Effects of a Protectant Versus a Systemic Fungicide on Disease Components of Peanut Late Leaf Spot

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ABSTRACT

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The epidemiological effects of a protectant versus a systemic, sterol-inhibiting fungicide on disease components of *Cercosporidium personatum* were quantified by linear regression and/or probit analysis. The effects of chlorothalonil (a protectant fungicide) and tebuconazole (a systemic, sterol-inhibiting fungicide) were quantified for the following disease components: 1) infection frequency, 2) incubation period, 3) lesion size, and 4) sporulation. An inoculation technique to quantify the protectant versus systemic effects of fungicides on disease components was developed. Both fungicides greatly reduced spore germination when applied to peanut leaf surfaces prior to inoculation. However, in addition to its action as a protectant, the systemic activity of tebuconazole also reduced infection frequency on nontreated leaves. Chlorothalonil had no effect on infection frequency after *C. personatum* germ tubes entered the peanut leaves. Tebuconazole also increased the incubation period and reduced the size of *C. personatum* lesions, while chlorothalonil had no effect on these components. In field experiments, chlorothalonil and tebuconazole reduced sporulation by up to 60 and 97%, respectively. Because of its systemic activity, tebuconazole used alone, or in combination with chlorothalonil, may provide more flexibility and less risk compared to the exclusive use of protectant fungicides when used in conjunction with late leaf spot spray advisories and/or chemigation systems.

Additional keywords: epidemiology, sterol biosynthesis inhibitors

Late leaf spot, caused by *Cercosporidium personatum* (Berk. & M.A. Curtis) Deighton, is one of the most important diseases of peanut (*Arachis hypogaea* L.) worldwide (1,24,28). More than 90% of the peanut growers in the southeastern United States rely on a single fungicide (chlorothalonil) to control late leaf spot. In addition, more than 80% of the peanut acreage is planted to a single cultivar (Florunner) (21,23,24, 28). This is a potentially dangerous situation, because the Environmental Protection Agency is reviewing pesticides used in peanut production (7), and because there is a genetic uniformity to the crop. The extensive use of a single cultivar that is susceptible to one or more plant pathogens has been a prime contributor to severe outbreaks of plant diseases throughout history (29).

The Florunner–chlorothalonil production system has been utilized for more than 15 yr, but not without cost to the producer (16,21). An average of 7–8 fungicide sprays per season are applied to control late leaf spot, at a cost of approximately $60 per acre in fungicide costs alone (18,21). Alternative leaf spot control systems that reduce the amount of fungicide required without increasing the risk of pod loss or reduced quality would greatly benefit peanut producers. The epidemiological effects of peanut genotypes (12,16,17,22) and unfavorable weather (16,23) on disease components (infection frequency, latent period, lesion size, and sporulation capacity) of early leaf spot (*Cercospora arachidicola* S. Hori) and late leaf spot have been studied in recent years, but the effects of fungicides on disease components of late leaf spot have been largely neglected (15,16,18,20,28). Zadoks (30) stated that when protectant fungicides are employed, there is good reason for this neglect. Protectant fungicides such as chlorothalonil reduce infection frequency by inhibiting germ tube growth and development (28). Thus, when chlorothalonil is present on leaf surfaces throughout the growing season, few spores are able to complete the infection process, and epidemiological analyses of subsequent disease components are seldom needed. However, if resistant cultivars and weather-based disease forecasting schedules reduce the need for the continuous presence of fungicide on peanut leaf surfaces, then a relatively larger proportion of the pathogen population may successfully complete the infection process during the season. It follows that epidemiological analyses of the effects of fungicides on postinfection disease components of late leaf spot, such as lesion size, incubation and latent periods, and sporulation per unit lesion area, are then both possible and necessary.

The objectives of this research were to 1) quantify the effect of chlorothalonil (a protectant fungicide) versus tebuconazole (a systemic, sterol-inhibiting fungicide) on percent germination of *C. personatum* spores when these fungicides are applied to leaf surfaces as protectants, 2) quantify the systemic activity of tebuconazole on subsequent disease components of late leaf spot, and 3) determine the effect of different concentrations of chlorothalonil and tebuconazole on disease development of late leaf spot in the field.

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MATERIALS AND METHODS

Greenhouse experiments 1986–1989. *Protectant effects on spore germination.* A modified version of the detached leaf method developed by Gobina et al (15) was used to quantify the effect of the protectant fungicide chlorothalonil (Bravo 720 F) versus a systemic, ergosterol biosynthesis inhibiting fungicide, tebuconazole (Folicur 1.2 EC), on spore germination. The second and third fully expanded leaves from 6-wk-old greenhouse-grown peanut plants (cv. Florunner) were cut at the base of the petiole and placed in 13-mm-diameter tubes containing 10% Hoagland's solution. Treatments consisted of the following chlorothalonil and tebuconazole concentrations applied to leaf surfaces: 0.05×, 0.13×, 0.25×, 0.50×, 1.00×, and 2.00× the recommended field rates, which are 1.25 kg a.i./ha and 0.42 kg a.i./ha, respectively. A control treatment (no fungicide, distilled water) was also included. Separate chromatography sprayers were used to apply each fungicide, and the sprayers were rinsed twice with distilled water between applications to prevent contamination among the concentrations used. Peanut leaves for each treatment and replication were distributed over a 900-cm^2 area, and 50 ml of the appropriate fungicide concentration was applied to leaf surfaces by uniformly passing the sprayer over the designated area until the 50 ml was dispensed (minimum of four passes made). Experimental units in the 900-cm^2 area were arranged in randomized complete blocks with 20 leaves (80 leaflets) per replication. Six replications were employed for this experiment. Following the fungicide treatment, the peanut leaves were returned to test tubes and the fungicide was allowed to dry on leaf surfaces.

Peanut leaves were inoculated 24 hr later with a spore suspension (5×10^3 spores per milliliter) of *C. personatum* using a Devilbiss atomizer. Twenty milliliters of spore suspension was used to inoculate each replication, which occupied a 900-cm^2 area. Following inoculation, peanut leaves were placed in a dew chamber (relative humidity greater than 95% at 25 C and darkness) for 36 hr to provide optimum conditions for spore germination and germ tube growth. To quantify the protectant effect of tebuconazole and chlorothalonil on spore germination, eight leaflets from each replication were arbitrarily sampled 12, 24, and 36 hr after inoculation. Leaflets were placed in 70% ethanol overnight and then stained with aniline blue. Twenty-five spores per leaflet were observed microscopically (100×) to determine percent germination. Spores with germ tubes longer than one-half the length of the spore were considered germinated. Percent germination was averaged over the eight leaflets sampled from each replication.

Systemic versus protectant effects on spore germination and penetration. Individual peanut seeds (cv. Florunner) were planted in 250-cm^3 plastic containers and grown in the greenhouse for 5 wk at 20–26 C. Peanut plants with six fully expanded leaves per plant were used in this experiment. Treatments consisted of 0.05×, 0.13×, 0.25×, 0.50×, 1.00×, and 2.00× the active ingredient concentration of the recommended field rate of tebuconazole, and a 1.00× treatment of chlorothalonil as a control. There were five plants per replication and six replications per treatment. For each treatment, 30 plants were placed in 1×2 m plastic trays (five per tray); and the trays were evenly distributed within a 3×10 m area on a flat asphalt surface. The top three leaves of peanut plants were covered with nonpermeable polyethylene plastic bags to prevent the deposition of fungicides. Fungicide applications were made outdoors with a hand-held, CO_2-powered boom sprayer with a single 8003 flat fan nozzle. The sprayer was operated at 210 kPa and delivered the equivalent of 540 L/ha of water. After the fungicide had dried, the bags were removed. Plants were inoculated with a spore suspension of *C. personatum* 24 hr after fungicide treatments were applied. Thirty milliliters of

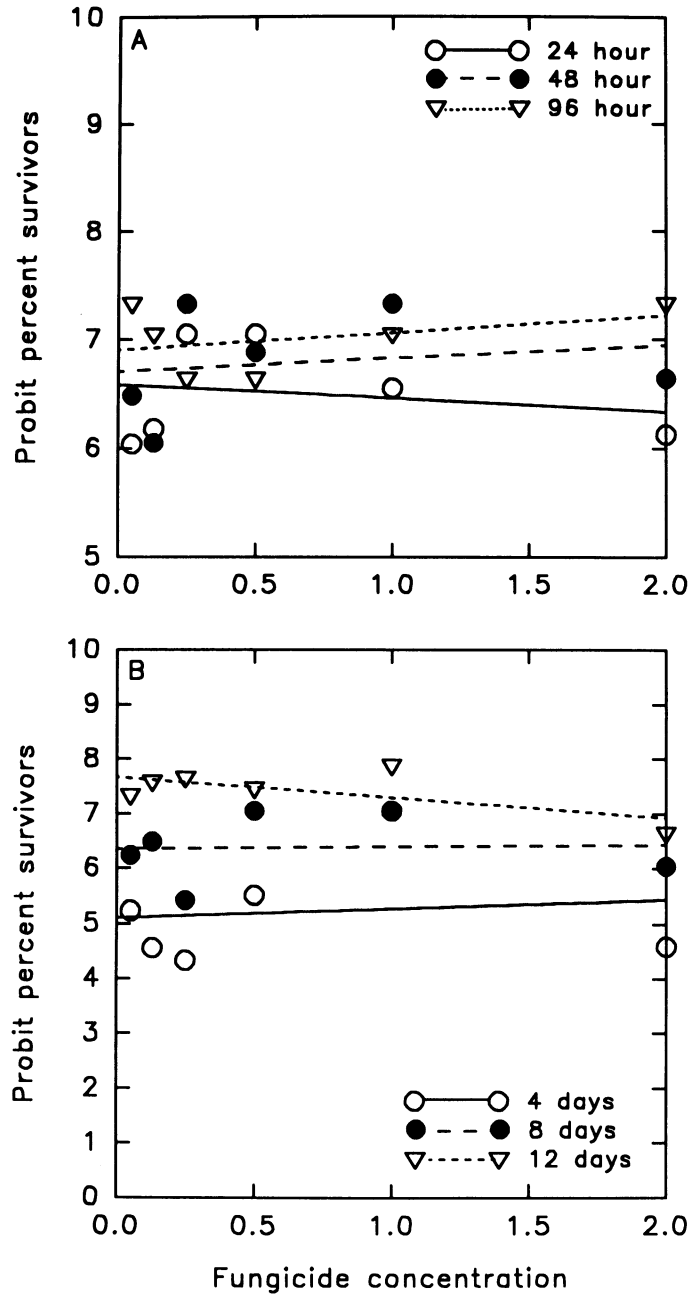

Fig. 1. Systemic effects of tebuconazole dosage on **(A)** germination (probit percent survivors) of *Cercospora personatum* on peanut leaf surfaces measured 24, 48, and 96 hours after inoculation and **(B)** germ tube penetration (probit percent survivors) of peanut stomates measured 4, 8, and 12 days after inoculation. All but the top three peanut leaves of each plant were treated with fungicide 24 hours prior to inoculation.

spore suspension (5×10^3 spores per milliliter) was uniformly applied to each replication. The plants were then placed in a dew chamber for 36 hr as described previously. After 36 hr, plants were subjected to 8-hr periods of 60–70% relative humidity with fluorescent light followed by 16-hr periods of high relative humidity (RH > 95%) and darkness at 25 C. To quantify the systemic fungistatic or fungicidal effects of tebuconazole versus chlorothalonil, leaflets from the top three leaves were sampled and examined for percent spore germination and percentage of germ tubes entering host stomata. Ten leaflets (two per plant) were sampled from each treatment within each replication at 4, 8, and 12 days after inoculation. The sampled leaflets were placed in a 70% ethanol solution overnight and then stained with aniline blue. Thirty-five spores per leaflet were examined (100×) for germination and germ tube penetration into the host leaf surface. Spores were considered to have germinated if germ tubes were longer than one-half the length of the spore. Germ tubes were considered to have penetrated the host leaf when germ tubes were observed to have entered through stomata. No direct penetrations were observed throughout the study. Data were averaged over the ten leaflets sampled, and the mean values for each replication were used for data analysis.

Systemic versus protectant effects on infection frequency, incubation period, and lesion size. Peanut plants were produced in the same manner as described in the previous experiment. The top three leaves were again covered with nonpermeable polyethylene plastic prior to the application of fungicides, to prevent fungicide deposition. Treatments included the same active ingredient fungicide concentrations used in the previous experiment except that the 2.00× concentration was omitted due to space limitations. The polyethylene covers were removed after the fungicide had dried on leaf surfaces, and the plants were inoculated 24 hr later and incubated as previously described. Lesions on the upper three leaves were counted 14, 15, 16, 17, 18, 20, 22, and 24 days after inoculation. Infection frequency (lesions per square centimeter on the upper three leaves) was determined for each date that lesion counts were made. The time required for 50% of the final lesion population to appear and be counted (T_{50}) was used as an estimate of the incubation period as affected by fungicide concentration. T_{50} values were calculated for each fungicide concentration by using the logistic model to describe the increase in lesion appearance over time and solving for time (t) when $y = 0.5$ (i.e., 50%). The logistic model was used because it provided the best fit based on coefficients of determination, F tests for model significance, and plots of residuals versus time (19). On the 24th day after inoculation, the leaf area (cm^2) of the top three leaves on every plant was measured with a Delta-T area measurement system.

The effect of chlorothalonil and tebuconazole on lesion size (mm^2) was determined by measuring the diameters of 25 lesions per replication sampled from the top three leaves. Lesion diameters were measured the 24th day after inoculation with a stereomicroscope equipped with a micrometer (40×) and converted to lesion area by using the equation for a circle.

Statistical analysis. Each greenhouse–dew chamber experiment was conducted twice. Probit percent survivors for spore germination was calculated as follows: probit [1 − (number of spores germinated as affected by dosage/number of spores germinated for the nonfungicide control × 100)]. Probit percent survivors for percentage of germ tubes penetrating stomata was calculated in a similar fashion.

Linear regression analyses were performed to determine the relationships between fungicide dosage (stimulus) and probit percent reduction (survivors) for specific disease components (response) as affected by tebuconazole and chlorothalonil (2,10,27). The probit transformation was used because the relationship between fungicide dose (x) and response (y) was often nonlinear. For example,

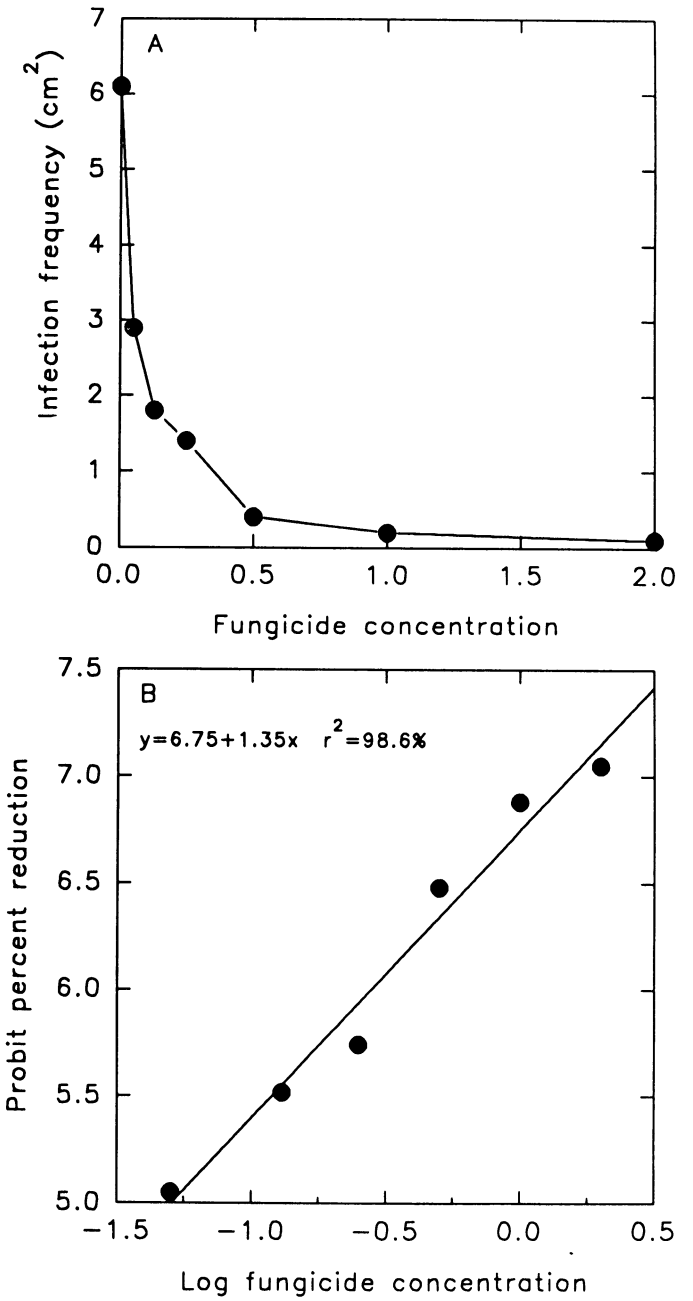

Fig. 2. Systemic effects of tebuconazole dosage on **(A)** infection frequency (lesions/cm^2) of *Cercosporidium personatum* and **(B)** probit percent reduction in infection frequency versus logarithm fungicide concentration relative to the nontreated control. All but the top three peanut leaves of each plant were treated with fungicide 24 hours prior to inoculation.

probit percent reduction in infection frequency was regressed against logarithm fungicide concentration to obtain a linear relationship between fungicide concentration (dose) and reduction in infection frequency (response).

Field experiments 1986–1987. Field experiments were conducted in 1986 and 1987 at the Southwest Georgia Experiment Station in Plains to quantify the effect of different active ingredient concentrations on percent defoliation and disease-incidence levels caused by late leaf spot, and the effect of increasing fungicide concentrations on sporulation and pod yield. These experiments were located in a field planted continuously to peanuts for the previous 20 yr to ensure the availability of naturally occurring inoculum to initiate leaf spot epidemics. All standard cultural practices recommended by the Georgia Cooperative Extension Service were followed (18). Experiments in both years were irrigated weekly to encourage leaf spot development. Florunner peanut was planted on 1 May 1986 and on 28 April 1987 in rows 0.97 cm apart at a rate of 100 kg/ha (approximately 5 seeds per 30 centimeters). A split-plot, randomized complete block design with four replications was used both years. Whole plots consisted of either chlorothalonil or tebuconazole. Subplots (six rows wide and 8.3 m long) consisted of different concentrations of fungicides, to differentially reduce the rate of leaf spot infection within subplots. Active ingredient concentrations for chlorothalonil were 0.15, 0.31, 0.63, 1.26, and 2.52 kg/ha; and for tebuconazole were 0.025, 0.05, 0.10, 0.21, and 0.42 kg/ha delivered in 2 L of water per subplot. These rates corresponded to 0.13×, 0.25×, 0.50×, 1.00×, and 2.00× the active ingredient concentrations recommended for each fungicide, respectively. Late leaf spot epidemics were allowed to progress without any fungicide until 1–5% disease incidence was attained. Fungicide treatments began 103 days after planting in 1986 and 59 days after planting in 1987. After the first application, fungicide treatments were applied to subplots at 7-day intervals in 1986 (five total sprays) and at 10-day intervals in 1987 (seven total sprays). Each whole plot also included a nonsprayed control and a second control treatment sprayed with chlorothalonil according to the fungicide schedule recommended by the Georgia Cooperative Extension Service (18). This schedule calls for the first fungicide application to begin 30 days after planting, with subsequent spray applications at 10–14 day intervals. Fungicide was applied with a hand-held, CO_2-powered boom sprayer with 8003 flat fan nozzles. The sprayer was operated at 210 kPa.

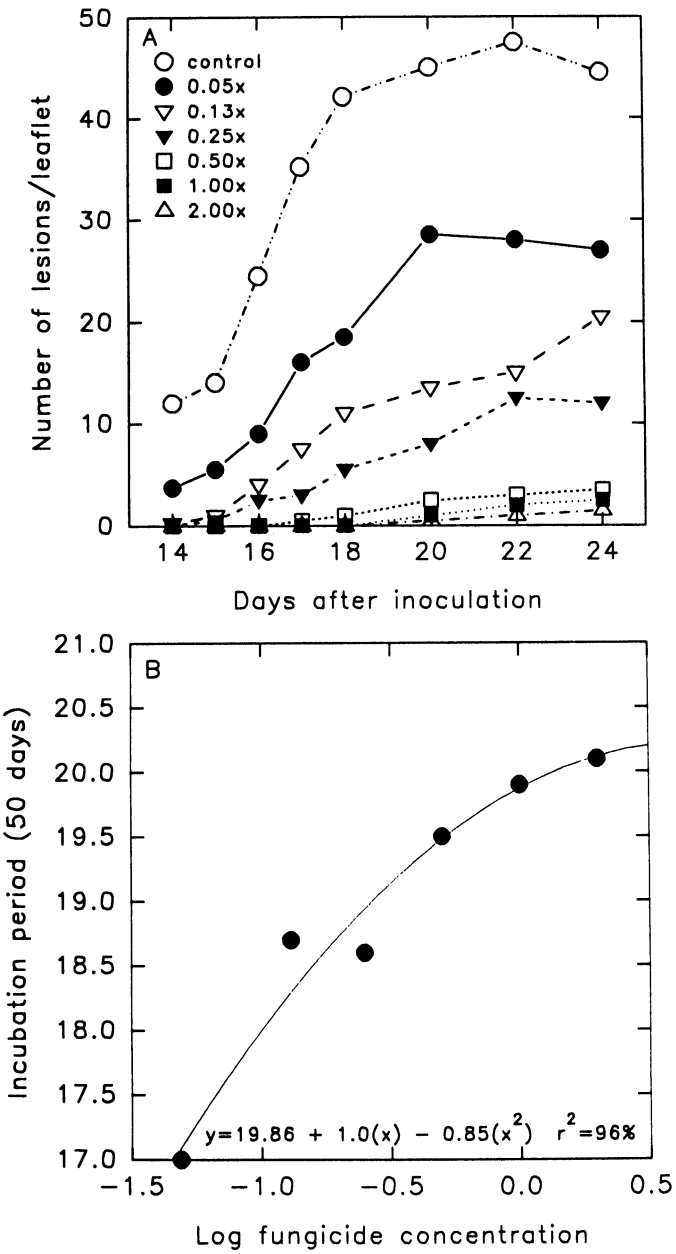

Fig. 3. Systemic effect of tebuconazole dosage on **(A)** time required for 50% of the final lesion population to appear and be counted (T_{50}) and **(B)** the incubation period of *Cercosporidium personatum*. All but the top three peanut leaves of each plant were treated with fungicide 24 hours prior to inoculation.

To measure the effect of increasing active ingredient concentrations of chlorothalonil and tebuconazole on pathogen sporulation, 25 lesions were randomly sampled from each subplot 145 days after planting in 1986 and 126 days after planting in 1987. With a cork borer, lesions were excised from peanut leaves by punching out 0.7-cm-diameter leaf areas containing a single lesion. Lesion disks were placed in petri dishes with moistened filter paper to maintain high relative humidity (RH > 95%) at 25 C for four days. Spores were harvested by stirring the 25 lesions in 30 ml of a 1% cupric sulfate solution with an 8 × 1.5 mm micro-stir bar for 2 min. Six 0.9-ml subsamples were drawn from each subplot sample with a micropipette, and the number of spores in each subsample was determined with a hemacytometer. Spore counts from each subsample were averaged for each subplot sample, and sporulation was expressed as the number of spores produced per lesion.

Percent defoliation and percent incidence were determined for each subplot beginning in mid-June and every 7 days until harvest. Ten lateral branch stems were randomly selected from each subplot, and percent defoliation was calculated for each stem according to the following formula: percent defoliation = $[(4NN - NL)/4NN] \times 100$, where NN = number of nodes and NL = number of leaflets present. Percent incidence was calculated as the following: (number of leaflets with at least one lesion/$4NN$) × 100. Defoliated leaflets were counted as infected. Percent defoliation and incidence values were calculated individually for each branch stem and averaged over the 10 stems to provide defoliation and

incidence values for each subplot and sampling date. Area under the disease progress curve (AUDPC) data were calculated for each subplot by using the procedure of Johnson et al (17).

Peanuts were dug 160 days after planting in 1986 (8 October) and 149 days after planting in 1987 (24 September). Peanuts were combined 5–7 days after digging, dried to 8% moisture, cleaned, and weighed. Pod weights from each subplot were converted to kilograms per hectare.

RESULTS

Protectant effects on spore germination. Application of either fungicide to peanut leaf surfaces 24 hr prior to inoculation greatly reduced the germination of spores. Spore germination on nontreated leaves measured 12, 24, and 36 hr after inoculation was 16.7, 56.3, and 80.3%, respectively. All chlorothalonil and tebuconazole dosages completely inhibited spore germination, except for the 0.05× concentrations, in which percent germination was 2.7% for chlorothalonil and 11.7% for tebuconazole 48 hr after inoculation. The slopes of the regression lines relating probit percent inhibition of spore germination to logarithm fungicide concentration were not significantly different from zero, indicating that there was no response to increasing fungicide concentrations of chlorothalonil and tebuconazole when plants were inoculated 24 hr after fungicide dosages were applied.

Systemic versus protectant effects on spore germination and penetration. Tebuconazole, when systemically present within the plant but not on inoculated leaf surfaces, did not reduce percent germination of *C. personatum* spores (Fig. 1A). The slopes of the regression lines relating probit percent survivors (germinated spores) measured 24, 48, and 96 hours after inoculation to tebuconazole dosage were not significantly different from zero. By 96 hours after inoculation, spore germination across all tebuconazole dosages ranged from 94.3 to 99.2%. Percent germination on the nontreated control was 98.7% after 96 hours. A control 1.00× concentration of chlorothalonil applied to the lower leaves had no effect on spore germination, as shown by germination on the upper, nontreated leaves that was not significantly different from the nontreated control.

The presence of tebuconazole systemically within the plant did not affect the ability of spores to penetrate host stomata (Fig. 1B). Eight days after inoculation, percent germ tube penetration ranged from 34 to 52% (nontreated control = 51.4%), but there was no significant effect of tebuconazole dosage on penetration as determined by probit-regression analysis. Twelve days after inoculation, germ tube penetration ranged from 88 to 99% (control = 89.2%). There was no effect of tebuconazole dosage on percent stomatal penetration, as shown by slopes that were not significantly different from zero ($P \leq 0.05$) and F statistics for regression models that were not significant ($P \leq 0.05$). A 1.00× chlorothalonil control treatment applied to the lower leaves did not affect germ tube development and penetration on the upper three leaves.

Increasing tebuconazole concentrations significantly reduced infection frequency on the top three nontreated leaves (Fig. 2A). Probit regression analysis showed that logarithm fungicide concentration of tebuconazole dosage explained 98.6% of the variation in probit reduction of infection frequency (Fig. 2B). A 1.00× chlorothalonil control treatment applied to lower leaves had no effect on infection frequency of the upper three leaves, as shown by infection frequency for the chlorothalonil treatment (6.0 lesions per square centimeter) that was not significantly different from the nonfungicide treated control (6.3 lesions per square centimeter).

Increasing tebuconazole concentrations applied to lower leaves increased the amount of time required for 50% of the lesions to form on the top three leaves (Fig. 3A). A regression equation relating the number of lesions counted over time for each tebuconazole dosage was used to calculate the time required for 50% of the lesion population to appear. The incubation period increased from 15 days

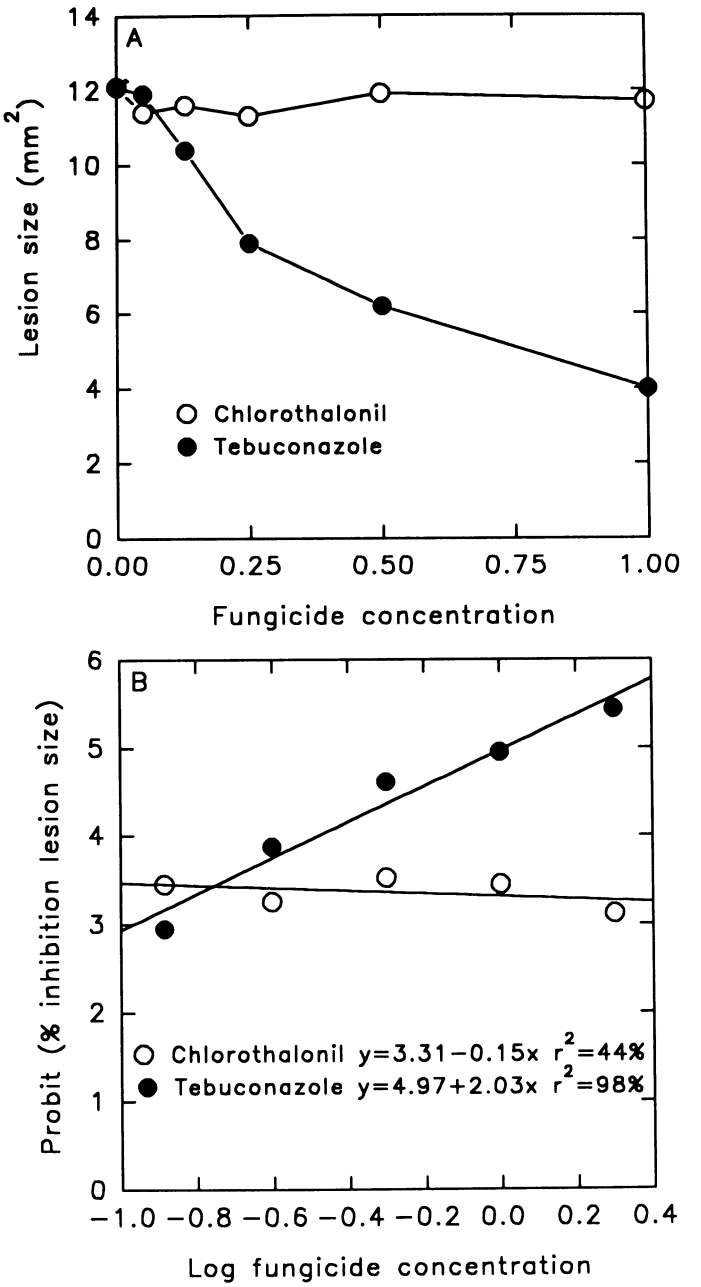

Fig. 4. Effect of tebuconazole and chlorothalonil dosage on **(A)** lesion size (mm^2) and **(B)** probit percent reduction in lesion size relative to the nontreated control. All but the top three peanut leaves of each plant were treated with fungicide 24 hours prior to inoculation.

for the nonfungicide and 1.00× chlorothalonil control treatments to 20 days for the 2.00× treatment of tebuconazole (Fig. 3B). A quadratic relationship was observed between logarithm fungicide concentration and incubation period measured as T_{50} ($R^2 = 96\%$).

Lesion size decreased as concentrations of tebuconazole increased (Fig. 4A). Logarithm tebuconazole dosage explained 98% of the variation in probit percent inhibition of lesion size, and the slope for tebuconazole (2.03) was significantly different from zero (Fig. 4B). However, the slope for chlorothalonil (-0.15) was not significantly different from zero ($P \leq 0.01$), indicating that there was no systemic effect of increasing chlorothalonil dosage on lesion size.

Fungicide effects in field experiments. As concentrations increased, both fungicides had an increasing negative effect on sporulation of *C. personatum* lesions sampled from the field (Fig. 5). Tebuconazole, however, reduced sporulation to a much greater extent than did chlorothalonil in both years, as shown by slopes relating probit inhibition to logarithm fungicide concentration that were significantly higher ($P \leq 0.01$) for tebuconazole than for chlorothalonil in both years (1.42 versus 0.95 in 1986 and 2.52 versus 1.56 in 1987) (Fig. 5A and B).

Increasing dosages of both fungicides decreased AUDPC in both years, but the lower concentrations of tebuconazole reduced AUDPC more than did the corresponding field rates of chlorothalonil (Fig. 6A and B).

Increasing fungicide concentrations of both fungicides increased pod yield (Fig. 7). In 1987, the 2.00×, 1.00×, 0.50×, and 0.25× tebuconazole treatments resulted in significantly higher yields ($P \leq 0.01$) than did the chlorothalonil treatment applied according to the growers' schedule, which yielded 2,862 kg/ha. Pod yields (dependent variable) were regressed against percent defoliation and percent incidence assessments (independent variables) made each week during the growing season. The best relationship between yield and these assessments occurred when percent defoliation was assessed approximately 14 days prior to the date of harvest (Table 1). Slopes and intercepts for tebuconazole versus chlorothalonil were not significantly different ($P \leq 0.01$) in 1986, indicating a common yield loss equation could be used for both fungicides to estimate yield loss due to defoliation. In 1987, however, slopes and intercepts were different, and therefore a common yield loss equation could not be developed.

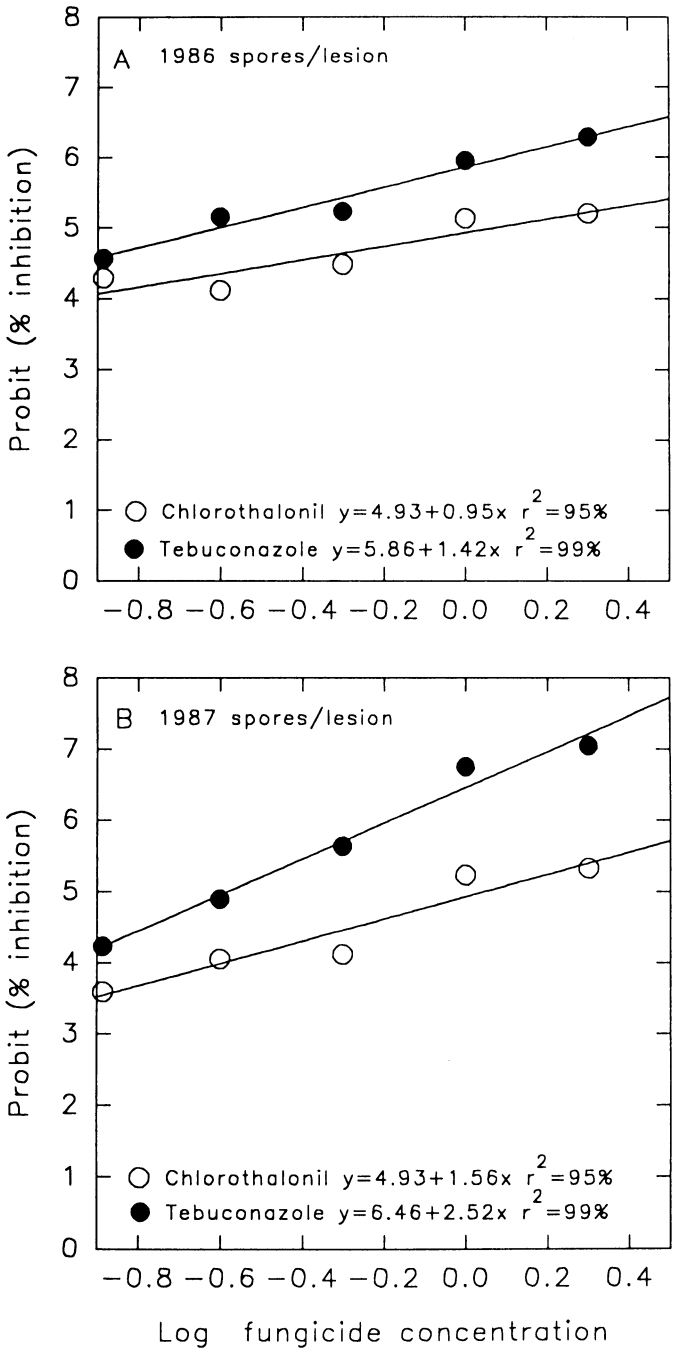

Fig. 5. Effect of logarithm tebuconazole and chlorothalonil dosage on probit percent inhibition of *Cercosporidium personatum* sporulation in field experiments conducted in Plains, Georgia, in **(A)** 1986 and **(B)** 1987. Percent probit inhibition was calculated relative to sporulation (spores/lesion) for the nontreated control.

DISCUSSION

Presently, there is a lack of quantitative information concerning the epidemiological effects of protectant and systemic fungicides on specific disease components of late leaf spot and how these components relate to disease progress (9,20,28). This information would be valuable in the development and implementation of integrated pest management programs for control of late leaf spot. In the present study, probit analysis was used to obtain a linear relationship between fungicide dosage (x) and the following disease components (y): 1) infection frequency (number of lesions per unit leaf area), 2) lesion size (mm^2), and 3) sporulation (number of spores produced per lesion). Shaner (27) previously used probit analysis to quantify latent period data in studies involving slow rusting resistance, and probit analysis has often been used to determine the lethal dose (LD_{50}) for 50% inhibition of pathogen populations (2,29). However, probit analysis has not previously been used to quantify and compare the effects of protectant versus systemic sterol-inhibiting fungicides on postgermination disease components of late leaf spot of peanut.

Brenneman and Murphy used an in vitro method to quantify the effects of chlorothalonil and tebuconazole on spore germination (4). They found that even low concentrations of chloro-

thalonil-amended water agar greatly inhibited spore germination. We obtained similar results in our study, when different fungicide dosages were applied to peanut leaf surfaces prior to inoculation with *C. personatum* spores. However, the systemic effects of tebuconazole cannot be quantified by Brenneman and Murphy's method. By applying different concentrations of tebuconazole to all but the top three leaves of peanut plants and inoculating plants 24 hr later, we were able to quantify the systemic effects of fungicide dosage on pre- and postinfection disease components of late leaf spot.

The infection process may be broken down into the following three measurable subprocesses: 1) germination of *C. personatum* spores; 2) penetration of germ tubes into stomates; and 3) successful colonization, which involves penetration of host cells and establishment of functional haustoria. Both fungicides greatly reduced the germination of *C. personatum* spores when present as protectants on peanut leaf surfaces 24 hr prior to inoculation. Because no systemic fungicidal activity of chlorothalonil has been reported in the literature, the fungicidal effect of chlorothalonil has been attributed solely to its protectant activity. The presence of tebuconazole systemically within the plant reduced infection frequency of late leaf spot by affecting different infection frequency subprocesses from those involved when tebuconazole was applied as a protectant, i.e., by inhibiting the initial stages of colonization.

It is not likely that tebuconazole applied to the lower leaves volatilized and reduced infection frequency on the upper three leaves, because neither spore germination nor germ tube penetration were affected by tebuconazole dosage. However, the higher the tebuconazole concentration applied to lower leaves, the greater the reduction in infection frequency. This is understandable because the chemical must come in contact with the target pathogen to exert a fungicidal or fungistatic effect. With protectant fungicides such as chlorothalonil, once a spore has germinated and entered a stomate, there is a high probability it will develop into a lesion. However, plants treated with tebuconazole have the additional benefit of its systemic action. Infection frequency on nontreated leaves was reduced by 97% when plants were treated systemically with tebuconazole. Schein et al (26) reported that concentrations of triadimefon as low as 0.01× greatly reduced the infection frequency of *Erysiphe graminis* DC. in wheat; however, they did not attempt to separate the protectant versus the systemic presence of this fungicide.

Several other studies have shown that systemic fungicides exert their inhibitory effects on the pathogen only after penetration into host cells (5,6,8). Cohen et al (8) found that metalaxyl was only slightly toxic to zoospores and did not inhibit the initial penetration of *Phytophthora infestans* (Mont.) de Bary into leaves of tomato (*Lycopersicon esculentum* Mill.), but the fungitoxic effect was evidenced by the appearance of minute sterile lesions. Bowen and Pedersen (3) previously reported that the rate of lesion expansion of *Exserohilum turcicum* (Pass.) K.J. Leonard & E.G. Suggs in corn (*Zea mays* L.) was inversely proportional to the dosage of propiconazole (Tilt).

The systemic activity of tebuconazole was also found to impact negatively on other disease components for late leaf spot which may have contributed to lower AUDPC levels for the lower dosages of tebuconazole compared to chlorothalonil. The length of the incubation period increased with increasing tebuconazole concentrations, whereas chlorothalonil had no effect on this component. A delay in the incubation period should contribute to a slower rate of disease progress in the field (29).

The observed reduction in number of spores produced per lesion on tebuconazole-treated plants should also have a large negative effect on disease progress in the field (17,26). Tebuconazole concentrations between 0.25× and 0.50× reduced sporulation by 50%. A reduction in spore production was also observed on chlorothalonil-treated plants, but not to the degree tebuconazole treatments reduced sporulation. Approximately 1.00× concentrations of chlorothalonil were required to reduce sporulation by 50%. The fact that chlorothalonil

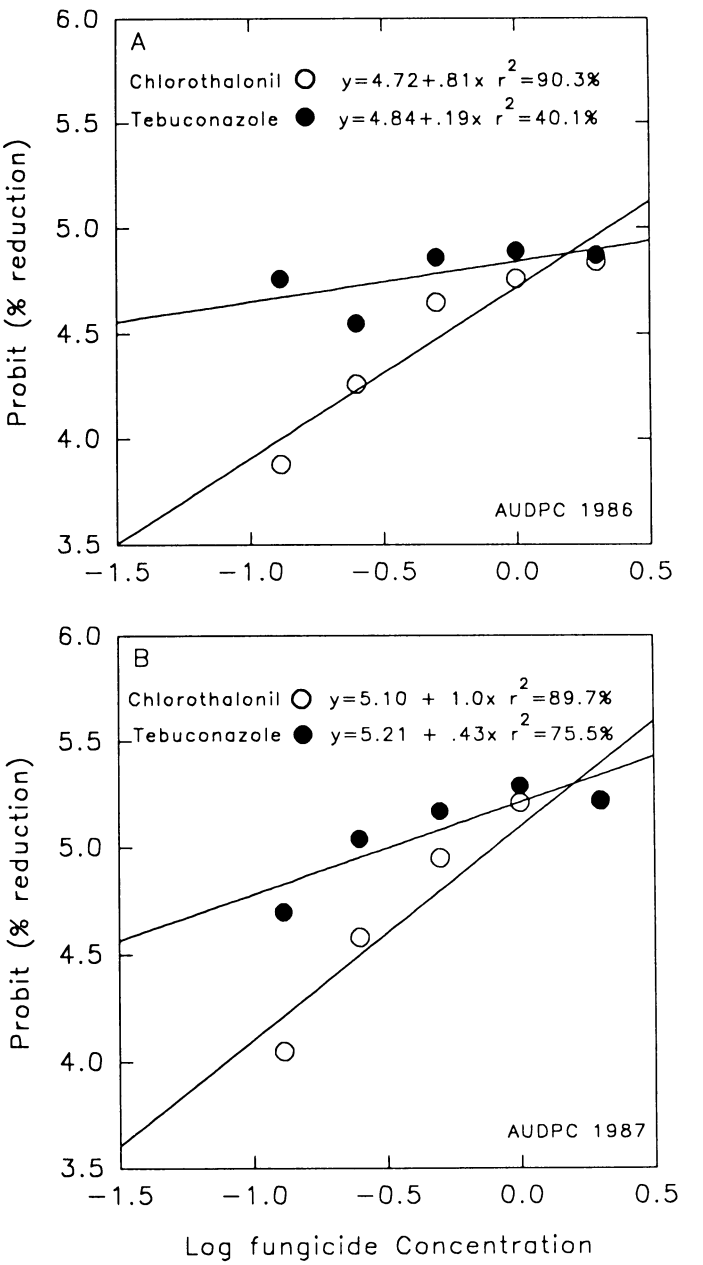

Fig. 6. Effect of logarithm tebuconazole and chlorothalonil dosage on AUDPC (incidence) in Plains, Georgia, in **(A)** 1986 and **(B)** 1987.

reduced sporulation was surprising since chlorothalonil was thought to have only protectant activity against *C. personatum*. Chlorothalonil, when applied directly onto sporulating lesions in the field, reduced spore production by up to 50% at the 1.00× and 2.00× concentrations. However, the systemic effects of tebuconazole reduced sporulation by more than 95% at the same concentrations. This agrees with in vitro results obtained by Brenneman and Murphy (4), who reported that sporulation was greatly inhibited by tebuconazole at rates as low as 1.0 μg/ml.

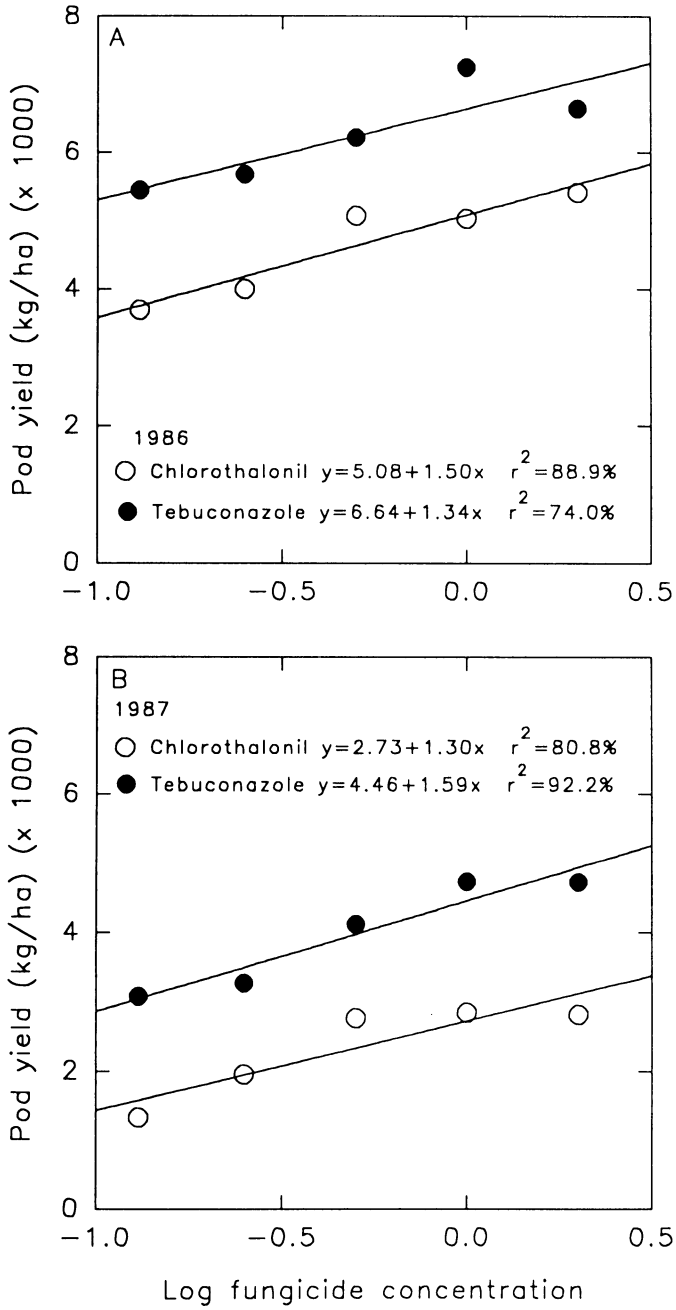

Fig. 7. Effect of logarithm tebuconazole and chlorothalonil dosage on pod yield in Plains, Georgia, in **(A)** 1986 and **(B)** 1987.

Table 1. Critical point models[a] relating defoliation levels assessed 14 days before digging to yield of Florunner peanut in 1986 and 1987

	Chlorothalonil				Tebuconazole			
Year	Intercept	Slope	R^2	Standard error of estimate	Intercept	Slope	R^2	Standard error of estimate
1986	6,821.2	−62.2	0.43	1,031	7,384.8	−63.7	0.72	881
1987	3,557.3	−32.2	0.58	792	5,403.3	−50.3	0.59	580

[a] F tests for both models were significant ($P \leq 0.001$). Intercepts and slopes were not significantly different for tebuconazole- and chlorothalonil-treated plots in 1986. Slopes and intercepts for tebuconazole and chlorothalonil were significantly different ($P \leq 0.01$) in 1987.

Lower infection frequency, a longer incubation period, smaller lesions, and reduced sporulation should result in a significant reduction in the apparent infection rate (17,29). Therefore, systemic fungicides that impact negatively on several disease components may have rate-reducing effects that mimic the effects of resistance components. Johnson et al (17) reported that there was a good relationship between components of resistance to *Cercospora arachidicola* and disease progress of early leaf spot epidemics in Virginia-type peanut. The fact that the presence of tebuconazole systemically within peanut leaves had a significant effect on all disease components measured offers a mechanistic explanation why tebuconazole-treated subplots had a lower range of AUDPC values than did chlorothalonil-treated subplots.

The highest yield during both years was achieved by the use of tebuconazole at the 1.00× recommended field rate. The 2.00× concentration of tebuconazole yielded slightly lower than the 1.00× concentration, which might indicate phytotoxicity. The best relationship between percent defoliation and yield occurred when defoliation was assessed 14 days before digging. Backman and Crawford (1) also reported a good relationship between late leaf spot defoliation and yield when defoliation was assessed a few weeks prior to harvest.

During both years, pod yields from tebuconazole-treated plots were much higher than those from corresponding treatments of chlorothalonil. The slopes of regression lines for relating AUDPC to yield were different for these two fungicides in 1987. These slopes indicate that peanut plants treated with tebuconazole yield more than those treated with chlorothalonil when AUDPC values are similar. Therefore, the increase in yield cannot be entirely explained by reduced AUDPC values in the tebuconazole-treated plots. One explanation is that tebuconazole is controlling other yield-reducing pathogens that are not affected by the presence of chlorothalonil (4). Another possible explanation is that tebuconazole has plant-growth regulating properties that increase attainable yield. The intercepts of the regression lines relating percent defoliation (x) to yield (y) were also significantly different for the two fungicides in 1987. Again, this may be another indication that tebuconazole has an effect on other pathogens or that it has a growth regulator effect or both. More studies are needed to determine the mechanisms of these nontarget effects on yield.

Information at the disease components level should provide a sound basis for the integration of management tactics (fungicides, resistant cultivars, disease forecasting) to more efficiently control

late leaf spot (25). Fry (13,14) found that a combination of control tactics can provide adequate disease control but with a fewer number of fungicide applications required per season. Ellis et al (11) also showed that the curative effects of fungicides (reduction in infection frequency) could be better utilized when a forecasting model was used to schedule fungicide applications rather than calendar spray schedules. This study provides quantitative information on the effects that chlorothalonil and tebuconazole have on specific disease components of late leaf spot. This information should help in developing management programs that integrate fungicide use with weather-based forecasting systems and resistant cultivars.

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