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AN INVESTIGATION INTO THE NATURE OF RESISTANCE TO THE FUNGICIDE TRIADIMEFON IN POPULATIONS OF *ERYSIPHE GRAMINIS* F.SP. *TRITICI*

by

KHALIL I. AL-MUGHRABI

Submitted in partial fulfilment of the requirements

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The undersigned hereby certify that they have read and recommend to the Faculty of Graduate Studies for acceptance a thesis entitled "An Investigation into the Nature of Resistance to the Fungicide Triadimefon in Populations of *Erysiphe graminis* f.sp. *tritici*"

by Khalil Al-Mughrabi

in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Dated May 2, 1994

External Examiner

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LIST OF ABBREVIATIONS

a.i.	Active Ingredient
ANOVA	Analysis of Variance
AV	Annapolis Valley
BT	Before Treatment
CC	Colchester County
cv(s)	Cultivar(s)
DF	Degree of Freedom
DMI(s)	Demethylation Inhibitor(s)
EBI(s)	Ergosterol Biosynthesis Inhibitor(s)
EC_{50}	Effective Concentration to Prevent Growth of 50% of a Fungal Isolate
G	Generation Cycle
ha	Hectare
h	Hour(s)
LSD	Least Significant Difference
R	Resistant
S	Sensitive
SAS	Statistical Analysis System
SBI(s)	Sterol Biosynthesis Inhibitor(s)
SE	Standard Error
UV	Ultraviolet
WK(s)	Week(s)
WP	Wettable Powder

ABSTRACT

The sensitivity of wheat powdery mildew (*Erysiphe graminis* DC. f.sp. *tritici* E. Marchal) populations to triadimefon for single spore-derived isolates collected from triadimefon-treated and -untreated wheat (cvs. Absolvent and Borden) fields in the Annapolis Valley (AV) and Colchester County (CC) of Nova Scotia, Canada, was studied *in vitro*. Results indicated that the populations of the fungus from the AV, where the fungicide has been used intensively, had higher mean EC_{50} values and higher frequencies of isolates resistant to triadimefon than those from CC. The wheat cv. Absolvent was more favourable for the development of resistant isolates of the fungus than the cv. Borden. For both cultivars, the mean EC_{50} values and frequencies of resistant isolates were higher for populations collected from triadimefon-treated fields compared to those from untreated fields. In the study of build-up of resistance, sensitivity tests were performed on powdery mildew isolates collected in 1992 from the AV and CC fields of the wheat cv. Absolvent before, and two and six weeks after triadimefon application. Results revealed that exposing powdery mildew populations to the selection pressure of triadimefon resulted in an increase in the EC_{50} values of populations and in the frequency of resistant isolates within each population. In 1991, the competitive abilities of randomly selected triadimefon-sensitive (S) and -resistant (R) isolates of powdery mildew from treated and untreated fields of the AV in mixed isolate inoculations (75:25, 50:50, 25:75) were tested in the absence of triadimefon on the wheat cv. Absolvent. In 1992, the 50:50 ratio was used in studying the competition between R and S isolates that were collected from treated fields in the AV. Results indicated that, in the absence of triadimefon, the mean EC_{50} values of mixtures of various R:S ratios decreased significantly after five generation cycles. Resistant isolates were less fit (less competitive) than sensitive ones in the absence of triadimefon.

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GENERAL INTRODUCTION

Wheat varieties grown in the Maritime region lack stable high-level resistance to powdery mildew (Gray, 1991). Therefore, effective chemical control of the disease has become an urgent necessity in the Maritimes in order to obtain optimal yield of high quality grain.

Kolbe (1976) reported that one single spray application of triadimefon (1-(4-chlorophenoxy)-3,3-dimethyl-1-(1*H*-1,2,4-triazol-1-yl)-2-butanone) at the onset of infection gave a yield increase of 28% on a mildew-susceptible cultivar of winter wheat, whereas the follow-up application at heading resulted in an additional increment of only 2%. In 1989, however, even a third triadimefon application in some fields in the Annapolis Valley of Nova Scotia failed to control the disease (Gray, 1991). The fear was that the population of the fungus had become resistant to the fungicide.

Most scientists agree on the fact that fungicide-resistant forms may be present in the population of a pathogen even before the fungicide is used. Their frequency, however, will be low at that time since the populations have not yet been subjected to the selection pressure of the fungicides. After a short period of exposing such population to a single-site fungicide, the frequency of resistant strains increases and these strains soon dominate the population.

Fitness of a pathogen strain is its ability to develop,

reproduce, and survive as compared to other strains under the same conditions (Wade, 1982; Skylakakis, 1987). With some fungicides, it has been found that, in the absence of the fungicide, resistant strains are less fit than the sensitive ones (Hollomon, 1975; Dekker, 1976; Gullino and Garibaldi, 1981; Buchenauer, *et al.*, 1984; Buchenauer and Hellwald, 1985; Moorman and Lease, 1992). This may have consequences for practice, as it may slow down the build-up of a resistant pathogen population in the field by favouring its decline in the absence of the fungicide.

Measuring fitness in the open field is a difficult task because of the migration of spores within the field and from outside sources. For this reason, a test tube method was adopted and used to test for the occurrence and build-up of resistance, and to test the competitive ability of resistant strains of *Erysiphe graminis* f.sp. *tritici* on winter wheat.

The objectives of this research project were as follows:

1. To give an account of the variation in the sensitivity of wheat powdery mildew fungus(*Erysiphe graminis* DC. f.sp. *tritici* E. Marchal) populations to triadimefon (Bayleton™) for samples collected from fungicide-treated and -untreated wheat (cvs. Absolvent and Borden) fields in the Annapolis Valley and Colchester County.
2. To study the build-up of resistance of *Erysiphe graminis* f.sp. *tritici* in Absolvent wheat fields for isolates collected before and after triadimefon application, in

both the Annapolis Valley and Colchester County, and to relate the results to disease progress curves for the two populations.

3. To study the competitive ability of triadimefon-resistant (R) and triadimefon-sensitive (S) isolates in mixed-isolate inoculation of the fungus.

Chapter 1.

GENERAL LITERATURE REVIEW

1.1 POWDERY MILDEW OF WHEAT

Powdery mildew, caused by *Erysiphe graminis* DC. f.sp. *tritici* E. Marchal, is one of several foliar diseases which occur throughout western Europe (Large and Doling, 1962; Wolfe and Barrett, 1977), North America (Cherewick, 1944; Jørgensen, 1988; McFadden, 1989) and other areas of the world (Moseman, 1973; Smith and Smith, 1974) where wheat (*Triticum aestivum* L.) is grown. It causes yield losses of up to 30% (Kasper and Kolbe, 1971) if infection occurs early in the crop cycle and conditions remain favourable for disease development (Prescott *et al.*, 1986).

Powdery mildew occurs in every province of Canada, being more prevalent in British Columbia and the five eastern provinces. In 1941, a general epidemic of powdery mildew occurred on wheat and barley in Manitoba (Cherewick, 1944). The fungus is an obligate parasite and often present on winter wheat in the fall (Christ and Frank, 1989). It overwinters on stubble and fall sown wheat crops. The presence of a local supply of inoculum in the spring, therefore, appears to be one of the determining factors in the establishment of an epiphytotic of powdery mildew in any part of Canada. The pathogen sporulates abundantly and is disseminated over

considerable distance by wind (Schnathorst, 1965; Spencer, 1978). The disease may occur at all stages of plant growth and the fungus may cover the leaves, shoots, and ears to give them a characteristic floury appearance. Infected plants become yellow and their photosynthesis is severely affected (Kolbe, 1976). Royse *et al.* (1980) found that infection of lower leaves reduces yield and leads to increased infection of the upper leaves.

1.2 TRIADIMEFON (BAYLETON®)

Triadimefon(1-(4-chlorophenoxy)-3,3-dimethyl-1-(1*H*-1,2,4-triazol-1-yl)-2-butanone) (Fig. 1.1) is a triazole fungicide which is reputed to be a very good protectant and eradicant fungicide against powdery mildew and rust fungi, and is claimed to move systemically in the apoplast after application to leaves of cereals. Its activity is probably enhanced as a result of considerable redistribution in the vapour phase (Clark *et al.*, 1978).

Triazoles are members of the demethylation inhibitor (DMI) group of compounds, which inhibit the C-14 demethylation step in the synthesis of ergosterol (Dekker, 1985a).

Agricultural practice of powdery mildew control in wheat in Nova Scotia may include one or two foliar spray applications of fungicides which inhibit ergosterol biosynthesis. Ergosterol biosynthesis inhibitors, or EBIs, have been used extensively to control powdery mildew of wheat

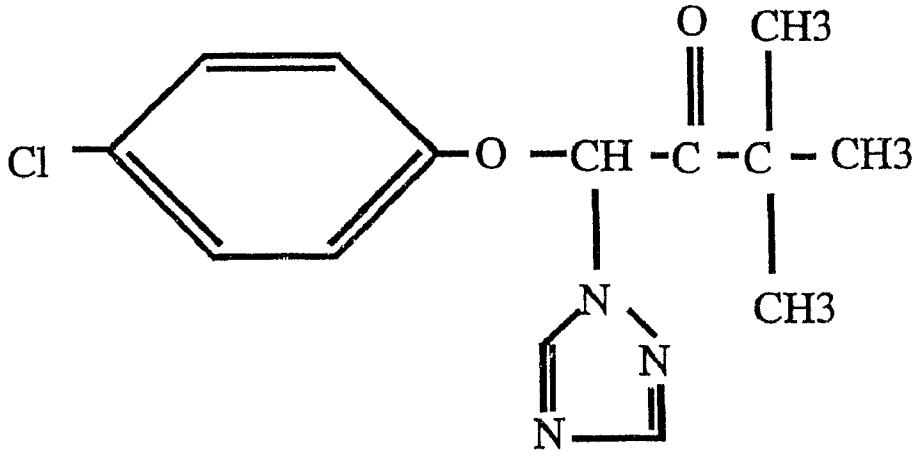


Fig. 1.1 Structural formula of triadimefon (Buchenauer, 1976).

and barley throughout the world (Butters *et al.*, 1984; De Waard *et al.*, 1986; Lasseron-De Falandre *et al.*, 1991).

When triadimefon was first introduced it was active against the cereal powdery mildew fungus (*Erysiphe graminis*) at extremely small concentrations (Rawlinson *et al.*, 1982). Subsequently, fewer less sensitive strains have arisen and are now widely distributed in the Netherlands (Wolfe 1981 & 1985), Hungary (Enisz, 1988), northern England (Fletcher and Griffin, 1981) and Germany (Buchenauer and Hellwald, 1985).

Farmers who saw that a fungicide was becoming less effective often increased the concentration, thus increasing the selection pressure and aggravating the problem (Dekker, 1986).

1.3 FUNGICIDE RESISTANCE

Resistance is defined as a decreased response of a plant pathogen to a fungicide or other disease control agent as a result of its application (Ogawa *et al.*, 1977). It describes the ability of pathogens to tolerate higher concentrations of chemicals (Ogawa *et al.*, 1976).

It has been argued that the resistance is less likely to occur to fungicides having more than one site of action than to those acting at a single site (Dekker, 1977; Georgopoulos, 1977). The key argument in support is that the introduction of site-specific systemic fungicides was soon followed by the appearance of resistance in the field.

1.3.1 Single-site vs. Multisite Inhibitors

Failure in disease control caused by fungicide resistance often occurs after the introduction of single site (i.e. specific-site) fungicides, since they act on only one metabolic site (Lasseron-De Falandre *et al.*, 1991). Apparently only one fungal gene is sufficient to induce a change at the target site leading to a decreased binding affinity of the inhibitor (Corbett, 1979; Delp, 1980; Dekker, 1985b; Köller and Scheinpflug, 1987). DMIs clearly belong to this group of fungicides, which is in general more prone to resistance than conventional multisite inhibitors (Dekker, 1985a).

Resistance to multisite inhibitors never can be achieved in this way, as there are too many sites of action which must be changed (Dekker, 1985a).

1.3.2 Resistance-risk Groups of Fungicides

The type of fungicide used is probably the most important factor which determines the likelihood of failure of fungicides due to resistance by the target organism. It has been suggested that the available agricultural fungicides can be classified into three main resistance-risk groups (Dekker, 1982; Georgopoulos, 1984):

1. Low-risk fungicides: In this group are most of the protectants which have remained effective despite long exposure of fungal populations to their activity.
2. Moderate-risk fungicides: Some systemics and a few

protectants form this group of fungicides.

3. High-risk fungicides: This group includes those losing effectiveness fairly quickly, including several systemics.

Fungicide resistance in target pathogen populations is a continuing problem associated with the use of systemic fungicides. The broad-spectrum systemic fungicides presently in use include benzimidazoles, dicarboximides, and EBIs (Sanders *et al.*, 1985).

The problem of resistance against EBIs was extensively studied in facultative parasites by Fuchs and Drandarevski (1976) and Fuchs *et al.* (1977) and De Waard and Van Nistelrooy (1979).

1.3.3 Case Studies on Fungicide Resistance

The frequency of fungicide resistance reports has accelerated since the mid-1960's, paralleling the introduction of new compounds that attack specific biochemical targets in the pathogen. Many of these are systemic fungicides, but the systemic property is not a requirement for resistance development, since several protectant fungicides such as dodine, fentin, and iprodione have shared the problem (Eckert, 1988).

One year after its introduction, a reduced effect of benomyl against cucumber powdery mildew was reported (Schoeder and Provvidenti, 1969). Disease control failures caused by

benzimidazole resistance began shortly after intensive use of these fungicides, and by 1976, Dekker (1976) listed 37 reports of benzimidazole resistance in various crops. In addition to benzimidazoles, high levels of resistance to acylalanine and carboximides (Georgopouplos and Ziogas, 1977), to polyoxin (Nishimura *et al.*, 1976), to kasugamycin (Taga *et al.*, 1979) and to members of the aromatic hydrocarbon group (Beever and Bryde, 1982) were also reported for different diseases.

Resistance of *Botrytis* strains to dicarboximide fungicides (Löcher, 1988), and to two sterol biosynthesis-inhibiting fungicides, namely fenetrazol and fenethanil (Elad, 1992), has been reported in many countries in crops such as strawberries, grapes, lettuce and tomatoes.

Development of resistance to dodine in *Venturia inaequalis*, the cause of apple scab, was only observed after 10 years of intensive and exclusive use of the compound in certain areas of the U.S.A. (Szkolnik and Gilpatrick, 1969). Dodine-resistant strains were later detected in Nova Scotia (Ross and Newbery, 1977) and Ontario (McKay and MacNeill, 1979).

The prolonged and widespread use of DMIs to control cereal mildews has selected increased resistance (Butters *et al.*, 1984; Heany *et al.*, 1984 & 1986; Wolfe, 1984; De Waard *et al.*, 1986; Andrivon and De Vallavieille-Pope, 1987; Fletcher *et al.*, 1987; Limpert, 1987; Enisz, 1988).

In European countries, resistance to triazole fungicides was first observed over a decade ago, only a few years after

their introduction (De Waard *et al.*, 1986). Fletcher and Wolfe (1981) reported an increased frequency of powdery mildew strains with a decreased sensitivity to triadimefon and triadimenol but could not show reduced performance. They expressed this as a shift towards insensitivity. In Nova Scotia, Al-Mughrabi and Gray (1993) also reported the development of resistance of powdery mildew of wheat to the fungicide triadimefon.

Isolates of *Erysiphe graminis* f.sp. *tritici* and *hordei* with decreased sensitivity to triadimefon showed cross-resistance to other inhibitors of sterol C-14 demethylation, such as triadimenol, propiconazol, diclobutrazol, prochloraz and nuarimol (Buchenauer and Hellwald, 1985).

1.4 BUILD-UP OF RESISTANCE

In a fungal population that is originally sensitive to a particular fungicide, resistant forms may arise, or be present at low frequency (Wolfe, 1975; Dekker, 1976; Georgopoulos, 1977; Dekker, 1985a). Growth and reproduction of these resistant forms is favoured by selection pressure of the fungicide, so that finally the entire pathogen population may become resistant (Wolfe, 1975; Dekker, 1985a).

In cereal powdery mildew, shifts in sensitivity to DMIs have been recorded, though on these crops correlation between poor control and reduced sensitivity has not been established (Fletcher and Wolfe, 1981). Wolfe (1971) stated that although

resistant isolates of barley powdery mildew can be obtained from ethirimol-treated crops, and although surveys (Shephard *et al.*; 1975) have indicated a slight decrease in sensitivity in these crops, the pyrimidine fungicide ethirimol continued to be effective and has given yield increase for a number of years.

Due to the frequent and almost exclusive use of the antibiotic kasugamycin for controlling rice blast (*Pyricularia oryzae*) in certain areas, kasugamycin has become less effective since 1971 (Miura *et al.*, 1975). After the use of kasugamycin was stopped during 1973, the percentage of resistant individuals rapidly decreased (Misato, 1975), suggesting that kasugamycin-resistant strains are less competitive than sensitive ones.

Horsten (1979) studied *in vitro* the influence of carbendazim, a benzimidazole fungicide applied to winter barley once per season, on the percentage of carbendazim-resistant isolates of *Pseudocercosporella herpotrichoides*, the cause of eye spot disease. His results revealed that the proportion of isolates with resistant spores increased from 17% in 1975 to 67% in 1977.

Resistance to carboxin has been detected in *Ustilago hordei* (Ben-Yephet *et al.*, 1975), but the fungicide continues to give effective control of this disease. In laboratory experiments, triforine-resistant strains of *Cladosporium cucumerinum* developed after several years of the use of triforine but

appeared to be less virulent. Resistance to fungicides that inhibit sterol biosynthesis might always be accompanied by decreased fitness (Fuchs and Drandarevski, 1976).

1.5 FITNESS OF RESISTANT STRAINS

Fitness of resistant strains of a pathogen is a term used to denote its virulence or competitive ability as compared to the other strains in the population under the same environment (Wade, 1982; Skylakakis, 1987). It can be estimated by growing mixtures of isolates or populations for many generations while monitoring the change in sensitivity (Jørgensen, 1988; Ulrich and Taehle-Csech, 1988).

Resistance to DMIs is often accompanied by poor germination and reduced growth which contribute to a lack of fitness and poor viability of the fungus (Fuchs and Prandarevski, 1976).

Many competition experiments, which test the relative ability of resistant strains to infect plants in the absence of fungicide, have been conducted on several pathogens (Gullino and Garibaldi, 1981; Buchenauer *et al.*, 1984; Schepers, 1985; Kadish and Cohen, 1988; Al-Mughrabi and Gray, 1993). Such experiments help determine whether resistant strains will become less fit and then disappear as a result of competition with the sensitive strains in the absence of the fungicide (Wade, 1982). The rate of increase of the resistant sub-population is reduced if it has to compete with the sensitive one for the occupation of a limited number of

available susceptible host sites (Skylakakis, 1980; Wolfe, 1982).

Sherpers (1985) found that EBI-resistant strains of *Sphaerotheca fuliginea* (powdery mildew of cucurbits) were more fit, and competed better than the sensitive ones and concluded that resistance is more likely to develop in practice. The competitive ability of mixtures of benomyl-resistant and sensitive strains of *S. fuliginea* (90:10, 50:50, 10:90) was tested in the absence of the fungicide. After five generations, the resistance had disappeared almost from the 90:10 mixture, and completely from the other mixtures (Dekker, 1976).

Phenylamide-resistant strains of *Phytophthora nicotianae*, on the other hand, were equally or even more fit than sensitive strains (Vigo *et al.*, 1986). Kadish and Cohen (1988) in Israel studied the competitive ability of metalaxyl-sensitive (MS) and -resistant (MR) isolates of *Phytophthora infestans* in the absence of metalaxyl. When mixtures of MR and MS (1:99, 10:90, 50:50) isolates were inoculated onto intact plants, all MR isolates exhibited a strong competitive ability: their proportion increased from 10 to 100% after eight to ten sporangial cycles. On the other hand, Dowely (1987) in Ireland found that MS isolates competed better, and were more fit than MR isolates of *P. infestans*. He concluded that this must account for the decrease in MR isolates from 75% of all isolates in 1981 to 6% in 1983.

Inoculating mixed cultures of strains of *Botrytis* sensitive and resistant (50:50, 10:90) to the dicarboximide fungicide vinclozolin on a fungicide-free medium for four generation cycles showed a disappearance of the resistant strain (Gullino and Garibaldi, 1981). Similar results were reported in Pennsylvania by Moorman and Lease (1992).

Repeated subculturing of a benomyl-resistant isolate of *Colletotrichum musae* on fungicide-free agar for 6 months in Britain did not change the degree of resistance (Griffee, 1973). In the case of kasugamycin resistance in *P. oryzae*, the rate of resistant strains decreased from 90% to 20% or less during three years after use of the antibiotic was discontinued (Miura and Takahashi, 1975). On the other hand, the rate of resistance of strains of *Cercospora beticola* to benzimidazole fungicides (Yamaguchi et al., 1976) did not decrease after the application of the fungicides was discontinued.

Different reports indicated that the competitive ability of DMI-resistant *Erysiphe graminis* f.sp. *tritici* and *hordei* isolates was inferior to that of the sensitive ones (Buchenauer *et al.*, 1984; Buchenauer and Hellwald, 1985). Isolates of *E. graminis*. f.sp. *tritici* and *hordei* with decreased sensitivity to triadimefon showed cross-resistance to other inhibitors of sterol C-14 demethylation, such as triadimenol, propiconazol, diclobutrazol, prochlorazol and nuarimol. The competitive abilities of resistant *hordei* and

tritici were inferior to those of the sensitive ones (Buchenauer and Hellwald, 1985). The degree of resistance in the mixture of sensitive and resistant isolates (50:50 ratio) to the triazole fungicides decreased from 50% to 32% after five transfers.

Buchenauer *et al.* (1984) found that the triadimeton resistant isolates of barley powdery mildew fungi were more sensitive to ethirimol than the sensitive ones. The results of their competition experiments of a mixture of triadimefon-resistant and -sensitive isolates (50:50) of *E. graminis f.sp. hordei* showed that in the absence of triadimefon the portion of the resistant isolates in the population decreased after five passages. Ethirimol- and tridemorph-resistant isolates of barley powdery mildew (Hollomon, 1975; Wamsley-Woodward *et al.*, 1979) also showed reduced competitive abilities compared to the sensitive pathogens in the absence of the fungicides.

If resistant strains are almost as fit as, or more fit than the sensitive ones in the absence of fungicide, then this favours the establishment of a resistant sub-population and the breakdown of disease control. In this case the fungicide has to be withdrawn from use (Ulrish and Staehle-Csech, 1988). On the other hand, if resistant strains are less fit than the sensitive ones, then, if the product ever had to be withdrawn because of resistance, there is a possibility that it could be used again later. In the latter case, strategies should be managed and implemented for coping with fungicide resistance

(Dekker, 1981; Wade, 1982; Gindrat and Forrer, 1985).

1.6 METHODS OF ASSESSMENT OF FUNGICIDE RESISTANCE IN POWDERY MILDEW

Most studies on fungicide resistance are based on the degree of inhibition of growth of isolates of the fungus on agar media containing known concentrations of fungicide (Brent, 1981). Toxicity assays are then done by determining the radial growth of the facultative parasite (Buchenauer, 1979; De Waard and Van Nistelrooy, 1990). The minimal inhibiting concentration (MIC) value, that is the lowest concentration producing total inhibition (Buchenauer, 1979; Barug and Kerkenccar, 1984; De Waard and Van Nistelrooy, 1990) or the EC_{50} value, that is the effective concentration for 50% fungal growth inhibition, is then determined.

However, powdery mildew fungi are obligate parasites, and hence other techniques, which are more difficult, have to be used (Brent, 1981). Assessment of the germination of cucumber powdery mildew (Bent, 1971) and barley powdery mildew spores (Buchenauer, 1979) was determined using cellulose-acetate membranes impregnated with the fungicide and placed in Petri dishes. Young conidia of both powdery mildew fungi were dusted onto the membranes. After a period of incubation the germination rate was determined and either EC_{50} or MIC, or both, was then calculated.

The amount of visible growth of powdery mildew fungi on

detached leaves or on leaf pieces inoculated with spores and treated with fungicide at various concentrations is the common basis of the assessment methods of several workers. Schroeder and Provvidenti (1969) used detached cucumber leaves, some of which were taken from plants grown in soil drenched with a standard amount of benomyl solution. Bent *et al.* (1971) used a similar method, in which cucumber-leaf discs were floated on dimethirimol solutions at six concentrations (0.052 - 5.0 μg mL^{-1}) in Petri dishes, inoculated with conidia and incubated for 7-10 days. Visible mildew on each disc was assessed on a simple 0-4 scale according to the proportion of its area covered by mildew.

Floating leaf pieces also can be used to examine isolates of barley powdery mildew fungi, although growth of this mildew is even less and harder to assess than that of cucumber mildew. There is more variation in infection from piece to piece, and curling and senescence of the leaf pieces can cause difficulties (Brent, 1981). To avoid curling and senescence of cereal leaves, Schulz *et al.* (1986) placed detached wheat leaves in agar containing 10 mg L^{1} of benzimidazole as an antisenescence compound. The leaves were sprayed with solutions containing various concentrations of triadimefon, then inoculated with conidia of the powdery mildew fungus, and the EC_{50} values determined after one week of incubation. A similar method was followed by Brown *et al.* (1991).

Another approach used by several workers is to treat whole

plants with fungicides at various concentrations, and to apply conidia of a particular isolate to replicates of treated plants (Rouse *et al.*, 1980; Fletcher and Wolfe, 1981; Schein *et al.*, 1984; Buchenauer and Hellwald, 1985; De Waard *et al.*, 1986; Brown and Wolfe, 1991).

A bait plant method (mobile nurseries), in which test plants seed treated with different concentrations of a fungicide are placed in the field for one or two days and incubated in the greenhouse, was used to determine fungicide resistance (Fletcher and Wolfe, 1981; Scheinpflug, 1988). In another method, wind-impelled spore traps (WIST mobile nurseries) were used (Fletcher and Wolfe, 1981; Wolfe, 1985; Scheinpflug, 1988). In this latter method, seed-treated plants or detached leaves in Petri dishes are placed within a spore trap mounted on a car. When the car is driven, the spores are sucked into the trap and distributed onto the leaves. The plants or detached leaves are then incubated in a greenhouse and a growth chamber, respectively.

Staub *et al.* in 1988 (unpublished research) suggested a tube-test method for the assessment of propiconazole sensitivity in cereal powdery mildew isolates. Test plants were grown from seeds in glass test tubes containing perlite and Hewitt solution. The seedlings (7-day old) were then sprayed with fungicide and after 24 h one or two pieces, 5-10 cm each, infected with powdery mildew were added to each tube of host plants. The tubes were plugged, slightly shaken to

disseminate the available conidia and then incubated. After 14 days, percentage of infected leaf surface on the first leaf was estimated and the EC_{50} values calculated. A similar method was reported by Smith and Bolton (1987), and Scheinpflug (1988).

In all cases, the percentage of leaf tissue infected with powdery mildew was evaluated according to James (1971), and the data then converted to EC_{50} and EC_{95} values (Finney, 1971; Buchenauer, 1979; Schulz and Scheinpflug, 1986; Urich and Ursula, 1988; Kadish and Cohen, 1989). The difference in sensitivity can be defined by the resistance factor, expressed as the ratio of EC_{50} of resistant strain to EC_{50} of sensitive strain (Köller and Scheinpflug, 1987; Lassero-De Falandre et al., 1991).

1.7 STRATEGIES TO AVOID AND MANAGE FUNGICIDE RESISTANCE

Horsfall in 1956 warned that "the development of resistance by fungi to more and more specialized fungicides is almost sure to come and plague the practical man. It would seem worthwhile for someone to undertake to investigate this aspect of the matter in some detail. It seems unwise to await the arrival of resistant strains in the field. There is ample warning they may come." The best method to avoid the development of fungicide resistance is to prevent resistant strains from emerging. To achieve this goal, strategies have been suggested by many scientists (Dekker, 1976; Bent, 1979;

Dekker, 1981; Gangawane and Saler, 1981; Useugi, 1983; Gindrat and Forrer, 1985; Wade, 1988). These spray regime strategies may include:

1.7.1 Mixtures

A single-site fungicide may be used with a multisite conventional fungicide or other single-site compound without a potential for cross-resistance with its partner. Even in mixtures, a systemic fungicide should not be used when it is not aimed at least at a pathogen which is present to a significant level in the crop. For example, the mixture of carbendazim and triadimefon should not be used against eyespot without the presence of critical levels of powdery mildew or rust.

1.7.2 Alternations

A single-site fungicide should be used alternately with one or more multisite fungicides, or with single-site fungicides without cross-resistance potential, even in mixtures. Resistant strains that may emerge for one product will be eliminated or reduced by the other before resistance can build up. The likelihood of resistance emerging to two or more products simultaneously is very low.

1.7.3 Combination of Mixtures and Alternations.

To ensure that the "at-risk" fungicide is protected, a

combination of mixtures of fungicides and alternating spray regimes may be needed.

1.7.4 Limiting the Number of Sprays per Season

Control can be obtained at low selection pressure (one or two sprays/season) allowing the sensitive form of the pathogen to compete with the resistant one. The selection pressure by a fungicide can be reduced by:

a. Restricting the application of resistance-prone fungicides to one or two critical periods in season.
b. Reducing the amount applied, and the frequency of application to the minimum necessary for economic control.
c. Limiting the area treated with any one fungicide.

1.7.5 Timing of Application and Suitability of Products

Treatment with "at-risk" fungicides after disease has become established is to be avoided since putting a product under excessive disease pressure may result in resistance occurrences because there will be a greater pathogen population from which to select.

1.7.6 Withdrawal of the Product

In cases where resistance to a class of products is too severe, it may be best to withdraw them from use in that particular situation.

1.7.7 Reentry of the Product

If the resistant strains are not fit in the absence of the fungicide, reentry of the product might be possible. This strategy must be implemented with caution, following monitoring studies to indicate when this may be worthwhile. The risk of reentry should be minimized by the use of mixtures and careful timing.

Chapter 2.

RESISTANCE OF ISOLATES OF ERYSIPHE GRAMINIS F.SP. TRITICI COLLECTED FROM THE ANNAPOLIS VALLEY AND COLCHESTER COUNTY OF NOVA SCOTIA TO TRIADIMEFON

2.1 INTRODUCTION

Agricultural practice of powdery mildew control in winter wheat in Nova Scotia usually includes one or two foliar spray applications of fungicides which inhibit ergosterol biosynthesis. The triazole fungicide triadimefon (Bayleton™) has been available on a temporary registration to the Maritime wheat growers for several years (Anonymous, 1991). Triazoles are members of the demethylation inhibitor (DMI) group of compounds, which inhibit the C-14 demethylation step in the synthesis of ergosterol (Dekker, 1985b).

Triadimefon is a systemic fungicide which is reputed to be a very good protectant and eradicant fungicide against powdery mildew and rust fungi (Clark *et al.*, 1978).

When triadimefon was first introduced it was active against the cereal powdery mildew fungus (*Erysiphe graminis*) at extremely small concentrations (Rawlinson *et al.*, 1982). Subsequently, less sensitive strains have arisen and are now widely distributed in the Netherlands (Wolfe 1981 & 1985), Hungary (Enisz, 1988), northern England (Fletcher and Griffin, 1981) and Germany (Buchenauer and Hellwald, 1985).

In Nova Scotia, most wheat production is concentrated in the Annapolis Valley (AV) where the fungicide triadimefon has been used intensively compared to Colchester County (CC). This has led us to suggest that the populations of *Erysiphe graminis* f.sp. *tritici* from the Annapolis Valley are more resistant to triadimefon than are those from Colchester County. This study was undertaken to investigate this hypothesis.

2.2 MATERIALS AND METHODS

2.2.1 Collection and Transport of Samples

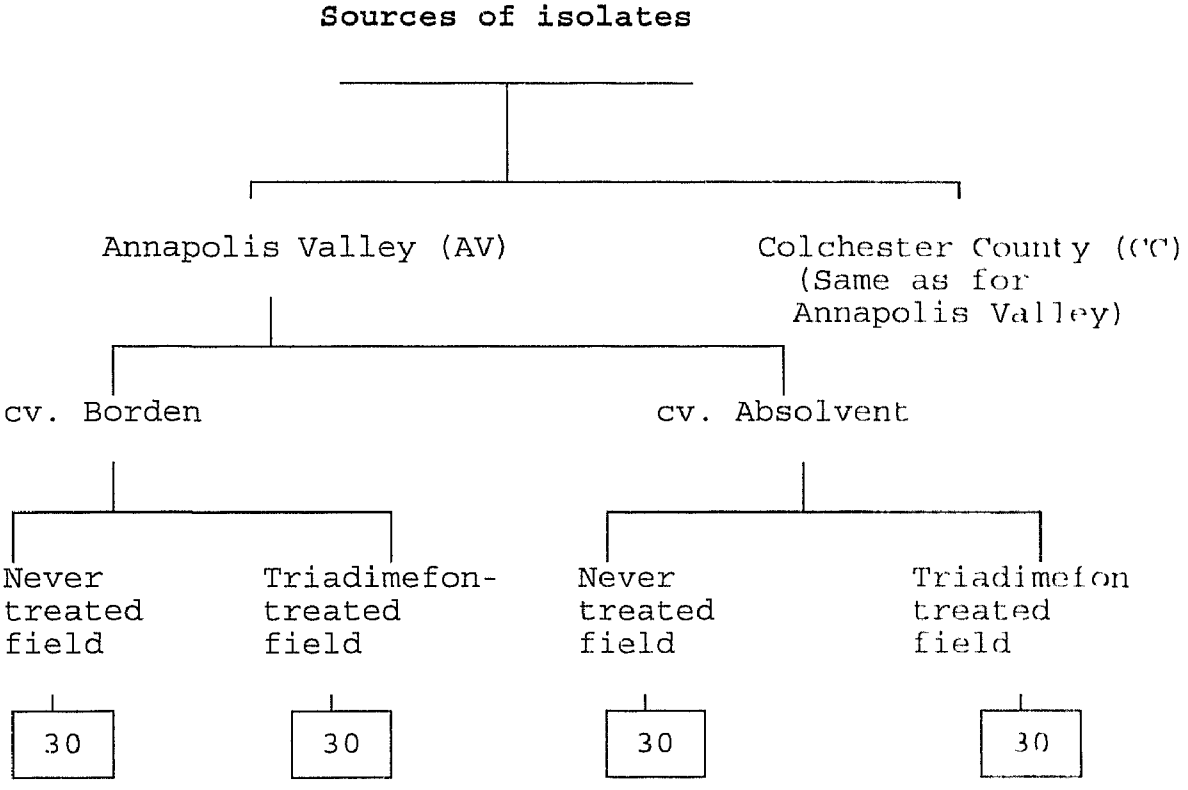
Plants and leaves with freshly sporulating pustules of *E. graminis* f.sp. *tritici* were collected in plastic bags from wheat fields (cvs. Borden and Absolvent) with and without a history of fungicide treatment, in both the Annapolis Valley (AV) and Colchester County (CC) areas (Fig. 2.1). Samples were brought to the laboratory and either transferred immediately to host plants growing in test tubes or kept at 4°C until the next day.

2.2.2 Host Plants

Test plants of two winter wheat cultivars (cvs. Borden and Absolvent) were grown in 25 × 250 mm glass test tubes with 25-mm size plastic closures. Each test tube was filled with 20 mL of perlite and 10 mL of Hoagland's solution (Dhingra and Sinclair, 1985). Fungicide-free wheat seeds were surface

Fig. 2.1 Description of sources of *Erysiphe graminis* f.sp. *tritici* isolates from winter wheat that were tested in the 1991 study.

Statistical Design: 2×2×2 Factorial Arrangement in a Complete-Randomized Design



† Thirty individual isolates.

disinfested by submerging them in 0.6% NaClO for 10 minutes then rinsing them in sterile distilled water for 10 minutes. After 12 h pregermination in sterile distilled water, the seeds were transferred to the tubes by placing five pregerminated seeds on the surface of the perlite in each tube. The tubes were then incubated in a growth chamber at 18°C and 12 h day light at 293-390 microensteis/m^2/sec. During the night, the temperature was lowered to 15°C. Tubes were kept in holders. Plants were ready for inoculation as soon as the first leaf was fully expanded (about 7 days).

2.2.3 Preparation of Inoculum

Powdery mildew isolates from a field of wheat (cv. Absolvent or Borden) were maintained on seedlings of the same cultivar. For each population of the fungus, the inoculum was prepared by inoculating 30 host plants in test tubes. Small sections of leaf, each bearing a single pustule, and selected at random, were cut from the seedlings and added to each tube. The tubes were plugged and shaken on a rotary shaker to disseminate the available conidia. Test tubes were then incubated as described in section 2.2.2.

The inoculum was ready to be used in a sensitivity test as soon as sporulating pustules occurred on the seedlings (approximately 14 days after inoculation). Old spores were dislodged from the leaves by shaking the tubes one day before inoculating the sensitivity test. Fresh sporulation was

normally produced within 24 h (Schein *et al.*, 1984).

2.2.4 Maintenance of the Isolates

Powdery mildew isolates were maintained on disease free wheat seedlings (cv. Borden or Absolvent) in tubes as described in the previous section. Subculturing of isolates onto disease-free seedlings was carried out every 2-3 weeks. In order to increase the amount of inoculum produced by single pustules, each isolate was subcultured onto host plants in two test tubes.

2.2.5 Fungicide Preparation

Foliar spray tests were carried out with the formulated product Bayleton® 50% WP (a.i. triadimefon). Bayleton suspensions (1 mg a.i. mL^{-1}) were freshly prepared in Hoagland's solution and diluted with a suspension of blank formulation of Bayleton so that each concentration (including the control) contained the same amount of the blank formulation. The concentration range adopted for concentration-response tests was 0, 0.1, 1.0, 10.0, and 100 μg a.i. mL^{-1}. Resistant isolates were expected to tolerate concentrations of fungicide 10 to 100-fold greater than those tolerated by sensitive ones (Georgopoulos and Dovas, 1973).

2.2.6 Application of the Fungicide

After the first leaf was fully expanded in the test tubes

(7 days), the fungicide was sprayed with an atomizer operated by compressed air. The plants were then left for 24 h in order to dry before inoculation with the fungus.

To ensure coverage of a large surface area of wheat leaves with the fungicide, a fluorescent dye was added to the Hoagland's solution before spraying the seedlings in test tubes using the same atomizer. After 24 h, seedlings were pulled out of tubes and checked in darkness under UV light. This experiment was conducted only once prior to the use of the sprayer in the experiments.

2.2.7 Sensitivity Tests

Infected wheat seedlings were cut into leaf sections of 3-5 cm. Two leaf sections, each with 3-5 similar-sized mildew pustules, were added to each tube of the host seedlings grown as described previously. For one test series with the concentrations 0, 0.1, 1.0, 10.0, and 100 μg a.i. mL^{-1} (5 tubes x 5 seedling x 2 replicates), two test tubes of single pustule-derived inoculum were needed for inoculation. After inoculation, tubes were capped, shaken and incubated at the conditions described previously.

2.2.8 Data Collection and Statistical Analysis

For each isolate, mean percent of surface area covered with powdery mildew on the primary leaf of five seedlings per test tube (2 tubes/isolate) treated with various

concentrations of triadimefon was estimated using standard area diagrams (James, 1971). Values estimated for treatments were expressed as percentages of the control. Average percentages of two replicates for each isolate were transformed to probits, and EC_{50} values were calculated by linear regression (i.e. probit analysis) from the concentration-response curves (Finney, 1971). Analyses were made with the Statistical Analysis System (SAS Institute, Inc., 1983).

The differences in sensitivity of powdery mildew populations from two locations (the Annapolis Valley and Colchester County), two treatments (treated and untreated fields) and two cultivars (Absolvent and Borden), were then compared based on the EC_{50} values. A 2×2×2 factorial arrangement in a complete-randomized design analysis was performed using the Analysis of Variance (ANOVA) Procedure. A log-transformation step was applied to all EC_{50} values (SAS Institute, Inc., 1983).

Frequencies of resistant isolates of *E. graminis* f.sp. *tritici* to triadimefon (expressed as a percentage of the number of isolates tested) were compared using Fisher's exact test (SAS Institute, Inc., 1983) for each location, treatment, and cultivar.

2.3. RESULTS

Using an atomizer operated by compressed air to spray wheat seedlings in test tubes with a fluorescent dye solution gave almost a complete coverage of leaves with the spray solution. This experiment was conducted in order to test the efficacy of the sprayer in distributing the spray solution to cover a large area of leaf surface. This procedure was conducted only once prior to using the sprayer in the experiments.

The EC_{50} value of each of the thirty individual isolates in each population was calculated using the Probit Analysis procedure. Isolates within each population were ranked as either resistant (R) or sensitive (S) to the fungicide triadimefon based on the EC_{50} value of the isolate. Isolates with EC_{50} value < 10 μg triadimefon mL^{-1} were considered sensitive (S), while those with EC_{50} value ≥ 10 μg triadimefon mL^{-1} were considered resistant (R).

2.3.1 Reaction of *E. graminis* f.sp. *tritici* Isolates to the Fungicide Triadimefon.

2.3.1.1 Isolates collected from the AV wheat fields.

The EC_{50} values of the 30 isolates collected from treated fields of the wheat cv. Absolvent ranged from 0.5 to 175.2 μg triadimefon mL^{-1} (Table 2.1), with a mean EC_{50} value of 44.6 ± 5.7 μg triadimefon mL^{-1}. Twenty (66.7%) of these 30 isolates were resistant to the fungicide triadimefon (Table 2.1).

Table 2.1. Effective concentration of triadimefon to prevent growth of 50% (EC_{50}) of each of 30 *Erysiphe graminis f.sp. tritici* isolates collected in 1991 from treated fields of the wheat cultivar Absolvent in the Annapolis Valley.

Isolate No.	EC_{50} Value ($\mu g\ mL^{-1}$)	Reaction*
01	47.3	R
02	113.2	R
03	1.1	S
04	39.8	R
05	51.0	R
06	71.3	R
07	10.9	R
08	1.4	S
09	2.2	S
10	10.4	R
11	70.0	R
12	64.8	R
13	0.5	S
14	10.0	R
15	32.8	R
16	31.5	R
17	1.6	S
18	151.6	R
19	71.5	R
20	175.2	R
21	133.2	R
22	3.0	S
23	0.9	S
24	119.0	R
25	33.0	R
26	0.6	S
27	3.2	S
28	69.8	R
29	1.0	S
30	15.1	R
Mean EC_{50} ± SE:	44.6 ± 5.7	
Frequency of Resistant Isolates (%):		66.7

* S = sensitive (EC_{50} < 10 $\mu g\ mL^{-1}$); and R = resistant (EC_{50} > 10 $\mu g\ mL^{-1}$).

On the other hand, the EC_{50} values of the 30 isolates collected from untreated fields of the same cultivar ranged from 0.5 to 270.7 μg triadimefon mL^{-1} (Table 2.2), with a mean EC_{50} value of 34.7 ± 5.7 μg triadimefon mL^{-1}. Seventeen (56.7%) of these 30 isolates were resistant to triadimefon (Table 2.2).

Table 2.3 presents the EC_{50} values of the 30 isolates collected from treated fields of the wheat cv. Borden. The EC_{50} values ranged from 0.1 to 100 μg triadimefon mL^{-1}, with a mean EC_{50} value of 19.2 ± 5.7 μg triadimefon mL^{-1}. Fourteen (46.7%) of these 30 isolates were resistant to triadimefon (Table 2.3). Meanwhile, the EC_{50} values of the 30 isolates collected from the untreated fields of the same cultivar ranged from 0.2 to 70.5 μg triadimefon mL^{-1} (Table 2.4), with a mean EC_{50} value of 13.0 ± 5.701 μg triadimefon mL^{-1}. Eight (26.7%) of the 30 isolates were resistant to triadimefon (Table 2.4).

2.3.1.2 Isolates collected from CC wheat fields.

Table 2.5 presents the EC_{50} values of the 30 individual isolates collected from treated fields of the wheat cv. Absolvent. The EC_{50} values ranged from 0.1 to 95 μg triadimefon mL^{1}, with a mean EC_{50} value of 18.7 ± 5.7 μg triadimefon mL^{-1}. Sixteen (53.3%) of these 30 isolates were resistant to triadimefon (Table 2.5). For untreated fields of the same cultivar, the EC_{50} values ranged from 0.4 to 31.5 μg triadimefon mL^{-1} (Table 2.6), with a mean EC_{50} value of 6.2 ± 5.7 μg triadimefon mL^{-1}. Only four (13.3%) of these 30 isolates

Table 2.2. Effective concentration of triadimefon to prevent growth of 50% (EC_{50}) of each of 30 *Erysiphe graminis f.sp. tritici* isolates collected in 1991 from untreated fields of the wheat cultivar Absolvent in the Annapolis Valley.

Isolate No.	EC_{50} Value ($\mu g\ mL^{-1}$)	Reaction*
01	16.0	R
02	3.2	S
03	6.8	S
04	12.6	R
05	16.8	R
06	31.5	R
07	117.2	R
08	69.3	R
09	13.0	R
10	2.4	S
11	21.6	R
12	27.3	R
13	4.6	S
14	11.7	R
15	65.4	R
16	2.1	S
17	1.0	S
18	6.4	S
19	180.8	R
20	5.0	S
21	56.4	R
22	6.2	S
23	9.8	S
24	11.1	R
25	270.7	R
26	3.2	S
27	2.1	S
28	19.2	R
29	0.5	S
30	47.0	R
Mean EC_{50} ± SE:	34.7 ± 5.7	
Frequency of Resistant Isolates (%):		56.7

* S = sensitive (EC_{50} < 10 $\mu g\ mL^{-1}$); and R = resistant (EC_{50} > 10 $\mu g\ mL^{-1}$).

Table 2.3. Effective concentration of triadimefon to prevent growth of 50% (EC_{50}) of each of 30 *Erysiphe graminis f.sp. tritici* isolates collected in 1991 from treated fields of the wheat cultivar Borden in the Annapolis Valley.

Isolate No.	EC_{50} Value ($\mu g\ mL^{-1}$)	Reaction*
01	9.8	S
02	31.5	R
03	11.0	R
04	0.4	S
05	13.2	R
06	61.7	R
07	4.6	S
08	31.6	R
09	10.2	R
10	65.6	R
11	2.4	S
12	1.8	S
13	1.6	S
14	100.0	R
15	32.9	R
16	10.9	R
17	61.2	R
18	3.7	S
19	0.1	S
20	68.5	R
21	10.3	R
22	2.9	S
23	3.8	S
24	0.3	S
25	18.6	R
26	0.6	S
27	7.6	S
28	0.7	S
29	2.0	S
30	5.1	S
Mean EC_{50} ± SE:	19.2 ± 5.7	
Frequency of Resistant Isolates (%):		46.7

* S = sensitive (EC_{50} < 10 $\mu g\ mL^{-1}$); and R = resistant (EC_{50} ≥ 10 $\mu g\ mL^{-1}$).

Table 2.4. Effective concentration of triadimefon to prevent growth of 50% (EC_{50}) of each of 30 *Erysiphe graminis f.sp. tritici* isolates collected in 1991 from untreated fields of the wheat cultivar Borden in the Annapolis Valley.

Isolate No.	EC_{50} Value ($\mu g\ mL^{-1}$)	Reaction*
01	6.3	S
02	8.2	S
03	0.9	S
04	6.1	S
05	6.4	S
06	2.9	S
07	70.5	R
08	18.4	R
09	59.8	R
10	31.5	R
11	8.3	S
12	7.6	S
13	0.3	S
14	9.0	S
15	20.3	R
16	0.2	S
17	4.1	S
18	7.7	S
19	31.5	R
20	6.6	S
21	1.3	S
22	9.7	S
23	4.4	S
24	8.7	S
25	5.9	S
26	22.5	R
27	10.2	R
28	9.3	S
29	1.0	S
30	9.2	S
Mean EC_{50} ± SE:	13.0 ± 5.7	
Frequency of Resistant Isolates (%):		26.7

* S = sensitive (EC_{50} < 10 $\mu g\ mL^{-1}$); and R = resistant (EC_{50} > 10 $\mu g\ mL^{-1}$).

Table 2.5. Effective concentration of triadimefon to prevent growth of 50% (EC_{50}) of each of 30 *Erysiphe graminis f.sp. tritici* isolates collected in 1991 from treated fields of the wheat cultivar Absolvent in the Colchester County.

Isolate No.	EC_{50} Value ($\mu g\ mL^{-1}$)	Reaction*
01	6.7	S
02	2.0	S
03	0.5	S
04	3.8	S
05	10.8	R
06	61.2	R
07	34.6	R
08	3.8	S
09	2.9	S
10	9.8	S
11	1.0	S
12	4.1	S
13	31.5	R
14	31.5	R
15	95.0	R
16	13.0	R
17	8.8	S
18	70.3	R
19	10.2	R
20	7.3	S
21	0.1	S
22	15.1	R
23	13.1	R
24	4.1	S
25	31.5	R
26	0.9	S
27	31.5	R
28	10.4	R
29	31.5	R
30	12.4	R
Mean EC_{50} ± SE:	18.6 ± 5.7	
Frequency of Resistant Isolates (%):		53.3

* S = sensitive (EC_{50} < 10 $\mu g\ mL^{-1}$); and R = resistant (EC_{50} ≥ 10 $\mu g\ mL^{-1}$).

Table 2.6. Effective concentration of triadimefon to prevent growth of 50% (EC_{50}) of each of 30 *Erysiphe graminis f.sp. tritici* isolates collected in 1991 from untreated fields of the wheat cultivar Absolvent in the Colchester County.

Isolate No.	EC_{50} Value ($\mu g\ mL^{-1}$)	Reaction*
01	6.6	S
02	7.1	S
03	7.3	S
04	1.7	S
05	13.2	R
06	1.0	S
07	0.9	S
08	3.4	S
09	4.4	S
10	31.5	R
11	1.9	S
12	0.5	S
13	2.0	S
14	7.2	S
15	0.4	S
16	7.8	S
17	1.6	S
18	1.1	S
19	1.4	S
20	6.7	S
21	2.5	S
22	1.1	S
23	8.7	S
24	2.3	S
25	3.9	S
26	31.5	R
27	1.8	S
28	8.5	S
29	10.2	R
30	8.5	S
Mean EC_{50} ± SE:	6.2 ± 5.7	
Frequency of Resistant Isolates (%):		13.3

* S = sensitive (EC_{50} < 10 $\mu g\ mL^{-1}$); and R = resistant (EC_{50} ≥ 10 $\mu g\ mL^{-1}$).

were resistant to triadimefon (Table 2.6). For the 30 isolates collected from treated wheat fields of the wheat cv. Borden, the EC_{50} values ranged from 0.1 to 31.7 μg triadimefon mL^{-1} (Table 2.7) with a mean of 11.1 ± 5.7 μg triadimefon mL^{-1}. Thirteen (40%) of these 30 isolates were resistant to triadimefon (Table 2.7). On the other hand, the EC_{50} values of the 30 isolates collected from untreated fields of the same wheat cultivar ranged from 0.1 to 31.5 μg triadimefon mL^{-1} (Table 2.8), with a mean EC_{50} value of 4.6 ± 5.7 μg triadimefon mL^{-1}. Only three (10%) of these 30 isolates were resistant to triadimefon (Table 2.8).

2.3.2. Comparison Between Locations, Treatments, Cultivars, and their Interactions with Regards to Log(EC_{50}) Values of Isolates of *E. graminis* f.sp. *tritici*.

Results of the ANOVA performed on log(EC_{50}) values are presented in Table 2.9. There was a significant difference among cultivars ($P=3\times10^{-5}$) in their effect on the log(EC_{50}) values of powdery mildew fungal populations (Table 2.9). The mean value of log(EC_{50}) for isolates collected from fields of the wheat cultivar Absolvent was higher (2.14 ± 0.16 μg triadimefon mL^{-1}) than that of those collected from fields of the wheat cultivar Borden (1.21 ± 0.16 μg triadimefon mL^{-1}) (Table 2.10). Results of the ANOVA (Table 2.9) demonstrated

Table 2.7. Effective concentration of triadimeton to prevent growth of 50% (EC_{50}) of each of 30 *Erysiphe graminis f.sp. tritici* isolates collected in 1991 from treated fields of the wheat cultivar Borden in the Colchester County.

Isolate No.	EC_{50} Value ($\mu g\ mL^{-1}$)	Reaction*
01	31.5	R
02	2.1	S
03	0.4	S
04	0.3	S
05	10.2	R
06	2.8	S
07	6.6	S
08	0.1	S
09	31.5	R
10	3.1	S
11	1.0	S
12	31.5	R
13	2.5	S
14	0.1	S
15	1.5	S
16	3.2	S
17	31.5	R
18	0.4	S
19	31.5	R
20	11.8	R
21	8.9	S
22	29.5	R
23	15.0	R
24	15.2	R
25	5.9	S
26	0.4	S
27	2.4	S
28	10.1	R
29	31.7	R
30	11.0	R
Mean EC_{50} ± SE:	11.1 ± 5.7	
Frequency of Resistant Isolates (%):		40.0

* S = sensitive (EC_{50} < 10 $\mu g\ mL^{-1}$); and R = resistant (EC_{50} > 10 $\mu g\ mL^{-1}$).

Table 2.8. Effective concentration of triadimefon to prevent growth of 50% (EC_{50}) of each of 30 *Erysiphe graminis f.sp. tritici* isolates collected in 1991 from untreated fields of the wheat cultivar Borden in the Colchester County.

Isolate No.	EC_{50} Value ($\mu g\ mL^{-1}$)	Reaction*
01	3.2	S
02	3.2	S
03	31.5	R
04	0.1	S
05	0.1	S
06	0.1	S
07	1.0	S
08	0.2	S
09	3.0	S
10	5.9	S
11	31.5	R
12	0.1	S
13	0.1	S
14	4.1	S
15	1.1	S
16	0.9	S
17	6.7	S
18	0.1	S
19	1.0	S
20	3.2	S
21	0.3	S
22	0.4	S
23	0.4	S
24	31.5	R
25	0.3	S
26	1.0	S
27	0.3	S
28	6.7	S
29	0.3	S
30	1.1	S
Mean EC_{50} ± SE:	4.6 ± 5.7	
Frequency of Resistant Isolates (%):		10.0

* S = sensitive (EC_{50} < 10 $\mu g\ mL^{-1}$); and R = resistant (EC_{50} ≥ 10 $\mu g\ mL^{-1}$).

Table 2.9. Analysis of Variance (ANOVA) for log(EC_{50}) values of *Erysiphe graminis f.sp. tritici* isolates collected in 1991 from two locations, two treatments, and two wheat cultivars.

Source of Variation	DF	Mean Square	Pr > F
Location	1	70.031	2×10^{6}
Treatment	1	22.824	0.0054
Cultivar	1	52.201	3×10^{5}
Location × Treatment	1	19.903	0.0093
Location × Cultivar	1	1.947	0.4128
Treatment × Cultivar	1	0.604	0.6480
Location × Treatment × Cultivar	1	2.963	0.3125
ERROR	232	2.892	

Table 2.10. Mean log(EC_{50}) values and their standard error for *E. graminis* f.sp. *tritici* populations of isolates collected in 1991 from two wheat cultivars.

Variable*	log(EC_{50})** Mean
CULTIVAR	
Absolvent	2.14
Borden	1.21
Standard Error = 0.16	

* Refer to the ANOVA table (Table 2.10).

** Mean value of log(EC_{50}) of 120 individual isolates of *E. graminis* f.sp. *tritici* per component per variable.

that there was a significant interaction ($P=0.0094$) between locations and treatments in their effect on the $\log(EC_{50})$ values of populations of *E. graminis* f.sp. *tritici*. Results of the LSD test (Table 2.11) showed that the mean value of $\log(EC_{50})$ for isolates collected from the AV-treated fields was significantly higher (2.23 ± 0.22 μg triadimefon mL^{-1}) than that of those collected from the CC-treated fields (1.73 ± 0.22 μg triadimefon mL^{-1}). A similar trend was found in the case of isolates collected from untreated fields of both locations. For the AV's fungal populations, the mean value of $\log(EC_{50})$ was significantly higher (2.19 ± 0.22 μg triadimefon mL^{-1}) than that of those from CC (0.54 ± 0.22 μg triadimefon mL^{-1}). The mean value of $\log(EC_{50})$ for isolates from treated fields in CC was significantly higher (1.73 ± 0.22 μg triadimefon mL^{-1}) than that of those from untreated fields (0.54 ± 0.22 μg triadimefon mL^{-1}). No significant difference in the mean values of $\log(EC_{50})$ was found between powdery mildew populations from treated and untreated fields of the AV.

No significant interaction was found between location × cultivar, cultivar × treatment, or location × cultivar × treatment (Table 2.9).

Table 2.11. Mean log(EC_{50}) values and their standard error for location x treatment interaction.

Interaction Variable	log(EC_{50})* Mean
Annapolis Valley x Treated Fields	2.23a**
Annapolis Valley x Untreated Fields	2.19a
Colchester County x Treated Fields	1.73b
Colchester County x Untreated Fields	0.54c
Standard Error = 0.22	

* Mean value of log(EC_{50}) of 60 individual isolates of *E. graminis* f.sp. *tritici* per interaction variable.

** Means followed by the same letter are not significantly different from each other at *P*= 0.05 according to LSD test.

2.3.3. Comparison Between Locations, Treatments, and Cultivars Based on the Frequencies of Resistant Isolates of *E. graminis* f.sp. *tritici*

Fisher's exact test was performed to test if there is a significant difference in the frequency of resistant isolates of *E. graminis* f.sp. *tritici* from the two locations, the AV and CC. There was a highly significant difference (P=0.0023) in the frequency of resistant isolates collected from the AV and CC (Table 2.12). The frequency of resistant isolates collected from the AV was higher (49.2%) than that of those collected from CC (29.2%).

Results of the same test performed on the frequency of resistant isolates of *E. graminis* f.sp. *tritici* from treated and untreated wheat fields (Table 2.12) indicated that there was a highly significant difference (P=0.0001) in the frequency of resistant isolates collected from treated and untreated fields. Treated fields had a higher frequency (51.7%) of resistant isolates compared to untreated fields (26.7%) (Table 2.12). Similarly, Fisher's exact test was also performed to test if the frequency of resistant isolates collected from fields of the wheat cultivar Absolvent was significantly different from that of those collected from fields of cultivar Borden. Results (Table 2.12) revealed that there was a significant difference (P=0.012) in the frequency of resistant isolates collected from fields of the wheat

Table 2.12. Comparisons between the frequencies of resistant isolates of *E. graminis* f.sp. *tritici* for two locations, two treatments, and two wheat cultivars using Fisher's exact test.

Source of Isolates	No. of Isolates Tested	No. of Resistant Isolates	Frequency of Resistant Isolates (%)	*P* Value
Annapolis Valley	120	59	49.2	
vs.				0.0023
Colchester County	120	35	29.2	
Treated Fields	120	62	51.7	
vs.				0.0001
Untreated Fields	120	32	26.7	
Cultivar Absolvent	120	57	47.5	
vs.				0.0120
Cultivar Borden	120	37	30.8	

cultivars Absolvent and Borden. A significantly higher frequency of *E. graminis* f.sp. *tritici* isolates that tested resistant to triadimefon came from fields of cultivar Absolvent (47.5%). Only 30.8% of the isolates collected from fields of cultivar Borden were resistant to triadimefon (Table 2.12).

2.4. DISCUSSION

Results (Tables 2.1 - 2.8) showed that isolates within each population fell within a wide range of EC_{50} values. Some of these single spore-derived isolates had EC_{50} values < 1 μg triadimefon mL^{-1}. A few others had EC_{50} values > 100 μg triadimefon mL^{-1}; the rest were in between. These results suggest that isolates within each of the 8 populations (Tables 2.1 - 2.8) varied from each other in terms of their response to the fungicide triadimefon. The distribution of sensitive and resistant isolates was not uniform among populations. Isolates were ranked as either sensitive (S) or resistant (R) to triadimefon based on their EC_{50} values. When responses of all single spore-derived isolates of *E. graminis* f.sp. *tritici* were tested over a range of concentrations (0, 0.1, 1, 10, and 100 μg triadimefon mL^{-1}), the difference in response of isolates to the fungicide was best shown at 10 μg triadimefon mL^{-1}. Therefore, for this reason, and based on the EC_{50} values of all isolates in all fungal populations, isolates with EC_{50} values < 10 μg triadimefon mL^{-1} were considered sensitive (S),

and those with EC_{50} values ≥ 10 μg triadimefon mL^{-1} were considered resistant (R).

A log-transformation step was applied to all EC_{50} values prior to performing the statistical analysis in order to ensure a better normality of distribution of isolates within populations.

The significant interaction between location and treatment in the mean values of $\log(EC_{50})$ for *E. graminis* f.sp. *tritici* isolates (Tables 2.9 & 2.11) indicated that fungal populations from the CC-treated fields were more resistant to the fungicide triadimefon than those from untreated fields. On the other hand, no significant difference in the mean values of $\log(EC_{50})$ was found between powdery mildew fungal populations from the AV-treated fields and those from untreated fields (Tables 2.9 & 2.11). These results met our speculations about the differences in the sensitivity of powdery mildew populations from wheat fields in both locations.

Normally, one single application of the fungicide triadimefon at the onset of infection is sufficient to stop disease progress (Kolbe, 1976). In 1989, however, even a third triadimefon application in some fields in the AV failed to control the disease (Gray, 1991). Results of this study explain the poorer performance of triadimefon towards controlling powdery mildew disease in some wheat fields in the AV.

The significantly higher mean value of $\log(EC_{50})$, and higher frequency of resistant isolates associated with powdery mildew populations from the AV is most likely to have been a response to the continuing widespread use of triadimefon fungicide by the AV wheat growers, and due to the greater area of wheat fields, compared to CC. In CC wheat cultivation is limited, and thus the fungicide use is also limited. Similar results were reported in different crops and locations throughout the world (Butters *et al.*, 1984; Heany *et al.*, 1984 & 1986; Wolfe, 1984; De Waard *et al.*, 1986; Wolfe and De Waard, 1986; Andrivon and De Vallavieille-pope, 1987; Fletcher *et al.*, 1987; Limpert, 1987; Enisz, 1988). All these reports indicated that a continued selection pressure of fungicides in space and time selected for significantly higher degrees of resistance.

In Nova Scotia, the triazole fungicides triadimefon and propiconazole have been available to the wheat growers for several years (Anonymous, 1991). These fungicides are single site inhibitors, and therefore resistance was more likely to have occurred toward them soon after their introduction and use in a wide scale and over a greater area of wheat fields such as in the AV. Only one fungal gene is sufficient to induce a change at the target site leading to a decreased binding affinity of the fungicide. This results in the emergence of isolates less sensitive to the fungicide (Corbett, 1979; Delp, 1980; Dekker, 1985b; Köller and Scheinpflug, 1987).

Erysiphe graminis f.sp. *tritici* populations collected from treated wheat fields in the AV and CC had a significantly higher frequency of resistant isolates (Table 2.12) than those collected from untreated wheat fields. Exposing powdery mildew populations to a continued selection pressure of triadimefon may have readily selected for significantly higher degrees of resistance in terms of the mean value of $\log(EC_{50})$ and frequency of resistant isolates.

Resistant strains of *E. graminis* f.sp. *tritici* might have existed in wheat fields in low frequencies before the introduction of triadimefon to the area. After a short period of intensive and repeated use of the fungicide, the frequency of resistant strains is expected to have increased in treated fields. When the mutants of the pathogen arose that were little affected by the fungicide, farmers complained that triadimefon was no longer effective. Wheat growers who saw that triadimefon was becoming less effective often increased the concentration, thus increasing the selection pressure and aggravating the problem.

Our results are in agreement with those of Shephard *et al.* (1975). They reported that in each survey, samples of barley powdery mildew from ethirimol treated fields were generally more resistant than samples from nearby untreated fields. Similar results were reported by Fletcher and Wolfe (1981) who studied the fungicide triadimefon in barley fields.

Elad (1992) reported that the frequencies of isolates of *Botrytis cinerea* insensitive to the fungicides fenetrazole and fenthanil (SBIs) were 3.4 and 1.8 times higher, respectively, at the site where control failed, compared with another site where SBI fungicides had never been applied to control gray mould. On the other hand, Kendall *et al.* (1993) in England found that isolates of *Rhynchosporium secalis* from DMI treated and untreated barley were not significantly different in their sensitivity.

The significant difference in the mean values of $\log(EC_{50})$ (Tables 2.9 & 2.10) and in the frequency of resistant isolates (Table 2.11) for *E. graminis* f.sp. *tritici* populations associated with the two wheat cultivars indicated that the wheat cv. Absolvent was more favourable for the development of resistant populations of the fungus. Absolvent is known to be susceptible to powdery mildew, while Borden is moderately resistant to this fungus (Anonymous, 1991).

It is possible that the higher resistance of the fungal populations and the higher frequency of resistant isolates associated with Absolvent are a response to the intensive usage of triadimefon to control the disease. Meanwhile, the moderately resistant Borden requires fewer applications of triadimefon in order to control powdery mildew and obtain an economic yield. It is recommended to use the cv. Borden since it is more resistant to resistant populations of the fungus. These results agree with those of Fletcher and Wolfe (1981)

who reported that the frequency of insensitive isolates of powdery mildew of barley was higher on the highly susceptible cv. Golden Promise, and lower on the highly resistant cvs. Triumph and Atem.

However, there may also be more specific interactions between a particular pathogen population and fungicide resistance. Such effects could be of considerable value in strategies for cultivar and fungicide use aimed at preserving the effectiveness of both. Therefore, possible relationships between cultivars and fungicide resistance deserve further study.

Significant interaction between locations and treatments (Tables 2.9 & 2.11) indicated that powdery mildew populations from both treated and untreated wheat fields in the AV had a similar response to triadimefon. These results suggest that the resistant isolates have spread primarily within the treated fields. It is, however, possible that the resistant spores have moved over wide areas in extended periods and spread throughout the AV. On the other hand, fungal populations from treated and untreated fields in CC were significantly different in their response to the fungicide (Table 2.11). The absence of the selection pressure of the fungicide in the untreated fields in CC resulted in fewer resistant individuals. Due to limited wheat cultivation in CC, the resistant spores of the fungus from the treated fields

were unable to survive over extended periods, which in turn, prevented their spread.

The AV is about 100 km apart from CC. Therefore, the possibility that triadimefon-resistant spores from the AV would move to CC is unlikely. Powdery mildew spores are very sensitive to UV light and adverse environmental conditions. Such unfavourable conditions are able to destroy these spores before they reach their host.

Fungal populations from treated fields in the AV were more resistant (Table 2.11) than those from treated fields in CC. Similar results were found for populations from untreated fields in both locations. This is due to the widespread use of triadimefon over a greater area in the AV compared to CC.

Studies using wheat differentials are needed to determine what physiological races are present in the resistant and sensitive subpopulations.

Chapter 3.

BUILD-UP OF RESISTANT ISOLATES OF ERYSIPHE GRAMINIS F.SP.TRITICI TO TRIADIMEFON

3.1 INTRODUCTION

In a fungal population that is originally sensitive to a particular fungicide, resistant forms may arise, or be present at low frequency (Wolfe, 1975; Dekker, 1976 & 1985; Georgopoulos, 1977). Growth and reproduction of these resistant forms will be favoured by the selection pressure of the fungicide, so that finally the entire pathogen population may become resistant (Wolfe, 1975; Dekker, 1985).

In cereal powdery mildew, shifts in sensitivity to DMIs have been recorded, although on these crops correlation between poor control and reduced sensitivity has not been established (Fletcher and Wolfe, 1981).

In this study, we expect that the resistant isolates might be present at low frequency in the field during winter. In early spring, the resistant isolates are expected to dominate the sensitive ones a certain time after fungicide application, a process known as resistance build-up (Wolfe, 1985). We also expect that there is a relationship between the severity of powdery mildew disease and the build-up of resistant isolates. This study was undertaken in order to investigate these hypotheses.

3.2 MATERIALS AND METHODS

3.2.1 Build-up of Resistance

Based on the results of the year 1991, the wheat cultivar Absolvent is more favourable for the development of resistant isolates of *E. graminis* f.sp. *tritici*, in the sense that higher mean log(EC_{50}) values, and higher frequencies of resistant isolates to the fungicide triadimefon, were found for samples collected from Absolvent fields in both of the AV and CC compared to the cultivar Borden. Therefore, the cultivar Absolvent was selected to be the emphasis of this study.

Samples were collected from Absolvent wheat fields in both the AV and CC. Thirty individual isolates were collected three times from each location: before triadimefon application, two weeks after triadimefon application, and six weeks after treatment. Materials and methods used to determine the EC_{50} values and frequency of resistant isolates in this experiment were the same as those described in chapter 2.

3.2.1.1 Statistical analysis

The experiment was 2 x 3 factorial arrangement in a complete-randomised design (2 locations x 3 sampling dates). Analysis was performed using the analysis of variance (ANOVA) procedure on SAS (SAS Institute, Inc., 1983). A log transformation step was applied to all EC_{50} values prior to statistical analysis. Least significant difference test (LSD)

was applied to separate experimental means (Chew, 1976; Petersen, 1977). Fisher's exact test was applied to compare frequencies of resistant isolates in fungal populations.

3.2.2 Disease Progress Curves of Powdery Mildew of Wheat

To test if there is a relationship between the build-up of resistant isolates of powdery mildew and disease severity, disease progress curves were established for both the AV and CC.

3.2.2.1 Annapolis Valley

Disease in wheat fields was rated several times before and after triadimefon application. Disease in two fields of total area of 21 ha was rated six times, once every two weeks, during the season; the first three ratings were done before triadimefon application. The top four leaves of 10 tillers were rated in 10 random locations for each field. Standard area diagrams (James, 1971) were used to determine the percentage of leaf area infected with powdery mildew. The average percentage of leaf area infection of 800 leaves was then calculated in each of the six dates and used to draw the disease progress curve.

3.2.2.2 Colchester County

Disease severity was assessed eight times, once per week, for both treated and untreated wheat plots. The average

percentage of disease severity of 160 leaves was then calculated in each of the eight dates and for both treated and untreated plots. Disease rating was done as described in section 3.2.2.2.

3.2.2.2.1 Statistical analysis

A logit-transformation step was applied to percentages of leaf area infected prior to statistical analysis using the SAS package. Logit disease severity was calculated according to the following equation (Cox and Snell, 1989):

Logit disease severity= log (severity/ (severity 100))

Since the disease severity in the first two ratings was 0%, we set the logit severity to start at time 3. Data of logit transformation of mean disease severity were analyzed in a 2 x 6 factorial arrangement in a complete-randomised design (2 treatments x 6 times). Analysis was performed using the analysis of variance (ANOVA) procedure on SAS (SAS Institute, Inc., 1983).

3.3 RESULTS

3.3.1 Build-up of Resistance

3.3.1.1. Reaction of *E. graminis* f.sp. *tritici* isolates to triadimefon

3.3.1.1.1. Annapolis Valley (AV)

The EC_{50} values for the 30 single spore-derived isolates collected from the AV before triadimefon application ranged from 0.2 to 62.8 μg triadimefon mL^{-1}, with a mean EC_{50} value of 6.2 ± 3.9 μg triadimefon mL^{-1} (Table 3.1). Five (16.7%) of these 30 isolates were considered resistant to the fungicide (Table 3.1). The EC_{50} values for isolates collected 3 wks after triadimefon application ranged from 0.3 to 98 μg triadimefon mL^{-1} (Table 3.2) with a mean EC_{50} value of 17.6 ± 3.9 μg triadimefon mL^{-1}. Eleven (36.7%) of these isolates were resistant to triadimefon (Table 3.2). Table 3.3 shows the EC_{50} values for the 30 isolates collected 6 wks after fungicide application. The EC_{50} values ranged from 0.2 to 100 μg triadimefon mL^{-1}, with a mean EC_{50} value of 23.8 ± 3.9 μg triadimefon mL^{-1} (Table 3.3). Fourteen (46.7%) of these 30 isolates tested resistant to triadimefon (Table 3.3).

3.3.1.1.2 Colchester County (CC)

Table 3.4 shows that the EC_{50} values for the 30 single spore-derived isolates collected from the AV before triadimefon application ranged from 0.2 to 31.6 μg triadimefon mL^{-1}, with a mean EC_{50} value of 4.2 ± 3.9 μg triadimefon mL^{-1}

Table 3.1. Effective concentration of triadimefon to prevent growth of 50% (EC_{50}) of each of 30 *Erysiphe graminis f.sp. tritici* isolates collected in 1992 before treatment from fields of the wheat cultivar Absolvent in the Annapolis Valley.

Isolate No.	EC_{50} Value ($\mu g\ mL^{-1}$)	Reaction*
01	5.6	S
02	0.3	S
03	3.2	S
04	1.3	S
05	4.6	S
06	16.0	R
07	0.4	S
08	12.4	R
09	3.2	S
10	0.8	S
11	4.5	S
12	18.1	R
13	4.9	S
14	0.4	S
15	0.2	S
16	1.0	S
17	0.5	S
18	0.3	S
19	3.6	S
20	0.8	S
21	14.8	R
22	0.5	S
23	0.6	S
24	0.2	S
25	62.8	R
26	0.5	S
27	10.5	R
28	6.2	S
29	8.1	S
30	0.9	S
Mean EC_{50} ± SE:	6.2 ± 3.9	
Frequency of Resistant Isolates (%):		16.7

* S = sensitive (EC_{50} < 10 $\mu g\ mL^{-1}$); and R = resistant (EC_{50} > 10 $\mu g\ mL^{-1}$).

Table 3.2. Effective concentration of triadimefon to prevent growth of 50% (EC_{50}) of each of 30 *Erysiphe graminis f.sp. tritici* isolates collected in 1992 2 wks after treatment from fields of the wheat cultivar Absolvent in the Annapolis Valley.

Isolate No.	EC_{50} Value ($\mu g\ mL^{-1}$)	Reaction*
01	7.9	S
02	1.9	S
03	4.2	S
04	11.2	R
05	1.5	S
06	5.3	S
07	1.6	S
08	31.6	R
09	12.2	R
10	1.4	S
11	10.8	R
12	0.6	S
13	8.5	S
14	7.5	S
15	0.6	S
16	6.2	S
17	62.6	R
18	0.3	S
19	3.2	S
20	46.6	R
21	31.6	R
22	69.7	R
23	20.6	R
24	3.2	S
25	60.9	R
26	4.0	S
27	9.7	S
28	98.0	R
29	0.5	S
30	3.2	S
Mean EC_{50} ± SE:	17.6 ± 3.9	
Frequency of Resistant Isolates (%):		36.7

* S = sensitive (EC_{50} < 10 $\mu g\ mL^{-1}$); and R = resistant (EC_{50} ≥ 10 $\mu g\ mL^{-1}$).

Table 3.3. Effective concentration of triadimefon to prevent growth of 50% (EC_{50}) of each of 30 *Erysiphe graminis f.sp. tritici* isolates collected in 1992 6 wks after treatment from fields of the wheat cultivar Absolvent in the Annapolis Valley.

Isolate No.	EC_{50} Value ($\mu g\ mL^{-1}$)	Reaction*
01	80.6	R
02	100.0	R
03	2.5	S
04	0.6	S
05	13.3	R
06	18.9	R
07	5.6	S
08	10.5	R
09	39.7	R
10	91.2	R
11	3.9	S
12	4.6	S
13	26.9	R
14	6.4	S
15	2.8	S
16	10.6	R
17	1.5	S
18	0.4	S
19	87.5	R
20	60.3	R
21	0.2	S
22	98.5	R
23	0.6	S
24	15.1	R
25	4.0	S
26	17.8	R
27	5.0	S
28	1.0	S
29	4.6	S
30	0.7	S
Mean EC_{50} ± SE:	23.8 ± 3.9	
Frequency of Resistant Isolates (%):		46.7

* S = sensitive (EC_{50} < 10 $\mu g\ mL^{-1}$); and R = resistant (EC_{50} > 10 $\mu g\ mL^{-1}$).

Table 3.4. Effective concentration of triadimefon to prevent growth of 50% (EC_{50}) of each of 30 *Erysiphe graminis f.sp. tritici* isolates collected in 1992 before treatment from fields of the wheat cultivar Absolvent in Colchester County.

Isolate No.	EC_{50} Value ($\mu g\ mL^{-1}$)	Reaction*
01	0.8	S
02	3.2	S
03	2.1	S
04	1.0	S
05	0.9	S
06	5.9	S
07	2.3	S
08	5.5	S
09	1.2	S
10	9.8	S
11	0.3	S
12	5.1	S
13	7.4	S
14	3.2	S
15	3.8	S
16	0.3	S
17	1.4	S
18	1.3	S
19	2.8	S
20	3.7	S
21	0.3	S
22	7.2	S
23	1.0	S
24	0.2	S
25	1.0	S
26	10.7	R
27	31.6	R
28	9.1	S
29	3.2	S
30	1.0	S
Mean EC_{50} ± SE:	4.2 ± 3.9	
Frequency of Resistant Isolates (%):		7.14

* S = sensitive (EC_{50} < 10 $\mu g\ mL^{-1}$); and R = resistant (EC_{50} ≥ 10 $\mu g\ mL^{-1}$).

Two (6.7%) of these 30 isolates were considered resistant to the fungicide (Table 3.4). The EC_{50} values for isolates collected 3 wks after triadimefon application ranged from 0.1 to 73.4 μg triadimefon mL^{-1} (Table 3.5) with a mean EC_{50} value of 5.7 ± 3.9 μg triadimefon mL^{-1}. Three (10%) of these isolates were resistant to triadimefon (Table 3.5). Table 3.6 shows the EC_{50} values for the 30 isolates collected 6 wks after fungicide application. The EC_{50} values ranged from 1.2 to 90 μg triadimefon mL^{-1}, with a mean EC_{50} value of 20.2 ± 3.9 μg triadimefon mL^{-1} (Table 3.6). Twelve (40%) of these 30 isolates tested resistant to triadimefon (Table 3.6).

3.3.1.2 Comparison between sampling dates, locations, and their interaction based on the log(EC_{50}) values of isolates of E. graminis f.sp. tritici.

Results of the analysis of variance (Table 3.7) demonstrated that there was a highly significant difference (P=0.0001) among sampling dates in their effect on the log(EC_{50}) values of powdery mildew populations collected from both the AC and CC.

Table 3.7 shows that there was a highly significant interaction (P=0.0019) between sampling dates and locations in their effect on the log(EC_{50}) values of mildew populations. Results (Table 3.8) demonstrated that, for fungal populations of isolates collected from the AV, the mean value of log(EC_{50})

Table 3.5. Effective concentration of triadimefon to prevent growth of 50% (EC_{50}) of each of 30 *Erysiphe graminis f.sp. tritici* isolates collected in 1992 2 wks after treatment from fields of the wheat cultivar Absolvent in Colchester County.

Isolate No.	EC_{50} Value ($\mu g\ mL^{-1}$)	Reaction*
01	9.5	S
02	0.5	S
03	1.1	S
04	1.1	S
05	0.1	S
06	10.7	R
07	4.0	S
08	0.5	S
09	0.8	S
10	3.2	S
11	0.6	S
12	1.1	S
13	0.5	S
14	0.3	S
15	2.6	S
16	0.5	S
17	3.2	S
18	31.6	R
19	7.2	S
20	2.5	S
21	0.2	S
22	73.4	R
23	1.0	S
24	1.5	S
25	0.4	S
26	0.4	S
27	8.7	S
28	3.2	S
29	1.0	S
30	0.6	S
Mean EC_{50} ± SE:	5.7 ± 3.9	
Frequency of Resistant Isolates (%):		10.0

* S = sensitive (EC_{50} < 10 $\mu g\ mL^{-1}$); and R = resistant (EC_{50} ≥ 10 $\mu g\ mL^{-1}$).

Table 3.6. Effective concentration of triadimefon to prevent growth of 50% (EC_{50}) of each of 30 *Erysiphe graminis f.sp. tritici* isolates collected in 1992 6 wks after treatment from fields of the wheat cultivar Absolvent in Colchester County.

Isolate No.	EC_{50} Value ($\mu g\ mL^{-1}$)	Reaction*
01	5.8	S
02	31.6	R
03	3.3	S
04	22.1	R
05	73.8	R
06	2.0	S
07	31.6	R
08	31.6	R
09	1.2	S
10	3.2	S
11	8.2	S
12	90.0	R
13	90.0	R
14	6.7	S
15	2.6	S
16	31.6	R
17	9.2	R
18	4.5	S
19	31.6	R
20	9.1	S
21	2.4	S
22	4.5	S
23	9.0	S
24	9.7	S
25	8.4	S
26	7.6	S
27	14.7	R
28	18.7	R
29	9.2	S
30	31.6	R
Mean EC_{50} ± SE:	20.2 ± 3.9	
Frequency of Resistant Isolates (%):		40.0

* S = sensitive (EC_{50} < 10 $\mu g\ mL^{-1}$); and R = resistant (EC_{50} > 10 $\mu g\ mL^{-1}$).

Table 3.7. Analysis of Variance (ANOVA) for log(EC_{50}) values of *Erysiphe graminis f.sp. tritici* isolates collected in 1992 from two locations (the Annapolis Valley & Colchester County) on three sampling dates (before, 2 wks after and 6 wks after triadimefon application).

Source of Variation	DF	Mean Square	Pr > F
Location	1	4.013	0.1782
Sampling date	2	33.461	0.0001
Location × Sampling date	2	14.233	0.0019
ERROR	174	2.196	

increased from 0.69 ± 0.27 μg triadimefon mL^{1} 3 wks before triadimefon application to 1.84 ± 0.27 and 1.97 ± 0.27 μg triadimefon mL^{-1}, respectively, two and six weeks after triadimefon application. Results of the LSD test (Table 3.8) indicated that there was a significant difference in the mean value of $\log(EC_{50})$ for the mildew populations collected from the AV before fungicide application and 2 wks after application. No significant difference in the mean value of $\log(EC_{50})$ was found between isolates collected 2 wks after triadimefon application and those collected 6 wks after application (Table 3.8).

On the other hand, results of the LSD test (Table 3.8) indicated that there was no significant difference in the mean value of $\log(EC_{50})$ for populations of isolates collected from CC before and 2 wks after triadimefon application. Meanwhile, a highly significant difference in the mean value of $\log(EC_{50})$ was found between isolates collected before (0.78 ± 0.27 μg triadimefon mL^{-1}) and 2 wks after (0.44 ± 0.27 μg triadimefon mL^{-1}) triadimefon application, and those collected 6 wks (2.40 ± 0.27 μg triadimefon mL^{-1}) after triadimefon application (Table 3.8). No significant difference was found between locations (Table 3.7) in their effect on the mean values of $\log(EC_{50})$.

Table 3.8. Mean log(EC_{50})* values and their standard error for location x sampling date interaction.

Location	Sampling date**		
	BT	WK+2	WK+6
Annapolis Valley	0.69a	1.84b	1.97bc***
Colchester County	0.78a	0.44a	2.405b

Standard Error = 0.27

* Mean value of log(EC_{50}) of 30 individual isolates of *E. graminis* f.sp. *tritici* per sampling date per location. Each individual isolate was replicated twice.

** BT= before treatment; WK+2 and WK+6= two and six weeks after triadimefon application, respectively.

*** Means followed by the same letter within a row are not significantly different from each other at p = 0.05 according to LSD test.

3.3.1.3 Comparisons among sampling dates in both the AV and CC, based on the frequencies of resistant isolates of *E. graminis* f.sp. *tritici*.

Fisher's exact test was performed on data of frequencies of resistant mildew isolates for populations from the AV and CC. Table 3.9 indicated that there was a highly significant difference (P=0.0004) among the frequencies of resistant isolates collected before triadimefon application, 2 wks and 6 wks after application. The frequency of resistant isolates increased from 11.7% before triadimefon was applied (Table 3.9) to 23.3 and 43.3%, 2 wks and 6 wks, respectively, after triadimefon application. For isolates collected from wheat fields of the AV, results of the Fisher's exact test also demonstrated that there was a significant difference (P=0.039) in the frequency of resistant isolates for populations collected on three sampling dates (Fig. 3.1). The frequency of resistant isolates increased from 16.7% before triadimefon application, to 36.7% and 46.7%, 2 wks and 6 wks, respectively, after treatment (Fig. 3.1). A highly significant difference (P=0.0029) was found in the frequency of resistant isolates for populations collected from wheat fields of CC over time (Fig. 3.1). The frequency of resistant isolates increased very slightly from 7.14% to 10%, respectively, before, and 2 wks after triadimefon application (Fig. 3.1).

Table 3.9. Comparisons among the frequencies of resistant isolates of *E. graminis* f.sp. *tritici* for populations from the Annapolis Valley and Colchester County using Fisher's exact test.

Source of Isolates	No. of Isolates Tested	No. of Resistant Isolates	Frequency of Resistant Isolates (%)
Before treatment	60	7	11.7
2 wks after treatment	60	14	23.3
6 wks after treatment	60	26	43.3

P-value = 0.0004

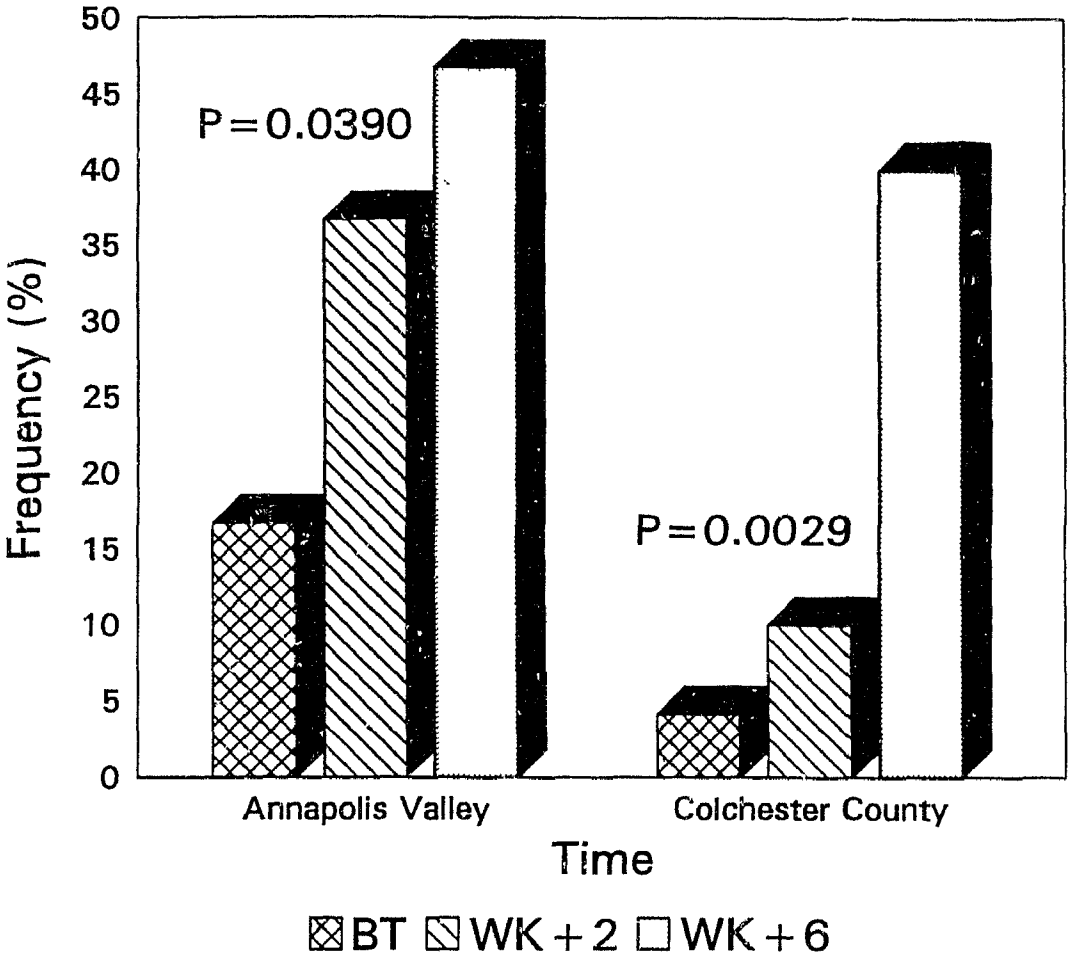


Fig. 3.1 Frequencies of isolates of *Erysiphe graminis* f.sp. *tritici* tested resistant to triadimefon for populations of isolates collected in 1992 from the Annapolis Valley and Colchester County before treatment (BT), and two (WK+2) and six weeks (WK+6), respectively, after triadimefon application.

Six weeks after treatment, the frequency of resistant isolates increased to 40%.

3.3.2. Disease Progress Curves of Powdery Mildew of Wheat

3.3.2.1. Annapolis Valley

Results of the mean percentages of disease severity (Fig. 3.2) of powdery mildew rated over six times showed that the disease severity increased from 24.1% two days before triadimefon application, to 30.4%, 2 wks after triadimefon was applied. The severity of powdery mildew increased at a slow rate during that period and reached 49.1% 6 wks after triadimefon application (Fig. 3.2).

3.3.2.2 Colchester County

Results of the untransformed data (Fig. 3.3) indicated that treatment with triadimefon decreased disease severity but did not stop disease development.

Results of the ANOVA (Table 3.10) demonstrated that there was significant difference among rating dates (P=0.0001) in terms of disease severity. Table 3.10 also indicated that there was a significant difference between treatments (P=0.0001) in their effect on disease severity. The mean logit severity was higher in untreated fields (-2.26 ± 0.18%) compared to treated fields (-3.45 ± 0.18%). No significant interaction was found between rating date and treatment in their effect on disease severity.

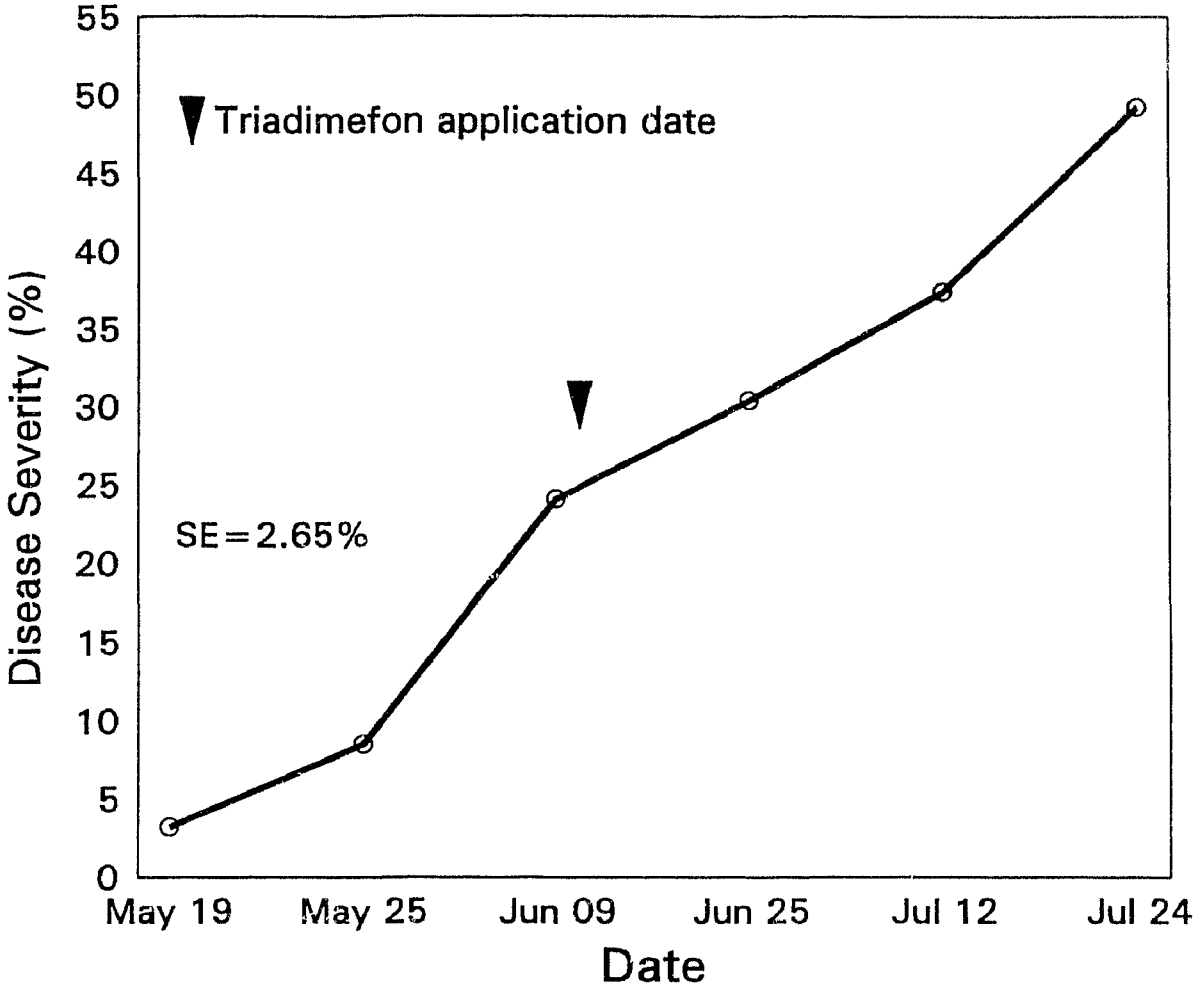


Fig. 3.2 Disease progress curve of powdery mildew of wheat (cv. Absolvent) in the Annapolis Valley in 1992.

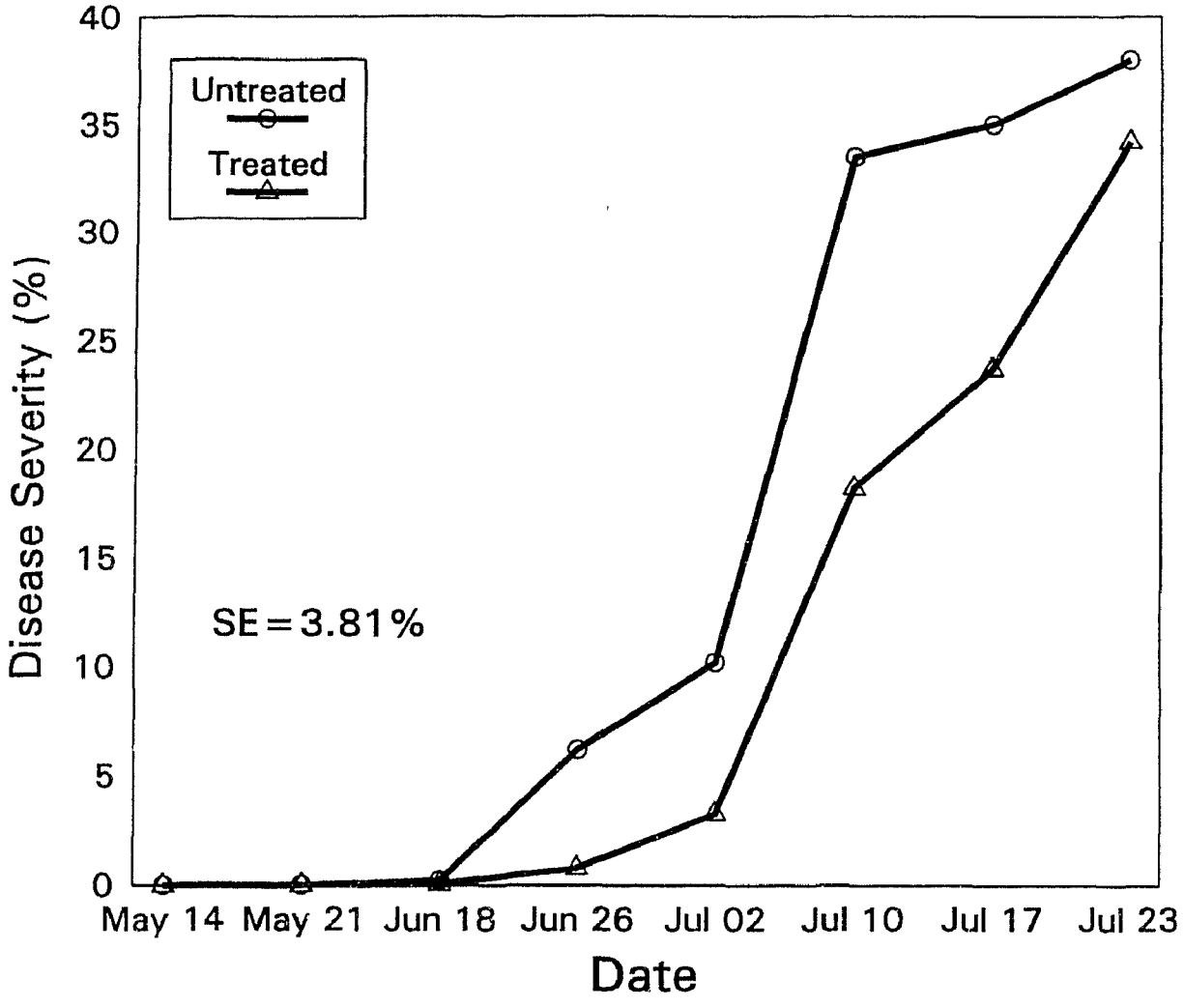


Fig. 3.3 Disease progress curve of powdery mildew of wheat (cv. Absolvent) for treated and untreated fields in the Colchester County in 1992.

Table 3.10 . Analysis of Variance (ANOVA) for logit disease severity values of *Erysiphe graminis f.sp. tritici* rated in 1992 over 6 time periods (rating dates) in treated and untreated fields of the wheat cv. Absolvent in Colchester County.

Source of Variation	DF	Mean Square	Pr > F
Rating date	5	41.331	0.0001
Treatment	1	16.591	0.0001
Rating date × Treatment	5	0.809	0.4060
ERROR	36	0.773	

3.4 DISCUSSION

3.4.1 Build-up of Resistance

Results (Tables 3.1 - 3.6) demonstrated that, within each population of mildew isolates collected before and after triadimefon application, isolates fell into a wide range of EC_{50} values. Those with EC_{50} value < 10 μg triadimefon mL^{-1} were considered sensitive (S), and those with EC_{50} values ≥ 10 μg triadimefon mL^{-1} were considered resistant (R) to triadimefon.

A log-transformation step was applied to all EC_{50} values prior to performing the statistical analysis in order to ensure a better normality of distribution of isolates within populations of powdery mildew.

The highly significant difference among sampling dates in terms of the mean values of $\log(EC_{50})$ (Table 3.7) and the frequencies of resistant isolates (Table 3.9) suggested that, triadimefon application for controlling the mildew populations has resulted in an increase in the resistance of populations when it was assessed 2 wks and 6 wks after treatment. These results also showed that the resistant isolates were present in the field in low frequencies (Table 3.9 & Figs. 3.1 & 3.2) before triadimefon application. Exposing the fungal populations to the selection pressure of triadimefon might have eliminated a proportion of the sensitive isolates allowing the resistant isolates to increase and dominate the fungal populations. Our results are in agreement with those of Fletcher *et al.* (1987) who found that two triadimefon sprays

resulted in a large decrease in the sensitivity of the *E. graminis* f.sp. *tritici* population when it was assessed shortly after the second spray. The mean EC_{50} values of the whole population increased from 22.5 μg triadimefon mL^{-1} to 32.7 μg triadimefon mL^{-1} after the second spray, and to 408.3 μg triadimefon mL^{-1} at the fourth spray.

The highly significant interaction among sampling dates and locations in their effect on the $\log(EC_{50})$ values (Table 3.8) suggested that, in the AV populations, the fungus built up a resistance to triadimefon 2 wks after it was applied. Meanwhile, 6 wks after treatment, a very slow, but not significant, increase in resistance in terms of a mean value of $\log(EC_{50})$ was observed. On the other hand, although there was no significant increase in the mean value of $\log(EC_{50})$ between populations collected 2 wks and 6 wks after triadimefon application, results indicated that there was a significant increase in the frequency of resistant isolates for populations collected in both times (Table 3.9). This suggests that there was a change in the distribution of isolates of powdery mildew fungus within the range of EC_{50} values. Some isolates acquired more resistance to the fungicide ($EC_{50} \geq 10$ μg triadimefon mL^{-1}) and others became more sensitive and had lower EC_{50} values (Tables 3.1 – 3.6). However, it was clear that the fungicide application has caused the resistant isolates to dominate the sensitive ones, and since the powdery mildew fungus has a short life cycle,

resistant isolates are expected to propagate and produce new generations of resistant spores in the same growing season. This process might have contributed to the increase in the frequency of resistant isolates after the fungicide application.

In the case of populations of the fungus from the CC, both of the mean values of log(EC_{50}) and the frequencies of resistant isolates collected before and 2 wks after triadimefon application didn't differ significantly (Table 3.8 & Fig. 3.1). However, these values and percentages were significantly different from those for isolates collected 6 wks after treatment. These results suggest that in CC, since the fungus is not well established compared to the AV, and since the favourable environmental conditions do not exist until later in the season, the build-up of resistance was very slow until 6 wks after treatment. Dekker (1986) indicated that environmental conditions that increase the severity of the disease may also speed the development of resistance.

In order to determine if there is a relationship between the build-up of resistance and the disease severity, disease progress curves were established for populations of *E. graminis* f.sp. *tritici* from the AV and CC.

3.4.2 Disease progress curves of powdery mildew of wheat

The disease progress curve for powdery mildew in the AV (Fig. 3.2) suggested that the application of triadimefon

didn't stop the development of the disease. Disease severity in untreated fields was not assessed in the AV, since all Absolvent fields were treated with triadimefon. However, unpublished data from the Maritime Provinces Production/ Management Cereal Trials in 1992 indicated that triadimefon provided some control of powdery mildew disease in the management trials in the AV. The disease progress curve for powdery mildew in the AV and the build-up of resistant isolates (Fig. 3.4) suggest that the reduction in sensitivity of field populations tends to be associated with the survival of the less sensitive elements of a mixed population after a period of disease control, rather than with the failure of control.

Brent (1981) reported that there was no correlation between the ethirimol sensitivity of powdery mildew isolates and the levels of mildew in their fields of origin at the time of sampling. When all data from the 1975 survey were amalgamated, there was a low significance, positive correlation coefficient (P=0.10) between sensitivity and the amount of field mildew. On the other hand, Eniz (1988) reported that a reduced sensitivity in some isolates of wheat powdery mildew was correlated with poor control by prochloraz and triadimefon. Similar results were reported by Elad (1992) who found that a reduced sensitivity of *Botrytis cinerea* to two SBI fungicides, fenetrazole and fenethanil, was associated with poor disease control.

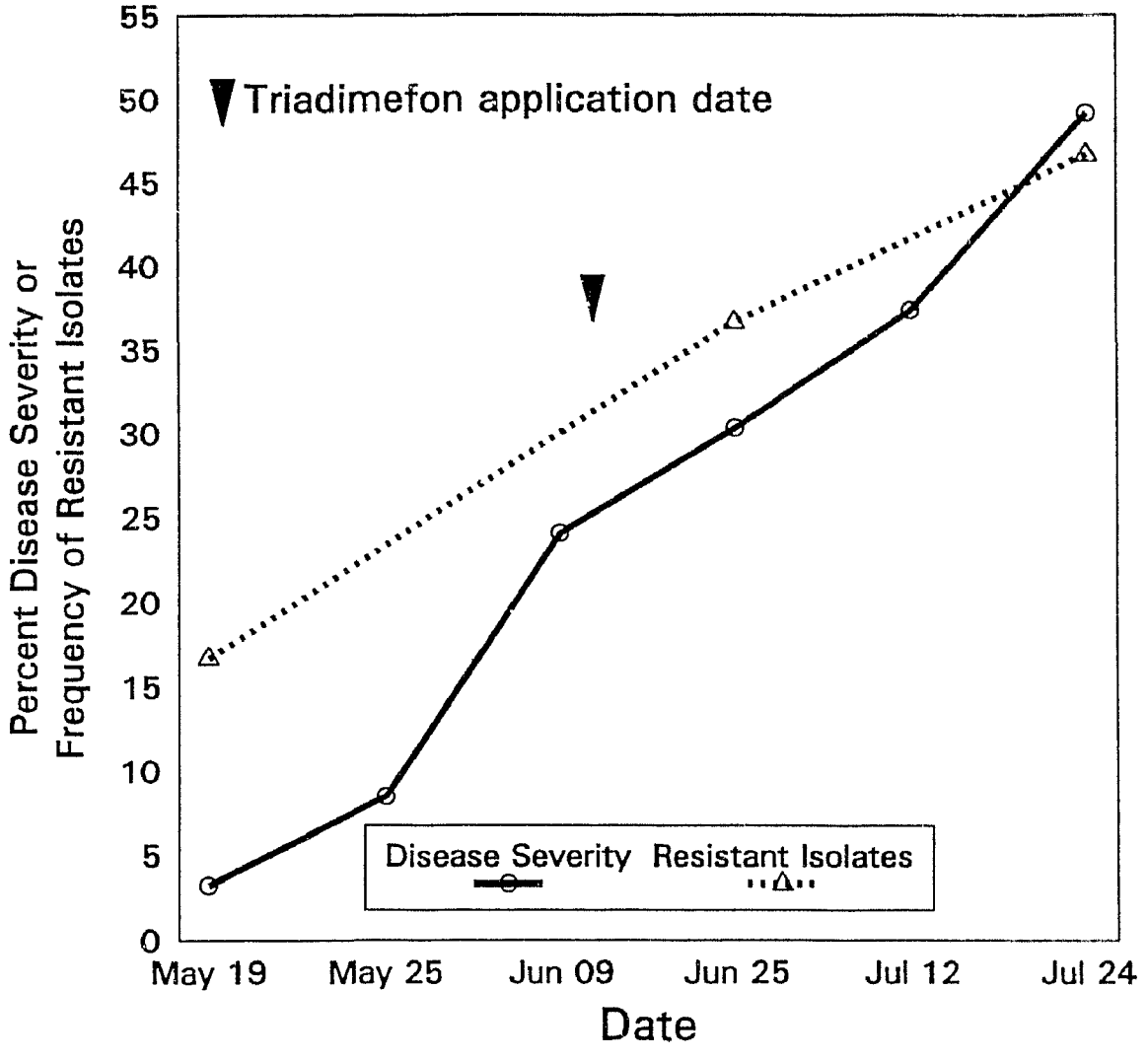


Fig. 3.4 Relationship between the frequency of isolates resistant to triadimefon and disease severity of powdery mildew of wheat (cv. Absolvent) in the Annapolis Valley in 1992.

In the case of CC, the highly significant difference between treatments (Table 3.10) revealed that triadimefon application in wheat fields reduced the disease severity of powdery mildew. However, triadimefon didn't stop the disease development (Fig. 3.3). A relationship between the increase in the frequency of resistant isolates after triadimefon application and the increase in disease severity can be drawn (Fig. 3.5).

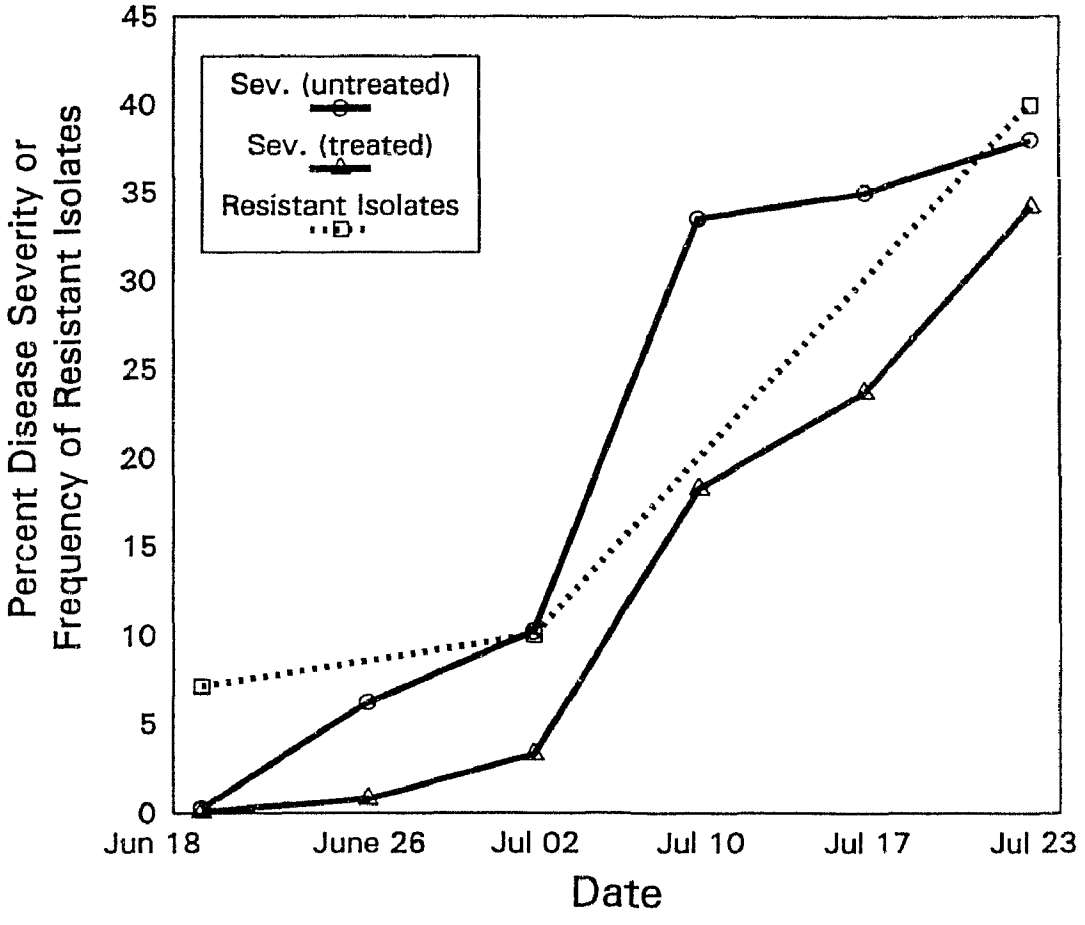


Fig. 3.5 Relationship between the frequency of isolates resistant to triadimefon and disease severity of powdery mildew of wheat (cv. Absolvent) in the Colchester County in 1992.

Chapter 4.

COMPETITION BETWEEN TRIADIMEFON-SENSITIVE AND TRIADIMEFON-RESISTANT ISOLATES OF ERYSIPHE GRAMINIS F.SP. TRITICI

4.1 INTRODUCTION

Resistance to DMIs is often accompanied by poor germination and reduced growth which contribute to a lack of fitness and poor viability of the fungus (Fuchs and Drandarevski, 1976). Fitness of resistant isolates of a pathogen is a term used to denote its virulence or competitive ability as compared to the other strains in the population in the same environment (Wade, 1982; Skylakakis, 1987).

Many competition experiments, which test the ability of resistant strains to infect plants in the absence of fungicide, have been conducted on different pathogens (Gullino and Garibaldi, 1981; Buchenauer *et al.*, 1984; Schepers, 1985; Kadish and Cohen, 1988). Such experiments help determine whether resistant strains will become less frequent and then disappear as a result of competition with the sensitive strains in the absence of the fungicide (Wade, 1982).

Fitness can be estimated by growing mixtures of isolates or populations for many generations while monitoring the frequency of each isolate (Jørgensen, 1988; Ulrich and Taehle-Csech, 1988).

Different reports indicated that the competitive ability of DMI-resistant *E. graminis* f.sp. *tritici* and *hordei* isolates was inferior to that of the sensitive ones (Buchenauer *et al.*, 1984; Buchenauer and Hellwald, 1985).

This investigation was undertaken to study the competitive ability of triadimefon-resistant (R) and triadimefon-sensitive (S) isolates in mixed-isolate inoculations of the fungus.

4.2 MATERIALS AND METHODS

4.2.1 1991 Experiment

Thirty individual isolates of *E. graminis* f.sp. *tritici* were collected during the fall of 1991 from each of treated and untreated fields of the wheat cultivar Absolvent in the Annapolis Valley. Materials and Methods described in Chapter 2 were used in this experiment to test the resistance of these isolates to the fungicide triadimefon.

Based on the responses of these isolates to various concentrations (0, 0.1, 1.0, 10, and 100 μg a.i. mL^{-1}) of triadimefon, isolates that showed growth at 10 μg triadimefon mL^{-1} were considered resistant (R), and those that did not grow were considered sensitive (S). For isolates from each of the treated and untreated fields, two R and two S isolates were randomly selected and used in this study. For isolates from treated fields, each isolate-mixture was made by randomly selecting and mixing one R and one S isolate. The same procedure was followed for isolates from untreated fields.

Mixtures were made by randomly mixing one resistant and one sensitive isolate from each treatment in different ratios of R and S isolates (Table 4.1).

Disease-free wheat seedlings were inoculated with various mixtures of pustules of S and R isolates (Table 4.1) to produce ratios of 0, 25, 50, 75, and 100% of resistant isolate (two tubes/ ratio). After sporulation, the mixed cultures were used to inoculate untreated disease-free seedlings. After 14 days, the resulting conidia were used to inoculate fresh untreated disease-free seedlings (two replicates per S:R mixture). This procedure was repeated four times (i.e. five generation cycles), and the competitive ability of the resistant and sensitive isolates (expressed as EC_{50} value) was tested for generation 1, 3, and 5 on triadimefon-treated (0, 0.1, 1.0, 10, and 100 μg triadimefon mL^{-1}) wheat seedlings. Fungicide preparation and application, and inoculation of treatments with the various S:R mixtures was done as described in Chapter 2.

The change in sensitivity of S:R isolates grown in the absence of the fungicide was tested after 1, 3, and 5 generation cycles (two replicates per S:R mixture) in the absence of triadimefon. The two replicates were not averaged for each mixture. The results were evaluated as described in section 2.2.8.

4.2.1.1 Statistical analysis

The experiment was split-split-plot design, with the whole

Table 4.1. Percentages of resistant isolates and numbers of pustules of *E. graminis* f.sp. *tritici* involved in preparing mixtures of resistant and sensitive isolates that were used in the 1991 experiment.

Mixture* no.	% of resistant isolate	No. of pustules of S:R
1	0	4:0
2	25	3:1
3	50	2:2
4	75	1:3
5	100	0:4

* Five mixtures per isolate per treatment; two isolates per treatment.

plot in a complete-randomized design arrangement. Treatments (treated and untreated fields) were considered as the main plot factor. Isolates were nested within treatments and were tested as an error term for the main plot factor. Mixtures (5 mixtures) were considered as the split-plot factor. The isolates x mixture interaction was nested within treatment and was tested as an error term for the split plot factor (mixture) and for the treatment x mixture interaction. Generations (3 generation cycles) were considered as the split-split-plot factor. The overall error was tested as an error term for the spilt-split factor and its interactions. All factors and their interactions were considered fixed effects, while error terms were considered random effects. Analyses were done using the General Linear Model (GLM) procedure on SAS (SAS Institute, Inc., 1983). All EC_{50} values (two replicates/ mixture) were transformed to log(EC_{50}) values prior to analysis. Least significant difference (LSD) test was applied to separate experimental means (Chew, 1976; Petersen, 1977).

4.2.2 1992 Experiment

Based on the results of the competition experiment of 1991, no significant difference was found between treated and untreated fields in terms of fitness of isolates collected from each of these fields (Table 4.2). Therefore, isolates in this study were collected from treated fields.

Thirty individual isolates of *E. graminis* f.sp. *tritici* were collected during the summer of 1992 from treated fields of the wheat cultivar Absolvent in the Annapolis Valley. Materials and Methods described in Chapter 2 were used in this experiment to test the response of these isolates to the fungicide triadimefon. Based on the response of these isolates to various concentrations (0, 0.1, 1.0, 10, and 100 μg a.i. mL^{-1}) of triadimefon, isolates that showed growth at 10 μg triadimefon mL^{-1} were consider resistant (R), and those that didn't grow were considered sensitive (S).

Based on the results of the competition of 1991, no difference (Table 4.2) was found among the mixtures of ratios of R:S isolates (25:75, 50:50, 75:25) with regards to the fitness of the R isolate in each mixture. Therefore, the 50:50 ratio was chosen to be used in studying the competition between the R and S isolates in this experiment.

Five R and five S isolates were randomly selected and used in this study. Five isolate-mixtures were made by mixing five different R and S isolates (50:50 ratio). Each isolate-mixture received only one R and one S isolate.

4.2.2.1 Statistical analysis

The effect of keeping mixtures of R and S (50:50) isolates in the absence of triadimefon over three generation cycles (G1, G3, and G5) on the EC_{50} values was compared in a randomized complete-block experiment, with generation cycles

considered as treatments and isolates (5 isolates x 2 replicates) considered as blocks; the two replicates were averaged for each isolate. The analysis was done using the analysis of variance procedure (ANOVA) on SAS (SAS Institute, Inc., 1983). All EC_{50} values were transformed to $\log(EC_{50})$ values prior to analysis. Least significant difference (LSD) test was applied to separate experimental means (Chew, 1976; Petersen, 1977).

4.3 RESULTS

4.3.1 1991 Experiment

Results of the ANOVA (Table 4.2) revealed that there was a highly significant difference (P=0.0001) among generation cycles of powdery mildew of wheat in terms of $\log(EC_{50})$ values of mixtures of resistant and sensitive isolates. Results of the LSD test (Fig. 4.1) demonstrated that the mean value of $\log(EC_{50})$ of all mixtures of resistant and sensitive isolates for isolate-mixtures tested on the first generation cycle of the fungus was 0.02 ± 0.09 μg triadimefon mL^{-1}. This mean value of $\log(EC_{50})$ then significantly decreased to -1.26 ± 0.09 and -2.46 ± 0.09 μg triadimefon mL^{-1} after keeping the mixtures of resistant and sensitive isolates for three and five generation cycles, respectively, in the absence of triadimefon (Fig.4.1).

Results (Table 4.2) also indicated that there was a significant interaction (P=0.0276) among treatments and generations in their effect on the $\log(EC_{50})$ values of

Table 4.2. Analysis of Variance (ANOVA) for log(EC_{90}) values of mixtures of ratios of triadimefon-resistant and triadimefon sensitive strains of two isolate- mixtures of *Erysiphe graminis f.sp. tritici* selected from populations collected in 1991 from treated and untreated fields of the wheat cv. Absolvent in the Annapolis Valley and tested in 3 generation cycles in the absence of triadimefon.

Source of Variation	DF	Mean Square	Pr > F
Treatment	1	26.481	0.1434
Isolate (Treatment)	2	4.806	0.0530
Mixture	4	1.495	0.3322
Treatment × Mixture	4	0.406	0.8263
Isolate × Mixture (Treatment)	8	1.108	0.0059
Generation	2	61.337	0.0001
Treatment × Generation	2	1.404	0.0276
Mixture × Generation	8	0.934	0.0178
Treatment × Mixture × Generation	8	0.644	0.1058
ERROR	80	0.374	

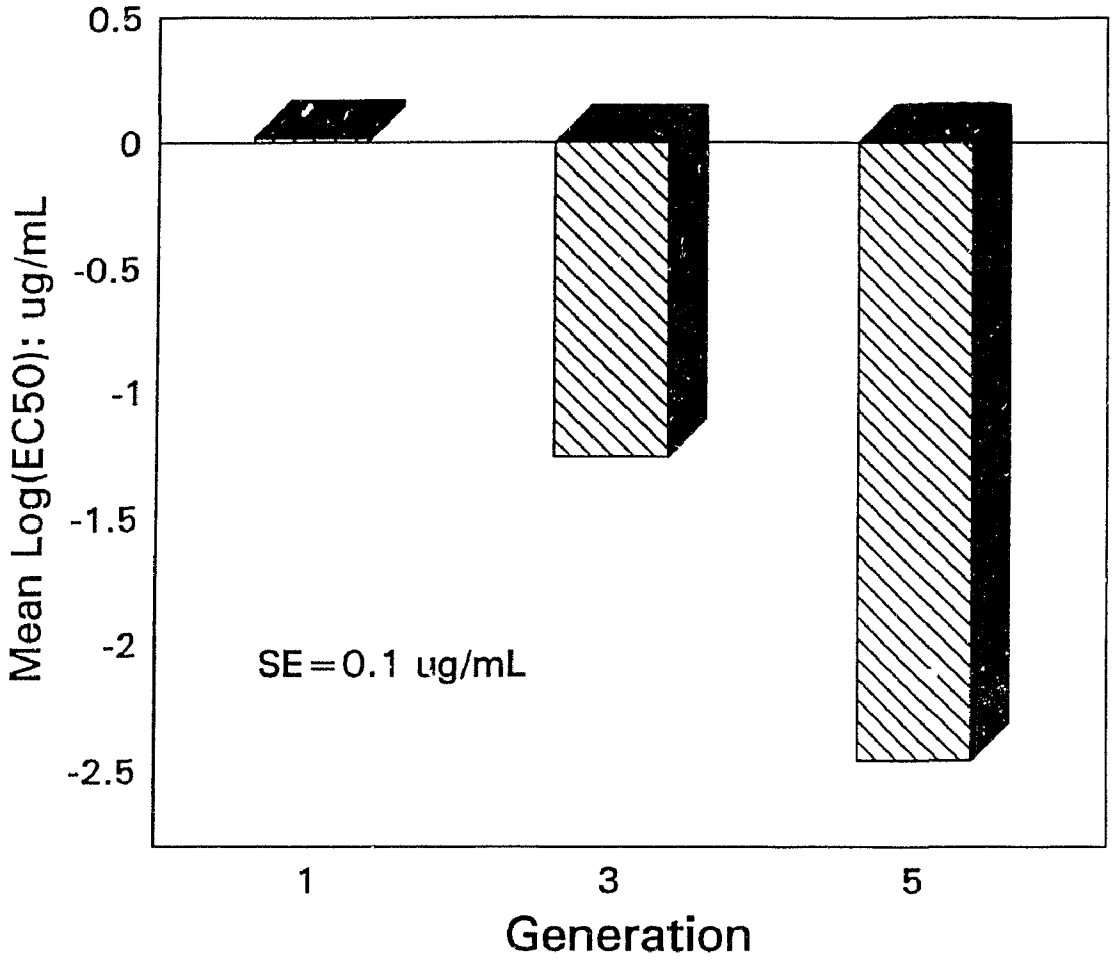


Fig. 4.1 Change in the mean values of log(EC_{50}) of all mixtures of resistant and sensitive isolates for isolate-mixtures of powdery mildew from the Annapolis Valley tested in 1991 on the first, third, and fifth generation cycles, respectively, in the absence of triadimefon.

resistant and sensitive isolates. Results of the LSD (Fig. 4.2) indicated that for mixtures of resistant and sensitive isolates from populations collected from wheat fields treated and untreated with triadimefon, the mean values of $\log(EC_{50})$ decreased significantly after keeping the mixtures in the absence of the fungicide for five generation cycles. In the case of those from treated fields, the $\log(EC_{50})$ value decreased from 0.70 ± 0.14 μg triadimefon mL^{-1} in the first generation cycle, to -0.94 ± 0.14 and -2.04 ± 0.14 μg triadimefon mL^{-1} after three and five generations, respectively, in the absence of triadimefon (Fig. 4.2). A similar trend was observed in case of mixtures of resistant and sensitive isolates from mildew populations collected from untreated wheat fields (Fig. 4.2).

Fig. 4.2 shows that for isolates from untreated fields, the mean value of $\log(EC_{50})$ of mixtures of resistant and sensitive isolates decreased from -0.66 ± 0.14 μg triadimefon mL^{-1} in the first generation cycle, to -1.57 ± 0.14 and -2.87 ± 0.14 μg triadimefon mL^{-1} in the third and fifth generation cycles, respectively. The mean values of $\log(EC_{50})$ were generally lower in mixtures of resistant and sensitive isolates for populations from untreated fields as compared to those for populations from treated fields (Fig. 4.2).

Results (Table 4.2) also revealed that there was a significant interaction (P=0.0178) between mixtures of resistant and sensitive isolates and generation cycles of the

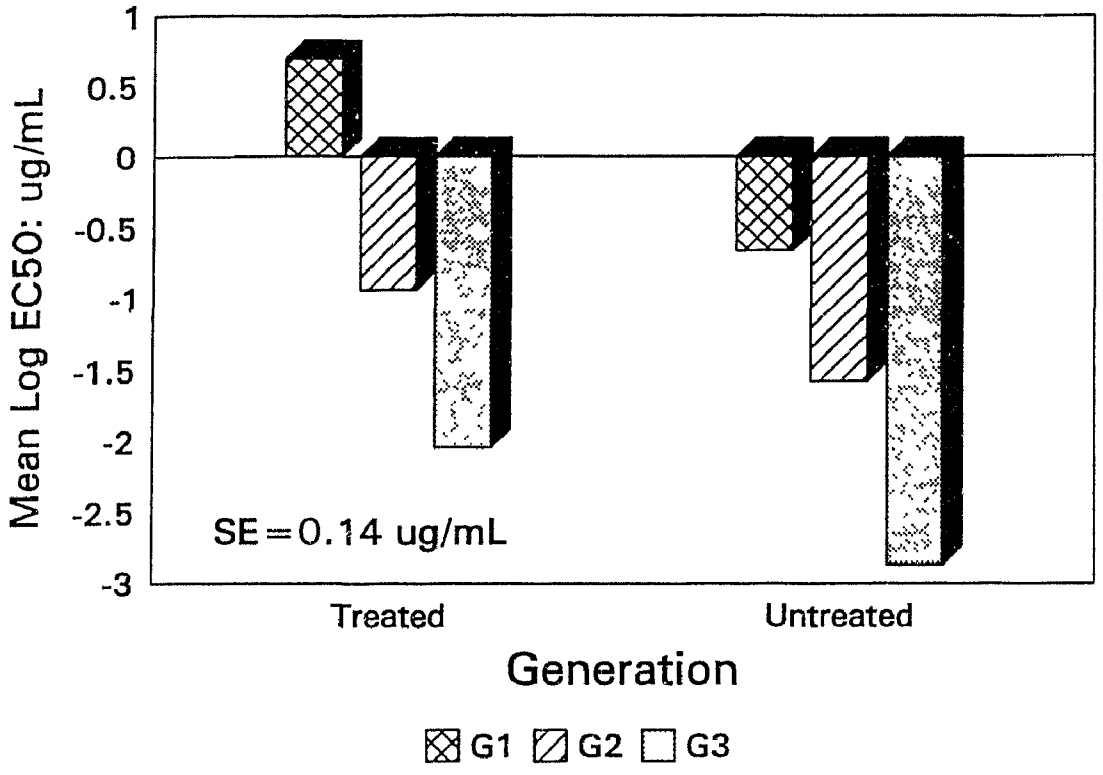


Fig. 4.2 Change in the mean values of log(EC_{50}) of all mixtures of resistant and sensitive isolates for isolate-mixtures of powdery mildew from treated and untreated fields of the Annapolis Valley tested in 1991 on the first, third, and fifth generation cycles, respectively, in the absence of triadimefon.

fungus in their effect on the mean values of log(EC_{50}). Table 4.3 shows the slopes of the mean values of log(EC_{50}) and the standard errors of the mean for mixtures of five R:S ratios. The slope was calculated as follows: slope of mean value of log(EC_{50})= [(mean log(EC_{50}) at G5) - (mean log(EC_{50})at G1)].

Results (Table 4.3) demonstrated that for each mixture of R:S ratios, the slope of the mean values of log(EC_{50}) was negative, indicating that the mean values of log(EC_{50}) decreased after keeping these mixtures for five generation cycles in the absence of the fungicide.

4.3.2 1992 Experiment

Results of the ANOVA (Table 4.4) revealed that there was a highly significant difference (P=0.0001) between generation cycles in their effect on the log(EC_{50}) values of all isolates tested. Results of the LSD test (Fig. 4.3) indicated that the mean value of the log(EC_{50}) of all isolate-mixtures decreased from 2.39 ± 0.22 μg triadimefon mL^{-1} in the first generation cycle, to 0.80 ± 0.22 and -0.38± 0.22 μg triadimefon mL^{-1} after keeping the mixtures of R and S isolates for three and five generation cycles, respectively. Table 4.4 also shows that there was a significant difference (P=0.0014) between isolates (blocks). This indicates that blocking isolates added to the precision of the ANOVA with regards to treatments (generations).

Table 4.3. Mean values of log(EC_{50}) and their slopes* for mixtures of ratios of triadimefon-resistant and triadimefon-sensitive strains of *E. graminis* f.sp. *tritici* tested in 1991 for tow treatments, two isolate mixtures at three generation cycles in the absence of triadimefon.

		Mean log(EC_{50})			
Mixture	Ratio (R:S)	G1**	G3	G5	Slope
1	0:100	-0.182	-1.314	-1.844	-1.662
2	25:75	0.168	-1.047	-2.248	-2.416
3	50:50	-0.094	-1.312	-2.746	-2.652
4	75:25	-0.447	-1.239	-3.100	-2.653
5	100:0	0.649	-1.369	-2.349	-2.998
Standard Error=					0.31

* Slope of mean value of log(EC_{50})= [(mean value of log(EC_{50}) at generation 5) - (mean value of log(EC_{50}) at generation 1)].

** G= generation cycle.

Table 4.4. Analysis of Variance (ANOVA) for log(EC_{50}) values of mixtures (50:50) of triadimefon-resistant and triadimefon sensitive strains of five isolate mixtures of *Erysiphe graminis* f.sp. *tritici* selected from population collected in 1992 2 wks after triadimefon application from fields of the wheat cv. Absolvent in the Annapolis Valley and tested at 3 generation cycles in the absence of triadimefon.

Source of Variation	DF	Mean Square	Pr > F
Generation	2	19.274	0.0001
Isolate	4	6.225	0.0014
ERROR	8	0.477	

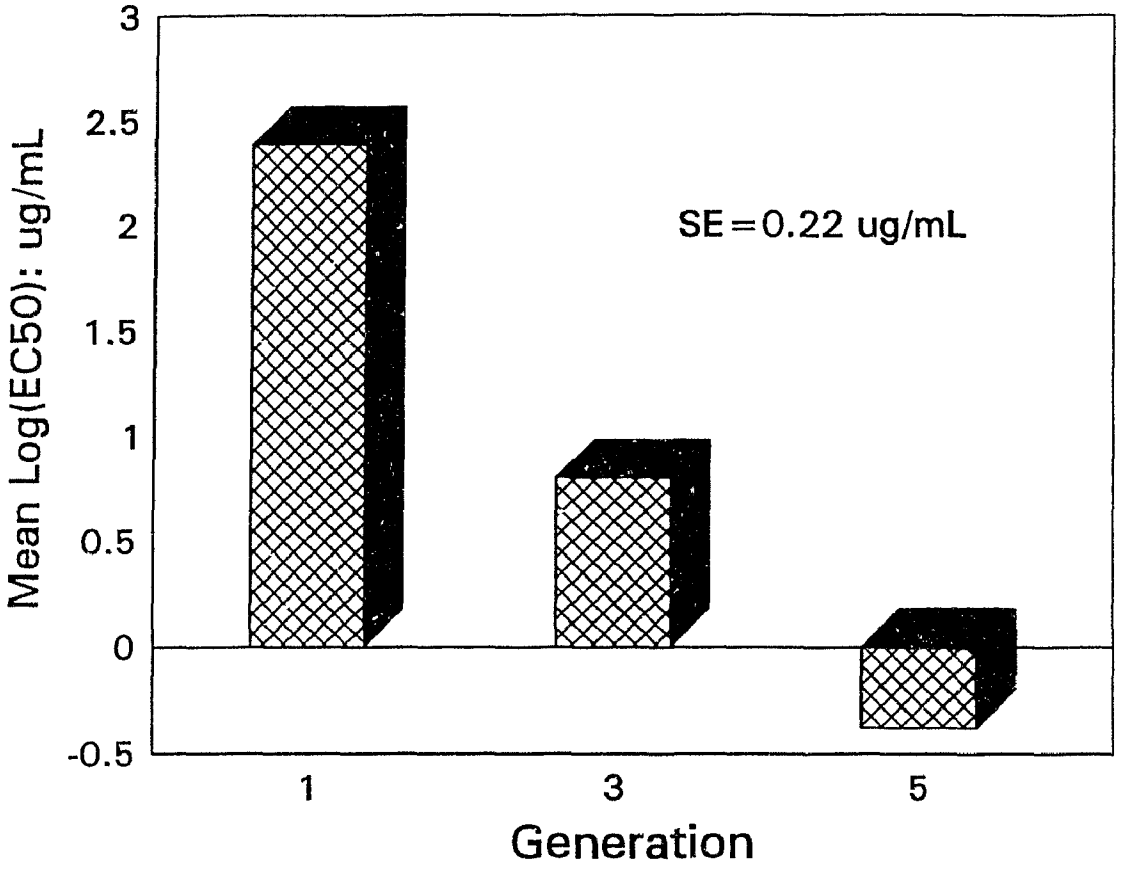


Fig. 4.3 Change in the mean values of log(EC_{50}) of all mixtures of resistant and sensitive isolates for isolate-mixtures of powdery mildew from the Annapolis Valley tested in 1992 on the first, third, and fifth generation cycles, respectively, in the absence of triadimefon.

4.4 DISCUSSION

4.4.1. 1991 Experiment

The highly significant difference between generation cycles of powdery mildew in terms of the mean values of $\log(EC_{50})$ (Tables 4.2 & 4.3) suggested that the resistant strains of *E. graminis* f.sp. *tritici* are less fit than the sensitive ones in the absence of the selection pressure of triadimefon. Regardless of the ratio of R:S in all mixtures, there was a significant increase in sensitivity of the fungus due to competition between R and S isolates over five generation cycles in the absence of the fungicide (Table 4.3). The significant interaction among generation cycles of powdery mildew and treatments (Table 4.2 & Fig. 4.2) also suggested that, for mixtures of R and S isolates from both treated and untreated wheat fields, there was a decline in resistance in terms of the mean values of $\log(EC_{50})$ after keeping the mixtures for five generation cycles in the absence of triadimefon. Significant interaction was also found among mixtures of R and S isolates and generation cycles of the fungus (Tables 4.2 & 4.3). Negative slopes of the mean values of $\log(EC_{50})$ for all the mixtures of R and S isolates over five generations indicated the decrease in resistance of the fungus in the absence of the fungicide. Table 4.3 also indicated that although the resistant isolates increased in the mixture from 0% R in mixture 1 to 100% R in mixture 5, their resistance decreased after five generation cycles in the absence of

triadimefon (Table 4.3). This suggests that even without the effect of competition process (100% R or 100% S), keeping fungal isolates of *E. graminis* f.sp. *tritici* for five generations in the absence of triadimefon caused them to become more sensitive to the fungicide.

Our results agree with those reported by Dekker (1976), Fuchs *et al.* (1977), and De Waard and Van Nistelrooy (1982). Dekker (1976) reported that the competitive ability of mixtures of benomyl-resistant and -sensitive isolates of *S. fuliginea* (90:10, 50:50, 10:90) has been tested in the absence of benomyl for five generations. He found that the resistance had declined and almost disappeared even from the mixture with 90% resistant isolate. Our results are not in variance with these results. Similar results were reported for triforine in the fungus *Cladosporium cucumerinum* (Fuchs *et al.*, 1977) and for fenarimol in *Penicillium italicum* (De Waard and Van Nistelrooy, 1982). In other cases, scientists reported that resistant strains were more fit, and competed better with sensitive ones in absence of fungicides (Shepers, 1985; Vigo *et al.*, 1986; Kadish and Cohen, 1988; Georgopoulos, 1977; Bollen and Van Zaayen, 1975; Bruin, 1988; Staub *et al.*, 1979). Staub *et al.* (1979) reported that cultures of *Phytophthora infestans* maintained their resistance for at least 2 years with repeated sub-culturing on media without the fungicide metalaxyl.

4.4.2. 1992 Experiment

The highly significant difference among generation cycles in terms of the mean values of log(EC_{50}) for mixtures of R and S isolates (Table 4.4 & Fig. 4.3) indicated 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 the selective pressure of triadimefon. Resistant isolates of *E. graminis* f.sp. *tritici* were less fit, and competed less, than sensitive ones in the absence of the fungicide. These results are similar to those reported by Buchenauer *et al.* (1984), who found that in mixtures of triadimefon-resistant and -sensitive isolates of barley powdery mildew (50:50), the proportion of resistant isolates decreased after five passages in the absence of triadimefon. Hollomon (1975) and Wamsley-Woodward *et al.* (1979) also indicated reduced competitive abilities of ethirimol- and tridemorph-resistant isolates of barley powdery mildew compared to sensitive isolates in the absence of the fungicides. Similar reports (Gullino and Garibaldi, 1981) indicated the disappearance of the resistant strain of *Botrytis* in mixtures of R and S isolates (50:50, 10:90) cultured on vinlozolin-free medium for four generation cycles. Moorman and Lease (1992) found similar results.

When fungicide resistance is accompanied by decreased fitness (our results), the risk of development of resistance problem in practice may be considered as moderate or low,

since low fitness counteracts the build-up of resistance. This is in agreement with Dekker (1985), Dekker and Gielink (1979), and Beever and Byrde (1982).

Chapter 5

GENERAL DISCUSSION AND CONCLUSIONS

The test-tube (*in vitro*) method used throughout these studies for the assessment of triadimefon sensitivity in wheat powdery mildew isolates proved to be successful. It provided a high degree of precision to these studies in the sense that all tests were conducted in a completely controlled environment, so that no other sources of the fungus contributed to the cultures of single spore-derived isolates of *E. graminis* f.sp. *tritici*. This allowed us to study the responses of various populations of the fungus through their single spore-derived isolates.

Effective concentrations of triadimefon to prevent growth of 50% (EC_{50}) of powdery mildew on wheat leaves varied within each population. This might indicate a heterogenous population consisting of resistant and sensitive spores. These results agree with those reported by Bruin (1980). Compared to Colchester County, the Annapolis Valley mildew populations were more resistant, and had a higher proportion of individual isolates resistant to triadimefon (Table 2.10 - 2.12). This may be due to the widespread use of the fungicide triadimefon in controlling the wheat powdery mildew, and due to the greater area of land cultivated with wheat. This suggests that the selective pressure of triadimefon in a large area of wheat

production has selected more resistant mildew isolates that spread throughout wheat fields in the Annapolis Valley (Tables 2.9-2.11). This study also indicated that powdery mildew fungal populations from treated fields in the AV were more resistant and had a higher proportion of resistant isolates compared to those from CC (Tables 2.9-2.11). However, there was no significant difference in the response of *E. graminis* f.sp. *tritici* populations from treated and untreated fields in the AV (Tables 2.9 and 2.12). This may be due to the migration of resistant spores from nearby treated wheat fields to the untreated ones. Resistance is known to spread more rapidly in a population of heavily sporulating fungi such as the powdery mildew of wheat, than in that of a slowly germinating pathogen, which does not produce aerial spores (e.g. *Ceratocystis ulmi* in elm) (Dekker, 1976). The resistant spores undoubtedly originated by mutation. The high level of resistance indicates that a decrease in the binding affinity of triadimefon at the target site might be involved when mutation has occurred (Corbett, 1979; Delp, 1980; Dekker, 1985b; Koller and Scheinpflug, 1987). The wheat cv. Absolvent was found to be more susceptible to resistant isolates of powdery mildew than the cv. Borden (Anonymous, 1991). This means that a higher concentration and more frequent applications of triadimefon might be needed to control powdery mildew on Absolvent compared to Borden, where the problem of powdery mildew disease is less severe. This practice might

have caused the resistant isolates to dominate the sensitive ones in Absolvent fields. Similar results were reported on powdery mildew of barley (Fletcher and Wolf, 1981). Brent (1981) stated that "given the marked selectivity of action against one group of fungi or at a particular biochemical site, and given the well-known ability of powdery mildew to adapt to resistant varieties of crop plants, it is not surprising that fungicide resistance should have arisen." The cv. Borden, therefore, is recommended to be grown in Nova Scotia as a replacement for the cv. Absolvent, but even then, strategies to cope with the fungicide resistance problem have to be implemented in order to prevent the fungicide resistance problem from growing worse.

This study indicated that resistant isolates of *E. graminis* f.sp. *tritici* were present in wheat fields in low frequencies early in the season before triadimefon application (Table 3.9 and Figs. 3.1 and 3.2). The frequencies of resistant isolates (Table 3.9) and the mean value of $\log(EC_{50})$ (Fig. 3.1) of mildew populations increased significantly after spraying Absolvent wheat fields with triadimefon. This build-up of resistance to triadimefon was faster (2 wks after application) in the case of the AV populations compared to the CC populations (Table 3.8 and Fig. 3.2). In the case of the CC fungal populations, the resistance towards triadimefon increased slowly after triadimefon application, and by the end of the season, (6 wks after application), both the mean value

of $\log(EC_{50})$ and the frequency of resistant isolates increased significantly (Table 3.8 and Fig. 3.2). This might be due to the fact that the fungus is not well established in the CC, and that the favourable environmental conditions do not exist until later in the season. Furthermore, since the frequency of resistant isolates in CC wheat fields was lower than that in AV wheat fields before triadimefon application, it took longer for the resistant isolates to reproduce and build-up (Fig. 3.2). However, in both locations, selection towards reduced sensitivity was clear after exposure to the fungicide, and was observed as a shift in the mean EC_{50} of the populations and the frequency of resistant isolates in each population. Emergence of resistant isolates may, presumably, occur with all systemic fungicides, and also with other, non-systemic, specific-site inhibitors (Dekker, 1985).

The disease progress curves (Figs. 3.3 and 3.4 and Table 3.10) indicated that triadimefon application decreased the severity of powdery mildew disease, but did not seem to stop its development. The increase in disease severity (Figs. 3.3 and 3.4) seems to be associated with the increase in the frequency of resistant isolates (Figs. 3.5 & 3.6) and in the mean values of EC_{50} of powdery mildew populations (Figs. 3.1 & 3.2). This has resulted in poor disease control rather than in failure of disease control. These results agreed with those reported by Brent (1981), Eniz (1988), and Elad (1992).

Nevertheless, it is important that the performance of this fungicide be kept under review.

This study also indicated that resistant isolates of *E. graminis* f.sp. *tritici* in both the AV and CC fungal populations were less fit than the sensitive ones after keeping mixtures of R and S isolates for five generation cycles in the absence of the selection pressure of triadimefon (Tables 4.2-4.4; Figs. 4.1-4.3). In the 1991 experiment, results indicated that for mixtures of resistant and sensitive isolates from treated and untreated wheat fields of the cv. Absolvent in the AV, there was a decline in resistance to triadimefon in terms of mean log (EC_{50}) values after five generation cycles in the absence of triadimefon (Fig. 4.2). All ratios of R:S isolates in all mixtures (Table 4.1) exhibited a clear decline in the mean log(EC_{50}) values after keeping the fungus mixtures for five generation cycles in the absence of triadimefon (Table 4.3). This indicates that regardless of the proportion of sensitive isolate in a mixed fungal population, the population becomes more sensitive to the fungicide if the use of triadimefon is discontinued for a certain period of time. In the 1992 experiment, similar results were found when the competitive abilities of mixtures (50:50) of resistant and sensitive isolates were tested in the absence of triadimefon for five generation cycles (Table 4.4 and Fig. 4.3). These results suggest that if the use of triadimefon in Nova Scotia is discontinued for a certain

period of time, and re-introduced thereafter with caution, this might help to decrease the proportion of resistant isolates and their frequency, and eventually, to achieve better disease control. Reentry of triadimefon must be done with caution, following monitoring studies to indicate when this might be worthwhile. The risk of reentry should be minimized by the use of fungicide mixture, careful timing, and growing resistant varieties of wheat. Therefore, further studies are needed in the area of implementing strategies to avoid or cope with fungicide resistance.

CLAIM OF ORIGINALITY

Original discoveries made in this investigation include:

1. Powdery mildew populations of wheat from the Annapolis Valley were more resistant to triadimefon than those from Colchester County.
2. The cultivar Absolvent was more favourable for resistant isolates of powdery mildew than the cultivar Borden.
3. Mildew populations from triadimefon-treated fields were more resistant to triadimefon than those from untreated fields.
4. In the absence of triadimefon, the proportion of resistant isolates in various mixtures of resistant and sensitive isloates decreased significantly after five generation cycles.

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