Effect of sterol-biosythesis inhibiting fungicides on take-all of spring wheat caused by Gaeumannomyces graminis var. tritici
by Celsa Garcia

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Plant Pathology
Montana State University

Abstract:
Take-all of wheat caused by Gaeumannomyces graminisis var. tritici (Ggt) is a very important root rot disease of wheat around the world. In Montana it is prevalent in irrigated spring wheat. Eight sterol-biosynthesis inhibiting fungicides (triadimenol, bitertanol, propi conazol, etaconazol, imazalil, prochloraz, nuarimol, and XE-779) were tested for their in vitro effect on mycelial growth of Ggt. Only five of the fungicides (triadimenol, propiconazol, XE-779, prochloraz, and imazalil) were tested in the field under artificial inoculation (infested oat kernels). Effect of soil fumigation, inoculum rate, and inoculum placement on disease level were also tested in the field. In the greenhouse, the efficacy of triadimenol as a seed treatment was evaluated as influenced by Inoculum level, inoculum location, soil fumigation, soil reaction, and wheat and barley cultivars. All 8 fungicides at 1000, 100, and 10 uM inhibited mycelial growth on PDA. At the lowest concentration tested, 0.01uM, prochloraz and imazalil inhibited growth by 70% while the remaining compounds were only minimally inhibitory. At 1 000 uM, nuarimol, imazalil, and prochloraz were fungicidal. The other compounds were only fungistatic but caused abnormal and restricted growth. None of the fungicides affected the virulence of Ggt. In the field, with 2g of inoculum/3m of row, the lowest disease index for 6 week old plants was obtained where seed was treated with triadimenol at 0.31 and 0.47 6 a.i./kg, with imazalil at 0.1 g a.i./kg, with XE-779 at 0.22 g a.i./kg, and with prochloraz at 0.2 and 0.4 g a.i./kg. At 5 g of inoculum/row, only triadimenol lowered significantly the disease index. At 2 g of inoculum/row only triadimenol had grain yield comparable to the non-inoculated check, but at 5 g of inoculum/row, none of the fungicides significantly increased yield. Fumigation of the soil had a marked effect on disease severity. Grain yield of the inoculated untreated fumigated check was reduced more than 90$. Seed treatment wwith triadimenol or propiconazol in this experiment failed to provide a protective effect in fumigated plots. Level of infection in plots in which the inoculum was rototilled into the soil was not as high as when it was placed in close contact with the seed. Inoculum location had a differential effect on the performance of triadimenol. The disease index of triadimenol seed treated seedlings when the inoculum was above the seed was 3.0 in a scale from 1 to 5. With inoculum below the seed, the DI was 1.1. Infection severity of seedlings grown in the greenhouse was not affected by reaction of the soils with pHs varying from 5.0 to 7.2. Barley cultivars had a lower infection than wheat cultivars. Triadimenol seed treatment significantly reduced infection level for all cultivars of wheat and barley.

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Celsa Garcia

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APPROVAL

of a thesis submitted by

Celsa Garcia

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citation, bibliographic style and consistency, and is ready for submission to the College of Graduate Studies.

Oct 31, 1986
Date

Donald E Mathre
Chairperson, Graduate Committee

Approved for the Major Department

Oct 31, 1986
Date

E. N. Sharp
Head, Dept. of Plant Pathology

Approved for the College of Graduate Studies

12/8/86
Date

Henry L Parsons
Graduate Dean

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TABLE OF CONTENTS

	Page
APPROVAL PAGE	ii
STATEMENT OF PERMISSION TO USE	iii
LIST OF TABLES	vi
LIST OF FIGURES	x
ABSTRACT	xi
INTRODUCTION	1
LITERATURE REVIEW	4
Infection Process	5
Field Symptoms	8
Chemical Control	9
Sterol-Biosynthesis Inhibiting Fungicides	12
MATERIALS AND METHODS	14
Poison Food Tests	14
In Vitro Mycelial Growth Tests	14
Fungicidal/Fungistatic Activity Tests	16
Virulence Tests	16
Field Trials	18
Inoculum Preparation	18
Seed Treatments	20
Planting	20
Evaluation of Fungicides	21
Effect of Soil Fumigation	22
Effect of Inoculum Placement	24
Greenhouse Tests	26
Inoculum Location.	26
Soil pH	28
Host Species and Cultivars	30
RESULTS	32
Poison Food Tests	32
Field Trials	36
Evaluation of Fungicides	36
Effect of Soil Fumigation	47
Effect of Inoculum Placement	53
Greenhouse Tests	58
Inoculum Location	58
Soil pH	61
Host Species and Cultivars	62

TABLE OF CONTENTS (cont.)

Page

DISCUSSION..... 63

LITERATURE CITED..... 69

LIST OF TABLES

Table	Title	Page
1	Sterol-biosynthesis inhibiting fungicides tested for activity against *Gaeumannomyces graminis* var. *tritici*	15
2	Scale used to assess infection by *Gaeumannomyces graminis* var. *tritici* on wheat plants	19
3	Sterol-biosynthesis inhibiting fungicides tested as seed treatments against *Gaeumannomyces graminis* var. *tritici*	20
4	Effect of various sterol-biosynthesis inhibiting fungicides on in vitro mycelial growth of *Gaeumannomyces graminis* var. *tritici* on potato dextrose agar	33
5	Propensity of various sterol-biosynthesis inhibiting compounds to be fungicidal or fungistatic to *Gaeumannomyces graminis* var. *tritici*	34
6	Effect of previous mycelium exposure to various sterol-biosynthesis inhibiting fungicides on virulence of *Gaeumannomyces graminis* var. *tritici* to Pondera spring wheat	35
7	Effect of various sterol-biosynthesis inhibiting fungicides on in vitro mycelial growth of *Gaeumannomyces graminis* var. *tritici* on Czapek's agar	36
8	Effect of sterol-biosynthesis inhibiting fungicides applied as seed treatments to Pondera spring wheat on severity of take-all disease after 6 weeks in the field	38

LIST OF TABLES (cont.)

Table	Title	Page
9	Effect of sterol-biosynthesis inhibiting fungicides applied as seed treatments to Pondera spring wheat on shoot dry weight of 6 week old plants in the field artificially infested with Gaeumannomyces graminis var. tritici.....	39
10	Effect of sterol-biosynthesis inhibiting fungicides applied as seed treatments to Pondera spring wheat on number of tillers per plant of 6 week old plants in the field artificially infested with Gaeumannomyces graminis var. tritici.....	41
11	Effect of sterol-biosynthesis inhibiting fungicides applied as seed treatments to Pondera spring wheat on severity of take-all after 8 and 12 weeks in the field.....	43
12	Effect of sterol-biosynthesis inhibiting fungicides applied as seed treatments to Pondera spring wheat on number of live plants/m in plots artificially infested with Gaeumannomyces graminis var. tritici.....	44
13	Effect of sterol-biosynthesis inhibiting fungicides applied as seed treatments to Pondera spring wheat on number of tillers/m in plots artificially infested with Gaumannomyces graminis var. tritici.....	46
14	Effect of sterol-biosynthesis inhibiting fungicides applied as seed treatments to Pondera spring wheat on grain yield of plants in field plots artificially infested with Guamannomyces graminis var. tritici.....	47

LIST OF TABLES (cont.)

Table	Title	Page
15	Analyses of variance of seven parameters that measure infection by Gaeumannomyces graminis var. tritici on Pondera spring wheat in artificially infested field plots.....	49
16	Effect of soil fumigation on take-all disease index and performance of sterol-biosynthesis inhibiting fungicides applied as seed treatments to Pondera spring wheat.....	50
17	Effect of soil fumigation with methyl bromide on grain yield in field plots artificially infested with Gaeumannomyces graminis and performance of sterol-biosynthesis inhibiting fungicides applied as seed treatments to Pondera spring wheat.....	52
18	Effect of inoculum level and placement on severity of take-all of 6 week old Pondera spring wheat in the field.....	54
19	Effect of inoculum level and placement on dry weight of take-all of 6 week old Pondera spring wheat in the field.....	55
20	Effect of inoculum level and placement on severity of take-all of 12 week old Pondera spring wheat in the field.....	56
21	Interaction of seed treatment and inoculum rate and Ggt placement on grain yield of Pondera spring wheat.....	57
22	Effect of amount of broadcast Gaeumannomyces graminis inoculum on percentage of white heads of Pondera spring wheat as affected by seed treatment.....	58

LIST OF TABLES (cont.)

Table	Title	Page
23	Effect of triadimenol on severity of take-all of seedlings of Pondera spring wheat in the greenhouse as affected by inoculum position.....	59
24	Effect of soil pH and triadimenol seed treatment on severity of take-all of artificially inoculated Pondera spring wheat seedlings in the greenhouse.....	61
25	Effect of triadimenol seed treatment of wheat and barley cultivars on severity of take-all.....	62

LIST OF FIGURES

Figure	Title	Page
1	Flow sheet showing sequence of tests to determine the effect of sterol-biosynthesis fungicides on virulence of *Gaeumannomyces graminis* var. *tritici* on wheat seedlings.....	17
2	Location of Ggt inoculum with respect to Pondera wheat seed used to test activity of triadimenol.....	27

Abstract

Take-all of wheat caused by Gaeumannomyces graminis var. tritici (Ggt) is a very important root rot disease of wheat around the world. In Montana it is prevalent in irrigated spring wheat. Eight sterol-biosynthesis inhibiting fungicides (triadimenol, bitertanol, propiconazol, etaconazol, imazalil, prochloraz, nuarimol, and XE-779) were tested for their in vitro effect on mycelial growth of Ggt. Only five of the fungicides (triadimenol, propiconazol, XE-779, prochloraz, and imazalil) were tested in the field under artificial inoculation (infested oat kernels). Effect of soil fumigation, inoculum rate, and inoculum placement on disease level were also tested in the field. In the greenhouse, the efficacy of triadimenol as a seed treatment was evaluated as influenced by inoculum level, inoculum location, soil fumigation, soil reaction, and wheat and barley cultivars. All 8 fungicides at 1000, 100, and 10 uM inhibited mycelial growth on PDA. At the lowest concentration tested, 0.01uM, prochloraz and imazalil inhibited growth by 70% while the remaining compounds were only minimally inhibitory. At 1000 uM, nuarimol, imazalil, and prochloraz were fungicidal. The other compounds were only fungistatic but caused abnormal and restricted growth. None of the fungicides affected the virulence of Ggt. In the field, with 2g of inoculum/3m of row, the lowest disease index for 6 week old plants was obtained where seed was treated with triadimenol at 0.31 and 0.47 g a.i./kg, with imazalil at 0.1 g a.i./kg, with XE-779 at 0.22 g a.i./kg, and with prochloraz at 0.2 and 0.4 g a.i./kg. At 5 g of inoculum/row, only triadimenol lowered significantly the disease index. At 2 g of inoculum/row only triadimenol had grain yield comparable to the non-inoculated check, but at 5 g of inoculum/row, none of the fungicides significantly increased yield. Fumigation of the soil had a marked effect on disease severity. Grain yield of the inoculated untreated fumigated check was reduced more than 90%. Seed treatment wwith triadimenol or propiconazol in this experiment failed to provide a protective effect in fumigated plots. Level of infection in plots in which the inoculum was rototilled into the soil was not as high as when it was placed in close contact with the seed. Inoculum location had a differential effect on the performance of triadimenol. The disease index of triadimenol seed treated seedlings when the inoculum was above the seed was 3.0 in a scale from 1 to 5. With

inoculum below the seed, the DI was 1.1. Infection severity of seedlings grown in the greenhouse was not affected by reaction of the soils with pHs varying from 5.0 to 7.2. Barley cultivars had a lower infection than wheat cultivars. Triadimenol seed treatment significantly reduced infection level for all cultivars of wheat and barley.

INTRODUCTION

Take-all is an old problem in the production of wheat. It has a worldwide distribution and has been reported from all temperate climates and tropical regions where wheat can be grown. Take-all is a serious problem in winter wheat in Australia, Europe, South Africa, Japan, North America and South America (Garrett, 1981). The disease is caused by Gaeumannomyces graminis (Sacc.) v. Arx and Olivier var. tritici. The fungus causes root and crown rot and is favored by high levels of soil moisture. Crop damage is extensive in irrigated or high rainfall areas, and yield losses can be as high as 50% (Wiese, 1977) and sometimes greater. World wheat production in general, and in North America in particular, consists largely of winter wheat. In the United States, winter wheat represents 70% of total wheat production (Reitz, 1967). Thus, most of the reports of take-all are from winter wheat areas, and most of the work dealing with take-all has been done on winter wheat. In Montana, however, take-all is primarily a disease of spring wheat produced under irrigation, where conditions are very conducive for the disease. Juhnke (1983) rated take-all as the second most important yield limiting factor for irrigated wheat. The irrigated acreage in Montana has

increased considerably in recent years, totalling 46,378 ha in 1984 (Montana Agricultural Statistics, 1985).

Control of take-all has been accomplished through cultural practices, particularly crop rotation. In the early 1900's, crop rotation for take-all control was a common practice in Europe and Australia (Yarham, 1981). However, more recently, rotation has been less attractive due to economic constraints on growers. In Montana, rotation of irrigated areas is restricted due to lack of suitable alternative crops which can provide an economic return to the grower.

In Oregon and Idaho, some reduction in losses due to take-all have been obtained through the use of ammonium based fertilizers (Smiley and Cook, 1973) and/or chloride fertilizers (Christensen et al., 1981). Suppression of the disease by ammonium and chloride fertilizers may be associated with changes in the rhizosphere microflora. This phenomenon and the occurrence of disease decline with continuous cropping in the same soil have led to the emphasis in research on biological control.

To allow maximum development of the decline phenomenon (=disease suppression), it would be useful to have available other control measures for take-all that would reduce the losses that a grower suffers. Such control may include the use of fungicides as soil drenches or seed

treatments. Bockus (1983), by using triadimenol as a seed treatment, observed a reduction of 60-75% in yield losses due to take-all in winter wheat.

The purpose of this study was to evaluate eight sterol-biosynthesis inhibiting fungicides for their potential in controlling take-all when used as seed treatments on spring wheat.

LITERATURE REVIEW

Gaumannomyces graminis (Sacc.) v. Arx & Olivier causes root rot in several grasses and cultivated cereals. *Gaeumannomyces graminis* (Sacc.) v. Arx & Olivier var. *avenae* (Turner) Dennis (Gga) is commonly associated with oats and turf grasses; *G. graminis* (Sacc.) v. Arx & Olivier var. *graminis* (Ggg) is a weak pathogen that has been reported from rice causing crown and sheath rot, and from several grasses; *G. graminis* (Sacc.) v. Arx & Olivier var. *tritici* (Walker) (Ggt) is primarily a pathogen of wheat and barley, but it has been reported to attack many other gramineae (Scott, 1981). *Gaeumannomyces graminis* characteristically forms dark mycelial strands, called runner hyphae, on the host root surface (Garret, 1934). The runner hyphae branch to produce lighter and finer infectious hyphae which penetrate the root. Simple and lobed swellings, called hyphopodia, are produced on infected host tissue and sometimes in culture (Fellows, 1928). Ggg produces simple and lobed hyphopodia, whereas Gga and Ggt produce only simple hyphopodia. A more detailed morphological description of *G. graminis* is given by Walker (1981).

Ggt causes the disease known as Take-all that affects both barley and wheat. As a rule, however, wheat is more susceptible than barley, and extensive damage to the root system of wheat plants is reported from many wheat growing areas around the world (Cook, et al., 1968; Diehl, et al., Gorska-Poczopko, 1971; Heyne, 1977; Jarham, 1981; Kirby, 1925; Lescar and Caron, 1980; Lester, 1967; Scott, 1978; 1984; Suzuki, et al., 1957). The pathogen survives saprophytically in the soil as mycelium in infested crop debris (Hornby, 1975; Shipton, 1981). Mycelium from the infested debris grows tropically toward roots of actively growing plants (Brown and Hornby, 1971; Wildermuth, et al., 1982). Once the mycelium reaches the roots, it can grow on any part of the plant below ground.

Infection Process

In noncompatible host-parasite systems, such as oats and Ggt, the fungus will grow as runner hyphae on the root surface without establishing infection. In a susceptible host such as wheat, runner hyphae usually grow longitudinally over the root surface, and from the runner hyphae infection hyphae develop that penetrate epidermal cells. Once the infection hyphae are in contact with the cell wall of epidermal cells, they form a penetration peg.

Penetration is largely the result of cell wall disintegration due to enzymes exuded from the peg (Skou,

1981). The host cell responds by depositing ligneous substances in the innermost part of the cell wall at the point of infection (Fellows, 1928; Russell, 1934; Holland and Fulcher, 1971). As the hyphae advance disintegrating the deposited materials, an inner tube of ligneous material (lignitubers) is formed around the hyphae (Fellows, 1928). Lignitubers give a positive reaction with safranin, but do not stain with fuchsin, indicating that the ligneous material is different from that of healthy cell walls (Skou, 1981). So long as the root cell remains alive, it produces new matter to compensate for that decomposed, lengthening the lignituber. Eventually, the root tissues are not able to prevent the hyphae from penetrating, and die (Skou, 1975).

Cell contents rapidly disintegrate following entry of the pathogen resulting in cell death. Infection hyphae then either grow forward to attack the opposite cell wall or fill the invaded cell with mycelium. This mycelium may develop into crust-shaped sclerotium-like structures, or into small spherical bodies of tightly interwovum hyphae, like microsclerotia (Fellows, 1928; Holland and Fulcher, 1971).

Hyphae spread into the cortex from the site of infection. In weak attacks, several lignitubers are found

in the epidermis and outer cell layers where they constitute a palisade. In severe attacks, fewer lignitubers are found in these layers (Skou, 1975). Deeper in the tissue, lignitubers are scattered. Palisades of lignitubers are, again, common in the endodermis where it constitutes a barrier, and the rate of infection slows down (Fellows, 1928; Skou, 1975). According to Skou (1975), the development of lignitubers is not considered a decisive resistance mechanism, but a general protective mechanism against weak or moderate attacks.

Once the endodermis is crossed and the fungus reaches the stele, the hyphae grow rapidly. With invasion of the xylem vessels, rupture of cell walls and breakdown of all stelar tissues occurs (Clarkson et al., 1975; Holden, 1976). The phloem usually disintegrates more rapidly than other stelar tissues. Clarkson et al. (1975), estimated that only 5 to 10% of the xylem is occupied by the pathogen. Disintegration of the phloem leads to restriction and eventual cessation of phloem translocation and root enlongation of the infected root. Ion transportation in the xylem can continue, but ceases after metabolic compounds are consumed (Clarkson et al., 1975).

All parts of susceptible plants at and below the soil surface may be attacked by Ggt. The subcoronal internode is attacked in the same way as seminal and adventitious

roots but is probably more resistant, perhaps because of a higher content of ligneous substances (Fellows, 1928; Robertson, 1932). The crown is invaded through the subcoronal internode and the adventitious roots (Fellows, 1938).

Infection and destruction of seminal roots promotes growth of additional adventitious roots (Skou, 1975; Clarkson et al., 1974). Skou (1975) attributes the resistance of barley to Ggt to the ability to produce additional adventitious roots during attack. In field conditions this is an important survival mechanism, but if infection occurs in very young plants, nutrient and water supply to the shoot will be inadequate and production of additional adventitious roots may be inadequate to sustain growth of the plant.

Field Symptoms

The nature and severity of symptoms observed in the field will depend on the virulence of the pathogen and the ability of the host to produce new roots more quickly than the fungus can destroy them. In severe attacks on young plants, there is no effective root replacement and the plant dies. This is the 'take-all' phase and goes largely unnoticed in the field. The death of plants at an early stage will result in patches sparsely populated with

stunted plants. In older plants, the most conspicuous symptom of take-all is the prematurely ripened white heads. At this stage, the straw base and lower leaf sheath are always brown to black, which is caused by runner hyphae and mycelial crusts (Fellows, 1928).

Chemical Control

The control of the take-all disease of wheat is a hard problem. The first approach and best known control measure is crop rotation. Although take-all was present in Britain during the last century, it was not recognized as a big problem on wheat. Yarham (1981) states that "take-all certainly caused nineteenth-century British farmers far less trouble than it did their Australian counterparts. This may have been due in part to the rotational practices of the day". To this day, crop rotation is by far the most common (if not the only one in some areas) control measure for this disease.

Use of ammoniacal sources of N (Smiley and Cook, 1973) are reported to decrease the effect of take-all on yield. This effect, however, is not universal and will vary with soil pH and soil type. Continuous cropping of wheat promotes build-up of Ggt antagonistic microorganisms in the soil that results in a decrease of the disease after several years of wheat. For many wheat producers, however, this take-all decline is not economically feasible as a

control measure.

Until recently, chemical control of the take-all was of limited value because of the lack of persistence or lack of efficacy of fungicides available. With the development of systemic fungicides, a new perspective on the control of take-all was available. Benomyl was one of the first systemic fungicides tested as a seed treatment against Ggt. Gorska-Paczapko (1971), found benomyl to be the best in-vitro mycelial growth inhibitor (1 ppm. of a.i.) among several other systemic fungicides. It also had activity against Ggt in greenhouse tests, applied as a seed dressing (2g a.i/kg seed). Pren and McIntosh (1975), however, did not observe activity of benomyl against take-all in naturally infested fields. More recently, Ballinger and Kollmorgen (1986) found that benomyl significantly reduced the disease when tested in the greenhouse with doses as low as 0.5g a.i./kg seed, but not when tested in the field. Bateman (1980) also found benomyl, as well as several other compounds, to be toxic to Ggt on agar plates. He, however, chose soil drenching for testing the fungicides in the greenhouse and field (Bateman, 1980, 1981, 1982, 1984a, 1984b, 1985; Bateman and Nicholls, 1982). Soil drenching does not have a practical use in the field since benomyl is effective only in the area where it is applied. This means

that surfactants or large amounts of fungicide would have to be used to obtain a better coverage and protection of the roots.

Several other systemic fungicides have been tested against take-all, but, with less successful results as compared to benomyl (Gorska-Proczopho, 1971; Bateman, 1980). In 1981, Dolezal and Jones reported that seed treatment with triadimefon, a sterol-biosynthesis inhibiting systemic fungicide, significantly reduced yield losses of winter wheat in artificially infested fields. Triadimenol, another sterol-biosynthesis inhibiting fungicide used against bunts and early infection of rusts in cereals, was reported (Bockus, 1982) to protect winter wheat seedlings in the greenhouse for 8 weeks and increase yield by 38% in the field. Mathre et al., (1986), also found that triadimenol reduced infection and increased yield of spring wheat in naturally and artificially infested fields.

Ergosterol is the main sterol in most fungi, and is an important component of mycelial membranes. It is not, however, the main sterol in the Uridinales and it is not present at all in the Oomycetes Pythium and Phytophthora (Mercer, 1984).

The starting point in the synthesis of all sterols is acetyl-CoA. Squalene is the leading compound in the

synthesis of ergosterol, and is formed after a series of biosynthetic reactions. Squalene then undergoes cyclization. In non-photosynthetic organisms such as fungi, the product of cyclization of squalene is lanasterol, but in photosynthetic organisms, such as lighter plants and algae. it is cycloasterol. The conversion of lanasterol into ergosterol is a complex multistep process. The sequence of the reactions is not certain but in most of the ergosterol-synthesizing fungi, the first step can be methylation of C-24. This is then followed by dimethylation at C-14, C-4α, and C-4β. The sequence of reactions leading from cycloasterol to ergosterol in photosynthetic organism, the 4α-methyl group, is normally removed first, then followed by C-4β and C-14 (Mercer, 1984).

Sterol-Biosynthesis Inhibiting Fungicides

A large number of compounds developed in recent years for the control of fungal pathogens of plants are sterol-biosynthesis inhibiting fungicides. These fungicides control a large number of diseases caused by Ascomycetes, Basidiomycetes, and Deuteromycetes, but do not control diseases caused by Pythium and Phytophthora (Siegel, 1981). This group of fungicides inhibits the sterol 14-demethylation process in the ergosterol biosynthesis

pathway causing the accumulation of sterol intermediates (Siegel, 1981; Ragsdale and Sisler, 1973; Buchenauer, 1977).

There is a considerable diversity in the structures of compounds which inhibit C-14 demethylation. The ones with agricultural use are included in three classes. Triazoles, the largest group, includes the fungicides triademefon, triadimenol, bitertanol, diclobutrazol, and propiconozal; imazalil and prochloraz are imidazoles, while the pyrimidines include fenarimol and nuarimol (Siegel, 1981).

The precise function of sterols in fungi has not been well defined, but their principal role is generally believed to be as architectural components of membranes (Nes, 1974). Reduction in the amount of ergosterol or qualitative change in sterol composition results in altered membrane properties such as permeability and enzyme activity (Sancholle et al., 1984), causing abnormal growth patterns, swollen hyphae, and/or excessive hyphal branching. All of these compounds do not inhibit spore germination but can be fungicidal at high concentrations. Propiconazol, though, appears to be fungistatic rather than fungicidal (Sancholle et al, 1984).

MATERIALS AND METHODS

To determine the activity of sterol biosynthesis inhibiting fungicides on Ggt, and therefore on disease development, several different experiments were conducted in the laboratory, greenhouse, and field.

Poison Food Tests

In Vitro Mycelial Growth Tests

Eight sterol-biosynthesis inhibiting chemicals were tested for their effect on mycelial growth of Ggt to determine the most toxic compounds to later be used in field and greenhouse tests (Table 1). Many of these materials are also reported to have systemic activity. Each fungicide was tested at 1000, 100, 10, 1.0, 0.1, and 0.01 µM of active ingredient in potato dextrose agar (PDA) or Czapek agar (CA). Two experiments were conducted with PDA (PDA-1 and PDA-2) and one with CA. Triadimenol was not included in the CA test. Czapek agar was used because it does not contain sterols. Dilutions of the fungicides were prepared in sterile water except for bitertanol, which was diluted with 95% ethanol. The solution/suspension was added to molten agar medium at 50° C, mixed, and then poured into 4 100 x 15mm petri plates. After solidification, each plate was inoculated with an 11mm disc from an 8 day-old culture of Ggt on PDA, or from a 15 day-

old culture on CA. Following incubation at room temperature, mycelial growth was measured on the second, fourth, sixth, eighth, and tenth day after inoculation for PDA-1 and PDA-2. For CA, only one measurement was taken on the 8th day.

Table 1. Sterol-biosynthesis inhibiting fungicides tested for activity against Gaeumannomyces graminis var. tritici.

Chemical Group	Compound Name	Formulation	Source
Triazole	Triadimenol	Baytan 30[1]	Gustafson, Inc.
	Bitertanol	Baycor 10% DS[1]	Bayer
	Propiconazol	Tilt 3.6E	Ciba Geigy
	Etaconazol	CGA 64251 0.846EH	Ciba Geigy
Imidazole	Imazalil	Fungaflor 5.8% or Imazalil 75%	Janssen Pharmaceuticals
	Prochloraz	Prochloraz 40% E C	Boots Hercules
Pyrimidine	Nuarimol	EL-228 5%	Eli Lilly
Unknown	XE-779	XE-779 25WP	Chevron

[1]Formulated specifically as seed treatments.

Data (total accumulated growth) were subjected to a factorial analysis of variances, with fungicides and concentration as factors. Fungicides were compared within each concentration.

Fungicidal/Fungistatic Activity Tests

To determine if the highest dosages of the experimental materials were fungicidal, rather than merely fungistatic, the original four discs from plates showing very little or no mycelial growth were removed and used to inoculate fungicide-free PDA or CA plates (Fig. 1). These plates were incubated at room temperature and the extent of mycelial growth was determined on the seventh day. In the experiment using CA, two of the discs were replated onto PDA and the other two onto CA.

Virulence Tests

The ultimate purpose of these tests was to determine whether there was any carry over effect of the fungicides on the virulence of Ggt. Inoculum consisting of mycelium growing on fungicide-free medium was obtained either from treatments that allowed some mycelial growth in the in-vitro poison food tests (Fig. 1C) or from the subsequent transfer to fungicide-free medium in the study of fungicidal/fungistatic activity (Fig. 1D). In the first case, an 11mm disc of mycelium from each treatment was used to inoculate fungicide-free PDA plates; these plates were the source of inoculum for the virulence test (Fig. 1E). In the second case, those plates with some mycelial growth constituted the source of inoculum. From these plates, a 20mm disc was removed and used to inoculate wheat seedlings