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Sensitivity of Texas strains of *Ceratocystis fagacearum* to triazole fungicides

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Abstract: Ten geographically diverse Texas strains of the oak wilt fungus *Ceratocystis fagacearum* were tested in vitro for their sensitivity to five triazole fungicides based on accumulated linear growth, linear growth rates, and dry weight accumulation in response to fungicide concentrations of 0.1 to 600 parts per billion (ppb). None of the triazoles inhibited growth at 0.1 ppb, but four of the five fungicides were highly effective in totally inhibiting growth on homemade potato dextrose agar medium and in neopeptone broth cultures at minimum effective concentrations (MECs) in the 10–200 ppb range. Triadimefon did not prevent growth at concentrations up to 1100 ppb. The sensitivity of Texas strains to the triazoles was up to tenfold higher in aqueous broth culture than on solid medium. Exceptional strains exhibited tolerance to certain triazoles, requiring MECs up to 500 ppb or higher for total growth inhibition. The specific activity of individual triazoles was related to substituent R-groups attached to chiral carbons. The ketal triazoles, propiconazole and difenoconazole with the dioxolane ring, had the greatest effect on growth rates of the Texas strains. Myclobutanil with nitrilo and butyl R-groups and terbuconazole with dimethylethyl and hydroxyl R-groups had intermediate effects on growth rates, while triadimefon with a dimethyl butanone group had the least effect on growth rates of the fungus. Triazole sensitivity was not linked to specific mating incompatibility alleles. The triazole sensitivity of Texas strains of *Ceratocystis fagacearum* was much greater than the sensitivity of many other phytopathogenic fungi previously tested. The significance of these results relative to current fungicide application methods used for oak wilt control by the Texas Oak Wilt Suppression Project (TOWSP) are discussed.

Key Words: chemical control, ergosterol-biosynthesis inhibitors, live oak, oak wilt, systemic eradicants

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INTRODUCTION

Oak wilt, caused by the ascomycete *Ceratocystis fagacearum* (T. W. Bretz) J. Hunt, is one of the most important and potentially destructive diseases of oaks (*Quercus* spp.) in the eastern, central, and more recently, southern United States. The disease has caused high economic losses in localized areas, but its widespread damage to highly valued oak forests has not been severe in most regions except in Texas (MacDonald, 1995). The disease, first diagnosed in Texas in 1961 (Dooling, 1961), reached epidemic levels around 1980 resulting in the formation of hundreds of new infection centers affecting over 20 million acres in 35 counties by 1986 (Texas Forest Service, personal communication). Currently, it has been identified in at least 55 counties throughout the state, including most of the large metropolitan areas (Appel et al., 1995). Live oaks, *Q. virginiana* Mill. and *Q. fusiformis* Small, are the most highly valued shade trees in the state contributing to 13–19% of tax-appraised residential property values in Austin (Martin, 1986). A single, large oak tree may be valued at $5000-20 000 (Dewers, 1971). The cumulative economic losses attributed to the disease statewide have exceeded $100 million based on annual estimates (see McKinney and Billings, 1995).

There are 24 oak species in Texas that are presumed to be variably susceptible to oak wilt. Red oak species, particularly Spanish or Texas red oak *Q. texana* Buckley (= *Q. buckleyi* Dorr & Nixon), shumard oak *Q. shumardii* Buckley, and blackjack oak *Q. marilandica* Miinchh. are highly susceptible and rapidly killed by the disease often within a few months after infection. Native white oaks such as post oak *Q. stellata* Wangenh., bur oak *Q. macrocarpa* Michx., and chinkapin oak *Q. muehlenbergii* Engelm., are resistant and rarely killed by the disease. The semievergreen live oak species are intermediate in susceptibility, but

are the most seriously affected oaks in terms of disease incidence and rate of spread. The high incidence and rapid rate of spread within pure live oak stands are due to their tendency to form vast interconnected root systems and to vegetatively propagate by root sprouts. This growth habit provides effective avenues for the oak wilt fungus to spread rapidly, up to 30 m or more per year between adjacent trees, through root grafts and common root systems (Appel et al., 1995). Infection centers up to 80 ha in size have been observed within live oak stands.

The severity and magnitude of the problem prompted the Texas Forest Service (TFS) to initiate a five-year cooperative Oak Wilt Demonstration Project with the USDA Forest Service in 1982 to assess the extent and impact of the problem and the need for a disease suppression program. Public demand for assistance led to the organization of the Texas Oak Wilt Suppression Project (TOWSP) in 1988, administered by the Texas Forest Service (TFS). The principal suppression activities currently used by the TOWSP involve an integrated approach consisting of trenching in both urban and rural sites to sever root connections in advance of the fungus to prevent root transmission, sanitation measures to avoid inoculum sources and vector transmission, and preventative fungicide applications to high-value trees within urban areas and near homesteads in rural areas to protect against infections.

The systemic triazole fungicide propiconazole (Alamo@) has been used to control oak wilt in Texas since 1990 (Gehring, 1995). Propiconazole remains the only fungicide currently registered for oak wilt control in this state. The high cost of this material and the variable results associated with its use has led to questions of whether other triazoles or different application methods with Alamo should be tested for oak wilt control (Wilson, 1995). Early results of field tests with new application methods including two types of low-volume, high-concentration microinjections and a soil application method have been reported (Wilson and Lester, 1995, 1996). Initial triazole screening studies in vitro have indicated that the triazoles fungicides, in general, have very good fungistatic if not fungicidal activity against the oak wilt fungus in the parts per billion (ppb) range, despite the inconsistent field results of the TFS (Wilson, 1994). This preliminary work further indicated that some Texas strains of C. *fagacearum* have tolerance to certain triazole fungicides and that some triazoles have much lower fungistatic activity against this fungus than others.

This study is part of a larger effort to investigate and test ways to improve on existing fungicide application methods currently used by the TOWSP. The purpose was to screen selected triazole fungicides in vitro to identify new alternative materials that are effective and potentially useful for oak wilt control in Texas. This information will be useful in designing subsequent field trials for efficacy testing of each fungicide. The specific objectives were: 1) to determine the minimum effective concentrations (MECs) for five triazole fungicides; 2) to compare the effects of the triazoles on accumulated linear growth, linear growth rates, and dry weight accumulation of C. *fagacearum* on solid and in liquid culture to assess the relative effectiveness of each fungicide in inhibiting growth of the oak wilt fungus; and 3) to assess the range of variability in fungicide sensitivity and tolerance among ten strains of C. ***fagacearum*** isolated from infected oaks throughout the Hill Country of central Texas.

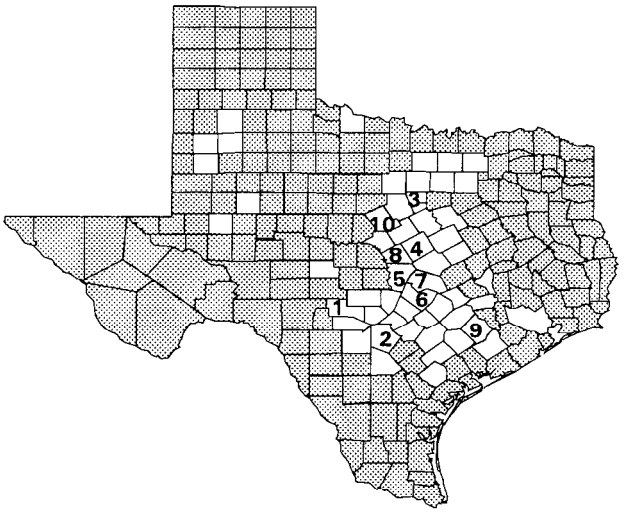

FIG. 1. Texas counties from which the ten isolates of C. *fagacearum* (numbered) were derived. Numbers refer to the isolate number as indicated in TABLE I. Unshaded areas without numbers indicate additional counties with confirmed cases of oak wilt.

MATERIALS AND METHODS

Isolation and culture.-Sapwood samples were collected from the boles and roots of declining, symptomatic live oaks and Spanish oaks from ten counties throughout the central Texas Hill Country on the Edwards Plateau (FIG. 1). Symptomatic live oaks were identified by the characteristic veinal chlorosis, veinal necrosis, and tip necrosis leaf symptoms diagnostic of oak wilt disease in this species. Spanish oaks exhibiting water soaking, chlorosis, bronzing, and marginal leaf necrosis were considered symptomatic and potentially infected with the oak wilt fungus. The flagging symptom of Spanish oaks caused by the death

TABLE I. Sources of Texas strains of C. fagacearum used in the triazole screening trials

Isolate* no.	Strain designation	County source	Host source	Mating type
1	SHL-TX-09	Kerr	*Quercus virginiana*	A_2
2	SHL-TX-10	Williamson	*Quercus virginiana*	A_2
3	SHL-TX-13	Bexar	*Quercus virginiana*	A_1
4	SHL-TX-15	Hood	*Quercus virginiana*	A_1
5	SHL-TX-17	Coryell	*Quercus texana*	A_2
6	SHL-TX-19	Burnet	*Quercus texana*	A_1
7	SHL-TX-22	Lampasas	*Quercus virginiana*	A_1
8	SHL-TX-24	Colorado	*Quercus virginiana*	A_2
9	SHL-TX-25	Comanche	*Quercus virginiana*	A_1
10	SHL-TX-36	Travis	*Quercus virginiana*	A_2

*Isolate numbers correspond with numbers shown at county locations on the Texas map (Fig. 1).

of leaves on individual limbs could often be observed from a distance. The presence of fungal mats on red oaks provided conclusive evidence of oak wilt. Bole samples were collected with 2.5 X 15.0 cm cuts 1.5-2.0 cm deep into the sapwood of symptomatic trees using a wood chisel sterilized with 0.525% NaClO (10% commercial bleach). Root segments were collected from l-4 cmdiam roots located up to a meter deep using a backhoe for digging and lopping shears to cut the segments. In addition, fungal mats forming under the bark of declining Spanish oaks were collected for hyphal and conidial isolations from conidiogenous growth developing on the surface of the mats in the spring. All samples were placed in sealed plastic bags or wide-mouth bottles on ice before they were transported back to the laboratory.

Bole and root samples were processed by removing the outer and inner bark from each sample, cutting the xylem tissue into 2-5 mm-square pieces, surface sterilizing with agitation 3-7 min in a solution of 0.525% NaClO (10% commercial bleach) for bole samples and 1.05% NaClO (20% commercial bleach) for root samples. The disinfested wood pieces were blotted on sterile tissues to remove excess bleach solution and plated without rinsing by embedding the pieces into homemade potatodextrose agar isolation medium (PDA), prepared as described by Dhingra and Sinclair (1985) for medium no. 153, amended with 0.05% w/v streptomycin sulfate after autoclaving at 15 psi for 35 min. All dishes were sealed with Parafilm® to protect them from contamination and excess drying. The dishes were incubated at 25 C in a dark growth chamber. The mycelium of C. *fagacearurn* usually grew from the wood chips within 3-7 d after plating, although 2-3 wk incubations usually were required before conidial sporulation began. Fungal mat isolations from Spanish oaks were obtained by picking off small masses of conidia or hyphae from the mat surface and transferring them to the PDA isolation medium. Colonies growing on isolation medium were subcultured from hyphal tips to ensure purity. All isolates were maintained and stored at 4 C on PDA slants and on agar plugs stored in sterile distilled water within cryovials as described by Burdsall and Dorworth (1994). Cultures of the ten strains of C. *fagacearum* used in this study have been deposited with the American Type Culture Collection (ATCC 200423-200432).

Triazole sensitivity studies.-The ten strains of C. *fagacearum,* isolated from live oaks and Spanish oaks in ten counties throughout the Edwards Plateau Region of Texas (TABLE I), were tested for their sensitivity in vitro to five triazole fungicides including: propiconazole (Alamo), difenoconazole (Dividend), myclobutanil (Systhane) , terbuconazole (Folicur) , and triadimefon (Bayleton) (TABLE II, FIG. 2). The effects of these triazoles on linear (radial) growth of the isolates on PDA, without antibiotic added, and on dry weight accumulation in Neopeptone broth (NPB) after a 6-wk incubation period at 24 C were compared. Two stock solutions of each fungicide were prepared using either emulsifiable concentrate, flowable sus-

TABLE II. Triazole fungicides screened in C. fagacearum sensitivity tests

Common name	Trade name	Distributor	Formulation[a]	Solubility[b]	Empirical formula	Structure[c]
Propiconazole	Alamo	Ciba Geigy	14.3% MEC	110	$C_{15}H_{17}Cl_2N_3O_2$	A
Difenoconazole	Dividend	Ciba Geigy	3 FS	3	$C_{19}H_{17}Cl_2N_3O_3$	B
Myclobutanil	Systhane	Rohm & Haas	2 EC	142*	$C_{15}H_{17}ClN_4$	C
Terbuconazole	Folicur	Bayer	3.6 F	32	$C_{16}H_{22}ClN_3O$	D
Triadimefon	Bayleton	Bayer	95.6% TG	64	$C_{14}H_{16}ClN_3O_2$	E

[a] Formulations: MEC = microencapsulated emulsifiable concentrate, FS = flowable suspension, F = flowable, TG = technical grade crystals.

[b] ppm in water at 20 C or 25 C (*).

[c] Chemical structures of triazoles (A-E) correspond to those presented in Fig. 2 A-E.

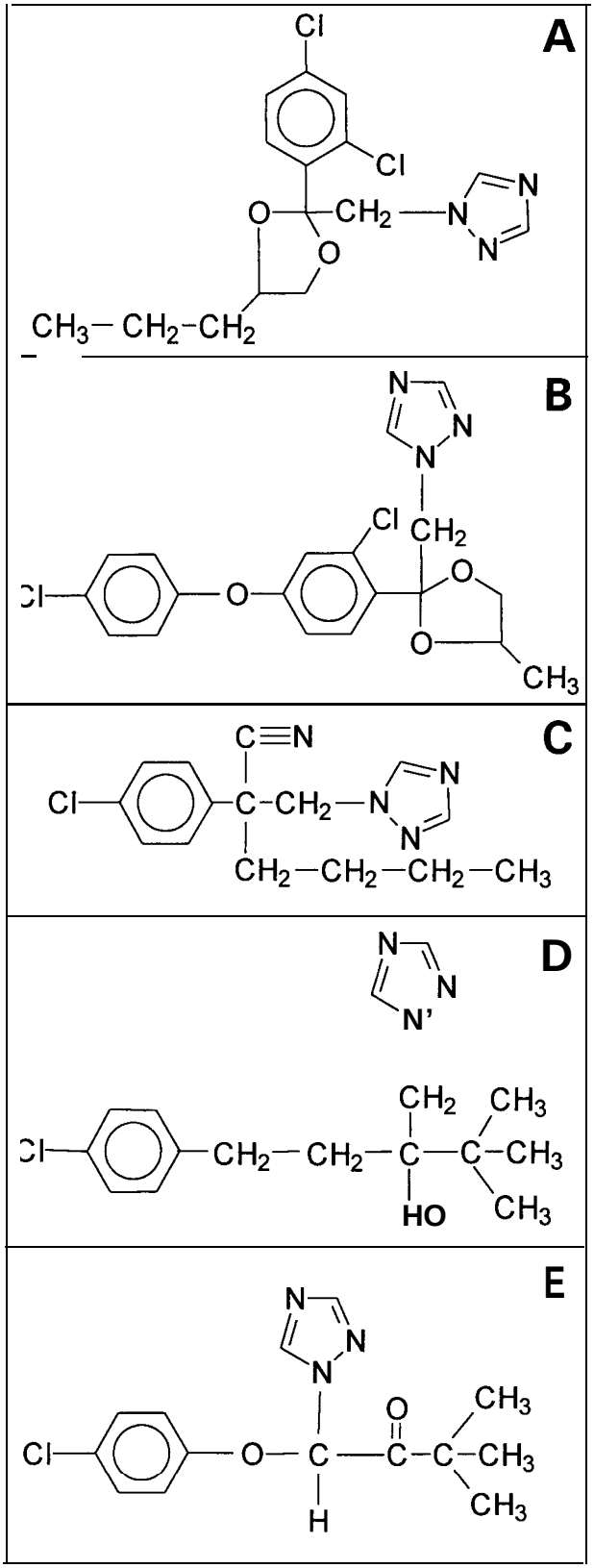

FIG. 2. Chemical structures of triazoles: (A) propiconazole; **(B) difenoconazole; (C) myclobutanil; (D)** terbuconazole; **(E) triadimefon.**

pensions, or pure technical grade material in sterile distilled water at concentrations of 10 ppm and 1000 ppm, one for each final test concentration in the ranges of 0.1-10 and 100-1000 ppb, respectively. An appropriate aliquot of a stock solution was added singly with vigorous vortexing after autoclaving to separate 1-L flasks of homemade PDA medium to final concentrations of 0.1, 1, 10 and 100-600 ppb active ingredient. Additional tests at higher rates allowed determinations of minimum effective concentrations (MEC) for total growth inhibition when growth occurred within the initial test range. A 5 mm PDA inoculum plug from a 1-wk-old PDA culture was placed inoculum down onto the surface at the edge of each plate containing one of the respective triazole fungicides. Four measurements per isolate were recorded for each treatment from two replicate plates of each strain. Two linear measurements separated by 45" were recorded for each plate by measuring the distance between the edge of the agar plug and the edge of the colony. Accumulated linear growth, weekly linear growth rates, and growth inhibition in response to each fungicide were measured and calculated at weekly intervals for 6 wk and analyzed using standard errors of means (mean ± SEM). Percent growth inhibition (relative to controls) was calculated by taking the difference between mean growth on control and fungicide-amended plates and expressing the difference as a percentage of mean growth of the control cultures. Accumulated growth responses of strains from each mating type also were compared using t-tests analyses to determine whether triazole sensitivity could be linked with mating incompatibility alleles. Statistical comparisons of mean linear growth rate responses of individual isolates relative to fungicide concentration were made using polynomial regression analyses of growth rates during the first 2 wk of exponential growth. Agar plugs were taken from plates with no growth and transferred inoculum down onto the surface of fresh PDA plates without fungicide (unamended) and observed for 3 wk to determine if the fungicide was fungistatic or fungicidal at each concentrated tested at or above the MEC.

The effect of the five triazoles on dry weight accumulation of fungal mycelium in unshaken Neopeptone broth cultures after 6-wk incubation at 24 C was determined to compare with growth on solid medium. The NPB medium was prepared according to Waksman (1991) in 50-mL aliquots in separate 125-mL Erlenmeyer flasks. Appropriate volumes of fungicide stock solutions, as described previously, were added to final concentrations of 1.0 to 500 ppb. A 5 mm PDA inoculum plug from 1-wk-old PDA cultures was placed into each flask containing one of

TABLE III. Effects of triazole fungicides on in vitro accumulated linear growth of C. *fagacearum*

	Accumulated mean linear growth (mm)[a]								
	Fungicide concentration (ppb)								
Fungicide	0.1	1	10	100	200	300	400	500	600
Propiconazole	M	M	25.6 ± 2.2	0.1 ± 0.1	—	—	—	—	—
Difenoconazole	M	M	29.0 ± 1.7	7.5 ± 1.0	3.8 ± 0.7	2.8 ± 0.5	2.8 ± 0.5	1.5 ± 0.3	1.9 ± 0.3
Myclobutanil	M	M	47.9 ± 3.6	0.2 ± 0.1	—	—	—	—	—
Terbuconazole	M	M	68.3 ± 2.4	6.5 ± 1.8	0.2 ± 0.1	—	—	—	—
Triadimefon	M	M	M	M	M	63.2 ± 3.9	60.2 ± 4.8	44.5 ± 3.5	44.5 ± 3.0

*Accumulated linear growth ($\bar{x}$ ± SEM) of ten isolates (four replicate measurements per isolate) at 24 C after 6wk incubation on homemade PDA amended with the respective fungicide to the indicated concentrations (ppb). M = growth exceeded the edge of the plate (90 mm) and was not measured. Dash (—) indicates no growth.

the respective triazole fungicides. One flask was prepared for each of the ten isolates (serving as replications) per fungicide concentration. The flasks were incubated without shaking because previous tests indicated that there was no significant difference in accumulated growth between shaken and unshaken NPB cultures. After the cultures had incubated for 6 wk, the mycelial growth was filtered onto a preweighed 5.5 cm No. 1 Whatman filter paper and dried in an oven at 100 C overnight. Dry weight measurements were determined to 0.1 mg for each treatment and statistically compared using standard errors of means.

RESULTS

Triazole effects on accumulated growth.-The sensitivity of the Texas strains of C. *fagacearum* to five triazoles was compared based on mean accumulated linear growth responses during prolonged exposure over a 6-wk period on PDA. None of the triazoles had measurable affects on accumulated linear growth at concentrations of 0.1 ppb and 1.0 ppb after 6-wk incubation (TABLE III). Growth responses to the fungicides were compared with growth on control plates (lacking fungicides) only during the first 3 wk of incubation since the growth of controls exceeded the edge of the plate within 3 wk after plating. All of the five triazoles tested, with the exception of triadimefon, significantly reduced accumulated growth in the 10–100 ppb range. Little or no accumulated growth occurred in response to propiconazole, myclobutanil, and terbuconazole at 200 ppb. Very low amounts of growth accrued in the presence of difenoconazole at concentrations of 200 ppb up to 1100 ppb. Triadimefon began to appreciably reduce accumulated growth at concentrations >400 ppb, but substantial mean linear growth accumulation (22.7 mm) was measured at 1100 ppb after 6 wk.

A comparison of accumulated growth responses among the ten isolates during 6-wk exposure to the triazoles at a single concentration of 10 ppb indicated considerable variability in sensitivity of individual strains to the fungicides (TABLE IV). For example, isolate no. 8 (strain SHL-TX-24) exhibited the highest sensitivity to the fungicides with no growth at all on PDA amended with propiconazole and myclobutanil, and had a slower growth response to four of the five triazoles than the other isolates. Isolate no. 2 (strain SHL-TX-10) also displayed higher than average sensitivity to the triazoles. Isolates no. 4 and 9 (strains SHL-TX-15 and 25) exhibited intermediate sensitivity to the triazoles, while isolates no. 3 and 5 (strains SHL-TX-13 and 17) showed some tolerance to the triazoles at 10 ppb with lower than average sensitivity relative to the other isolates. None of the isolates were appreciably affected by triadimefon at 10 ppb, including isolate no. 8. T-test analyses for each fungicide, comparing the sensitivity of strains with different mating types (see TARLE I), indicated no significant difference in the sensitivity of strains of one mating type over the other for any of the five triazoles at the concentrations tested ($P > 0.37$).

The triazoles had similar effects on accumulated growth of C. *fagacearum* isolates in NPB culture, although the isolates generally exhibited greater sensitivity to the triazoles in liquid culture as evident by the lower MECs required for total growth inhibition (TABLE V) . Propiconazole totally inhibited growth and dry weight accumulation after 6-wk incubation at 10 ppb in liquid culture, and only slight accumulated growth was measured at 10 ppb and 100 ppb concentrations for difenoconazole. Difenoconazole slowed growth accumulation more effectively at 10

TABLE IV. Effects of triazole fungicides on in vitro accumulated linear growth of individual isolates of *C. fagacearum*

Fungicide[b]	Accumulated mean linear growth (mm)[a]									
	Isolate									
	1	2	3	4	5	6	7	8	9	10
Propiconazole	33.0 ± 0.7	12.5 ± 1.4	42.0 ± 6.7	21.0 ± 2.7	37.5 ± 0.5	35.8 ± 3.9	33.3 ± 2.5	0.0	21.5 ± 2.2	25.0 ± 3.3
Difenoconazole	38.8 ± 1.9	20.8 ± 1.4	44.0 ± 7.5	16.5 ± 1.9	39.8 ± 2.3	25.3 ± 2.1	30.5 ± 0.5	16.8 ± 0.9	31.8 ± 1.3	26.3 ± 1.4
Myclobutanil	50.5 ± 6.2	30.5 ± 3.5	60.3 ± 1.0	50.0 ± 9.7	53.3 ± 2.0	51.0 ± 3.0	74.0 ± 1.5	0.0	33.5 ± 0.5	59.8 ± 4.3
Terbuconazole	M	58.8 ± 2.5	M	74.8 ± 1.9	76.0 ± 0.4	75.8 ± 0.8	75.0 ± 1.1	48.3 ± 7.8	71.5 ± 1.5	M
Triadimefon	M	M	M	M	M	M	M	M	M	M

[a] Accumulated linear growth ($\bar{x}$ ± SEM) of each isolate (four replicate measurements per isolate) after 6 wk incubation at 24 C on homemade PDA amended with the respective fungicide to a concentration of 10 ppb. M = growth exceeded the edge of the plate (90 mm) and was not measured.

ppb than myclobutanil or terbuconazole, but a higher MEC was required to totally inhibit growth with difenoconazole than with the other two triazoles. Triadimefon did not prevent growth accumulation up to 500 ppb, although dry weight accumulation at 500 ppb was less than half of the amount measured for control cultures.

Growth inhibition.-The effects of each fungicide on growth at concentrations of 0.1-600 ppb after 2-wk incubation at 24 C on PDA were expressed in terms of percent growth inhibition relative to controls to further evaluate the minimum effective concentrations required for total growth inhibition. Propiconazole and myclobutanil totally stopped growth of all isolates at 100 ppb (TABLE VI). Similarly, difenoconazole and terbuconazole inhibited growth in the 100-200 ppb range, but there was more variability of sensitivity among individual isolates as indicated by a range of MECs for these triazoles. The MEC of triadimefon required for total inhibition of growth of the isolates was >600 ppb. Subsequent measurements indicated that the MEC for triadimefon was actually >1100 ppb, at least five to ten times higher than the MECs of the other triazoles tested.

Triazole effects on growth rates.-The effects of each triazole on mean weekly growth rates were determined during 6-wk incubation at 24 C on PDA to assess the variability of growth response to exposure over time. Control colonies expanded linearly at rates of 25-37 mm/wk and typically grew to the edge of the plate within 3 wk after plating (TABLE VII). All of the triazoles increased the duration of the lag phase of growth as fungicide concentration increased. This proportional increase in the length of the lag phase with increasing fungicide concentration resulted in an increase in the time required to reach the maximum exponential growth rate as fungicide concentration increased. For example, the maximum exponential growth rates achieved at fungicide concentrations up to 1 ppb for all of the triazoles occurred at 2 wk after plating. The maximum exponential growth rates attained when exposed to each fungicide at concentrations of 10 ppb and 100 ppb, with the exception of triadimefon, occurred at 4-6 wk after incubation. For triadimefon, maximum exponential growth of the isolates occurred at 2 wk after plating when exposed at the 10 ppb and 100 ppb concentrations, and at six wk after plating when exposed at the 1000 ppb concentration.

Cumulative mean weekly growth rates, averaged over the 6-wk incubation period, decreased proportionally with increasing concentration of each fungicide. Propiconazole and myclobutanil caused similar patterns of reductions in weekly growth rates as con-

TABLE V. Effects of triazole fungicides on in vitro accumulated dry weight of C. *fagacearum* in neopeptone broth culture

	Dry weight accumulation (mg)[a]								
	Fungicide concentration (ppb)								
Fungicide	Control	1	10	100	200	300	400	500	MEC (ppb)
Propiconazole	52.3 ± 1.2	58.4 ± 2.6	—	—	—	—	—	—	10
Difenoconazole	67.1 ± 1.5	34.4 ± 8.4	1.3 ± 0.3	0.7 ± 0.2	—	—	—	—	100-200
Myclobutanil	44.8 ± 3.8	49.1 ± 5.2	16.8 ± 7.1	—	—	—	—	—	100
Terbuconazole	64.1 ± 1.2	56.9 ± 3.4	10.2 ± 5.5	—	—	—	—	—	100
Triadimefon	48.4 ± 2.8	46.8 ± 2.5	45.7 ± 2.1	33.2 ± 3.8	32.6 ± 3.2	24.4 ± 4.0	18.3 ± 3.1	19.8 ± 4.5	>500

[a] Dry weight accumulation ($\bar{x} \pm$ SEM) of ten isolates after 6 wk of growth at 24 C in 50 mL of unshaken Neopeptone broth (NPB) amended with the respective fungicide to the indicated concentrations. Dash (—) indicates no growth.

centration increased up to 100 ppb. Very low growth rates were achieved by the sixth week of incubation in the presence of these two triazoles at the 100 ppb concentration. Similarly, difenoconazole and terbuconazole caused a comparable pattern in growth rate reductions vs. concentration, although the decrease in growth rates occurred at a less accelerated rate with increasing concentrations up to 100 ppb when compared with propiconazole and myclobutanil. The effects of triadimefon on linear growth rates were relatively consistent and invariable up to 100 ppb. A tenfold higher concentration (1000 ppb) was required to have a comparable effect on growth rates with triadimefon than with the other triazoles at 100 ppb. All of the Texas strains of C. *fagacearum* had much higher tolerance and less sensitivity to this triazole.

The growth responses of the Texas strains to each triazole were further compared by polynomial regression analyses to establish best-fit equations to describe growth rates as a function of concentration for each fungicide during the first 2 wk of linear growth on PDA at 24 C. The resulting growth response curves constructed by plotting linear growth rate vs. fungicide concentration demonstrated significant differences in the rates of decline in growth rates with increasing concentrations of the five triazoles (FIG. 3). The polynomial regression equations that best predicted growth rates of the Texas strains when exposed to the range of fungicide concentrations tested here are presented in TABLE VIII. Growth rate responses to tested concentrations of propiconazole and difenoconazole were best described with third order (cubic) regression equations. Second order (quadratic) regression equations provided the best fit of the data for growth rate responses to concentrations of myclobutanil and terbuconazole. The growth rate responses to concentrations of triadimefon were relatively linear and best described with a first-order regression equation. Pair-wise comparisons of the negative slopes of regression curves for each fungicide indicated that all of the slopes of the regression curves for the five triazoles were significantly different from each other ($P < 0.01$). The order of magnitude by which the rates of decline in growth rates occurred in response to increasing concentrations of the fungicides were as follows (from highest to lowest): difenoconazole, propiconazole, myclobutanil, terbuconazole, and triadimefon. However, all of the triazoles except triadimefon either totally stopped growth or reduced growth rates to very low levels at 100 ppb.

The results of transfers of agar plugs from plates with no growth, in cases where the triazole concentrations were at or above the MEC, provided evidence that the fungistatic vs. fungicidal effects of the triazoles were dependent on fungicide concentration and individual responses of each strain. The triazoles were invariably fungistatic at the lower concentrations at or immediately above the MEC as indicated by resumed growth on unamended, fungicide-free PDA. However, the fungicidal effect appeared to increase or at least sufficiently impair anabolic processes to prevent growth as concentration increased above the MEC. Again, there was considerable variability in the effects of fungicide exposure at concentrations above the MEC on growth of individual isolates after transfer to unamended PDA. In general, propiconazole, myclobutanil, and terbuconazole were apparently fungicidal at the higher concentrations (400-600 ppb) tested based on no growth for 3 wk after transfer, although difenoconazole continued to have only fungistatic carryover effects on growth after agar plugs were transferred from these higher concentrations. No results were available for

TABLE VI. Growth inhibition of *C. fagacearum* strains by triazole fungicides

Fungicide	Percent growth inhibition[a]										
	Fungicide concentration (ppb)										
	0.1	1	10	100	200	300	400	500	600	MEC (ppb)
Propiconazole	15.8 ± 1.4	43.0 ± 1.4	93.6 ± 0.8	—	—	—	—	—	—	10–100[b]
Difenoconazole	12.5 ± 0.9	47.3 ± 1.0	90.3 ± 0.9	99.4 ± 0.4	—	—	—	—	—	100–200
Myclobutanil	13.0 ± 1.4	24.2 ± 1.1	79.6 ± 2.5	—	—	—	—	—	—	100
Terbuconazole	3.8 ± 0.6	30.9 ± 1.5	67.2 ± 1.8	98.8 ± 0.4	—	—	—	—	—	100–200
Triadimefon	0.0	0.0	0.0	6.0 ± 1.2	28.4 ± 2.4	48.7 ± 2.9	61.1 ± 3.0	71.0 ± 3.0	80.0 ± 1.6	>600

[a] Percent growth inhibition ($\bar{x}$ ± SEM) of ten isolates (relative to controls) after 2 wk growth at 24 C on homemade PDA amended with the respective fungicide to the indicated concentrations (ppb). Dash (—) indicates no growth.

[b] Ranges are provided when the MEC for individual strains varied significantly.

triadimefon since growth occurred at all concentrations up to 1100 ppb and the **MEC** was not determined for this fungicide.

DISCUSSION

Fungicide sensitivity tests with ten geographically diverse strains of C. *fagacearum* from locations throughout Texas confirmed preliminary results (Wilson, 1994) indicating that the triazole class of fungicides is extremely effective in inhibiting vegetative growth of the fungus in vitro at very low concentrations. The very high sensitivity of Texas strains to four of the five triazoles tested demonstrated that these fungicides should be very effective in preventing growth of the oak wilt fungus in vivo if an adequate prophylactic treatment is applied to assure that sufficient systemic distribution to all susceptible tissues, especially the root system, is achieved prior to infection. These results indicate that difenoconazole, myclobutanil, and terbuconazole are additional triazoles besides propiconazole that are potentially useful for oak wilt control. All of the triazoles except triadimefon significantly reduced accumulated growth after 6 wk incubation when exposed to concentrations of ≤200 ppb active ingredient. The range of triazole concentrations (lo-200 ppb) required for total growth inhibition of the Texas strains was considerably lower than the concentrations required to effectively stop growth of many other pathogenic fungi with the same or similar triazoles (Kuck and Scheinpflug, 1986; Whitson and Hine, 1986), although similar sensitivities have been reported for certain other plant pathogens (Comstock et al., 1984).

A major objective of this study was to determine the MECs of alternative triazoles required for total growth inhibition of C. *fagacearum.* Determinations of MECs for each fungicide screened are necessary for a highly virulent necrotrophic pathogen such as the oak wilt fungus because of its capability of causing rapid death of its hosts within a few months after infection. Since much less inoculum potential is needed to initiate infections with exceptionally virulent pathogens, a reduction in the growth of the fungus by 50%, the median effective concentration ($EC_{,,,}$), is not sufficient to achieve effective control in field situations. Appel and Kurdyla (1992) reported that the $EC_{,,,}$ of propiconazole required for four Texas isolates of the fungus ranged from 2 ppb to 15.7 ppb. This is useful information, but not very practical in terms of providing the information needed for effective control. A determination of an application rate that kills 50% of the inoculum (LD_{50}), although more difficult to measure, would be more useful, yet this application rate still may not prevent

TABLE VII. Effects of triazole fungicides on in vitro weekly growth rates of C. *fagacearum* strains

Fungicide	Conc.[b] (ppb)	Mean weekly growth rates (mm/wk)[a] Weeks 1	2	3	4	5	6	Cumul. Mean
Propiconazole	Control	28.9 ± 0.9	35.1 ± 0.9	M	M	M	M	31.8 ± 0.7
	0.1	24.4 ± 0.5	30.3 ± 0.6	M	M	M	M	27.4 ± 0.5
	1	15.8 ± 0.6	21.3 ± 1.0	18.5 ± 1.1	M	M	M	18.5 ± 0.6
	10	0.8 ± 0.2	3.5 ± 0.5	4.2 ± 0.5	5.9 ± 0.7	6.0 ± 0.7	6.1 ± 0.6	4.4 ± 0.3
	100	0.0	0.0	0.0	0.0	0.0	0.4 ± 0.2	0.1 ± 0.0
Difenoconazole	Control	28.7 ± 0.8	33.6 ± 0.8	M	M	M	M	31.2 ± 0.6
	0.1	25.4 ± 0.6	29.6 ± 0.7	M	M	M	M	27.5 ± 0.5
	1	13.9 ± 0.4	18.8 ± 0.4	15.9 ± 0.6	15.1 ± 0.4	M	M	15.9 ± 0.3
	10	2.0 ± 0.3	4.1 ± 0.4	4.2 ± 0.4	5.3 ± 0.4	6.7 ± 0.5	7.0 ± 0.6	4.9 ± 0.2
	100	0.0	0.3 ± 0.2	3.0 ± 0.6	1.0 ± 0.2	1.3 ± 0.2	2.0 ± 0.4	1.3 ± 0.1
Myclobutanil	Control	25.4 ± 0.6	31.9 ± 0.8	M	M	M	M	28.7 ± 0.6
	0.1	23.0 ± 0.5	26.9 ± 0.7	M	M	M	M	24.9 ± 0.5
	1	20.4 ± 0.5	23.3 ± 0.9	20.0 ± 0.7	M	M	M	21.2 ± 0.4
	10	4.1 ± 0.4	6.2 ± 0.5	9.4 ± 0.9	9.9 ± 1.1	8.3 ± 0.8	9.1 ± 0.9	7.8 ± 0.3
	100	0.0	0.0	0.0	0.0	0.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.2
Terbuconazole	Control	25.2 ± 0.6	32.2 ± 0.8	M	M	M	M	28.7 ± 0.6
	0.1	24.6 ± 0.6	32.1 ± 0.6	M	M	M	M	28.4 ± 0.6
	1	16.5 ± 0.5	22.7 ± 0.4	23.0 ± 0.4	M	M	M	20.8 ± 0.4
	10	6.9 ± 0.5	11.8 ± 0.6	15.6 ± 0.7	15.8 ± 0.8	13.9 ± 1.2	10.1 ± 1.8	12.4 ± 0.4
	100	0.3 ± 0.1	0.4 ± 0.1	1.0 ± 0.4	1.4 ± 0.4	1.3 ± 0.4	2.2 ± 0.6	1.1 ± 0.2
Triadimefon	Control	20.7 ± 0.4	24.7 ± 0.5	25.5 ± 0.5	M	M	M	23.6 ± 0.3
	0.1	21.5 ± 0.5	25.6 ± 0.8	24.8 ± 0.9	M	M	M	24.0 ± 0.5
	1	25.8 ± 0.5	30.7 ± 0.7	20.8 ± 0.8	M	M	M	25.8 ± 0.5
	10	25.2 ± 0.6	29.6 ± 0.7	21.2 ± 0.9	M	M	M	25.3 ± 0.5
	100	19.1 ± 0.8	25.3 ± 0.8	24.7 ± 0.6	M	M	M	23.0 ± 0.5
	1000	1.3 ± 0.2	2.5 ± 0.3	3.7 ± 0.5	4.7 ± 0.8	6.4 ± 0.9	4.6 ± 0.8	3.8 ± 0.3

*Mean linear growth ($\bar{x}$ ± SEM) of ten isolates during 6-wk incubation at 24 C on homemade PDA amended with the respective fungicide to the indicated concentrations. M = growth exceeded edge of plate (90 mm) and a value could not be calculated.

[b] Separate control plates were used for each batch of agar medium prepared for each fungicide treatment.

infection. Consequently, MEC determinations are essential for identifying the minimum target concentrations required in vivo for complete chemical protection. Knowing the MEC target for each fungicide is useful in efficacy testing for comparisons against actual concentrations measured in plant tissues via residue analyses following specific fungicide application methods. The MECs of the five triazoles tested here ranged from 10–100 ppb for total growth inhibition with propiconazole to >1100 ppb with triadimefon, considerably higher concentrations than those needed for 50% growth inhibition of C. *fagacearum* with each respective fungicide. Measures of growth rates of each strain as a function of fungicide concentration along with MEC data provided a much more complete indication of fungicide sensitivity. The regression equations (TABLE VIII) allow accurate predictions of expected growth rates of C. *fagacearum* strains for each fungicide concentration. This information is useful for predicting disease progress in individual trees and developing fungicide dosage-response models from residue data following fungicide applications with different injection methods.

The notable variability in sensitivity of individual strains to the triazoles was indicated by MEC ranges for propiconazole, difenoconazole, and terbuconazole, and differences in accumulated linear growth, linear growth rates, and growth inhibition in response to each fungicide. The sensitivity and growth response of any one strain relative to the other strains were consistent for all five triazoles tested, indicating reciprocal cross-susceptibility to compounds having very similar if not identical modes of action. Certain strains, notably SHL-TX-10 and 24, exhibited higher than average sensitivity to the triazoles, while other strains (SHL-TX-13 and 1'7) were relatively tolerant

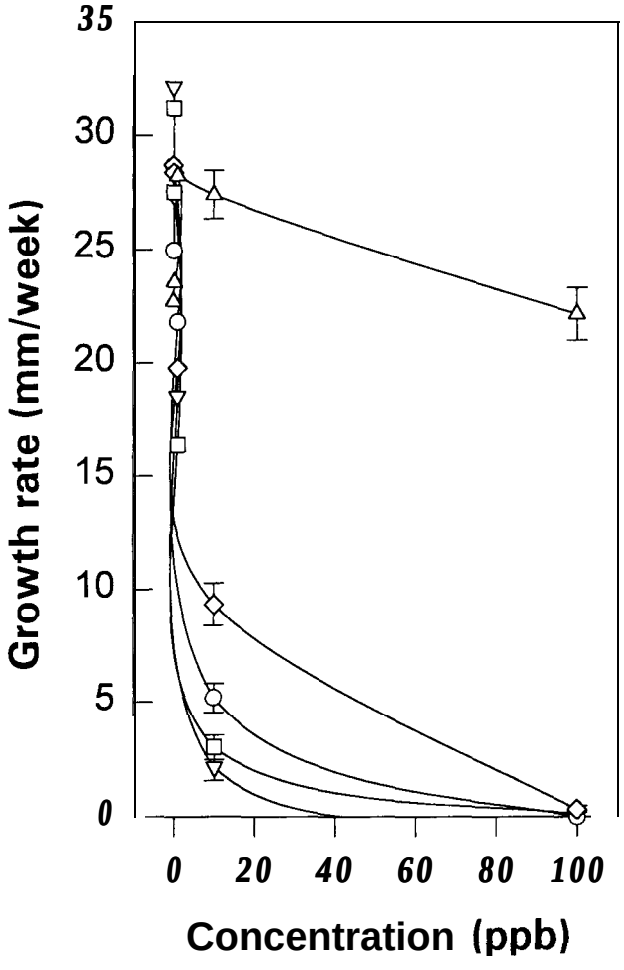

FIG. 3. Effects of triazole fungicides at concentrations of O-100 ppb in homemade PDA on linear growth rate of C. fagacearum at 24 C during the first 2 wk of exponential growth. Growth rate (mm/week) in response to propiconazole (▽), difenoconazole (□), myclobutanil (0), terbuconazole (0), and triadimefon (A). Error bars indicate standard deviation (95% confidence interval).

to four of the triazoles at 10 ppb. The relative tolerance of certain strains does not imply that they have resistance to these triazoles because of the very low concentrations involved. It is inappropriate to use the term "fungicide resistance" to describe strains that are sensitive to these materials in the parts per billion range. All of the isolates showed greater sensitivity in liquid culture than on solid culture medium, probably due to higher rates of fungicide diffusion under aqueous conditions. Fungicide sensitivities in liquid culture are probably more representative of sensitivities expected *in vivo* because triazoles are mostly translocated in the aqueous environment of the transpiration stream within the xylem. As a vascular wilt pathogen, the oak wilt fungus also predominantly resides in the apoplast of the outer sapwood. This offers promising protection of plant tissues located upward from the injection point, but provides little protection of root tissues since very limited symplastic movement of triazoles have been observed (Kuck and Scheinpflug, 1986).

Fungicide resistance to triazole fungicides does not yet appear to be a significant problem threatening oak wilt management in Texas. Despite observations of variable sensitivities and differential growth responses of individual strains to the triazoles tested, no shifts in propiconazole sensitivity have been reported in Texas since 1990 when this material was first used for oak wilt control. Furthermore, none of the Texas strains showed evidence of appreciable resistance to the triazoles. The majority of reported cases of triazole resistance since the early 1980's have occurred with powdery mildews on various crops receiving intensive spray treatments (Butters et al., 1984; Huggenberger et al., 1984; Schepers, 1985). Most other phytopathogenic fungi targeted by triazoles and other sterol Cl4 demethylation inhibitors (DMIs) have not shown decreased sensitivities to these materials with exposure over time (Staub, 1991). However, the detection of less sensitive strains

TABLE VIII. Regression equations describing linear growth response of C. *fagacearum* strains to concentrations of triazole fungicides during the first 2 wk of growth on homemade PDA at 24 C

Fungicide	Polynomial regression equation	n	R^{2b}	F[c]	SEE[d]
Propiconazole	$y = 30.5 - 13.3x + 1.15x^2 - 0.0102x^3$	100	0.89	270.4	4.54
Difenoconazole	$y = 30.3 - 14.8x + 1.33x^2 - 0.0119x^3$	100	0.91	332.1	3.94
Myclobutanil	$y = 26.2 - 2.26x + 0.0200x^2$	100	0.87	332.3	4.35
Terbuconazole	$y = 26.6 - 1.88x + 0.0162x^2$	100	0.81	208.7	5.22
Triadimefon	$y = 25.4 - 0.0235x$	120	0.78	419.7	4.60

[a] Predicts linear growth rate (y) relative to fungicide concentration (x) in ppb. Probability values for all equations were ($P < 0.0001$).

[b] Coefficient of determination or square of the correlation coefficient.

[c] F statistic.

[d] Standard error of the estimate.

of certain pathogens can occur without any clear effects on fungicide performance (Köller and Scheinpflug, 1987; Stanis and Jones, 1985). The current absence of propiconazole resistance in Texas strains of C. *fagacearum* might be explained by the low frequency of exposures, since most trees usually are injected only once, or by the low incidence of resistant strains in the population. Resistance to demethylation inhibitors of ergosterol biosynthesis is usually a polygenic trait acquired via small genetic steps (Schepers, 1985; van Tuyl, 1977). These genetic steps involve changes in genetic (fungicide-resistance) factors that have an additive effect as they accumulate over time in the population; significant resistance is observed when the frequencies of incidence of the most resistant strains in the population become high enough (Georgopoulos and Skylakakis, 1986; Leroux, 1991; Skylakakis and Hollomon, 1987). Dose-response curves from field situations usually indicate a continuum of sensitivities to DMIs, reflecting the presence of many phenotypic levels of fungicide resistance (De Waard et al., 1993). The biochemical mechanisms of this polygenic resistance are not yet known (Skylakakis and Hollomon, 1987).

The levels of fungicide sensitivity, considered in terms of whether the resulting effects are fungistatic or fungicidal, also have important implications for control. If the fungicide is merely fungistatic, the fungus could recolonize the host once the fungicide has been metabolized, degraded, or diluted to ineffective concentrations by the transpiration stream. A reapplication of the fungicide would be required in this case. The transfer of agar plugs from fungicide-amended plates with no growth to unamended plates mimicked this dilution effect in vitro in this study by allowing residual fungicide in the agar plugs to diffuse out into the larger volume of unamended agar medium, presumably permitting growth of inoculum remaining viable following fungicide exposure. The results tentatively suggest that the ultimate effect (fungistatic vs. fungicidal) is concentration-dependent since several triazoles appeared to become increasingly fungicidal as concentrations increased above the MEC. However, the results do not conclusively demonstrate fungicidal activity, but may only indicate fungistasis since the final fungicide concentrations that the inocula were exposed to after transfer and incubation were not known. Residual fungicide remaining in the agar plugs after transfer from media with high fungicide concentrations could have been sufficient to inhibit growth from the plugs. Difenoconazole remained fungistatic to most isolates at concentrations up to 600 ppb. Assuming adequate distribution, fungicidal dosages should kill all inoculum that the fungicide comes in contact with, requiring retreatment only if new infections occur. Unfortunately, no field data are available that indicate how long fungistatic vs. fungicidal dosages of triazoles provide protection against oak wilt infections in uninfected oaks that are challenged by the pathogen.

The variability in activity attributed to individual triazoles, indicated by differences in growth inhibition and accumulated dry weight, largely can be explained by structural differences between these compounds, particularly in substituent R-groups adjacent to chiral carbons. The triazoles, in general, are a group of relatively high molecular weight (see **TABLE** II), low volatility systemic fungicides, distinguished by the presence of the trinitrogen azole group, that are predominantly translocated acropetally in the apoplast. The principal mode of action of the triazole fungicides involves the inhibition of ergosterol biosynthesis essential for the physical stabilization of fungal cell wall structure (Berg, 1986). Ergosterol is the main sterol found in higher fungi (Kato, 1986) which explains the broad spectrum of activity against many phytopathogenic fungi. The triazole ring is the necessary component (toxophore) required for inhibition of ergosterol biosynthesis (Büchel, 1986). The R-groups attached to chiral carbons modify the activity of the molecule and impart characteristics through their influence on the solubility and mobility of the molecule, and the stereochemistry of the toxophore. The inhibitory effects of triazoles on several demethylation steps in the biosynthesis of sterols from squalene via the isoprenoid pathway are partially mediated by these substituent groups that affect stereospecific binding of triazoles to sterol demethylases and possibly sterol carrier proteins on the smooth portion of the endoplasmic reticulum (Ragsdale, 1977; Siegel, 1981; Büchel, 1986). The interaction is sufficiently specific that differences in activity have been found even between individual geometrical isomers (enantiomeric forms) of several triazoles. For example, a comparison of the fungicidal activities of the four stereoisomeric forms of triadimenol and bitertanol, both with two chiral carbons, indicated markedly higher growth-inhibitory activity with the (−)−1S,2R diastereomere than with the other three isomers of these triazoles (Biichel, 1986). In the current study, differences in growth inhibition observed in response to the five triazoles appeared to be related to the presence of specific R-groups attached to chiral carbons. The two ketal triazoles, propiconazole and difenoconazole with the dioxolane ring (see FIG. 2a, **b)**, had the greatest fungicidal activity and largest effect on growth rates among the triazoles tested against the Texas strains. Myclobutanil with nitrilo and butyl R-groups and terbuconazole with dimethylethyl and hydroxyl R-groups had intermediate activ-

ity, while triadimefon with a dimethyl butanone group had the lowest activity against the oak wilt fungus (see FIG. 2c–e). The similarities of the chlorophenyl groups on these materials suggested that these substituents did not contribute significantly to differences in fungicidal activity. The variability in fungicidal activity also could not be explained by differences in triazole formulation and solubility because all of the triazoles are soluble to concentrations much higher than the concentrations tested (see TABLE II).

The limited available knowledge of environmental factors affecting fungicide injections, the distribution and interactions of systemic fungicide materials with host transport and biochemical processes, and the effectiveness of different fungicide application methods suggest that further research is needed to improve on current chemical control methods to provide adequate treatment of the root system and to evaluate new fungicide application methods and materials for oak wilt control. This effort should include investigations of other classes of sterol-biosynthesis inhibiting fungicides that are effective DMIs such as the piperazines, pyridines, pyrimidines, imidazoles, and morpholines.

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LITERATURE CITED

Appel, D. N., R. S. Cameron, A. D. Wilson, and J. D. Johnson. 1995. How to identify and manage oak wilt in Texas. USDA Forest Service, Southern Research Station, New Orleans, Louisiana. How-To SR-1, 8 pp.

——— and T. Kurdyla. 1992. Intravascular injection with prbpiconazole in live oak for oak wilt control. *Plant Dis. 76: 1120-1124.*

Berg, D. 1986. Biochemical mode of action of fungicides. Pp. 25-51. *In: Fungicide chemistry, advances and practical applications.* Eds., M. B. Grey and D. S. Spilker. American Chemical Society Symposium Series 304. American Chemical Society, Washington, D.C.

Büchel, K. H. 1986. The history of azole chemistry. Pp. 1–23. *In: Fungicide chemistry, advances and practical applications.* Eds., M. B. Grey and D. S. Spilker. American Chemical Society Symposium Series 304. American Chemical Society, Washington, D.C.

Burdsall, H. H., Jr., and E. B. Dorworth. 1994. Preserving cultures of wood-decaying Basidiomycotina using sterile distilled water in cryovials. *Mycologia 86:* 275–280.

Butters, J., J. Clark, and D. W. Holloman. 1984. Resistance to inhibitors of sterol biosynthesis in barley powdery mildew. *Meded. Fac. Landbouwwetensch. Rijksuniv. 49: 143-151.*

Comstock, J. C., S. A. Ferreira, S. A. Ching, and H. W. Hilton. 1984. Control of pineapple disease of sugarcane with propiconazole. *Plant* Dis. 68: 1072-1075.

De Waard, M. A., S. G. Georgopoulos, D. W. Hollomon, H. Ishii, P. Leroux, N. N. Ragsdale, and F. J. Schwinn. 1993. Chemical control of plant diseases: problems and prospects. *Ann. Rev. Phytopathol. 31:* 403–421.

Dewers, R. S. 1971. Shade tree evaluation. Texas Agric. Ext. Service. Fact Sheet L-958.

Dhingra, 0. D., and J. B. Sinclair. 1985. *Basic plant pathology methods.* CRC Press, Inc. Boca Raton, Florida. 355 pp.

Dooling, 0. J. 1961. Oak wilt identified in Texas. *Plant Dis. Rept. 45: 749.*

Gehring, E. H. 1995. Evaluation of suppression project treatments. Pp. 147-154. *In: Oak wilt perspectives: The proceedings of the national oak wilt symposium.* June 22-25, 1992. Austin, Texas. Eds., D. N. Appel and R. F. Billings. Information Development, Inc., Houston.

Georgopoulos, S. G., and G. Skylakakis. 1986. Genetic variability in the fungi and the problem of fungicide resistance. *Crop Protect. 5:* 299–305.

Gibbs, J. N., and D. W. French. 1980. The transmission of oak wilt. USDA For. Serv. Res. Pap. NC-185. 17 pp.

Huggenberger, F., M. A. Collins, and G. Skylakakis. 1984. Decreased sensitivity of *Sphaerotheca fuliginea* to fenarimol and other ergosterol-biosynthesis inhibitors. *Crop Protect. 32:* 137-149.

Kato, T. 1986. Sterol biosynthesis in fungi, a target for broad spectrum fungicides. Pp. l-24. *In: Chemistry of plant protection. Vol. 1. Sterol biosynthesis inhibitors and anti-feeding compounds.* Eds., G. Haug and H. Hoffmann. Springer-Verlag, Berlin.

Köller, W., and H. Scheinpflug. 1987. Fungal resistance to sterol biosynthesis inhibitors: a new challenge. *Plant Dis. 71: 1066-1074.*

Kuck, K. H., and H. Scheinpflug. 1986. Biology of sterol-biosynthesis inhibiting fungicides. Pp. 65-96. *In: Chemistry of plant protection. Vol. 1. Sterol biosynthesis inhib itors and anti-feeding compounds.* Eds., G. Haug and H. Hoffmann. Springer-Verlag, Berlin.

Leroux, P. 1991. Resistance des champignons phytopathogenes aux fongicides. *Phytoma Def. Cult. 434: 20-26.*

MacDonald, W. L. 1995. Oak wilt: An historical perspective. Pp. 7-14. *In: Oak wilt perspectives: The proceedings of the national oak wilt symposium.* June 22-25, 1992. Austin, Texas. Eds., D. N. Appel and R. F. Billings. Information Development, Inc., Houston.

Martin, C. W. 1986. The value trees contribute to residential property in the Austin metropolitan area: A factor in assessing the impact of oak wilt. M.S. thesis. Department of Forestry, Texas A&M University, College Station. 64 pp.

McKinney, C., and R. F. Billings. 1995. Economic analysis of oak wilt suppression in Texas. Pp. 155–160. *In: Oak wilt perspectives: The proceedings of the national oak wilt symposium.* June 22-25, 1992. Austin, Texas. Eds., D. N. Appel and R. F. Billings. Information Development, Inc., Houston.

Ragsdale, N. N. 1977. Inhibitors of lipid synthesis. Pp. 333–

363. *In: Antifungal compounds. Vol. 2.* Eds., M. R. Siegel and H. D. Sisler. Marcel Dekker, New York.

Schepers, H. T. A. M. 1985. Changes during a three-year period in the sensitivity to ergosterol biosynthesis inhibitors of *Sphaerotheca fuliginea* in the Netherlands. *Netherlands J. Pl. Pathol. 91: 105-l 18.*

Siegel, M. R. 1981. Sterol-inhibiting fungicides: Effects on sterol biosynthesis and sites of action. *Plant Dis. 65: 986-989.*

Skylakakis, C., and D. W. Holloman. 1987. Epidemiology of fungicide resistance. Pp. 94–103. *In: Combatting resistance to xenobiotics.* Eds., M. G. Ford, D. W. Hollomon, B. P. S. Khambay, and R. M. Sawicki. Ellis Horwood, Chichester.

Stanis, V. F., and A. L. Jones. 1985. Reduced sensitivity to sterol-inhibiting fungicides in field isolates of *Venturia inaequalis. Phytopathology 75:* 1098-l 101.

Staub, T. 1991. Fungicide resistance: practical experience with antiresistance strategies and the role of integrated use. *Ann. Rev. Phytopathol. 29: 421-442.*

van Tuyl, J. M. 1977. Genetics of fungal resistance to systemic fungicides. *Meded. Landbouwhoogeschool 77:* 1–137.

Waksman, G. 1991. Purification and characterization of two endo-β-1,4-D-glucanases from *Sclerotinia sclerotiorum. Biochimica et biophysica acta. 1073: 49-55.*

Whitson, R. S., and R. B. Hine. 1986. Activity of propiconazole and other sterol-inhibiting fungicides against *Phymatotrichum omnivorum. Plant* Dis. 70: 130-133.

Wilson, A. D. 1994. Relative in vitro sensitivity of Texas strains of *Ceratocystis fagacearum* to selected triazole fungicides. *Phytopathology* 84:1127.

———. 1995. Future direction of USDA Forest Service oak wilt research. Pp. 181-186. *In: Oak wilt perspectives: The proceedings of the national oak wilt symposium.* June 22-25, 1992. Austin, Texas. Eds., D. N. Appel and R. F. Billings. Information Development, Inc., Houston.

———, and D. G. Lester. 1995. Application of propiconazole and *Pseudomonas chichorii* for control of oak wilt in Texas live oaks. *Fungic. & Nematic. Tests 50: 393.*

———, and ———. 1996. Evaluation of propiconazole application methods for control of oak wilt in Texas live oaks. *Fungic. & Nematic. Tests 51: 389.*