibited germination of uredospores at higher concentrations. Cereal rust species differed in their sensitivity to sulphur; for instance, *Puccinia striiformis* proved to be more sensitive than Puccinia *graminis* f.sp. *tritici* and *P. recondita*. At around the same time Gassner and Hassebrauk (1936) examined the therapeutic effectiveness of a great number of organic chemicals against *P. recondita* and *Puccinia simplex*. Pyric acid showed both protective and therapeutic activity against the brown rust fungus. However, concentrations necessary for sufficient disease control caused phytoxicity.

After the revolutionary chemotherapeutic effectiveness of the sulfonamides against bacterial diseases in humans and animals had been detected by Domagk (1935), the activity of these compounds against cereal rusts was tested by Hassebrauk (1938). Applications of some of these compounds (e.g. o- or p-toluene sulfonamide metanilic acid) provided complete control against the most important cereal rusts under greenhouse conditions. In the field trials however, p-toluene sulfonamide showed no effect on rust development, at the higher quantities applied and it inhibited host plant development. In further studies the sodium salt of sulfanilic acid, sulfamic acid and sulfanilic acid derivatives exhibited both protective and curative properties against stem and leaf rust of wheat (Livingston, 1953). Under field conditions, these compounds also provided partial control of rusts and increased yield, when applied together with wetting agents. However, at higher concentrations, sulfamic and sulfanilic acid derivatives caused phytotoxicity, and impaired baking behaviour and germination of the harvested grain (Mattern & Livingston, 1955). Further shortcomings of many of these compounds were their high costs and the number of applications required for sufficient control.

Dithiocarbamates were thoroughly tested against cereal rusts. While Zadoks (1958) was unable to demonstrate conclusive control of stripe rust with zineb and maneb, Nelson (1962) reported significantly diminished disease severity that simultaneously resulted in an increase of grain yields after spray treatment with maneb. Activity against stripe rust of wheat has also been established for metiram and propineb (Mundy, 1973).

The therapeutic activity of nickel salts against rusts was first recognised by Sempio (1936) who showed that nickel effectively suppressed development of wheat leaf rust. However, more than 20 years elapsed before interest in nickel salts was renewed. Greenhouse experiments with numerous organic nickel compounds established that some compounds exerted both protective and eradicative properties against leaf rust of rye and in field experiments, many of these nickel chemicals provided control against leaf and stem rust of wheat.

In field trials where dithiocarbamates and nickel salts had been applied simultaneously, the effectiveness against cereal rusts was superior to single applications of either compound. Treatment with both components resulted in additive effectiveness whereby the dithiocarbamates displayed exclusively protective and the nickel salts additional eradicative activity (Forsyth & Peturson, 1960). Nickel salts have not been used extensively for control of rusts in cereals because nickel accumulates readily in grains.

The discovery of the 1,4-oxathiin derivatives, carboxin and oxycarboxin, represented a major breakthrough in the chemotherapy of plant diseases (Schmeling & Kulka, 1966). Both chemicals possess systemic properties and display a highly selective toxic activity against Basidiomycetes. Oxycarboxin was found to be superior to carboxin in long-term effectiveness, eg. in controlling cereal rusts (Rowell, 1967). Since the introduction of oxathiins in practice, development of resistance to these chemicals had been easily obtained under laboratory conditions (Ragsdale & Sisler, 1970; Ben-Yephet, Henis & Dinoor, 1974). Carboxin and its derivatives exhibit high selectivity. Growth and metabolism of non-target organisms (such as plants and non-sensitive fungi) are only affected when high fungicidal concentrations are reached.

Among the numerous benzolic anilide derivatives tested, benodanil proved to be most active for rust control coupled with a great margin of crop safety. Benodanil has been extensively examined for its efficacy against rusts on cereals and grasses. Foliar applications of the chemical reduced incidences of different types of rust including brown rust of wheat (Frost & Hampel, 1976) and brown rust of barley (Frost, 1975; Frost & Hampel, 1976). Results indicated that benodanil predominantly displayed protective activity and had little eradicant action.

In the search for new fungicides with chemotherapeutic properties, Hardison (1971) examined various thiazole compounds against smut and rust pathogens of Kentucky bluegrass. By analogy with oxathiin fungicides, Hardison (1971) presumed that thiazole derivatives with oxidized sulphur in the heterocycle would represent further promising candidates with therapeutic activity against smut and rust diseases.

The development of systemic fungicides together with favourable economics of cereal growing have resulted in their widespread use to control cereal diseases. Some of these chemicals are highly specific in their control of the pathogen whereas others have a broad spectrum of activity that makes them attractive where other diseases such as powdery mildew (*Erysiphe graminis*) or yellow rust (*P. striiformis*) are present. Fungicides with a broader spectrum of activity against cereal pathogens that were developed more recently and are widely used in N.W. Europe include fenpropimorph, triadimefon, triadimenol, propiconazole, flutriafol, diclobutrazol and prochloraz.

Fenpropimorph

Fenpropimorph was introduced in 1979 by Maag and BASF under the trade names Mistral and Corbel respectively. It is used exclusively as a cereal fungicide especially for control of powdery mildews. Control of various *Puccinia* species in wheat, barley, oats and rye can also be achieved. Of special importance is the curative action and 'stopping' effect of fenpropimorph, which becomes visible shortly after application. Development of appressoria and haustoria is retarded and secondary hyphae grow in an uncoordinated manner.

Systemic properties

Fenpropimorph is systemic. It is mainly taken up by the roots but also by the foliage, and is translocated acropetally via the xylem. Untreated, newly developing leaves are protected to a certain extent. Basipetal translocation has not been found. Trials with radio-labelled material revealed a good distribution pattern in healthy leaves. Only a very low concentration of fenpropimorph has been found in guttation droplets. A remarkably high vapour pressure supports the activity of the compound, allowing distribution to unsprayed plant surfaces.

Insensitivity

Barley powdery mildew (*Erysiphe graminis* f.sp. *hordei*) isolates were identified from Scotland that had a low but stable level of insensitivity to fenpropimorph (Wolfe, Slater & Minchin, 1987).

Fenpropidin

Fenpropidin, a piperidine, has been shown to have a very similar mode of action to morpholine fungicides (Baloch, Mercer, Wiggins & Baldwin, 1984). In the present studies it has been grouped with the morpholine fenpropimorph. It is specifically for cereals and its main activity is against powdery mildew (*E. graminis*). Perfomance is comparable with that of other standards. Its effect against various Puccinia species is good but inferior to that of the market standards.

Systemic properties

Fenpropidin is rapidly absorbed by roots but only to a minor extent by other parts of the plant. Acropetal transport has been demonstrated but there is no basipetal transport.

Insensitivity

The risk of a build-up of insensitivity is claimed to be low because of a dual site blocking action in sterol biosynthesis (Bohnen, Pfiffner, Siegle & Zobrist, 1986).

Triadimefon

Triadimefon was announced in 1973 by Bayer under the trade name Bayleton. It was the first triazole to have a broad spectrum-activity against Ascomycetes, Basidiomycetes and Fungi Imperfecti in various crops. Powdery mildews and rusts, however, represent the core of its applications (Anon, 1983). Excellent activity against different rust species in cereals has been demonstrated in several trials (Line, 1976; Siebert, 1976; Sheridan & Dawson, 1982). The major effect of triadimefon is seen as suppression of appressoria and haustoria development, mycelial growth and spore production. As a seed treatment, triadimefon controls powdery mildew, loose smuts, bunt in wheat, oats and barley, seed-borne *Pyrenophora* species, *Typhula incarnata*, *Gaeumannomyces graminis* and also has a side effect against rusts (Frohberger, Berthier, Daurade & Garin, 1973).

Systemic properties

Triadimefon has been shown to be systemic. It is taken up by the leaf surface and transported mainly acropetally within the transpiration stream. Basipetal movement is slight. Triadimefon is also translocated laterally through the leaf sheaths. These qualities predestine the compound for use as a foliar spray, seed treatment or soil application.

Insensitivity

Studies in the Netherlands indicated that wheat powdery mildew isolates obtained in 1982 in the province of Limburg, where triazole fungicides had been used intensively for several years, were less sensitive than isolates from other regions. In the succeeding years, isolates with reduced sensitivity were also found in other parts of the country. There was a strong correlation between the intensity of use of triazole fungicides and the occurrence of such isolates (Schulz & Scheinpflug, 1988). Investigations in Germany in 1985 on the powdery mildew population in winter wheat revealed that triadimefon sensitivity varied during the course of the season even in the absence of fungicide pressure in the field (Schulz & Scheinpflug, 1988).

Triadimenol

Triadimenol was first developed as a cereal seed dressing and introduced into the market under the name Baytan in 1977. Triadimenol has been developed further as a foliar fungicide for various crops under the trade name Bayfidan. Its spectrum of activity is quite similar to that of Bayleton (triadimefon). It is particularly effective against rusts, powdery mildews, rhynchosporioses, septorioses, *Typhula* and other leaf blotch pathogens and has a protective and eradicative action (Anon. 1984).

Systemic properties

Triadimenol is highly systemic and therefore gives excellent control, not only of pathogens located on the surface of the seed but also of those developing within. It is translocated to the leaves and controls wind-borne diseases.

Insensitivity

The first reports of decreased sensitivity of cereal powdery mildew towards triadimenol were published in 1981 (Schulz & Scheinpflug,1988). Details were revealed about isolates 'insensitive' to triadimenol which were being found with increasing frequency in England, Wales and Scotland. The distribution of such isolates was concentrated in regions where cereal cultivars without known resistant genes were being grown, and it increased with a high frequency of fungicide applications. Triadimenol proved to be cross-resistant with other inhibitors of demethylation in the sterol biosynthesis pathway but not with the morpholines. Apart from this and other cases where there have been indications of 'insensitive' isolates to triadimenol there are various pathogens which can be controlled by triadimenol without any problems.

Propiconazole

Propiconazole was discovered by Jansen and further developed by Ciba-Geigy under the trade name Tilt. Tilt has a broad spectrum of activity against fungi from different systematic groups. Prophylactic use gives protection for up to five weeks depending on the pathogen. Remarkable curative and eradicative effects can be achieved. It controls powdery mildews, various rusts in wheat, barley, oats and rye (Sheridan & Dawson, 1982), *Septoria* in wheat, *Rhynchosporium* and *Pyrenophora teres* in barley and a broad range of fungi in rice.

Systemic properties

Propiconazole is highly systemic and it is taken up by the stem and foliage, transport being mainly acropetal. A limited protection of untreated, newly developing leaves can be achieved. Test results have indicated that propiconazole moves rather slowly relative to other triazoles (Shephard, 1985) and that it is not translocated to the ears (Anon, 1981). Remarkable activity via the vapour phase contributes to the good field performance.

Insensitivity

Sterol Biosynthesis Inhibitor (SBI) fungicides have been reported to select strains of plant pathogens with reduced sensitivity. Propiconazole is no exception in this respect and shows cross-sensitivity with triadimefon and triadimenol (Waard, Kipp, Horn & Nistelrooy, 1986).

Flutriafol

Flutriafol was announced by ICI in 1983. It controls all major diseases of cereals including *E. graminis*, *Puccinia* spp., *Septoria* spp., *Helminthosporium* (Drechslera) spp. and *Rhynchosporium secalis* (Dawson, Torcheux & Horellou, 1984). It has a three-fold activity: protective, curative and eradicative. It can be used as a seed treatment either alone or in various combinations, depending on the disease spectrum. It is available only in mixtures (Impact Excel) along with chlorothalonil, a non-systemic which is not listed for the control of rust fungi but controls *Septoria* spp. and *Rhyncosporium* on cereals, (Early Impact) along with carbendazim, (Vincit) along with imazil and other partners and (Ferrax) along with ethirimol and thiabendazole.

Systemic properties

Flutriafol is systemic. It is transported acropetally within the transpiration stream of the plant. A marked translaminar effect has been demonstrated. Good vapour phase activity has also been reported. The compound is quite fast moving and is rapidly translocated to the leaf tips.

Insensitivity

There are no published accounts of resistance in brown rust to flutriafol but field observations have indicated loss of effectiveness in some instances (P. Rylott, pers. comm.).

iii. MATERIALS AND METHODS

Leaves infected with brown rust were collected from barley and wheat crops, mainly from southern England during 1987-1989, and sent to Aberystwyth where the fungi were cultured on susceptible cultivars of the respective host. These isolates were screened against a standard set of differential cultivars and their virulence characteristics determined in accordance with the procedures used in the UK Cereal Pathogen Virulence Survey (Jones & Clifford, 1980, 1988). For comparison, some earlier isolates were included. Freeze-dried spores of the isolates were sent to Edinburgh.

To get fresh inoculum, the isolates were first bulked-up on susceptible seedlings of barley and wheat. These were raised in 3" square pots either in trays in a Fison's growth cabinet at 18°C or in trays covered with propagators in a greenhouse. Three plants were raised in each pot and 24 pots were sown for each isolate. These were grown until the first true leaf was fully unfolded (Growth stage 12 = GS12) when they were inoculated with a brown rust isolate mixed with talc using a fine brush. The inoculated plants were covered with misted propagators in trays which were half-filled with water (6 pots to a tray). The propagators were sealed to increase the relative humidity. The covered plants were left in the dark for 24 hours in a Fison's growth cabinet at 15°C and 100% r.h. The plants were then uncovered and placed in a Fison's cabinet at 18°C and 80% r.h. with a 12 hr photoperiod. During the incubation period it was ensured that there was water in the trays to maintain a high humidity.

After 10 days the inoculum was collected by tapping individual leaves either in a test tube or on to aluminium foil paper. When necessary the bulked fresh inoculum was stored (up to three weeks) in test tubes covered with cotton wool over 80% sulphuric acid in a desiccator in a cold store at 4°C. The freeze-dried spores in vials were also stored in the same desiccator. The barley brown rust isolates were bulked on either cv Midas or cv Golden Promise and the wheat isolates on either cv Fenman or cv Armada, all of which do not have resistant factors against the respective brown rust.

Altogether six fungicides were used in three experiments. These included fenpropimorph (morpholine), fenpropidin (grouped with the morpholine) and propiconazole, triadimenol, triadimefon and flutriafol, all of which are triazoles. For each test, 3-5 seedlings per pot were grown in 5" pots in a Burkhart isolation propagator. At GS12 the plants were exposed for 5 seconds to a series of fungicide concentrations in form of a fine spray with distilled water as control. Two pots were sprayed for each concentration, replication being provided by using two spray cabinets. Humbrol spray guns which were maintained at the same pressure were used. After spraying, the plants were left for 15 minutes to allow the fungicide cloud to settle. Two (experiment 3), six (experiment 1 & 2) or thirteen (experiment 2) fungicide concentrations were used: 1/16 C, 1/14 C, 1/10 C, 1/8 C, 1/6 C, 1/5 C, 1/4 C, 1/3 C, 1/2 C, C, 1.5 C and 2 C where C is the concentration of normally recommended field spray. Table 1 shows the active ingredients of the different fungicides used at each concentration.

Table 1. Amount (g/l) of Active Ingredient of the Fungicide at Different Concentrations.

FUNGICIDE	BAYLETON*	MISTRAL	PATROL	TILT	BAYFIDAN	IMPACT
FIELD SPRAY DOSE	0.5 kg/ha in 200 l water/ha	1 l/ha in 200 l water/ha	1 l/ha in 200 l water/ha	0.5 l/ha in 100 l water/ha	0.5 l/ha in 200 l water/ha	1.25l/ha in 200 l water/ha
1/16	0.0390	0.2344	0.2344	0.0781	0.0391	0.0367
1/14	-	0.2678	0.2678	0.0893	0.0446	0.0419
1/12	-	0.3125	0.3125	0.1045	0.0521	0.0490
1/10	-	0.3750	0.3750	0.1250	0.0625	0.0588
1/8	0.0780	0.4688	0.4688	0.1563	0.0781	0.0734
1/6	-	0.6249	0.6249	0.2083	0.1042	0.0979
1/5	-	0.7500	0.7500	0.2500	0.1250	0.1175
1/4	0.1560	0.9375	0.9375	0.3125	0.1563	0.1469
1/3	-	1.2498	1.2498	0.4166	0.2083	0.1958
1/2	0.3120	1.8750	1.8750	0.6250	0.3125	0.2938
1	0.6250	3.7500	3.7500	1.2500	0.6250	0.5875
1.5	-	5.6250	5.6250	1.8750	0.9375	0.8813
2	1.2500	7.500	7.500	2.500	1.2500	1.1750

* Experiment 1 only

Twenty-four hours after spraying, 8-16 replicate leaf segments (2-3 cm long) were cut from the middle of the first true leaves of the test plants and placed in 9 cm diameter or 10 cm square petri dishes of 80 ppm benzimidazole water agar. The proximal end of each segment was inserted in the benzimidazole agar, each petri dish holding eight leaf segments. All dishes required for one test were inoculated together in a large settling tower to ensure uniformity. Spores (0.00625 g) were diluted with talc (1:3) to increase the volume of powder and reduce the risk of spore clumping. A dilution of 25% by weight of spores in talc has been found most effective (Kellock & Lennard, 1985). The spore/talc mixture was put into a glass inoculating tube, bent at the tip to project upwards and inserted into the settling tower. A 5-seconds blast of air from a vacuum pump expelled the mixture up to the top of the tower in a cloud. After 15 minutes, the petri dishes were removed and placed in an incubator in the dark at 15°C for twenty four hours and then at 18°C in an incubator with a 12 hr photoperiod. Pustule numbers on each leaf segment were counted at 10-12, 14-16 and 19-21 days post inoculation (dpi) and then the results were standardised to pustules per square cm.

In experiments 1 and 2 sensitivities were expressed as EC50 values. Experiment 3 involved some isolates collected in 1988 and some collected in 1989. All the isolates were screened against the five fungicides used in Experiment 2. In order to screen as many isolates as possible, only two concentrations of the fungicides were used. Sensitivity was recorded as the ratio of median pustule numbers produced on leaf segments at each concentration to the median pustule numbers on the untreated control.

iv. RESULTS

Experiment 1(1987 and pre-1987 barley and wheat brown rust isolates)

Results (Appendix 1) of the sensitivities of barley and wheat brown rust isolates collected in 1987 or before, to triadimefon (Bayleton) and propiconazole (Tilt), given as EC50 values have been reported previously (Boyle, Gilmour & Lennard, 1988). Only a summary of the findings is presented here:-

(1) Assessments were made at 10 and 16 days after inoculation. After this time a significant proportion of leaf segments senesced so that later assessments would have been unreliable.

(2) The production of pustules appeared to be stimulated in some isolates by low concentrations of fungicide, which may relate to a host as well as a pathogen effect.

(3) The isolates showed a 20-fold variation of sensitivity to triadimefon but only a 6 to 10-fold variation to propiconazole.

(4) The earlier isolates of both barley and wheat brown rust with no known exposure to fungicides were the most sensitive to propiconazole, as were the wheat brown rust to triadimefon. However, the earlier isolates of barley brown rust were not the most sensitive to triadimefon.

(5) The sensitivity rankings of wheat brown rust isolates to the two fungicides were similar, but those of the barley isolates differed.

(6) There was no apparent relationship between sensitivity and virulence among the barley isolates. The sample of wheat isolates was too small to draw any conclusion on any relationship.

Experiment 2 (1988 barley and wheat brown rust isolates)

The second series of experiments were conducted using some of the barley and wheat brown rust isolates collected in 1988 against five fungicides. Problems in producing adequate amounts of inoculum and in achieving adequate levels of infection on controls reduced the number of tests that had been intended. In making assessments the median pustule numbers at 10-12 dpi were sometimes more than those counted at 14-16 dpi or 19-21 dpi because sometimes pustules merged together and sometimes some pustules became inactive at later times.

Barley brown rust isolates

Figures 1-5 illustrate the sensitivities of barley brown rust isolates collected in 1988 to five different fungicides as EC50 values. Fig. 4b is an example of the kind of irregular results obtained for many of the isolates for triadimenol.

In attempting to compare the responses of isolates to the different fungicides Table 2 (overleaf) gives the relative sensitivities based on EC50 values when colony development was maximum on the controls,

Table 2. Sensitivity of Barley Brown Rust to Five Fungicides (EC50 values (g/l) when maximum colony development occurred on controls).

ISOLATE	FENPROPIMORPH	FENPROPIDIN	PROPICONAZOLE	TRIADIMENOL	FLUTRIAFOL
BRS-88-195	0.42	0.47	0.78	0.82	0.09
BRS-88-120	0.12	0.19	0.42	0.20	-
BRS-88-213	0.30	0.10	-	-	-
BRS-88-108	0.19	0.24	0.50	0.40	-
BRS-88-207	0.82	-	0.17	0.60	-
BRS-88-202	0.40	0.24	-	-	1.00
BRS-88-197	-	-	0.20	-	-
BRS-88-211	-	0.20	-	-	-
BRS-88-196	-	-	-	0.10	-

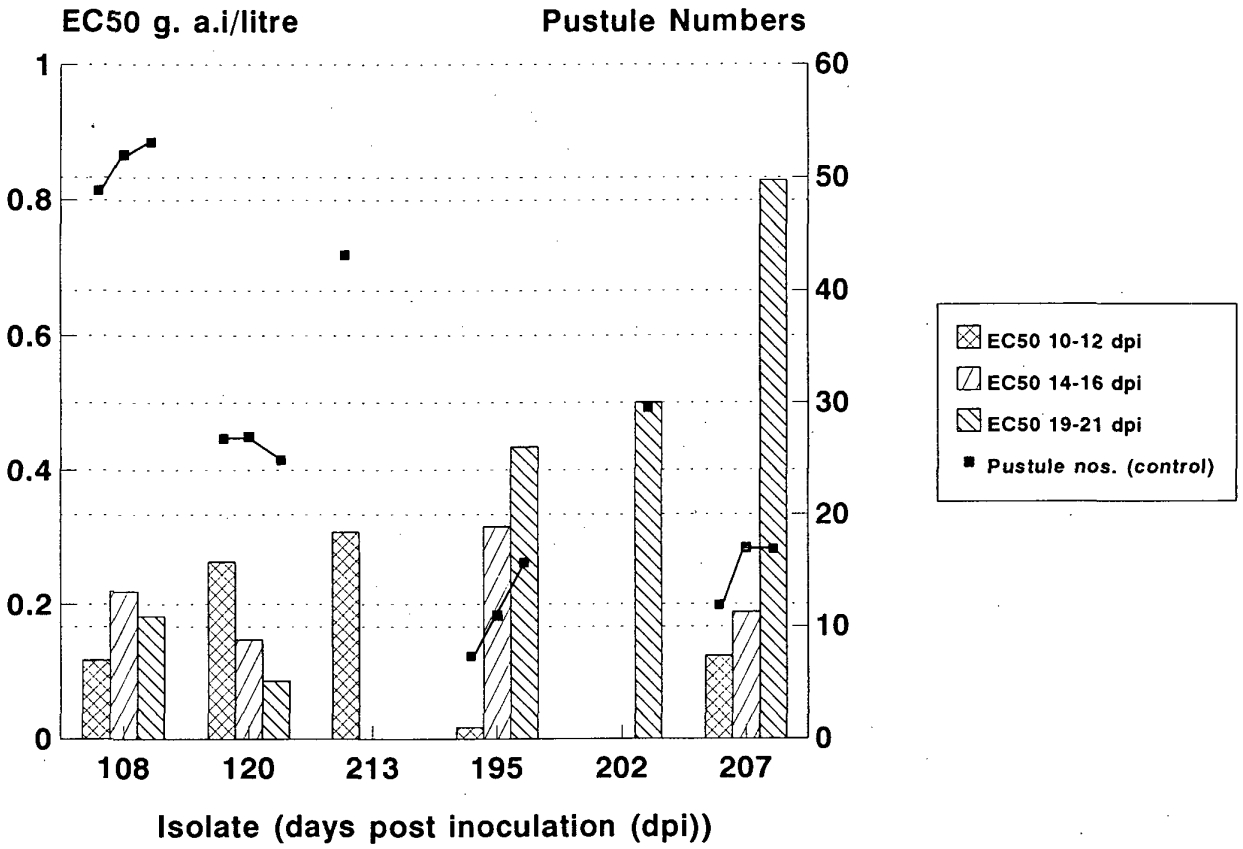


Fig. 1. Sensitivity of Barley Brown Rust (BRS-88-) to Fenpropimorph (Mistral).

Fig 1. illustrates the sensitivity of some of the isolates to fenpropimorph. Only six of the isolates were successfully screened in this test and not all the isolates screened were assessed at the three different days of post inoculation (dpi). Taking into account the dpi with the maximum control pustule leaf cover per centimetre for each isolate, there was a 6-fold difference between the most sensitive isolate, BRS-88-120 (14-16 dpi) and the least sensitive one, BRS-88-207 (19-21 dpi). However, the latter showed relatively low EC50 values.

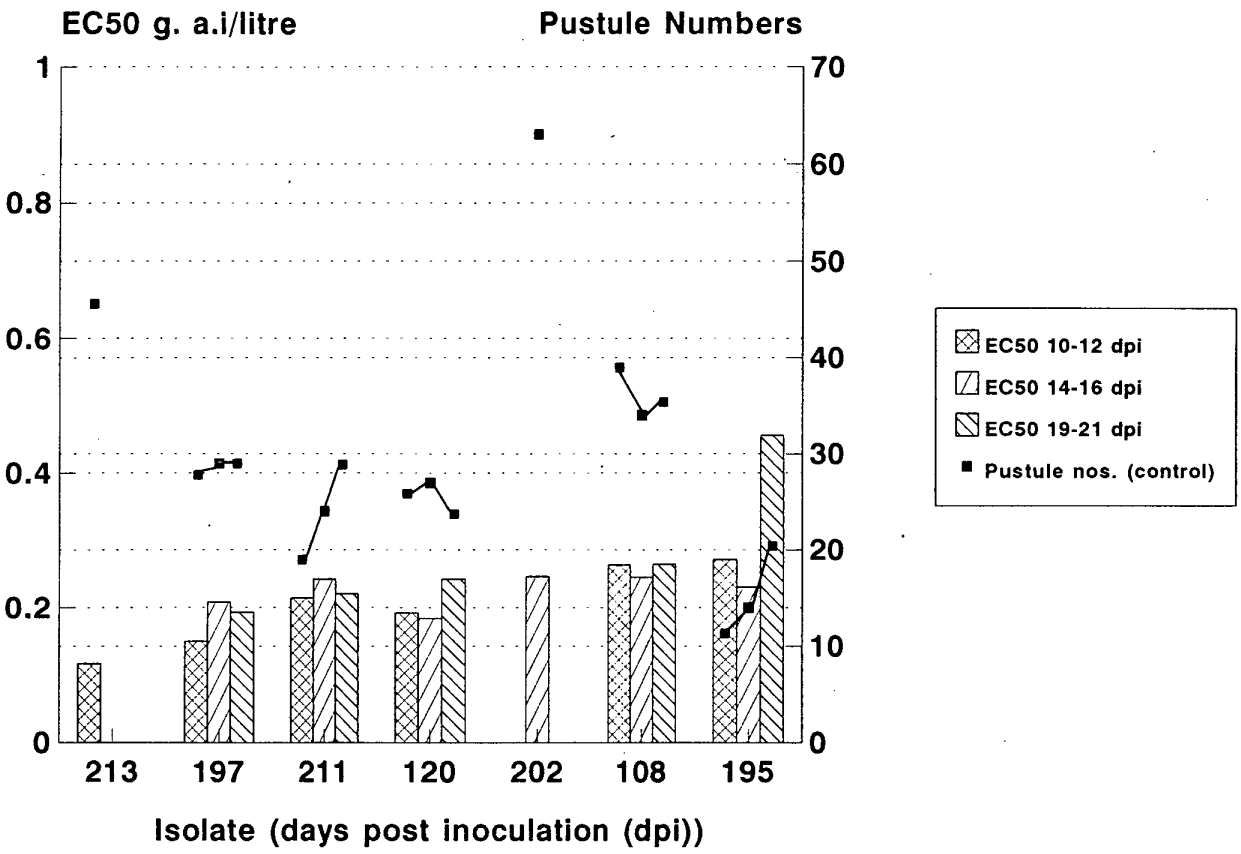


Fig. 2. Sensitivity of Barley Brown Rust to Fenpropidin (Patrol).

Fig. 2 shows that there was a 4-fold difference betweeen the most sensitive isolate, BRS-88-213 (10-12 dpi) and the least sensitive one, BRS-88-195 (19-21 dpi). The range of sensitivity for fenpropidin was lower than for fenpropimorph and the different isolates tested tended to show more or less similar responses to fenpropidin.

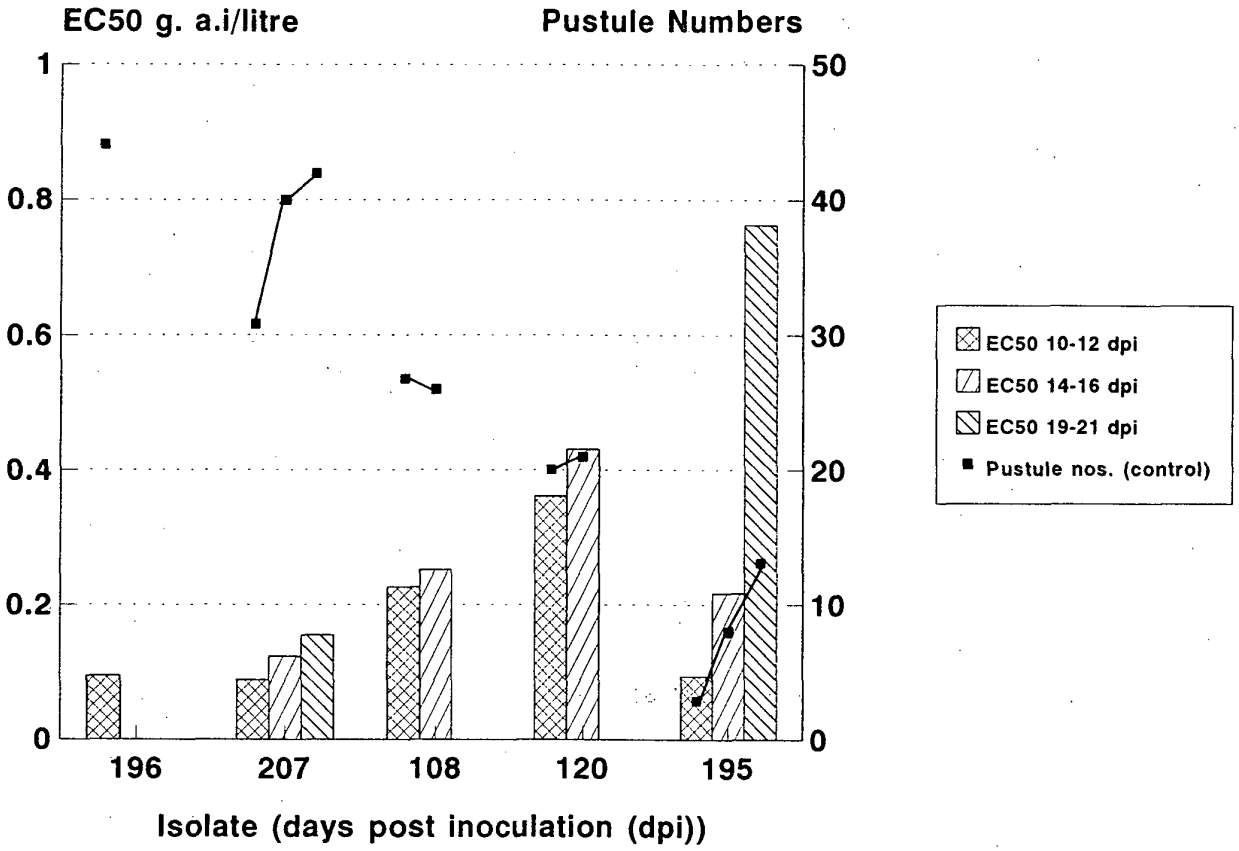


Fig. 3. Sensitivity of Barley Brown Rust (BRS-88-) to Propiconazole.

The range of sensitivity in Fig. 3 was 8-fold between BRS-88-196 (10-12 dpi), the most sensitive isolate and BRS-88-195 (19-21dpi), the least sensitive one although the EC50 values of BRS-88-195 showed a similar range of variation over the three assessment dates.

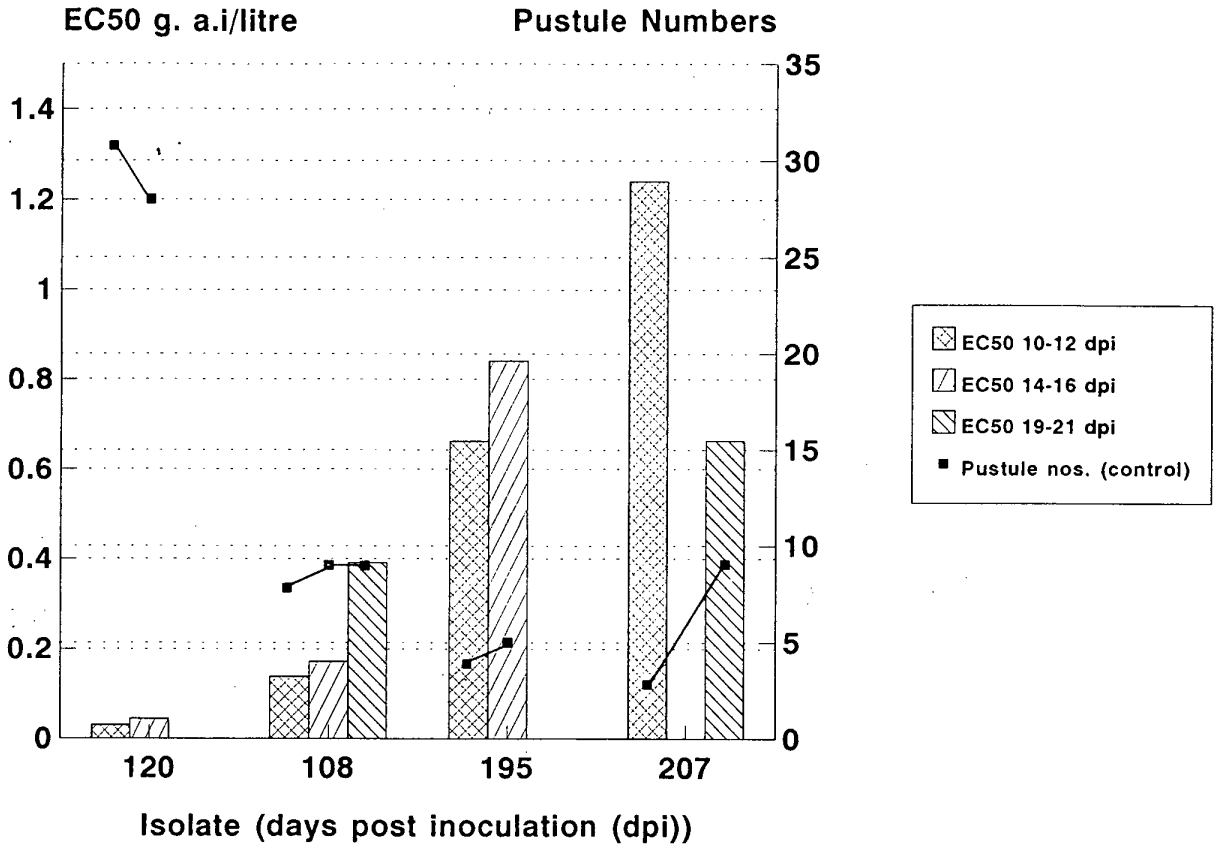
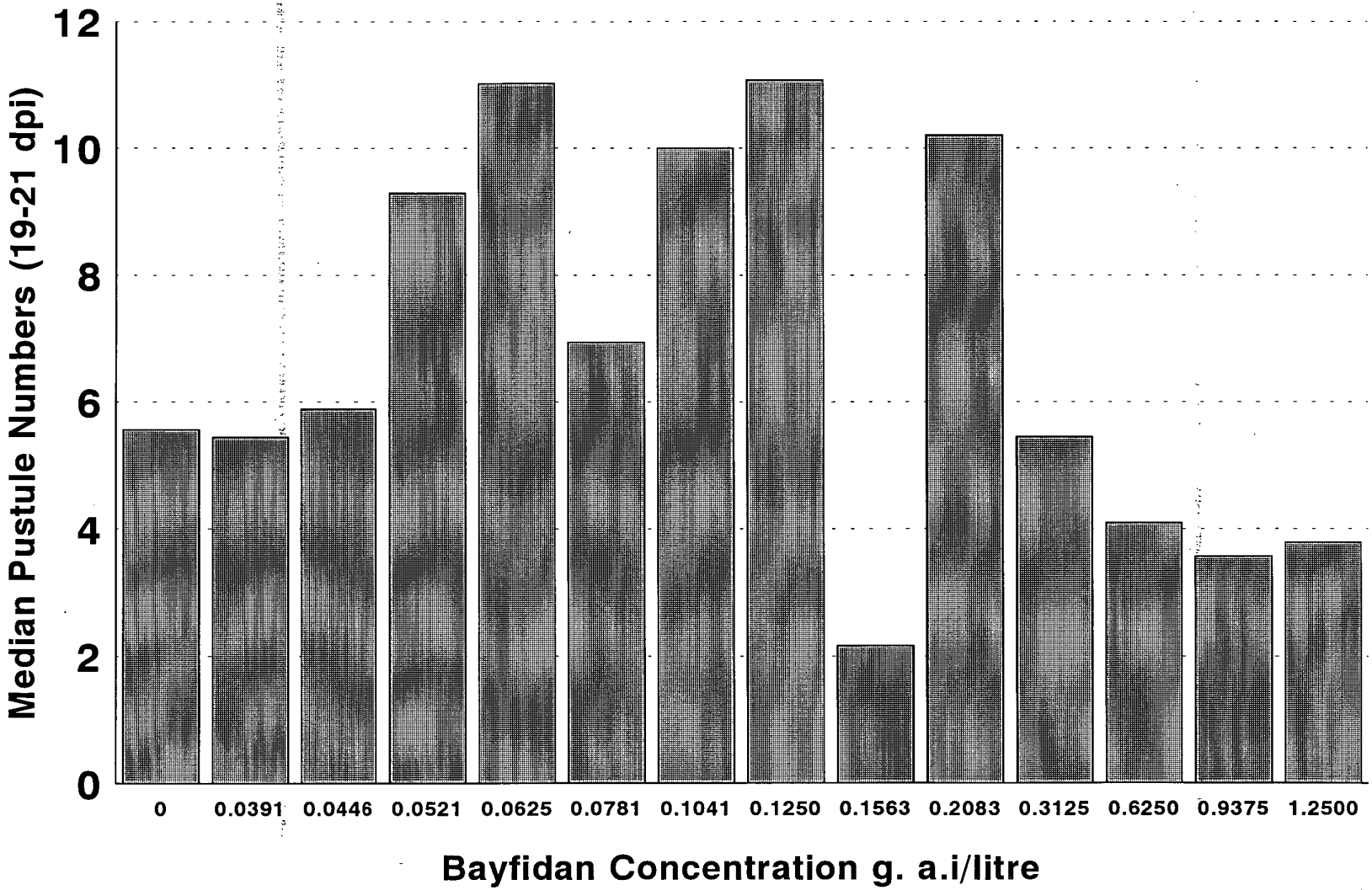


Fig. 4. Sensitivity of Barley Brown Rust (BRS-88-) to Triadimenol (Bayfidan).

Fig. 4 illustrates that the difference between the most sensitive isolate, BRS-88-120 and the least sensitive one, BRS-88-207 was 41-fold but the results for triadimenol were irregular. It was not possible to get EC50 values for some of the isolates at some or all different dpis because there were many more pustules produced on segments sprayed with different concentrations of the fungicide than on the untreated control. Moreover pustule numbers produced on controls, for all isolates except BRS-88-120, tended to be low. Fig. 4b (overleaf) is an example of the kind of irregular results obtained with triadimenol.

Fig. 4b. Median Pustule Numbers of BRS-88-195 at Control and different Concentrations of Bayfidan.



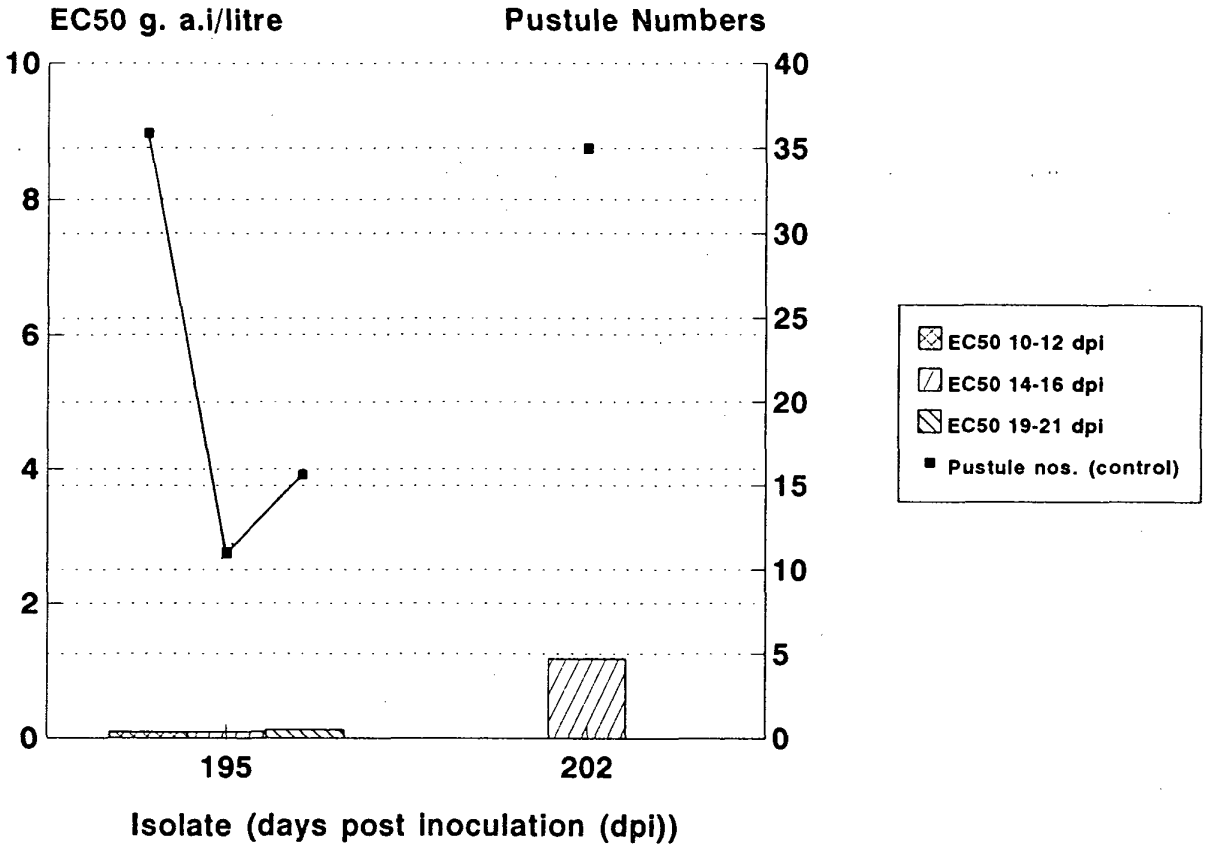


Fig. 5. Sensitivity of Barley Brown Rust (BRS-88-) to Flutriafol (Impact).

Only two isolates were successfully screened for sensitivity to flutriafol. Fig. 5 shows that there was a 13-fold difference between the two isolates. The less sensitive isolate BRS-88-202 grew at twice the recommended field spray concentration.

BRS-88-195 tended to be relatively insensitive to both the morpholine and the DMI fungicides but was sensitive to flutriafol (Impact). Both BRS-88-120 and BRS-88-108 were sensitive to fenpropimorph, fenpropidin and triadimenol and moderately insensitive to propiconazole. They were not tested against flutriafol. Isolate BRS-88-207 on the other hand had appeared to be sensitive to propiconazole but relatively insensitive to fenpropimorph and triadimenol. None of the other isolates was adequately tested against the full range of fungicides, but BRS-88-202 was remarkable in its ability to grow on high concentrations of flutriafol.

The sensitivity responses to the five fungicides did not appear to relate to distribution or the virulence characteristics of isolates. The apparently least sensitive isolates to most fungicides, tended to belong to octal race 1653 and the widely virulent race 1673 (Jones, 1987) see Appendix 2. BRS-88-195 also belonged to the widely virulent octal race 1673.

Wheat brown rust isolates

The numbers of pustules which developed on untreated controls was genenerally low as indicated in Figs. 6-10 which illustrate the sensitivity of wheat brown rust isolates to the five fungicides.

From Table 3 (overleaf), it may be seen that most isolates tended to be sensitive to most fungicides, an exception being WBRS-88-8 which showed a degree of insensitivity to fenpropidin and propiconazole. With respect to virulence factors present in the isolates, all exhibited 2,6 virulence factors at 10°C but differences occur between isolates at 25°C (Appendix 3). No relationship between the virulent factors present in isolates and their fungicide sensitivity characteristics was shown. Isolate WBRS-88-8 which was associated with some fungicide insensitivity, showed a virulence spectrum similar to WBRS-88-21 which was sensitive to all fungicides tested.

Experiment 3 (1988 and 1989 barley and wheat brown rust isolates)

Barley brown rust isolates

Again problems with levels of infection on controls reduced the number of successful tests and a further difficulty was contamination with mildew, which resulted in some tests being abandoned. Altogether sixteen barley brown rust isolates were screened in the third series of tests.

Ratios were calculated for the dpi with a higher number (>10) of pustules: nine isolates were tested at 10-12 dpi and seven at 14-16 dpi. Table 4 shows the number of isolates with more than 0.1 and 0.5 pustule numbers of the untreated control.

Table 3. Sensitivity of Wheat Brown Rust Isolates to Five Fungicides (EC50 values at dpi with maximum pustule numbers on controls).

ISOLATE	WB VIRULENCE FACTOR AT 25°C	FENPROPIMORPH	FENPROPIDIN	PROPICONAZOLE	TRIADIMENOL	FLUTRIAFOL
WBRS-88-5	2,6	<0.1	0.3	-	-	-
WBRS-88-6	2,6	0.4	0.3	0.2	-	0.1
WBRS-88-8	2,3,6	0.3	1.3	1.8	0.2	0.1
WBRS-88-16	2,3,4,6	0.5	0.4	0.4	-	-
WBRS-88-17	2,3,6	0.6	0.2	0.4	<0.1	0.1
WBRS-88-21	2,3,6	-	0.2	0.1	<0.1	<0.1

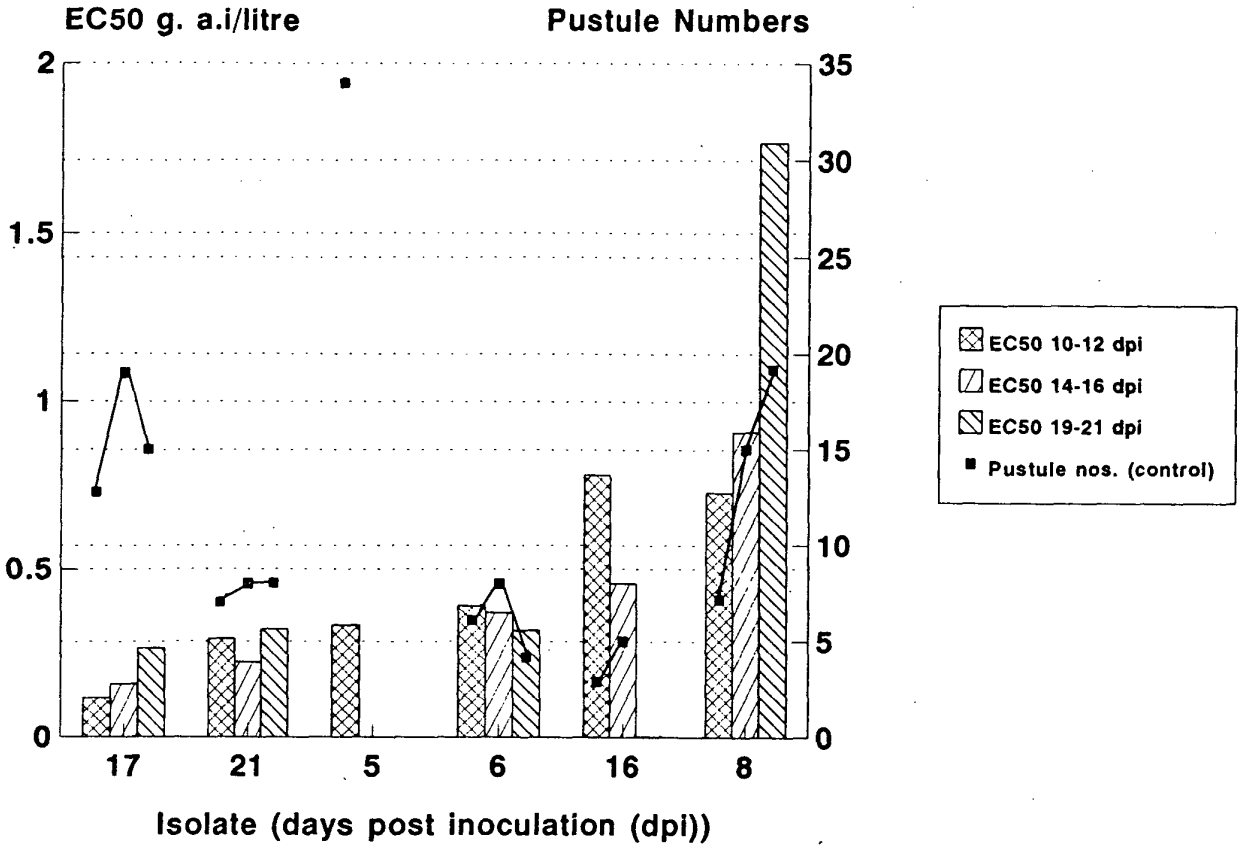


Fig. 6. Sensitivity of Wheat Brown Rust (WBRS-88-) to Fenpropimorph (Mistral).

Most isolates appeared to be sensitive (Fig. 6) but still showed some development at low dosage rates of the fungicide. There was an 11-fold difference between the most sensitive isolate WBRS-88-17 and the least sensitive one WBRS-88-8.

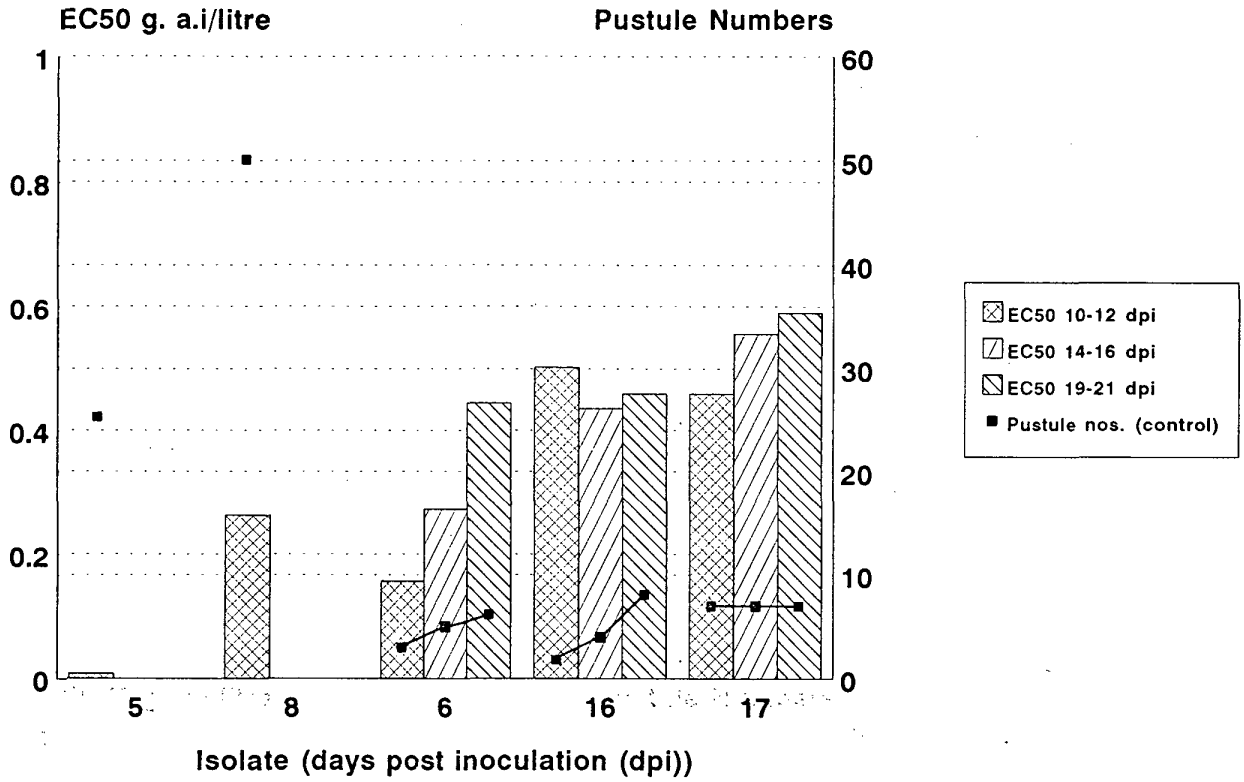


Fig. 7. Sensitivity of Wheat Brown Rust (WBRS-88-) to Fenpropidin (Patrol).

Fig. 7 illustrates that four of the five isolates screened fell within the same range of sensitivity to fenpropidin, but there was a 73-fold difference in sensitivity between the fifth, very sensitive isolate WBRS-88-5 and the least sensitive isolate, WBRS-88-17.

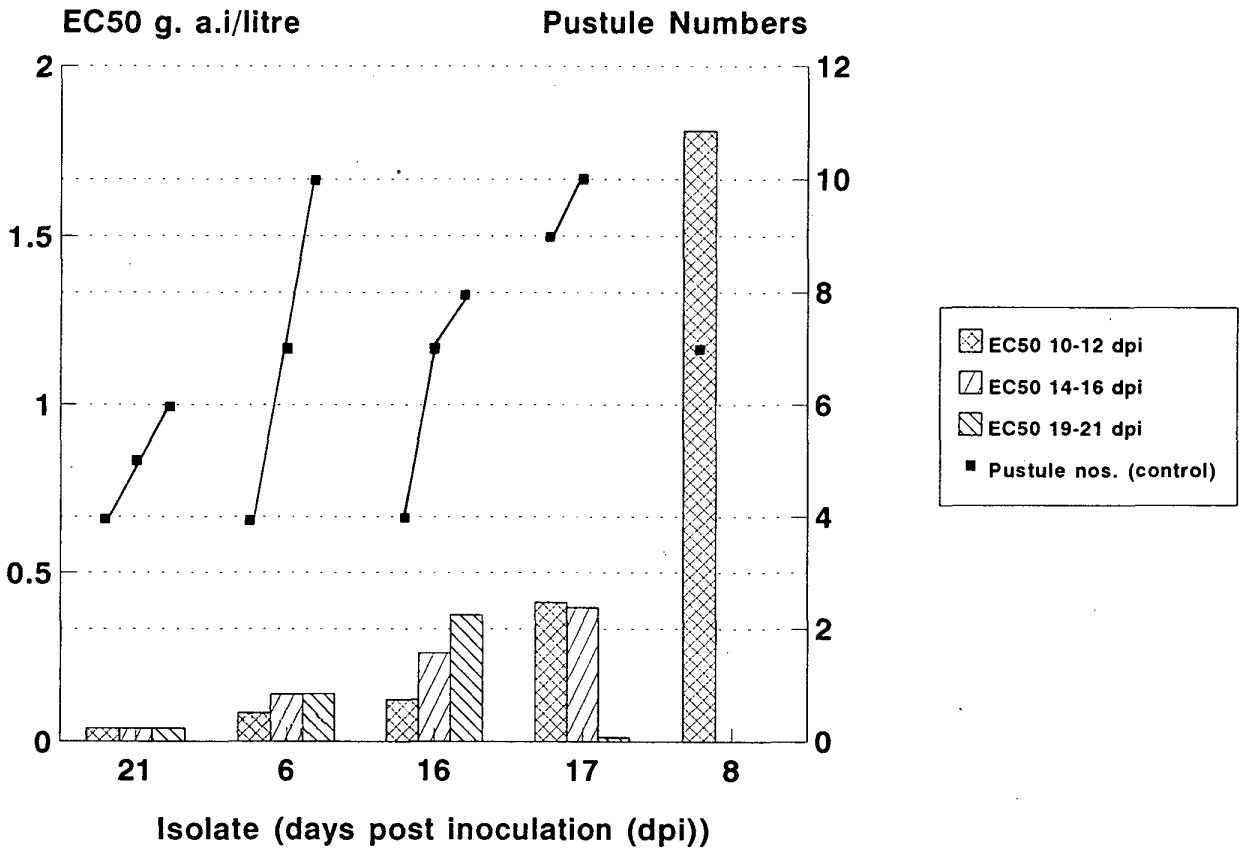


Fig. 8. Sensitivity of Wheat Brown Rust (WBRS-88-) to Propiconazole (Tilt).

With propiconazole one isolate WBRS-88-8 appeared less sensitive than the four others tested while isolate WBRS-88-21 appeared the most sensitive (Fig. 8). There was a 46-fold variation in sensitivity for these two extremes.

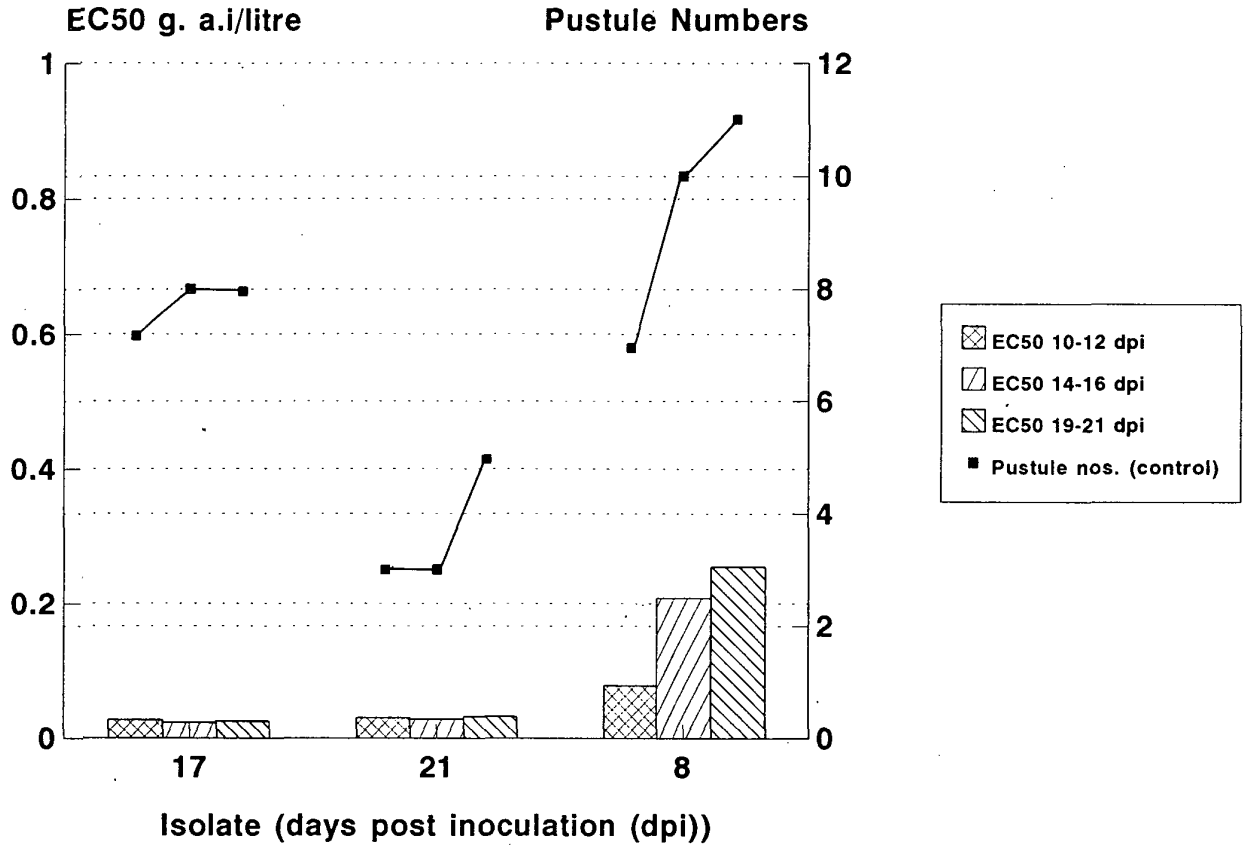


Fig. 9. Sensitivity of Wheat Brown Rust (WBRS-88-) to Triadimenol (Bayfidan).

Only three isolates were tested successfully against triadimenol. The results in Fig. 9 indicated that WBRS-88-8 was less sensitive than WBRS-88-21 and WBRS-88-17, with a 10-fold difference evidenced.

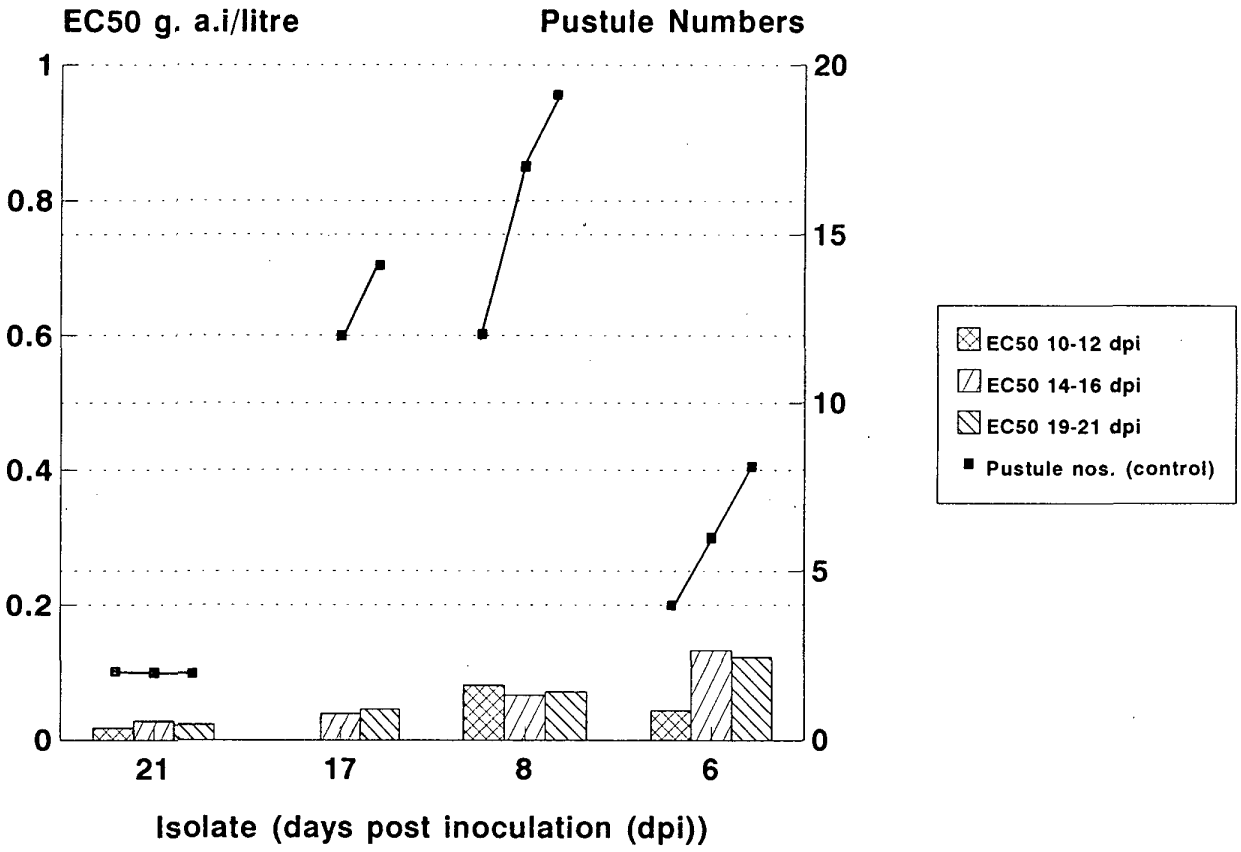


Fig. 10. Sensitivity of Wheat Brown Rust (WBRS-88-) to Flutriafol (Impact).

The four isolates tested against flutriafol all appeared to be sensitive (Fig. 10), with however, a 4-fold difference between the most sensitive isolate WBRS-88-21 and the least sensitive one WBRS-88-6.

Table 4. Number of Barley Brown Rust Isolates with more than 0.1 and 0.5 of untreated control pustules at different Fungicide Concentrations.

PERIOD	DOSE	FENPROPIMORPH		FENPROPIDIN		PROPICONAZOLE*		TRIADIMENOL*		FLUTRIAFOL*	
		>0.1	>0.5	>0.1	>0.5	>0.1	>0.5	>0.1	>0.5	>0.1	>0.5
10-12 Days (N=9)	1/8	0	0	0	0	8	4	8 (out of 8)	5	3 (out of 8)	1
	1 or 2*	0	0	0	0	1	0	8	3	2 (out of 7)	0
14-16 Days (N=7)	1/8	1	0	1	0	6	4	5	3	4	2
	1 or 2*	0	0	0	0	2 (out of 6)	0	3	2	3	1

* = 2x recommended field spray concentration at second dose

Overall, the isolates were more sensitive to the morpholines than to the triazoles. Only two isolates, one for either morpholine, produced more than 0.1 of the untreated control pustules. This was at 1/8th the recommended field spray concentration. The isolates were, BRS-89-47 (octal race 673) for fenpropimorph and BRS-88-103 (octal race 1673) for fenpropidin. None of the isolates produced more than 0.5 of the untreated control pustules for both morpholines.

There was a range of sensitivity to the triazoles. Isolates were least sensitive to triadimenol; some isolates produced more than 0.5 of the untreated control pustules at twice the recommended field spray concentration. These isolates were BRS-89-51 (octal race 1673), BRS-88-189 (octal race 1653), BRS-89-37 (octal race 673), BRS-89-46 (octal race 673) and BRS-88-103 (octal race 1673). Those which produced more than 0.1 of the untreated control at twice the recommended field spray concentration of triadimenol were eleven altogether. In addition to the above five the other six were, BRS-88-96 (octal race 673), BRS-88-113 (octal race 673), BRS-88-188 (octal race 1653), BRS-88-205 (octal race 1653) and BRS-89-39 (octal race 673).

Only three isolates; BRS-89-51, BRS-89-46, and BRS-88-103 produced more than 0.1 of the untreated control pustules at twice the recommended field spray concentration of propiconazole. None produced more than 0.5 of the untreated control at this concentration.

For flutriafol at twice the recommended field spray concentration, five isolates namely, BRS-88-96 (octal race 673), BRS-89-37, BRS-89-47, BRS-88-103 and BRS-89-45 (octal race 1673) had more than 0.1 of the untreated control pustules. Only one of these, BRS-89-47 , had more than 0.5 of the untreated control pustules.

Wheat brown rust isolates

Table 5 shows the number of wheat brown rust isolates with more than 0.1 and 0.5 of the untreated control pustule numbers for the two fungicide dosage rates. Altogether eleven isolates were screened. Four of the isolates were assessed at 14-16 dpi and the remainder at 10-12 dpi. Like the barley brown rust isolates these isolates were also more sensitive to the morpholines than to the triazoles.

Only two isolates, WBRS-89-6 and WBRS-88-23, produced more than 0.1 of the untreated control pustules at 1/8th the recommended field spray concentration of fenpropimorph. Of these only the first one produced more than 0.5 pustules at this concentration. None produced more than 0.1 of the untreated control pustules at the recommended field spray concentration.

Table 5. Number of Wheat Brown Rust Isolates with more than 0.1 and 0.5 of untreated Control Pustules at different Fungicide Concentrations.

PERIOD	DOSE	FENPROPIMORPH		FENPROPIDIN		PROPICONAZOLE*		TRIADIMENOL*		FLUTRIAFOL*	
		>0.1	>0.5	>0.1	>0.5	>0.1	>0.5	>0.1	>0.5	>0.1	>0.5
10-12 Days (N=7)	1/8	1	1	4	2	3	1	4	4	4	1
	1 or 2*	0	0	1	0	0 (out of 6)	0	3	0	1 (out of 6)	0
14-16 Days (N=4)	1/8	1	0	1	1	1	1	1	1	2	1
	1 or 2*	0	0	0	0	0	0	1	0	1	0

* = 2x recommended field spray concentration at second dose

With fenpropidin, five isolates produced more than 0.1 of the untreated control pustules at 1/8th the recommended field spray concentration. WBRS-89-6, WBRS-89-14 and WBRS-89-17 produced more than 0.5 at the same concentration but WBRS-89-14 produced more than 0.1 at the recommended dose.

Four isolates namely, WBRS-89-6, WBRS-89-14, WBRS-88-9 and WBRS-89-17 produced more than 0.1 at 1/8th the recommended field spray concentration of propiconazole, WBRS-89-14 and WBRS-89-17 producing more than 0.5. None of the isolates produced more than 0.1 at twice the recommended field spray concentration.

As for barley brown rust, wheat brown rust isolates were least sensitive to triadimenol. Five isolates produced more than 0.5 at 1/8th the recommended field spray concentration. WBRS-89-6, WBRS-89-14, WBRS-88-9 and WBRS-89-17 produced more than 0.1 at twice the recommended concentration.

With flutriafol, six isolates produced more than 0.1 at 1/8th the recommended concentration. Of these, WBRS-89-14 and WBRS-89-17 produced more than 0.5 at the same concentration. WBRS-86-11 and WBRS-88-23 produced more than 0.1 at twice the recommended field spray concentration.

v. DISCUSSION

Results from pre-1987 and 1987 barley and wheat brown isolates indicated a 20-fold variation of sensitivity to triadimefon and a 6 to 10-fold variation to propiconazole (Boyle, Gilmour & Lennard , 1988). It was found that the earlier isolates with no known exposure to fungicides, were the most sensitive to propiconazole as were the earlier isolates of wheat brown rust to triadimefon. However, the earlier isolates of barley brown rust were not the most sensitive to triadimefon. The sensitivity ranking of wheat brown rust isolates to the two fungicides were similar, but those of barley isolates differed markedly. There was no apparent relationship between sensitivity and virulence among the barley isolates, while the sample of wheat isolates tested was too small to draw any conclusion.

Fewer 1988 and 1989 isolates than 1987 isolates were tested against more fungicides as already indicated. Not all the isolates were screened against all the five fungicides as sometimes the amount of bulked inoculum was insufficient and, sometimes there were very few pustules (especially wheat isolates) on the untreated control. Moreover, many tests were abandoned in 1989 because of contamination with powdery mildew (*Erysiphe graminis*).

With the few barley isolates screened, variation of sensitivity to fenpropimorph was 6-fold, 4-fold to fenpropidin, 8-fold to propiconazole, 41-fold to triadimenol and 13-fold to flutriafol. The least sensitive isolates tended to belong to octal race 1653 and the widely virulent octal race 1673. However, this should be viewed with caution since relatively few isolates were screened. Isolates of octal race 1653 were collected from cv Magie and those of octal race 1673 were from cv Igri. With so few isolates originating from different sources, it is not possible to make any conclusion about the relationship between origin and sensitivity of isolates. Information about application of fungicides to the crops from which the isolates were obtained was scanty.

The barley brown rust isolates were more sensitive to the morpholines than to the triazoles and the range of variation was smaller for the morpholines than for the triazoles. Isolates were least sensitive to triadimenol. Results indicated a decline in sensitivity to triadimenol by brown rust isolates, which may have been the reason why results for triadimenol were very irregular (e.g. Fig. 4b). Insensitivity to triadimenol by *E. graminis* has been reported elsewhere (Fletcher, 1981; Hollowman, Butters & Clark, 1984). With the few isolates screened, there was no cross-sensitivity between triadimenol and the other inhibitors of demethylation in the sterol biosynthesis pathway namely, propiconazole and flutriafol.

With wheat brown rust isolates, the variation of sensitivity to fenpropimorph was 11-fold, 73-fold to fenpropidin, 46-fold to propiconazole, 10-fold to triadimenol and 4-fold to flutriafol. The wheat brown rust isolates were not necessarily more sensitive to the morpholines than to the triazoles as the barley

isolates. However they showed more variation in sensitivity than barley brown rust isolates. There was no apparent relationship between sensitivity, virulence and origin.

The results of the third series of tests were analysed differently because there were not enough points for the Genstat 5 programme which was used in the first two series of experiments to get EC50 values. Isolates of both wheat and barley brown rust were more sensitive to the morpholines than to the triazoles. Most isolates were least sensitive to triadimenol as with 1988 isolates. There was no single isolate that was not sensitive to all the fungicides indicating lack of cross-sensitivity to the fungicides.

Taking the results of all the three years and using any growth at the recommended dosage rate or above as an arbitrary criterion of field insensitivity, Table 6 may be taken as an index of the possible risk of insensitivity problems in practice. It may be seen that for all the fungicides, there were some isolates which grew on at least one of the replicates sprayed with the recommended dosage rate or above. In the case of triadimenol, there was some growth of barley brown rust on at least one of the replicates in all tests carried out. Triadimenol and flutriafol emerged as the most vulnerable to insensitivity. Triadimefon was shown as the least vulnerable to insensitivity but tests for this fungicide were carried out in 1988 and included isolates that had had no history of fungicide exposure.

The development of many isolates appeared to be stimulated by low concentration of the fungicide. Such a reaction has been reported in other sensitivity tests (Williamson, 1983; Boyle *et al.*, 1988; Robertson,Gilmour, Newman & Lennard 1990). Leaf segments used in tests with triazoles tended to remain green longer than in tests with morpholines. Similar stimulation has been reported in tests on other fungi; *Bortrytis cinerea* (Miller & Fletcher, 1974); *Erysiphe graminis* f.sp. *hordei* (Williamson, 1983); *Pseudocercosporella herpotrichoides* (Corrigan, 1984).

Conclusion

From the work carried out to date the following summary of observations may be made:

(1) Variation in sensitivity was found among isolates of both brown rust fungi with respect to all test fungicides.

(2) A greater range of variation in sensitivity was shown by wheat brown rust than by barley brown rust in the responses of isolates to fenpropidin and propiconazole. In contrast, barley brown rust isolates showed a greater range of variation to triadimenol.

(3) The frequency of insensitivity among isolates appeared to be greater for triazoles compared with morpholines, particularly in the case of barley brown rust.

(4) Insensitivity occurred most frequently in triadimenol tests. Again this trend was most evident for barley brown rust.

(5) There was no pattern in relationships between virulence characteristics, origin and insensitivity.

Table 6. Percentage of isolates growing at recommended dosage rates and above (1987-1989).

ISOLATES	FENPROPIMORPH	FENPROPIDIN	PROPICONAZOLE*	TRIADIMENOL*	FLUTRIAFOL*	TRIADIMEFON
Barley brown rust	46%	17%	40%	100%	90%	20%
Wheat brown rust	47%	55%	34%	69%	65%	29%

* Experiment 3 2X recommended dosage rate

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APPENDIX 1. Barley Brown Rust Isolates, their Octal Races and Source of Origin.

ISOLATE	OCTAL RACE	ORIGIN
BRS-88-96	673	Trawsgoed, Wales
BRS-88-103	1673	Reading
BRS-88-108	1673	East
BRS-88-113	673	Wolverhampton
BRS-88-120	1673	Trawsgoed, Wales
BRS-88-188	1653	Wolverhampton
BRS-88-189	1653	Wolverhampton
BRS-88-195	1673	Huntingdon
BRS-88-196	673	Gillingham
BRS-88-197	1673	Warminster, Wilts
BRS-88-202	1653	Wolverhampton
BRS-88-205	1653	Kings, Lynn
BRS-88-207	1653	Kirton, Lincoshire
BRS-88-211	1653	Colchester
BRS-88-213	1653	Norwich
BRS-89-37	673	Devon
BRS-89-39	673	Devon
BRS-89-45	1673	Devon
BRS-89-46	673	Devon
BRS-89-47	673	Devon
BRS-89-48	673	Bristol
BRS-89-49	1653	Reading
BRS-89-50	1673	Huntingdon
BRS-89-51	1673	Reading
BRS-89-84	1673	Trawsgoed, Wales

OCTAL RACE	BR VIRULENCE FACTOR
673	1,2,4,5,6,8,9,
1653	1,2,4,6,8,9,10
1673	1,2,4,5,6,8,9,10

APPENDIX 2. Wheat Brown Rust Isolates, their Virulence Characteristics and Source of origin.

ISOLATE	WB VIRULENCE FACTOR		ORIGIN
	10°C	25°C	
WBRS-86-11	-	-	-
WBRS-88-5	2,6	2,6	Reading
WBRS-88-6	2,6	2,6	Norwich
WBRS-88-8	2,6	2,3,6	Reading
WBRS-88-9	2,6	2,3,6	Cambridge
WBRS-88-13	2,6	2,3,6	Bristol
WBRS-88-15	2,6	2,6	Huntingdon
WBRS-88-16	2,6	2,3,4,6	Colchester
WBRS-88-17	2,6	2,3,6	Ipswich
WBRS-88-21	2,6	2,3,6	Cambridge
WBRS-88-23	2,6	2,6	Hertford
WBRS-89-6	Not known	-	Wye, Kent
WBRS-89-14	1,2,3	-	Somerset
WBRS-89-17	Not known	-	Wye, Kent
WBRS-89-19	Not known	-	Westbury-on-Seven
WBRS-89-22	Not known	-	-
WBRS-89-27	-	-	-