MODELING EFFECTS OF FUNGICIDE APPLICATION ON DYNAMICS OF WATERMELON ANHTRACNOSE IN TIME AND SPACE

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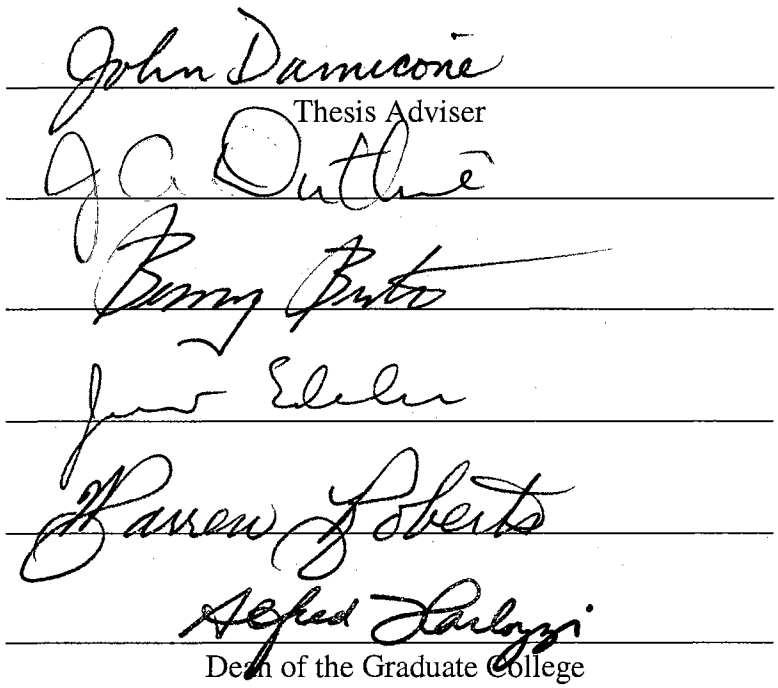

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NOMENCLATURE

D	total dose of fungicide applied during a cropping season
D_x	the direction parallel to crop row with point source of conidia (the abscissa)
D_{xy}	the direction at an angle of 45° to the row (the diagonal)
D_y	the direction perpendicular to the row (the ordinate)
g	the intrinsic rate of decrease of disease with respect to distance
N	number of applications of fungicide applied during a cropping season
N_d	the number of defoliated leaves in a quadrat
Ns	the number of leaves with anthracnose lesions in a quadrat
N_h	the number of leaves without anthracnose lesions in a quadrat
r	intrinsic temporal rate of disease progress
S	distance from a point source
S_x	distance from a point source in the direction parallel to the row
S_y	distance from a point source in the direction perpendicular to the row
T	days after inoculation
$T_{0.5}$	days required to increase an incidence of 0.5
v	velocity of disease spread
Y	mean incidence of disease over replicate plots
Z	mean incidence of disease over replicate plots at specific location in a field

CHAPTER I

Introduction

Occurrence of watermelon anthracnose

Anthracnose, caused by *Colletotrichum orbiculare* (Berk & Mont.) (synonym *Colletotrichum lagenarium* (pass.) Ellis & Halst, telemorph *Glomerella lagenarium* F. Stevens), was first described in Italy in 1867 (63). Since that time it has been reported in 82 countries and regions in the world (13). Anthracnose primarily occurs in North American, Asia, and Australia (13). In the United State of American, it happens in each of the regions in which watermelon is planted. In Oklahoma, over 300 farmers annually produce watermelon that is worth $ 5 million on approximately 5,000 hectares (52). In the southern region of the USA, watermelon worth $ 200 million is produced annually on about 81,000 hectares (52). Anthracnose is one of the most destructive diseases of watermelon in the south-central states (Oklahoma, Missouri, Arkansas, and central Texas) and Midwest (Indiana) (36). Anthracnose can occur on all aboveground parts of watermelon plants, leaves, stems and fruits, but most commonly on older-leaves (26). Loss of healthy foliage due to anthracnose significantly reduces the number, size, and quality of fruit (36). Entire vines may be killed under disease conductive environmental conditions. Anthracnose is difficult to manage once it becomes established (52). Up to 60 % of yield losses of watermelon fruits frequently occur (36).

Rational of the study

The main goal of integrated disease management (IPM) is to reduce the quantity of synthetic fungicides that is applied to crops by using integrated measures for maximizing profits of crop production and reducing risk of fungicide to human health and environment. However, in most cases, fungicide cannot be completely replaced by biological measures and often remains as the key component in the IPM. Therefore, improving understanding of the mechanisms underlying quantitative effects of fungicide application on disease control is essential for achieving this goal.

In cucurbit vegetables such as watermelon, use of fungicides still is the key component in integrated management of anthracnose because resistant cultivars and cultural practices are not effective or reliable. In the United States, fungicides are applied to approximately 70% of commercial watermelon crops and they account for 65% of all pesticides applied to the crop (2). Several protectant and systemic fungicides are registered and commonly applied to watermelon foliage. Chlorothalonil is a more effective protectant fungicide than others such as maneb, mancozeb, and cupric hydroxide. They are effective against a broad range of diseases. In contrast, systemic fungicides like benomyl and thiophanate-methyl are effective against a limited range of diseases. However, they have post-infection activity and are more resistant to weathering.

A large amount of fungicide application may lead to development of fungicide resistance and increases watermelon production cost. Resistance of *Didymella bryoniae* (Auersw.), the causal agent of both gummy stem blight and black rot of watermelon, to benomyl and thiophanate-methyl has been found in several commercial watermelon-grown regions in the USA (22, 37, 48). In the southern regions of the USA, control of foliar disease represents a large fraction of the cost of watermelon production. More

money is spent on fungicides to control foliar diseases than on pesticides to control arthropods or weeds (2).

Effects of type, dose at each application, and number of applications of fungicide on anthracnose have been evaluated extensively (3, 6, 16, 17, 26, 36, 43-46, 58, 65, 66, 75). However, knowledge of quantitative effects of total dose of fungicide and number of applications during a cropping season, and effects of pre- and post-infection applications of protectant and systemic fungicide on dynamics of anthracnose is inadequate. More importantly, effect on disease of varying the number of applications of a fungicide in a cropping season at a fixed total dose of fungicide is poorly understood. Fungicide-mediated variation in the rate of the spread of foliar disease in two dimensions has not been explored. Previous research on effects of fungicides on anthracnose has emphasized qualitative effects, and the impacts on temporal dynamics. Further work is needed to understand quantitative effects, and the impacts on spatial, and spatio-temporal dynamics.

A more detailed knowledge of effects of total dose of fungicide, number of applications, and the time of pre- and post-infection applications on dynamics of anthracnose in time and space would help efforts to improve our understanding of the basic mechanisms underlying the effects of chemical control, optimize the strategies for management of the disease, reduce fungicide use, and delay or prevent fungicide resistance. This study was focused on using a combination of experimental and modeling approaches to evaluate the effects of fungicide application on dynamics of diseases in time and space under the field conditions.

Purpose of the study

The purpose of this study is to evaluate effects of selected key factors of fungicide application on epidemics of watermelon anthracnose in order to provide the information necessary to support the construction of computerized, decision-aids for cucurbit production.

Objectives of the study

The objectives of this study include:

1. Evaluate the effects of total dose and number of fungicide applications during a cropping season on temporal dynamics of anthracnose.
2. Determine the effects of pre- and post-infection applications of chlorothalonil and benomyl on incidence of anthracnose.
3. Characterize the effects of fungicide application on spatio-temporal dynamics of watermelon anthracnose.
4. Evaluate fungicide-mediated variation in the rate of spread of anthracnose in two dimensions.

CHAPTER II

Distinguishing Effects of Total Dose and Number of Chlorothalonil Application on Watermelon Anthracnose

ABSTRACT

Incidence of a foliar disease tends to decline with total dose (D) of fungicide applied during a cropping season but it also depends on the number (N) of applications. Seldom are effects of D and N on incidence distinguished explicitly. To control anthracnose caused by *Colletotrichum orbiculare*, possible combinations of 3 levels of D and N of chlorothalonil were applied to watermelon canopies in field plots in an experiment that was conducted each of two years. Disease incidence (Y) at most assessments during an epidemic significantly decreased with increasing D and N in each year. The rate of the response to D was increased with N. Effects of D and N on variation in Y with time were characterized by $Y = f(D, N) / (1 + exp^{-r(T-t)})$, in which T represented the days after inoculation, r was the parameter for the relative temporal rate (day^{-1}); t was the parameter for the time required to reach 50% of the upper limit on disease, and $f(D,N)$ was $f(D,N) = a\, exp^{-(bD + cDN)}$, in which a was the parameter for the level of Y when no fungicide was applied, b and c were the parameters for the relative rate of decrease of Y with D and DN, respectively. Estimates of b or c for each year were all approximately to 0.01 per kg a.i. per ha (per application). Based on the model, reduction of anthracnose could be estimated by adjustments of D and N. The new model presented here may provide a valuable tool for optimizing the use of chlorothalonil on control of foliar diseases.

Introduction

Knowledge of the fungicidal control of foliar disease provides a basis for improving the economic benefits of crop production and minimizing the impact of fungicides on human health and the environment. Consequently, plant pathologists have given considerable effort to the problem of finding the minimum dose of fungicide that can prevent foliar disease from exceeding economically acceptable levels. Effects on disease of varying the dose of fungicide that is applied to a crop during a cropping season have been documented in many experiments (3, 6, 8, 14, 16, 17, 25, 41, 43, 65, 66). The total dose of fungicide that is applied to a crop is determined by the dose of fungicide at each application and the total number of applications during the cropping season. Thus, in one common approach to evaluating fungicidal control, the number of applications of fungicide during the season is held constant but the total dose is varied by varying the dose at each application (8, 14, 25, 41, 44, 66). Alternatively, the dose at each application is fixed and the total dose is varied by varying the number of applications (8, 9, 17, 25, 43, 75).

The number of applications of fungicide during a cropping season could have an effect on disease that is independent of the total dose during the season. That is, for a given total dose of fungicide, increasing the number of applications but reducing the dose at each application could reduce levels of disease. The effect on crop productivity of varying the number of applications of a macronutrient during a cropping season for a fixed total dose of the macronutrient are well understood (5, 29, 34, 61). In contrast, the analogous effect on disease of varying the number of applications of a fungicide during a cropping season

at a fixed total dose of fungicide is poorly understood. In most studies of the effect of varying the number of applications of fungicides (3, 8, 9, 35), the effect of varying the number of applications cannot be distinguished from the effect of varying the total dose.

The development of a more general understanding of quantitative relationships between increase of foliar diseases during a cropping season and dose of fungicide is hindered by some of the techniques that commonly are used to assess these effects. For example, many analyses of the effect of fungicide dose on disease consist of a comparison of disease levels among discrete doses (6, 16, 20, 46, 65, 66). In contrast, estimates of rates of change in the level of disease with respect to the total dose of fungicide during a cropping season, the dose per application, or the number of applications are rare. The lack of such estimates has hindered meaningful comparison of results among studies. Similarly, knowledge of the effect of fungicides on the temporal dynamics of foliar epidemics is limited. The characteristics of temporal dynamics that are affected by fungicides frequently are obscured because disease is measured at only one or two points during the cropping season (14, 41, 43, 75) or because the overall level of disease during the cropping season is measured by a response variable such as the area under the disease progress curve (8, 14, 20, 25, 41, 65).

In this study, we investigated some effects of chlorothalonil on epidemics of anthracnose of watermelon. Watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) was selected for detailed study because it is an economically important vegetable crop in the southern USA (2). Anthracnose, which is caused by *Colletotrichum orbiculare* (Berk & Halst) Arx (= *C. lagenarium*), is an important constraint on watermelon production worldwide (76). Protectant fungicides, such as chlorothalonil, remain key components of

management strategies for many diseases of vegetable crops including watermelon anthracnose. Use of fungicides accounts for about 65% of all pesticides applied to US grown watermelon (2).

The objective of this study was to distinguish the effects of two factors, the total dose of chlorothalonil that was applied during a cropping season and the number of applications of chlorothalonil on the temporal dynamics of watermelon anthracnose. Preliminary results of this work have been reported (71).

MATERIALS AND METHODS

Field plots. An experiment was conducted in the same field in 1997 and 1998 at the Wes Watkins Agricultural Research and Extension Center in Lane, OK [latitude (N): 34.3086°; longitude (E): -95.9975°]. The soil type was a Bernow fine sandy loam (fine-loamy, siliceous, thermic Glossic Paleudalf) with pH = 6.6 and N: P_2O_5: K_2O = 2: 9: 299 (kg /ha). The field was cropped to winter wheat during each previous winter season. Metam sodium (267 kg a.i. /ha, Vapam, Stauffer Chemical, Westport, CT) was applied to all plots 14 to 20 days before seeding. Before seeding, granular fertilizer was applied to a band of soil in which seeds would be sown, at the rate of 56, 61, 152 kg /ha, respectively, of N, P_2O_5, and K_2O. Following anthesis, a second application of 50 kg/ha of N was applied through drip irrigation lines. Plants were trickle irrigated as needed using drip tape. The herbicide ethalfluralin (0.9 kg a.i./ha, Curbit EC, Platte Chemical Co., Fremont, NE) and the insecticide carbofuran (1.1 kg a.i /ha, Furadan 4F, FMC, Philadelphia, PA) were applied for control of weeds and insects based on the commercial practices (52).

In each experiment, an experimental unit was a plot, 1.8 m wide in which one row of plants of watermelon cultivar Mickylee was centered along the length. In 1997, each plot was 9.1 m long and was planted on 25 May. In 1998, each plot was 8.1 m long and was planted on 18 June. An area 2 m long, which was planted to soybean, separated the ends of adjacent plots within a row of plots. A strip 6 m wide, which was planted to sorghum (1997) or soybean (1998), separated adjacent rows of plots on one side. On the other side, a strip 1.8 m wide separated adjacent rows. One row of plants of cultivar Sugar Baby was centered along the length of this strip. In each row of watermelon, 10 seeds/m were sown. Seedlings of cultivar Mickylee with 3 to 4 true leaves were thinned to 0.6 m between plants within the row.

Fungicide treatments. In this study, a unit dose of chlorothalonil (Bravo720, ISK Biotech Co., Menter, Ohio) was 1.68 kg a.i. /ha. In each of two experiments, treatments consisted of a factorial arrangement of two treatment factors, *D* (the total dose of chlorothalonil that was applied during the cropping season) and *N* (the total number of applications during the cropping season). The experiment comprised five treatment combinations in 1997 and nine combinations in 1998 (Table I).

Each treatment combination was assigned randomly to plots arranged in 6 (1997) or 4 (1998) complete blocks. Chlorothalonil was applied to the foliage of cultivar Mickylee through 5 flat fan nozzles (Model Tee-Jet #8003, Spraying Systems, Inc., Wheaton, III) using a compressed CO_2-type sprayer at a pressure of 279 kPa. Nozzles were spaced 46 cm apart on a 2.1-m boom that was mounted 46 cm above the canopy on the side of a manually propelled, wheel barrow-type sprayer. These nozzles were determined to deliver 271 L/ha.

Pathogen inoculation and disease assessment: A monoconidial culture of *C. orbiculare* was isolated from diseased watermelon plants in Oklahoma. The culture was inoculated into sterilized oat kernels and incubated for 14 days at room temperature with a 12-hr photoperiod. The colonized kernels were air-dried for 96 hours. When male flowers of watermelon plants first appeared, the dry oat kernels were distributed over the surface of soil along rows of cultivar Sugar Baby. The inoculum was distributed at the rate of approximately 10 g/m of row. In 1998, plots were irrigated with a sprinkler about 10 hrs every other night for a 2-wk period following inoculation to stimulate disease development. No overhead irrigation was made to plots in 1997 plots. To assess the concentration of conidia, 10 grams of the dry oat kernels were ground in 50 ml water. The conidial suspension was filtered through four layers of gauze. Conidia in each of ten, 10µl samples of the filtrate were counted under a light microscope. Concentration of conidia in the inoculum was estimated by the means of three samples (40). Each gram of inoculum contained about 10^8 and 10^6 conidia for 1997 and 1998, respectively.

Disease was measured by using a leaf-count technique. In each plot, a quadrat (0.5 × 0.5 m) was positioned at the crown of each of three, arbitrarily-selected, plants. In each quadrat, the number of defoliated leaves (N_d), the number of symptomatic leaves (N_s), and the number of asymptomatic leaves (N_h) were counted separately. Disease was assessed every 3-4 days in 1997 and every 7 days in 1998 for 7 times from anthesis to fruit maturity.

Data analysis and model development. For each quadrat, the proportion (Yq) of all leaves within a quadrat that were diseased was determined as:

$$Yq = (Ns + N_d)/(N_s + N_d + N_h) . \qquad (1)$$

The mean proportion of diseased leaves in a plot on each date of sampling on average over the three quadrats was then *Y*.

Effects of *D* and *N* on variation in *Y* with time (*T*; days) was evaluated using a modified logistic equation,

$$Y = f(D, N, T) = k_u / (1 + exp^{-r(T-t)}) \qquad (2)$$

in which $k_u = f(D, N) = a\ exp^{-(bD + cDN)}$. (3)

In the model defined by equation 2, *r* represents the relative rate (day $^{-1}$) of change of *Y* with respect to *T* and *t* represents the time required to reach 50% of the upper limit on disease. The upper limit on *Y* (parameter k_u) at large *T* decreases exponentially with *D* and *N* according to equation 3. Thus, in equation 3, *a* estimates the upper limit on *Y* in the absence of fungicide when *T* is large. The parameters *b* and *c*, respectively, estimate the relative rate of decrease of *Y* with *D* and *DN*.

Parameters of the model were estimated by non-linear regression using the derivative-free option DUD of the procedure NLIN of the Statistical Analysis System (SAS version 6.12, SAS Institute, Cary, NC). Overparameterization and fit of the models were evaluated by: (i) inspection of predicted values and observed values plotted against independent variables, (ii) standardized residual errors plotted against predicted values, (iii) significance of the estimated parameters, (iv) standard errors on parameter estimates, and (v) the asymptotic correlation between parameter estimates. Fisher's Least Significance Difference (LSD) was employed to compare differences in *Y* among the *D* or *N* treatments for each assessment and year (62).

RESULTS

Anthracnose decreased with increasing D and with increasing N (Fig. 1). In 1998, disease incidence significantly ($P \leq 0.05$) declined with the increase of D from 2 to 8 unit doses at 5 of 7 assessments for each N treatment (Fig. 1D-F). In 1997, similar effects appeared at the last two assessments, and at 5 of 7 assessments, respectively for the 1 (Fig. 1 A), and 5 (Fig. 1 C) -application treatment. Profound ($P \leq 0.05$) reduction of disease incidence with increasing N occurred at the same assessment dates as the effects of D on the decrease of disease did (Fig. 1).

In each year, the response of disease incidence to dose was increased by increasing N. In 1998, difference in Y among three D treatments increased significantly with increasing N from 2 to 8 applications at each of 5 assessments beginning with the third (Fig. 1 D-F). Similar dose responses were also observed in 1997 (Fig.1 A-C).

Effects on Y with increasing D and N as well as the dose response became more apparent with disease progress over time. In 1998 (Fig. 1 D-F), difference in Y among three D treatments for each N treatment was not significant ($P \geq 0.05$) at the first two assessments, became significant ($P \leq 0.05$) at the third one, and increased thereafter. In 1997, similar variation in Y between two D treatments with time was observed for the 5-application treatment (Fig.1 C). However, for the one-application treatment (Fig. 1A), the difference was not significant ($P \leq 0.05$) for the first five assessments, but was significant at the sixth. The dose responses became more pronounced over time in each year (Fig. 1).

The effects of D and N on variation in Y with time were characterized by the modified logistic model (equation 2) in which the upper limit was a function of D and N. Inspection of the plots of observed versus predicted values indicated that the model adequately characterized the effects of D and N for each year (Fig.1). Parameters were

precisely estimated (Table II) and asymptotic correlation coefficients were not large ($\leq$ 0.60). Standardized residuals in plots were randomly distributed. The effects of D and N were similar in 1997 and 1998 (Table II). Estimates of parameter b and c did not vary greatly between years and their differences fell within a very narrow range (Table II). However, disease incidence in the absence of fungicide, parameter a, was two-fold higher in 1998 than in 1997 (Table II).

In the regression analysis, equation 3 adequately characterized the effects of D and N on final disease incidence as well as average disease incidence over all assessments and relative area under disease progress (*AUDPC*) for each year (data not shown). To link with fungicide effects, simplifying assumptions were made for the logistic model by changing one or more parameters to variables that were D and N dependent. All these models with the exception of equation 2 failed to fit the observed data.

DISCUSSION

This is the first investigation to distinguish explicitly the effect on a foliar disease of varying the total dose of a fungicide that is applied during a cropping season from the effect of varying the number of applications of the fungicide. Clearly, either increasing total dose or increasing number of applications can reduce disease incidence. More importantly, variation in the dose response with increasing the number of applications was evaluated. The results of this study confirm that for a given total dose of chlorothalonil during a cropping season, the absolute rate of increase of watermelon anthracnose declines as the number of applications of fungicide during the season is increased. That is, if the total dose of fungicide during a season is fixed, then anthracnose

epidemics can be inhibited by increasing the frequency of application but decreasing the dose of chlorothalonil at each application. Conversely, the results also confirm that, if the number of applications of chlorothalonil during the season is fixed, then increasing the total dose of the fungicide reduces the absolute rate of increase of anthracnose. For the first time, a modified logistic model (equation 2) has been developed that characterizes the effects of total dose of a fungicide and the number of applications on variation in the incidence of a foliar disease with time.

Our study extended previous results of the effects of dose and number of applications of chlorothalonil on foliar diseases. Reduction of incidence of anthracnose with increasing dose per application of the fungicide has been observed on cucumber (66). Fry (25) found potato late blight decreased with increasing dose or frequency of the fungicide. Effects of dose or number of applications of chlorothalonil or other fungicides on foliar diseases were also evaluated (9, 14, 25, 41). However, previous investigations did not quantify the rate of dose response or effects of both total dose and application number. Our study not only evaluated but also clearly characterized these effects.

Fixing some model parameters by simplifying assumptions has been used to characterize effects of chemical control (27), host resistance (27, 69) and cultural practices (27) on the upper limit on disease. In this study, the model defined by equation 2 adequately characterized effects of chlorothalonil on the temporal dynamics of watermelon anthracnose. In this model, the absolute rate of increase of disease depended on the total dose of fungicide that was applied during a cropping season and on the number of applications of chlorothalonil because these factors affected the upper limit on the incidence of disease. These factors did not influence the intrinsic rate of increase of

disease or the length of the delay in the increase of disease. This model is consistent with the results of previous studies of effects of fungicide on the temporal dynamics of foliar diseases (3, 25, 27). Gilligan (27) found that disease control measures including fungicide application do not affect the relative temporal rates of the logistic model, but do affect the upper limit parameter. In an important study on effects of dose of mancozeb on potato late blight, Fry (25) observed the relative temporal rates in the linearized form of the logistic model were not significantly changed with increasing dose per application from 0.22 to 1.8 kg/ha. Amin and Ullasa (3) also reported that increasing total dose of thiophanate from 0.67 to 3.3 kg ha^{-1} did not change the temporal rates when the fungicide was applied for control of watermelon anthracnose. Good fit of the final form of equation 2 with observed data (Fig.1) also supports our assumption. The model as described worked well over two years with very different disease levels.

Characteristics of exponential reduction of disease levels with fungicide use as noted in this study seems to be supported by other related reports. Culbreath et al (14) evaluated effects of dose per application of cyproconazole alone or in combination with chlorothalonil on peanut leaf spot. They found a negative exponential equation appropriately characterized the response of the disease to the increase of dose of cyproconazole. However, some studies showed that linear function models might also be appropriate to quantify effects of dose or number of chlorothalonil or other fungicides on foliar diseases (9, 14, 41). The model developed from this study demonstrates a novel trait of the interaction of total dose and number of applications of a fungicide. Thus, it may provide new theoretical foundations for understanding how a foliar disease is quantitatively affected by a fungicide in field conditions.

Single application technique is a method whereby a single massive dose of fungicide is applied to crop canopies. Over time the fungicide is redistributed on plant surfaces by rain or irrigation so that the plant remains protected longer compared with standard applications of the fungicide. This technique has been recommended to control mango anthracnose (*Colletotrichum gloeosporioides* (60), cocoa black pod (*Phytopathora palmivora*) (59), and tomato black mold (*Alternaria alternata* (19). However, the results of this study indicate that this method may not effectively prevent epidemics of watermelon anthracnose. In 1997, single application of chlorothalonil at the rate of 2.5 or 10 kg a.i/ha at watermelon anthesis did not effectively reduce anthracnose levels (Fig. 1). This corroborates the results of the investigation on the effects of thiophanate on anthracnose (3). This can be explained by the persistence of chlorothalonil on plant canopies, which usually does not remain more than 3 weeks (10, 11, 21, 32, 47, 64). It is clear that a single dose of fungicide cannot protect plants from infections of the polycycle-life pathogen beyond the effective residual periods.

The effects of total dose and number of chlorothalonil application on watermelon anthracnose have been clearly described. However, this study does not account for difference in timing of applications of the fungicide, which is another important factor in determining the efficacy of a fungicide. Further studies are needed to determine if the model presented is appropriate for other fungicides or other crop-pathogen systems.

Table I. The combined schedules of total dose (*D*) and number (*N*) of chlorothalonil applications during two cropping seasons

Year	Total dose of fungicide [a]	Total number of applications	Date of application of fungicide (week following anthesis)							
			1	2	3	4	5	6	7	8
1997	1.5	5	× [b]	×	×	×	×			
	6.0	5	×	×	×	×	×			
	3.0	3	×		×		×			
	1.5	1	×							
	6.0	1	×							
1998	2.0	8	×	×	×	×	×	×	×	×
	4.0	8	×	×	×	×	×	×	×	×
	8.0	8	×	×	×	×	×	×	×	×
	2.0	4	×		×		×		×	
	4.0	4	×		×		×		×	
	8.0	4	×		×		×		×	
	2.0	2	×		×					
	4.0	2	×		×					
	8.0	2	×		×					

[a] Dose was measured as multiples of a standard dose of 1.68 kg chlorothalonil /ha.

[b] Fungicide was applied during each week indicated by an "×". The date of the application was 17, 24 and 31 July, 7 and 14 August in 1997, and 24 and 31 July, 7, 14, 21 and 28 August, 4 and 11 September in 199

Table II. Parameter estimates of the modified logistic model [x] that characterized the effects of total dose (D) and number (N) of chlorothalonil applications during a cropping season on variation in the incidence (Y) of watermelon anthracnose with time (T, days after inoculation) for each of 2 years

Year	Parameter estimate [x]				
	a	b	c	r	t
1997	0.418 ± 0.009 [y]	0.014 ± 0.006	0.011 ± 0.001	0.328 ± 0.019	20.0 ± 0.2
1998	1.009 ± 0.024	0.013 ± 0.005	0.008 ± 0.001	0.122 ± 0.007	30.4 ± 0.5

[x] Parameters were estimated by non-linear regression of the modified logistic model, $Y = f(D, N) / (1 + exp^{-r(T-t)})$, in which r is the parameter for the temporal rate (day^{-1}) of disease increase; t is the parameter for the time required to reach 50% of final disease levels; and $f(D, N)$ is a function describing the upper limit in Y that varies with D and N such that $f(d, N) = a\ exp^{-(bD+cDN)}$, in which a estimates the level of Y during a cropping season when no fungicide is applied; b and c are the parameters for the relative rate of decrease of Y with D and with the product DN, respectively. Dose was measured as multiples of a standard dose of 1.68 kg chlorothalonil /ha.

[y] Estimate ± Standard error.

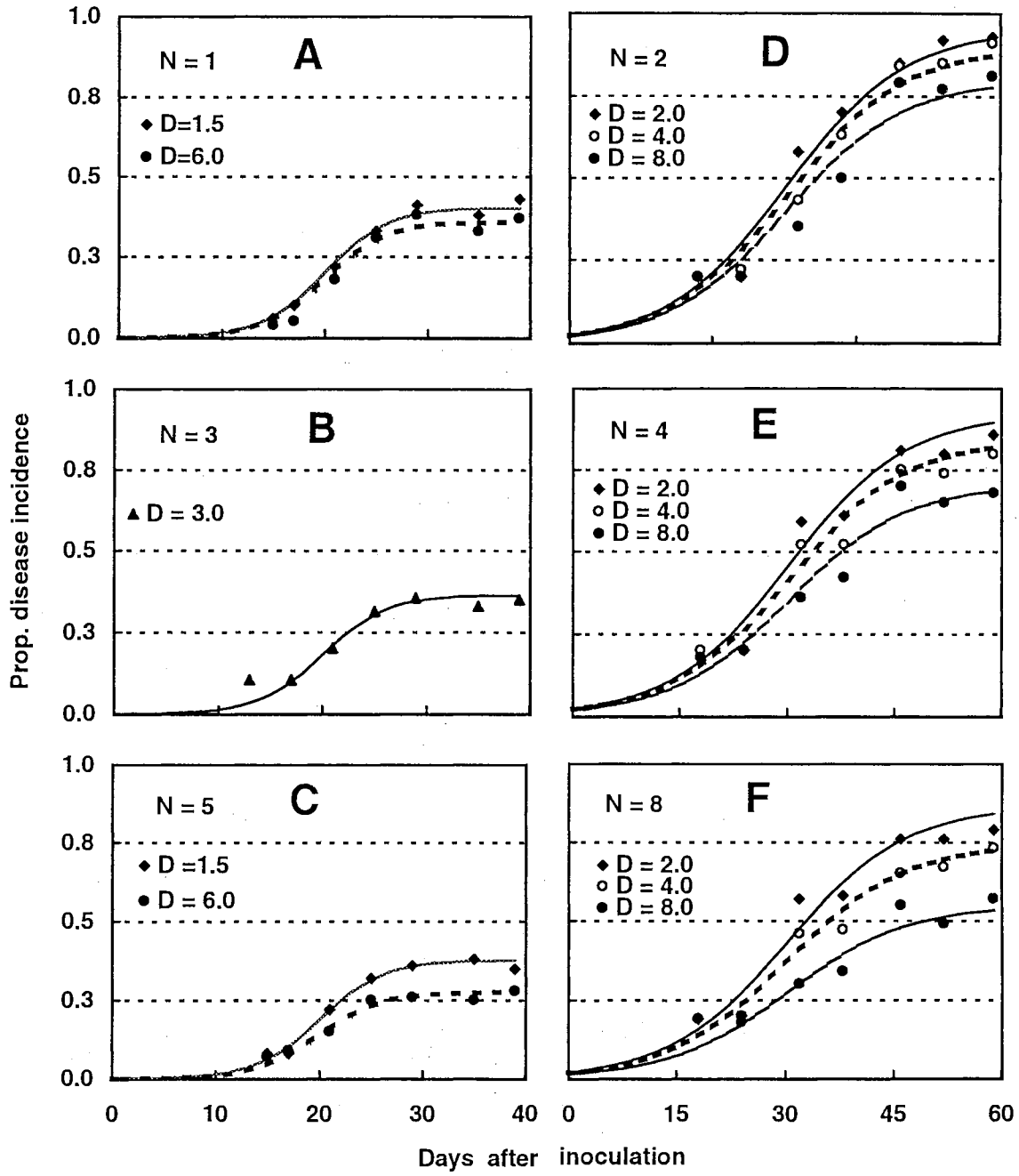

Fig. 1. Effects of total dose (D) and number (N) of chlorothalonil application during the 1997 (**A**-**C**) and 1998 (**D**-**E**) cropping seasons on variation in the incidence of watermelon with time (days after inoculation). Each point represented the mean of 18 ratings (3 quadrats x 6 replicate plots) in 1997 and 12 ratings (3 quadrats x 4 replicate plots) in 1998. There were a total of 7 assessments for each year. Curves represented the modified logistic model fitted to the data. Dose was measured as multiples of a standard dose of 1.68 kg chlorothalonil / ha.

CHAPTER III

Effects of Pre- and Post-Infection Application of Chlorothalonil and Benomyl on Incidence of Watermelon Anthracnose

ABSTRACT

An improved knowledge of effects on disease of the time of applications of protectant and systemic fungicide prior to or after infection would be helpful to design optimal timing schedule of fungicide application. Effects of pre- and post-infection applications of chlorothalonil (protectant), benomyl (systemic) and their mixture on watermelon anthracnose caused by *Colletotrichum orbiculare* were evaluated under field conditions over two years. Single application of each fungicide was made 1, 7, or 14 days prior to and 1, 3, or 7 days after inoculation. In each year, incidence of anthracnose increased with the length of time interval from the day of inoculation when the fungicides were applied prior to inoculation. Difference in incidence in plots treated with chlorothalonil and benomyl was small. However, the difference was large when they were applied after inoculation; incidence increased more quickly for chlorothalonil than for benomyl. No significant difference in incidence between benomyl and the mixture was observed when both fungicides were applied before or after inoculation.

INTRUDUCTION

Timing of a single application of a fungicide relative to infection is an important determinant of effectiveness of the fungicide. Disease incidence tends to increase with increasing time intervals of the application prior to or after infection. The magnitudes of change of incidence levels depend on fungicide activity (protective or curative) (41) and other factors (10, 11, 21, 32). A more detailed knowledge of effects of the timing of the applications of protectant and systemic fungicide prior to or after infection on disease would provide a basis for designing optimal fungicide application for control of plant disease.

Information concerning the effects of pre- and post-infection application of chlorothalonil (protectant) and benomyl (systemic) on foliar diseases are limited. Dahmen et al (15) reported one application of chlorothalonil up to 21 days before inoculation remained sufficient to control *Cercospora arachidicola* on peanut. Osorio et al (54), in a study of comparisons of effectiveness of timing of benomyl application, found severity of peach brown rot increased with the time of application before infection was increased. Grybauskas (31) observed that suppression of Fusarium head blight was greater when fungicide applications were made 3-4 days before inoculation rather than 3-4 days after inoculation. Most previous investigations of timing focus on greenhouse studies (15, 39, 41), pre-infection effects (41, 54) or comparisons of multiple-application during a cropping season without specifying infection periods (3, 14, 17, 35, 44, 45, 58, 65, 75). The lack of detailed analyses of the response of disease to the time of pre- and post-infection application of protectant and systemic fungicide under field conditions has hindered our understanding of effects of fungicide application on disease epidemics and

distinguishing differences between protectant and systemic fungicide in field performance.

Chlorothalonil and benomyl are two of the most effective fungicides for control of anthracnose, caused by *Colletotrichum orbiculare* (Berk & Halst.) Ark (= *C. lagenarium*), on watermelon (*Citrullus lanatus* (Thunb.) Matsum & Nakai) (36). Currently, repeated applications of these two fungicides and their mixture at 7- or 14-day intervals are recommended in Oklahoma for integrated management of the disease. A total of 2 to 10 applications throughout a growing season is often needed depending on year and location (36).

The objective of this research was to quantify the effects on incidence of watermelon anthracnose of pre- and post-infection applications of chlorothalonil, benomyl and their mixture on watermelon anthracnose under field conditions. Preliminary results have been reported (74).

MATERIALS AND METHODS

Field plot establishment. Field plots were established as described in chapter I. In each experiment, an experimental unit was a plot consisting of a planting bed that was 15 cm high, 1.8 m wide and 3.5 m long. Adjacent plots were separated by a 2-m long area in which soybean was planted. Adjacent blocks were separated by a 2-6 - m long area in which soybean or peanut was planted. Seeds of the cultivar Mickylee were sown on the center of each bed at a rate of approximately 10 seeds per m of row on June 18 (1998) or

July 7 (1999). Seedlings were finally thinned by hand to approximately 0.8 m apart within row for a total of 4 plants per plot.

Experiment design. The experiments conducted in each of 1998 and 1999 were arranged in a split-plot design. Three fungicide treatments were randomly to whole plots, which were arranged in four randomized complete blocks. The fungicide treatments consisted of a single application of chlorothalonil (Bravo720, 1.7 kg a.i./ha, ISK Biotech Co., Menter, Ohio), benomyl (Benlate 50W, 280 g a.i./ha, E. I. du Pont de Nemoirs Co.) or a mixture of chlorothalonil and benomyl (Bravo720, 1.7 kg a.i./ha plus Benlate 50W, 280 g a.i./ha). The subplot factor consisted of the time of application of fungicide. Each fungicide was applied to subplots 1, 7 or 14 days prior to inoculation, or 1, 3 or 7 days after inoculation. One plot in each block that was not applied with any fungicides was arranged as an additional control treatment.

Each fungicide was applied to the watermelon foliage through 5 flat fan nozzles (Model Tee-Jet #8003, Spraying Systems, Inc., Wheaton, III) using a compressed CO_2-type sprayer at a pressure of 279 kPa. Nozzles were spaced 46 cm apart on a 2.1-m boom that was mounted 46 cm above the canopy on the side of a manually propelled, wheel barrow-type spray rig. The sprayer was calibrated to deliver 271 L/ha.

Pathogen inoculation and disease assessment. A monoconidial isolate of *C. orbiculare* from diseased watermelon plants in Oklahoma was grown on sterilized green bean agar (GBA) in 9-cm petri dishes. The culture was incubated under a 12-hr photoperiod at room temperature for 14 days. To prepare GBA, 20 g of agar and 226g of macerated green bean (Gerber Production Co., Fort Smith, AR) were added to 1 L of double deionized water (27). The concentration of conidia was adjusted to 6.0×10^5 m^{-1} in

each year. The concentration of conidia was assessed as described in chapter I. The conidial suspension was applied to all watermelon foliage in each plot using the sprayer described above. In 1998 and 1999, plants at the fruit set stage were inoculated on August 14, and September 9, respectively. After inoculation, plants in each plot were irrigated with an overhead sprinkler at a rate of about 20 mm/hr for 5 hours beginning 8 p.m.

In each plot, disease was assessed in a quadrat (0.5×0.5 m) that was positioned at the crown of one arbitrarily selected plant. In each quadrat, the number of defoliated leaves (N_d), the number of leaves with anthracnose lesions (N_s), and the number of leaves without anthracnose lesions (N_h) were counted separately. Disease was assessed on five dates, 7, 11, 14, 17 and 21 days after inoculation.

Data analysis. For each quadrat, the proportion (Yq) of all leaves within a quadrat that were diseased on each date of disease assessment was

$$Yq = (Ns + N_d)/(N_s + N_d + N_h). \qquad (1)$$

The mean proportion of diseased leaves on average over four replicate plots was Y.

Effects of fungicide and timing on Y for each date of disease assessment were analyzed by variance of the procedure of GLM of The Statistic Analysis System (SAS System for Windows release 6.11, SAS Institute, Cary, NC). To minimize heteroscedasticity, the natural logarithm of Y_q was analyzed.

The increase of Y in time for each timing treatment was characterized by

$$Y = a \ exp^{bT} \qquad (2)$$

(12) in which T was the number of days after inoculation, a was the parameter for the overall disease incidence when $T = 0$, and b was the intrinsic rate of increase of disease

with respect to T (days $^{-1}$). Based on estimates of a and b, $1/T_{0.5}$, the predicted values of $1/T$ at $Y = 0.5$, was calculated.

Parameters of equation 2 were estimated by nonlinear regression using the derivative-free option DUD of the procedure NLIN of The Statistic Analysis System (SAS System for Windows release 6.11, SAS Institute, Cary, NC). Fit was evaluated by inspection of observed values and predicted values of Y plotted against T, standard errors on parameter estimates, and the asymptotic correlation between parameter estimates. Heteroscedasticity was evaluated by checking standardized residual errors plotted against predicted values. Multiple comparisons were made for Y assessed at the days 11 after inoculation, and for $T_{0.5}$ by the mixed procedure (PROC MIXED) using the Least Square Difference Test (LSD) of The Statistic Analysis System (SAS System for Windows release 6.11, SAS Institute, Cary, NC) (62).

RESULTS

Effects of fungicide type and time of application on *Y*. Fungicide type and time of application each affected the incidence of anthracnose, but the effect depended fungicide. In the analysis of variance for each year, the main effects of fungicide, timing and their interaction were all highly significant ($P \leq 0.0001$) at all disease assessments but the last (Table III).

Effects of chlorothalonil and benomyl on incidence were similar when they were applied prior to inoculation; however, the effects of benomyl were greater than that of chlorothalonil when they were applied after inoculation. For pre-inoculation applications, differences in incidence between chlorothalonil and benomyl were small in 1998 (Fig. 2

A) and 1999 (Fig. 2 B). In each year, levels of incidence in plots treated with benomyl were always lower ($P \leq 0.05$) than in plots treated with chlorothalonil when these applications were made after inoculation (Fig.2 A and B).

Effects of benomyl and the mixture on incidence were similar (Fig.2 A and B). Difference in incidence between benomyl and the mixture was negligible ($P \geq 0.05$) for each timing treatment in both years.

The effects of timing on anthracnose were similar in each year. Disease incidence increased with increasing time interval of application from the day of inoculation, but more quickly for post-infection applications than for pre-infection applications (Fig. 2 A and B). For chlorothalonil, these effects were more obvious. The disease was least when the application was made 1 or 7 days prior to inoculation. Disease incidence was not significantly ($P \geq 0.05$) reduced by applications made 3 or 7 days application after inoculation. For benomyl and the mixture, the change of disease with the time interval of post-infection application was less obvious compared with chlorothalonil. The least incidence occurred at the applications ranged from 7 days before to 3 days after inoculation (Fig. 2 A and B). Incidence was not significantly ($P \geq 0.05$) decreased by applications made 7 days after inoculation.

Effects of fungicide type and time of application on $1/T_{0.5}$. The exponential model (equation 2) fitted the disease progress curve for each subplot treatment (results not shown). Therefore, values of $1/T_{0.5}$ calculated from the model were used to further characterize effects of fungicide and timing on watermelon anthracnose.

Effects of fungicide type and time of application on $1/T_{0.5}$ were similar to the effects of fungicide type and time of application on Y, although the scale of the effects differed

(Fig. 3 A and B). In each year, there was no significant ($P \geq 0.05$) difference in the time required to reach an incidence of 0.5 between chlorothalonil and benomyl when they were applied prior to inoculation; however, there was significant ($P \leq 0.05$) difference when they were applied after inoculation. The response of $1/T_{0.5}$ to the time of applications was similar between benomyl and the mixture. For chlorothalonil, pre-infection applications were effective in delaying the epidemics. However, post-infection applications that were made 1 and 3 days after inoculation significantly ($P \leq 0.05$) delayed the epidemics. For benomyl and the mixture, all applications were effective in lengthening the time to reach an incidence of 0.5 although delays were small for the application made 7 days after inoculation.

Discussion

Most previous studies on effects of timing of fungicide applications on disease have focused on comparisons of effectiveness of different timing regimes of multiple-applications during a cropping season (3, 14, 17, 35, 44, 45, 58, 65, 75). In this study, attempts were made to quantify the effects of a single application of fungicides prior to and after inoculation under field conditions and to distinguish differences between protectant and systemic fungicides. The results of this study are important because they confirm that benomyl has good post-infection disease control activity while chlorothalonil provides a long-lasting protective activity. More importantly, we found that both chlorothalonil and benomyl have similar field performance against infection of

C. orbiculare when they were applied prior to inoculation, and that chlorothalonil can delay the epidemics when applied within a period from 1 to 3 days after inoculation.

Our results showed that anthracnose incidence increased with the time interval of pre-infection applications from the day of inoculation. These results are supported by other reports (15, 54, 64). Suheri and Latin (64) reported that spore inhibition of Alternaria leaf blight linearly decreased with increasing the length of time of application of chlorothalonil or other fungicides on muskmelon in greenhouse conditions. Dahmen et al (15) observed increase of *Cercospora arachidicola* lesion development on peanut as the time of application of chlorothalonil from the time of inoculation was increased in a greenhouse study. Osorio et al (54) also found severity of peach brown rot increased as the interval from the time of application of benomyl to the time of inoculation was extended. The results of this study extend previous results by evaluation of the time range extended from pre- to post-infection periods.

Variation in performance of post-infection applications between chlorothalonil and benomyl can be attributed to fungicidal activity. Chlorothalonil has no curative activity and cannot inhibit colonization of *C. orbiculare* in plant tissues (36, 41), but benomyl has systemic activity and eradicates the initial stages of the colonization (36). In contrast, similarity in effectiveness of pre-infection applications between chlorothalonil and benomyl might be explained partially by fungicide persistence. Other studies have showed that sufficient levels of chlorothalonil residues still remained on peanut (10, 21), potato (11, 32), and tomato (47) 14 days after application. Good persistence of benomyl might come from its systemic activity that facilities benomyl to move within a leave of watermelon. Our results are supported by previous studies that applications of

chlorothalonil and benomyl at 7-day intervals had the same control effects on watermelon anthracnose (44, 45).

Some effectiveness of delaying epidemics by post-infection applications of chlorothalonil (Fig. 3 A and B) observed in this study is reasonable. The post-inoculation applications did not inhibit the infection process, germination, penetration and colonization of *C. orbiculare*, which usually takes approximately 1-2 days under favorable field conditions (36). However, these applications could protect uncovered and new growth surfaces of watermelon plants from the infection. Inhibition of sporulation of the pathogen to some degree may also be explained for these effects (41).

Increasing control effectiveness of the combination of chlorothalonil with benomyl or other systemic fungicides has been documented for many crop diseases (14, 35, 36). However, the results in this study showed that the mixture has the same performance as benomyl and that there is no evidence of an increase in its effectiveness. The difference in effectiveness of the mixture seen in the present study and the previous reports might be contributed in part by the number of applications. Fungicide was applied only once in this study, but multiple-application was made in previous studies. Although there are no synergistic effects of the combination, it may be useful to prevent or delay buildup of populations of resistant pathogens against benomyl. Resistance of *Didymella bryoniae* to benomyl has been found on commercial production of watermelon in some states of the United States (22, 37, 48).

Our results indicated that application of benomyl provides more flexibility in a timing schedule of application than application of chlorothalonil. Benomyl and chlorothalonil have the same control effectiveness if they are applied at 7- or 14- day intervals. The

results of the current study have important implications for efforts to improve existing disease control advisory programs including weather-based forecasters on cucurbit crops.

Table III Summary of the results of the analysis of variance [a] for effects of field block (B), fungicide (F) and time (T) of application on incidence of watermelon for five disease assessments in each of 2 years

			Date of disease assessment (days after inoculation)									
			7		11		14		17		21	
Year	Source	df	*F*	*P > F*	*F*	*P>F*	*F*	*P>F*	*F*	*P>F*	*P*	*P>F*
1998	Wholeplots											
	B	3	8.05	0.0001	0.90	0.4464	1.20	0.3167	1.80	0.1562	0.49	0.6899
	F	3	64.46	0.0001	51.74	0.0001	21.83	0.0002	43.72	0.0001	3.55	0.0607
	Error (F× B)	9	2.11	0.0424	2.34	0.0251	4.84	0.0001	2.85	0.0073	1.5	0.1697
	Subplots											
	T	5	95.47	0.0001	45.78	0.0001	24.52	0.0001	25.78	0.0001	2.85	0.0224
	F × T	15	13.91	0.0001	8.33	0.0001	6.24	0.0001	5.84	0.0001	1.49	0.1365
	Error (T × F × B)	60										
1999	Wholeplots											
	B	3	14.05	0.0001	0.2	0.8893	3.29	0.0265	1.31	0.2796	0.76	0.5218
	F	3	75.76	0.0001	322.45	0.0001	618.05	0.0001	229.77	0.0001	8.54	0.0053
	Error (F× B)	9	1.61	0.1321	0.41	0.9228	0.58	0.8090	2.07	0.0466	0.63	0.7707
	Subplots											
	T	5	21.60	0.0001	30.39	0.0001	35.83	0.0001	29.93	0.0001	0.49	0.7813
	F × T	15	22.62	0.0001	4.61	0.0001	9.04	0.0001	4.72	0.0001	1.28	0.2420
	Error (T × F × B)	60										

[a] Proportion of disease incidence (Y) in each of four replicate plots was transformed into values of {ln(Y)} prior to analysis.

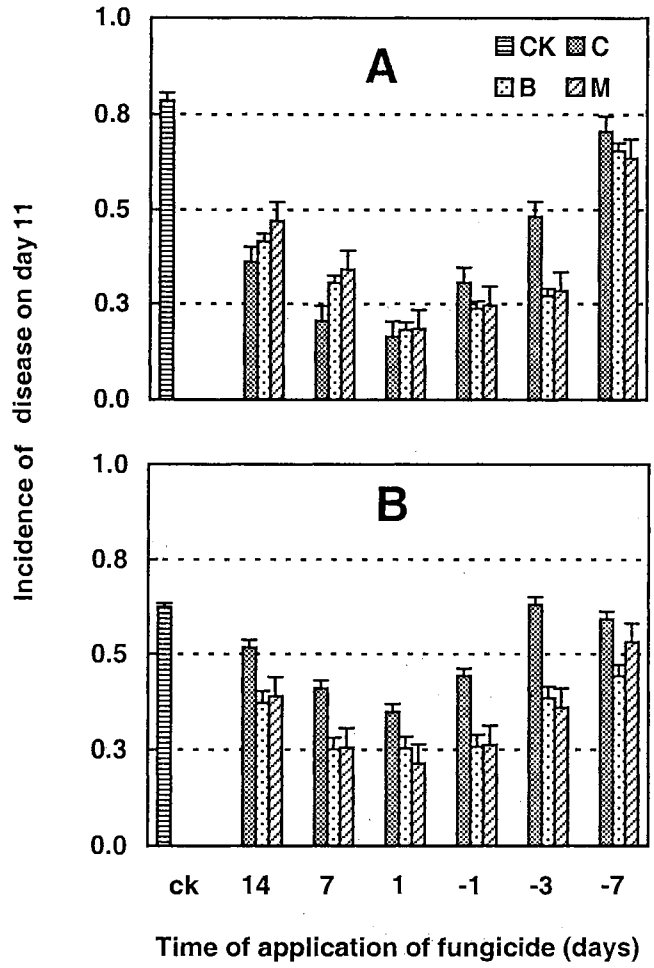

Fig. 2 Effects of the time of application of chlorothalonil (**C**), benomyl (**B**) and a mixture of chlorothalonil and benomyl (**M**) in relation to the time of inoculation on the incidence of watermelon anthracnose. Time of application (T) was the number of days before ($T > 0$) or after ($T < 0$) inoculation that fungicide was applied. The experiment was conducted in 1998 (**A**) and In 1999 (**B**). **CK** represented the control treatment that fungicide was not applied. The observed values with standard error represented the mean incidence on average over 4 replicate plots.

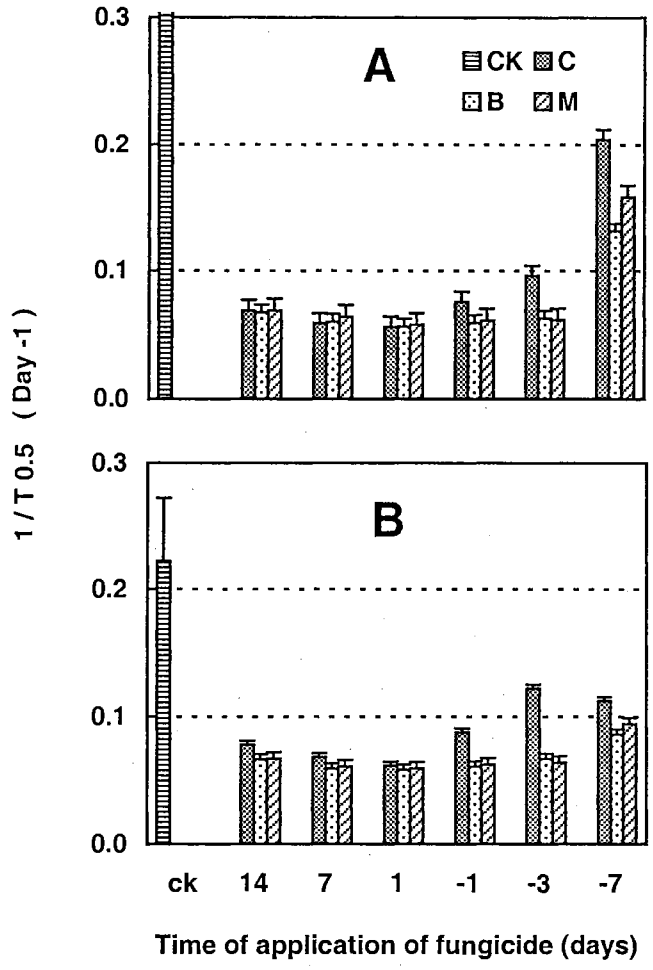

Fig. 3 Effects of the time of application of chlorpothalonil (**C**), benomyl (**B**), and a mixture of chlorothalonil and benomyl (**M**) in relation to the time of inoculation on $T\ 0.5$, the predicted value of T at an incidence of 0.5 of watermelon anthracnose. Time of application (T) was the number of days before ($T > 0$) or after ($T < 0$) inoculation that fungicide was applied. The experiment was conducted in 1998 (**A**) and in 1999 (**B**). **CK** represented the control treatment that fungicide was not applied. The observed values with stardard error represented the mean incidence on average over 4 replicate plots

CHAPTER IV

Fungicide-Mediated Spatio-Temporal Dynamics of Watermelon Anthracnose

ABSTRACT

Analyses of effects of fungicide on spatial and temporal dynamics of foliar disease could improve understanding of the mechanisms underlying disease control. The spread of watermelon anthracnose was evaluated in each of two years. Proportion of diseased leaves in quadrats was assessed on each of seven dates at distances from a point source in replicate plots (11×11 m) to which chlorothalonil was or was not applied. Effects of fungicide on temporal dynamics, on spatial dynamics, and on spatio-temporal dynamics were quantified by logistic models. At most distances, fungicide reduced the intrinsic temporal rate of disease progress (r). At each time, effects of fungicide on the intrinsic rate (g) of decrease in disease with respect to distance were small. These effects were characterized primarily by differences in parameters of the spatio-temporal model. In each year, the application of fungicide reduced r, but there was no evidence that fungicide reduced g and the time (t) at which disease incidence was increased to one half of the upper limit. The fungicide effects were further evaluated using data pooled over two treatments for each year. Parameter r was fungicide-dependent, but g and t were not. The effects of fungicide on the parameters were much more clear and the predicted incidence fitted the data. Chlorothalonil decreased velocities of disease spread (v) from 1.14 to 0.82 m/day in 1998, and from 0.65 to 0.38 m/day in 1999.

INTRODUCTION

Understanding effects of disease management practices on dynamics of plant disease in time and space is one of the major goals of botanical epidemiology. Consequently, effects of different control measures such as chemical control and host resistance on temporal and spatial dynamics of foliar fungal diseases have been evaluated in several studies (7, 18, 28, 50). Currently, the common approaches to evaluate the impacts of disease control measures are to use the same observation data twice, and to analyze temporal dynamics and spatial dynamics separately (7, 18, 50). This method does not completely account for the nature of epidemics (33) and effects of disease management. Jeger (33) proposed analytical spatio-temporal models based on the rate of isopath movement from a point of inoculation. These models not only give a means to describe progress of foliar diseases in time and space (4, 33, 53) but also make it possible to examine effects of disease management practices on spatio-temporal dynamics. However, there have been no studies addressing this important issue, and no studies comparing integrated analysis of spatial and temporal dynamics with separate analysis to evaluate the effects of measures for control of disease.

Comparisons of the response of model parameters to disease management practices are helpful in understanding the underlying mechanisms of disease control. Gilligan (27) applied a parallel curve regression method to determine the parameters of temporal dynamic models most related to the measures for disease control. He found that effects of fungicide treatments were characterized primarily by differences in the upper limit, but not the intrinsic rate of increase of disease with time. Limitation of his analyses is that

effects of fungicides on spatial dynamics were not evaluated. The sensitivity of the parameters of spatio-temporal models to disease management practices has not been assessed.

In this study, we examined effects of applications of a protectant fungicide on progress of a foliar disease in time and space. Anthracnose caused by *Colletotrichum orbiculare* (Berk & Halst.) Arx (= *C. lagenarium*) is one of the most important constraints on watermelon production worldwide (36, 76). Epidemics often begin with the appearance of distinct foci. As an epidemic develops, foci expand and coalesce (26, 36). To control anthracnose, repeated applications of chlorothalonil or other fungicides at 7- to 14-day intervals throughout a growing season are usually recommended in commercial production farms (52, 76).

The objective of the current study was to characterize the effects of application of chlorothalonil on spatio-temporal dynamics of watermelon anthracnose. Some preliminary results are available (72).

MATERIALS AND METHODS

Field plots. Field plots were established as described in chapter I. Each plot in this study was 11 m long and 11 m wide (Fig. 4). Each plot contained 11 rows of watermelon spaced 1 m apart. Rows were oriented east to west, approximately at a 45° angle to the prevailing southeasterly wind during the experiments. Planting beds were 30 cm high. Seeds of the watermelon cultivar Mickylee were planted on 18 June 1998, and on 14 July 1999, respectively. Seedlings at the 3 to 4 –leaf stage were thinned to approximately 1

plant per meter of row. Each plot was bordered on each side by a strip of 4 - 10 m in which soybean (1998) or peanut (1999) was planted.

A total of 6 plots were randomly divided into two treatments of three replicates each. Three plots were not treated with fungicide. The other three plots were applied with a total of three applications of chlorothalonil (Bravo720, 1.7 kg a.i./ha, ISK Biotech Co., Menter, Ohio) at 14-day intervals. The first application was made following appearance of anthracnose lesions on watermelon leaves at a point source. An area (0.5 × 0.5 m) around the point source in each fungicide-treated plot was not treated and thus provided a continuing inoculum source through the experiment. Chlorothalonil was applied using the method described in chapter I.

Inoculation and disease assessment. A monoconidial culture of *C. orbiculare* was isolated from diseased watermelon plants in Oklahoma. The culture was inoculated into sterilized oat kernels and incubated for 14 days at room temperature with a12-hr photoperiod. The colonized oat kernels were air-dried for 96 hours. When male flowers of watermelon plants first appeared, 50 g of the inoculum were distributed over the surface of soil in a 0.1 × 0.1 m area at the crown of one watermelon plant at the southeast corner of each plot (Fig. 4).

In each plot, disease was assessed in quadrats that were arranged along three transects (Fig. 4). The transects were orientated each of three compass directions (W, SW and S). Quadrats (0.5 × 0.5 m) were located at a distance of 0, 1, 3, 5, 7 and 9-m from the point of inoculation. In each quadrat, the number of defoliated leaves (N_d), the number of leaves with anthracnose lesions (N_s), and the number of leaves without anthracnose

lesions (N_h) were counted separately. Disease was assessed at 3- to 7-day intervals on five dates following the appearance of symptoms.

Data Analysis. The proportion of diseased leaves in each quadrat (Y_q) was calculated by

$$Y_q = (N_d + N_s) / (N_d + N_s + N_h) \qquad (1)$$

The mean value of Y_q for a given date and a given distance, on average over the three transects in a plot was then Y_p. The mean value of Y_p on average over 3 replicate plots was Y.

Variation in Y with respect to fungicide treatment and distance from a point of inoculation was evaluated for each date of disease assessments using PROC GLM of the Statistical Analysis System (SAS version 6.12, SAS Institute, Cary, NC). A split plot design was used for the analysis, with fungicide treatments as whole plots and distance from inoculum as subplots (18).

To separately analyze effects of fungicide on the temporal dynamics and temporal dynamics, variation in Y with time (T, days after inoculation) for each distance was evaluated by equation 2

$$Y = 1/(1+ exp^{-r(T-t)}) \qquad (2)$$

(67) in which r was the intrinsic rate of increase in Y with respect to T (day^{-1}) ; t estimated the time at which Y had increased to one half of the upper limit.

Variation in Y with distance (S) from a point of inoculation for each date of disease assessments was evaluated by equation 3 (38).

$$Y = 1/(1+ exp^{g(S-d)}) \qquad (3)$$

in which g was the intrinsic rate of decrease in Y with respect to S (m^{-1}) ; d estimated the distance at which Y was reduced to one half of the upper limit.

To integrally analyze effects of fungicide on spatial and temporal dynamics, spatial-temporal dynamics were evaluated

$$Y = 1/(1+ exp^{-r(T-t)+gS}) \qquad (4)$$

(33).

Effects of fungicide on the spatio-temporal dynamics of disease were evaluated by a modified form of equation 4 in which r, but not g and t, was fungicide dependent. The parameter r of equation 4 was rewritten as a linear function of the level of fungicide treatment (F),

$$r = r_0F_0 + r_1F_1 . \qquad (5)$$

The independent variables F_0, and F_1 together specified the level of the fungicide treatment. When no fungicide was applied, the values of F_0 and F_1 were 1 and 0, respectively. When fungicide was applied the values of F_0 and F_1 was 0 and 1, respectively. Thus, the parameter r_0 or r_1 estimated the intrinsic rate of change in Y with respect to time when fungicide was not or was applied, respectively.

T tests were employed to examine difference in parameter estimates between fungicide and non-fungicide treatment (12). Velocity of spread (v in m/day) for each treatment was estimated by the slope of the regression of the distance at which Y reached to one half of the upper limit on the number of the days after inoculation, or estimated by Minogue and Fry's equation (49):

$$v = r/g . \qquad (6)$$

Parameters were estimated by non-linear regression using the derivative-free option DUD of the procedure NLIN of the Statistical Analysis System (SAS version 6.12, SAS Institute, Cary, NC). Overparameterization and fit of each model were evaluated by inspection of observed values and predicted values of Y plotted against independent variables, standardized residual errors plotted against predicted values, standard errors of parameter estimates, and the asymptotic correlation between parameter estimates (62).

RESULTS

Based on the analysis of variance, chlorothalonil reduced anthracnose incidence, and incidence decreased with distance from the point source on each date of disease assessment. The main effects of fungicide (F) and distance (S) were significant ($P \leq 0.04$) at each sampling date event except for one time in each year. However, the rate of spread of disease from the point source was independent of fungicide treatment. The interaction of $F \times S$ was not significant ($P \geq 0.11$) in either year (results not shown).

Effects on temporal dynamics. Incidence of disease was lower in fungicide-treated plots than in untreated control plots in each year (Fig. 5). An obvious difference in disease was noted between the two treatments beginning 30 days after inoculation in1998 (Fig. 5 A-E) and 29 days after inoculation in 1999 (Fig. 5 F-J).

Effects of fungicide on the intrinsic temporal rate of disease increase (r) were great (Table IV). In each year, r was significantly lower ($P \leq 0.05$) at most distances in fungicide-treated plots than in untreated control plots (Table IV). Differences between the

two treatments in the length of the delay in the increase in disease, which was estimated by t, usually were small.

Effects on spatial dynamics. Fungicide applications reduced incidence of disease at most distances from the point source (Fig. 6). Difference in incidence between the two treatments increased with increasing distance. The magnitudes of reduction in disease by fungicide were greater in 1999 (Fig. 6 F-J) than in 1998 (Fig. 6 A-E).

Effects of fungicide on the intrinsic rate of decrease of disease with respect to distance (g) were small (Table V). Estimates of g did not differ significantly ($P \geq 0.05$) between the two treatments at most assessment dates in each year. Difference in estimates of the location parameter d between the two treatments was significant ($P \leq 0.05$) only at the last three assessments in 1999.

Effects on spatio-temporal dynamics. Effects of fungicide were characterized primarily by differences in r. The spatio-temporal logistic model (equation 4) was adequately fitted to the data pooled over all assessments for each treatment. In each year, r was greater for fungicide treatment than for untreated control ($P \leq 0.05$) (Table VI). The effects of fungicide on g and t were not significant ($P \geq 0.05$). Velocities of spread (ν) of anthracnose were consistently lower in fungicide-treated plots than in untreated control plots.

The effects of fungicide were further evaluated by a modified form of equation 4 in which r was fungicide dependent, but g and t were not (Table VII). The model fitted the data for each year (Fig. 5 and 6). Each parameter was precisely estimated (Table VII). The asymptotic correlation coefficients between parameters were not large. Estimates of r and ν were similar to those estimated from equation 4, but more precise.

DISCUSSION

Results of this study demonstrate that fungicide application most affected the intrinsic rate of increase of disease with respect to time, but less affected the intrinsic rate of decrease of disease with respect to distance. Comparisons of analyses for integration and separation of temporal and spatial dynamics were also made to evaluate the response of model parameters to fungicide. A modified logistic model was developed to characterize the effects of chlorothalonil on spatio-temporal dynamics of watermelon anthracnose.

To our knowledge, this is the first, detailed analysis of effects of fungicide application on spatio-temporal dynamics of a foliar disease. Our results extend previous investigations in which effects of fungicide on spatial and temporal dynamics are analyzed separately (28, 50). We further evaluate the effects of fungicide on disease progress in both space and time. Therefore, the effects of fungicide on spatio-temporal dynamics could be completely characterized and sensitivity of the response of model parameters to fungicide could be clearly distinguished.

The integrated analysis approach is superior to the separate analysis to evaluate effects of fungicide although these two approaches reach similar results. Firstly, the model (equation 4 and 5) developed by the first approach gives more descriptive information on dynamics of disease in time and space, which reflects whole process of natural epidemics (33). Secondly, effects of fungicide could be consistently and clearly characterized by differences in parameter estimates (Table VI and VII). In contrast, estimates for effects of fungicide using separate analysis approach were more variable, and more difficult to interpret (Table IV and V). Finally, the final model is also biologically and statistically

simple. Effects of fungicide on parameters in the model can be easily observed (Table VI and VII).

Our results indicated the logistic model (67) has adequate flexibility to fit the data. In general, the model described the temporal dynamics (Fig. 5) better than the spatial dynamics (Fig.6). However, it still could adequately fit the data for the spatial dynamics over all disease assessments. This is because the model produces an S-shaped curve with two bands (12). It could describe exponential decrease of disease with distance at early stages of disease development (Fig. 6 A-B or F-G), logistic decrease at middle stages (Fig. 36 C-D, or H-I), and monomolecular decrease at late stages (Fig. 6 E or F).

Effects of fungicide have been characterized by variation of temporal dynamics in the intrinsic rate of disease increase with time (28, 50), the upper limit of disease (27), and by variation of spatial dynamics in the intrinsic rate of disease decrease with distance (50). Results of the current study clearly support the first mechanism. Effect of fungicide on the intrinsic rate of disease increase with time was large. The effect on the intrinsic rate of disease decrease with distance was smaller and less consistent.

In this study, the intrinsic temporal rate of disease increase is sensitive to fungicide. This differs from that reported by Gilligan (27), who found that the intrinsic temporal rate was less sensitive than the upper limit of disease to the effects of fungicide. The difference in effects of fungicide treatments on parameters seen in this study and his study might be contributed partially to the structure of the logistic model. In the current study, the upper limit was not contained in the model (equation 3). This is because the observed data were not sufficient to estimate all parameters including the upper limit. Fixing the upper limit as maximum (1.0) by simplifying assumptions could allow

adequate estimations of parameters for the intrinsic temporal rate and epidemic delay. In his study, there was enough data available to be used to estimate all parameters of the model with the upper limit. Differences in fungicide, disease and host might also be explained for some of the discrepancies. Nevertheless, effects of chemical control on the intrinsic temporal rate of disease increase have been commonly observed in many foliar diseases (35, 50).

Our results indicated that watermelon anthracnose has a higher velocity of spread (ν) compared with other reported rain-splash fungal diseases. Our estimates, 0.65 to 1.14 m/day in untreated control plots (Table VII), were higher than the velocities of 0.27, 0.24, and 0.3 to 0.5 m/day that were reported, respectively, for *Septoria nodorum* of wheat (33), postbloom fruit drop of citrus (1), and septoria leaf spot of tomato (55). Additionally, results of this study also indicated that applications of chlorothalonil at the 2-week intervals can reduce velocity of spread (ν) of anthracnose, but the magnitudes of reduction seem to not be large especially in 1998 (Table VII).

Principle limitations of this study are that we did not account the direction of spread from a point of inoculation for variation in fungicide-mediated rates of disease development. In analyses of the data, the mean level of disease on average over all three directions was adopted. This may result in overestimation or underestimation of the rates of spread of disease. Effects of direction on fungicide-mediated rates will be addressed in chapter V. Another unexpected result is the comparative insensitivity of the intrinsic rate of disease decrease with respect to distance to fungicide. This was shown in each year (Table VI). Further work may need to test whether this is a real biological mechanism or due to the analysis method. Significant reduction of the intrinsic rate of increase of

disease with respect to time by chlorothalonil application has been reported on other crop disease (50).

The analysis approaches advanced here may also be useful in evaluating effects of other disease management practices such as cultivar resistance and cultural practices on spatio-temporal dynamics of soilborne and foliar disease.

Table IV. Estimates of the parameters of a logistic model [a] used to characterize effects of chlorothalonil applications on temporal dynamics of watermelon anthracnose at each of five distances from a point of inoculation in each of 2 years

Year	F [b]	Distance from inoculum (m)									
		1		3		5		7		9	
		t [a]	*r*	*t*	*r*	*t*	*r*	*t*	*r*	*t*	*r*
1998	-	26.1± 1.3 [c]	0.893± 0.936	27.0± 0.9	0.413± 0.150	28.4± 1.0	0.246± 0.063	29.9± 0.6	0.224± 0.030	31.7± 0.4	0.204± 0.017
	+	26.2± 1.0	0.586± 0.325	27.2± 1.3	0.245± 0.081	29.9± 1.1	0.156± 0.028	32.3± 0.9	0.143± 0.019	35.5± 0.5	0.154± 0.011
1999	-	18.3± 0.6	0.180± 0.019	26.1± 0.7	0.211± 0.031	28.9± 0.2	0.249± 0.014	31.0± 0.4	0.258± 0.023	33.0± 0.2	0.265± 0.013
	+	19.5± 0.4	0.161± 0.012	31.9± 0.6	0.165± 0.017	35.7± 0.3	0.155± 0.009	38.2± 0.4	0.166± 0.014	39.3± 0.6	0.187± 0.024

[a] Parameters were estimated by nonlinear regression of the logistic model, $Y = 1 / \{1 + exp^{-r(T-t)}\}$, in which Y was the mean of disease incidence; T was the number of days after inoculation; r estimated the intrinsic rate of disease increase (day^{-1}); and t estimated the time (days) required for Y to increase to 0.5.

[b] F represented fungicide ; '-' represents non-fungicide application; and '+'represents fungicide application.

[c] Estimate ± standard error.

Table V. Estimate of the parameters of a logistic model [a] used to characterize effects of chlorothalonil applications on spatial dynamics of watermelon anthracnose for each of five assessments in each of 2 years

Year	F [b]	Date of disease assessment [c]									
		1		2		3		4		5	
		d [a]	*g*	*d*	*g*	*d*	*g*	*d*	*g*	*d*	*g*
1998	-	-6.5 ± 3.3 [d]	0.147± 0.047	-20.7± 4.1	0.046± 0.007	7.4± 0.5	0.329± 0.071	11.2± 0.4	0.402± 0.047	14.7± 0.3	0.603± 0.330
	+	-6.0 ± 3.7	0.150± 0.055	-9.8± 2.6	0.078± 0.014	6.4± 0.4	0.347± 0.058	8.9± 0.5	0.320± 0.053	14.0± 0.7	0.285± 0.030
1999	-	0.9 ± 0.2	0.585± 0.086	2.7± 0.4	0.466± 0.085	5.4± 0.3	0.318± 0.037	11.7± 0.7	0.304± 0.044	14.2± 1.4	0.269± 0.056
	+	0.7 ± 0.1	0.867± 0.060	1.8± 0.3	0.825± 0.220	3.0± 0.6	0.438± 0.116	6.3± 0.7	0.300± 0.081	7.6± 0.9	0.278± 0.085

[a] Parameters were estimated by nonlinear regression of the logistic model, $Y = 1/\{1 + exp^{g(S-d)}\}$, in which Y was the mean of disease incidence; S was the distance from the point of inoculation (m); g estimated the intrinsic rate of decrease of disease with respect to distance (m^{-1}); and d estimated the distance (m) required for Y to increase to 0.5.

[b] '-' represents non-fungicide application; '+' represents fungicide application.

[c] Date of disease assessment 1 through 5 were , respectively, made18, 25, 30 ,37 and 44 days after inoculation in 1998, and 18, 25, 29, 36 and 39 days after inoculation in 1999.

[d] Estimate ± standard error.

Table VI. Estimates of the parameters of a logistic model [a] and velocity of disease spread (v) that characterized effects of chlorothalonil application on spatio-temporal dynamics of watermelon anthracnose in each of 2 years

Year	Fungicide [b]	t (days) [a]	r (day^{-1})	g (m^{-1})	v [c] (m/day)
1998	-	24.4 ± 1.0 [d]	0.257 ± 0.031	0.203 ± 0.049	1.12 ± 0.52
	+	23.2 ± 1.2	0.179 ± 0.019	0.238 ± 0.043	0.90 ± 0.26
1999	-	19.9 ± 0.8	0.231 ± 0.018	0.363 ± 0.040	0.66 ± 0.09
	+	21.1 ± 1.4	0.150 ± 0.019	0.376 ± 0.048	0.34 ± 0.04

[a] Parameters were estimated by nonlinear regression of the logistic model, $Y = 1 / \{1 + exp^{-r(T-t)+gS}\}$, in which Y was the mean of disease incidence; T was the number of days after inoculation; S was the distance from the point of inoculation; r estimated the intrinsic rate of increase of Y with respect to time; g estimated the intrinsic rate of decrease of Y with respect to distance; and t estimated the time required for Y to increase to 0.5.

[b] '-' represents non-fungicide application; '+'represents fungicide application.

[c] Estimated as the slope of the regression of the distance at which Y increased to 0.5 (S_{50}) on the number of the days after inoculation.

[d] Estimate ± standard error.

Table VII. Estimates of the parameters of a modified logistic model [a] and velocity of disease spread (ν) [b] that characterized effects of chlorothalonil applications on spatio-temporal dynamics of watermelon anthracnose in each of 2 years

Year	Parameter estimate [a]				Velocity [b]	
	t (days)	r_0 (day $^{-1}$)	r_1 (day $^{-1}$)	g (m $^{-1}$)	ν_0 (m/day)	ν_1 (m/day)
1998	23.9 ± 0.7 [c]	0.251 ± 0.025	0.180 ± 0.016	0.220 ± 0.031	1.14 ± 0.001	0.82 ± 0.001
1999	20.1 ± 0.7	0.242 ± 0.018	0.140 ± 0.012	0.374 ± 0.031	0.65 ± 0.002	0.38 ± 0.002

[a] Parameters were estimated by nonlinear regression of the modified spatial-temporal logistic model, $Y = 1 / \{1 + exp^{-r(T - t) + gS}\}$, in which Y was the mean of disease incidence; T was the number of days after inoculation; S was the distance from the point of inoculation (m); r estimated the intrinsic rate of increase of Y with respect to T; g estimated the intrinsic rate of decrease of Y with respect to S; and t estimated the time required for Y to increase to 0.5. The parameter r was described by $r = r_0 F_0 + r_1 F_1$. The independent variables F_0, and F_1 together specified the level of the fungicide treatment. When no fungicide was applied, the values of F_0 and F_1 were 1 and 0, respectively. When fungicide was applied the values of F_0 and F_1 was 0 and 1, respectively. The parameter r, but not the parameter t, and g, were assumed to be fungicide dependent as described in text.

[b] ν_0 and ν_1 were estimated by the form of r_0 / g and r_1 / g for non-fungicide and fungicide treatment, respectively.

[c] Estimate ± standard error.

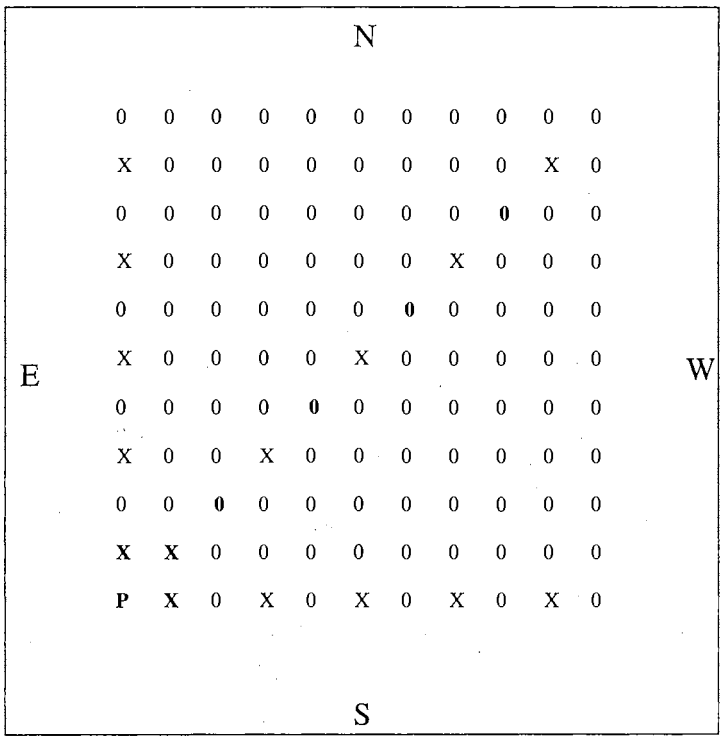

Fig. 4 Diagram of three sampling directions (W, SW and S) away from a point source relative to an inoculated plant (**P**). Rows were oriented east to east. Proportion of diseased leaves in a quadrat (**X**) was assessed. Spacing among plants (0 or **X**) was approximately 1 m. Distances for quadrats in the SW were not drawn to scale (see text).

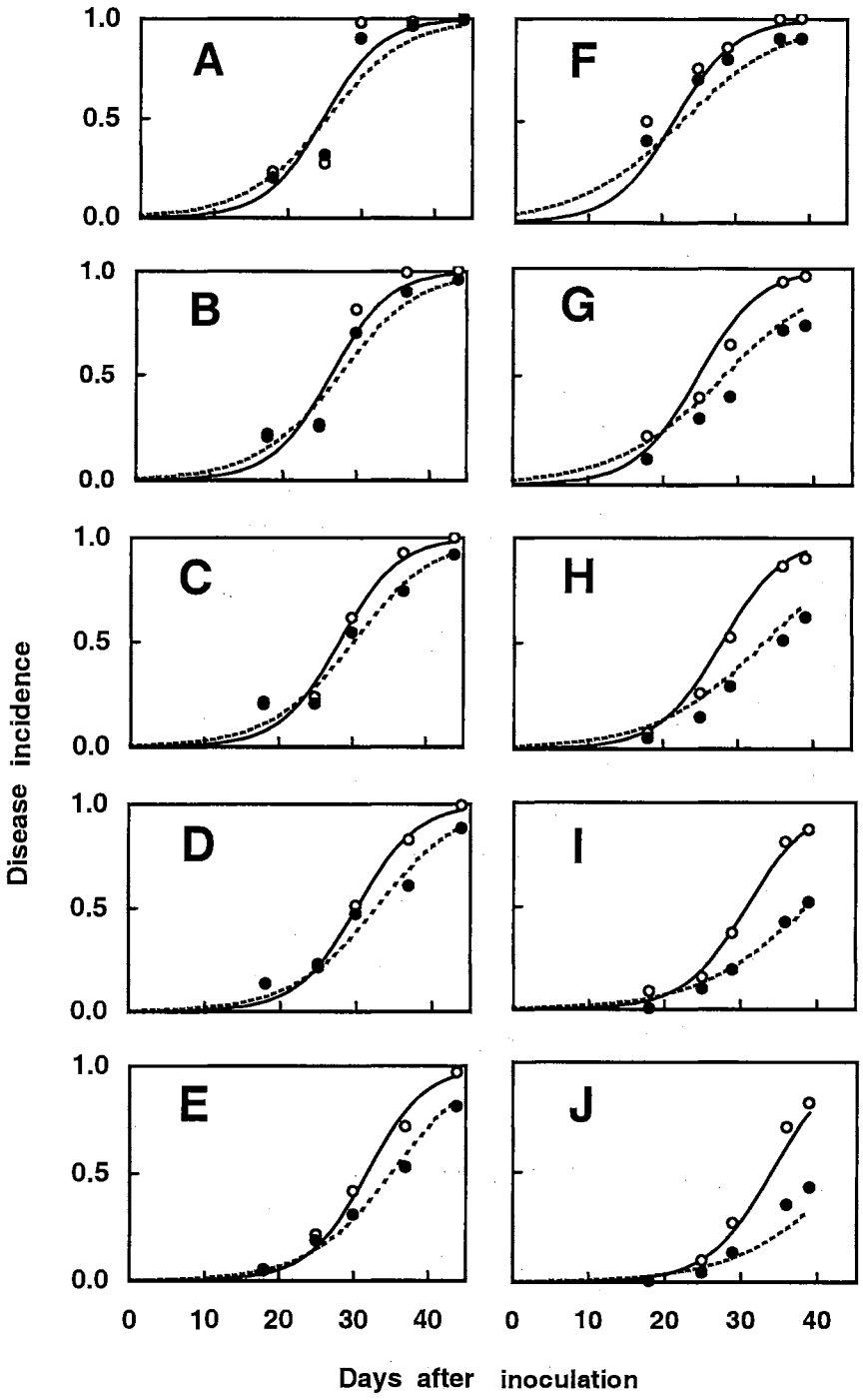

Fig. 5 Effects of application of chlorothalonil on the incidence of watermelon anthracnose with time after inoculation, 1, 3, 5, 7, and 9 m from a point source in 1998 (**A-E**, respectively) and in 1999 (**F-J**, respectively). The observed incidence was the mean of 9 measurements in plots treated with chlorothalonil (closed circles) and in untreated plots (open circles). Equation 4 was used to calculate the expected incidence for the treated plants (dashed lines) and untreated (solid lines).

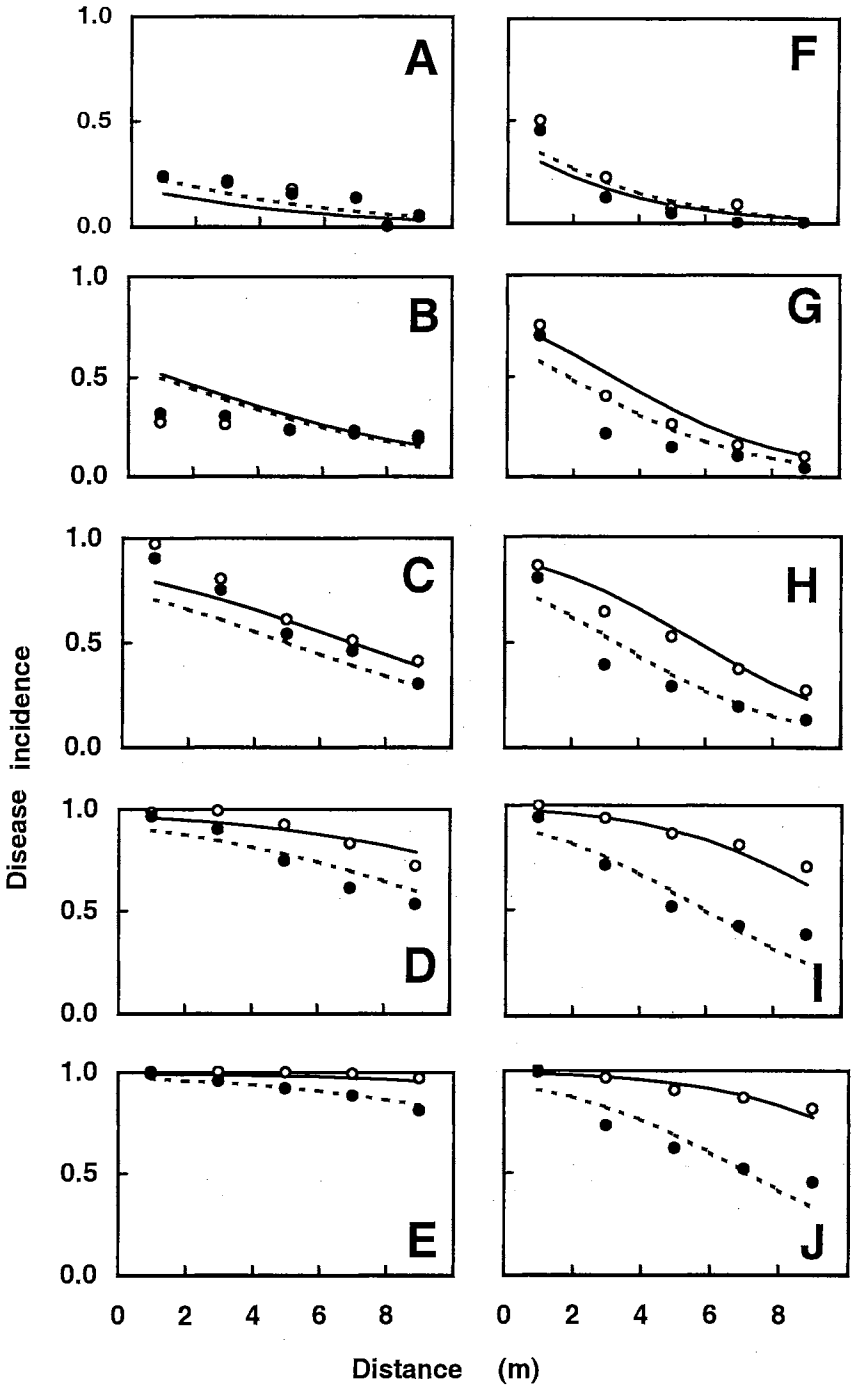

Fig. 6 Effects of application of chlorothalonil on the incidence of watermelon anthracnose with distance from a point source, 18, 25, 30, 37, and 44 days after inoculation in 1998 (**A-E**, respectively) and in 1999 (**F-J**, respectively). The observed incidence was the mean of 9 mesurements in plots treated with chlorothalonil (closed circles) and in untreated plots (open circles). Equation 4 was used to calculate the expected incidence for treated plants (dashed lines) and untreated (solid lines)

CHAPTER V

Fungicide-Mediated Variation in the Rate of Spread of Watermelon Anthracnose in Two Dimensions From a Point Source

ABSTRACT

Quantitative analyses of the effects of fungicide application on the rate of the spread of disease in two dimensions could improve understanding of dynamics of disease in space. In a previous study of effects of fungicide application, spread of watermelon anthracnose on average over all directions was evaluated. In the current study fungicide-mediated variation in the rate of the spread in two dimensions was quantified. Incidence of anthracnose arising from a point source was established in plots (11 x 11m) for each of two years. Two treatments, chlorothalonil application and no fungicide treatment, were randomly assigned to plots. Spread of disease along rows (at an angle of 0^{o} to the direction of the rows) and across rows (at an angle of 45^{o} or 90^{o}) in each plot was measured. Spread of disease was quantified by a spatial logistic model for each assessment time and by a spatio-temporal logistical model using data pooled over all times. A new three-dimensional model was developed to describe the spread of watermelon anthracnose in two dimensions in a field over time. Fungicide application increased the intrinsic rate of decrease of disease with respect to distance, and decreased the intrinsic rate of increase of disease with respect to time. However, the effect of fungicide application on the intrinsic rate of decrease of disease with distance varied with

direction. Fungicide application had a greater effect on the intrinsic rate of decrease of disease with distance along rows than across rows.

INTRUDUCTION

Quantitative knowledge of the effects of fungicide application on the spread of plant disease in two dimensions is important in improving understanding of the spread of disease in space and the mechanisms underlying chemical control (70). In a row-crop field, spread within rows and across rows are the two major directions of the spread of disease from a point source. Effects of fungicide application on the rate of spread of disease may vary with direction. The spread of plant disease may be different in the direction that is parallel to rows of plants and in the direction that is perpendicular to rows. The difference could be more significant for a wide-row crop such as watermelon (*Citrullus lanatus* (Thunb.) Matsum.& Nakai) (52).

Quantitative effects of fungicide application on the spread of foliar fungal disease in two dimensions remain poorly understood. Lambert et al (42) determined the gradients of rice blast from a focus using an equation. Fernando et al (23) characterized the spread of wheat head blight from a point source and Paulitz et al (56) further described the spread of the disease by a two-dimensional model. Other studies indicated that row direction affected directionality of the spread of disease (1, 18). These studies characterized the location of disease in two dimensions in a field, but they did not attempt to evaluate the effects of fungicide on variation of the rate of spread. Gottwald (28) evaluated effects of fungicides on spatio-temporal dynamics of citrus scab, but the effects on the spread of the

disease in two dimensions were not characterized clearly. In most other studies of spread, disease along a single row of plants (4, 50, 55, 57), or the mean level of disease on average over two or more directions (7, 18, 51) is evaluated. In these studies, the rate of spread of disease in two dimensions is not characterized.

Recently we have evaluated the effects of fungicide application on variation in incidence of watermelon anthracnose, caused by (*Colletotrichum orbiculare* (Berk & Halst) Arx (= *C. lagenarium*)), on average over three directions with space and with time (see chapter IV). Effects of fungicide were great on the intrinsic rate of increase of disease with respect to time, but were small on the intrinsic rate of decrease of disease with respect to distance. Comparisons of analyses for integration and separation of spatial and temporal dynamics were made to determine the response of model parameters to fungicide. However, fungicide-mediated variation in the rate of spread of watermelon anthracnose has not been assessed in two dimensions from a point source.

The objective of the current study was to quantify fungicide-mediated variation in the rate of spread of anthracnose in three directions including within rows and across rows of watermelon from a point of inoculation. Preliminary results of this work have been published (73).

MATERIALS AND METHODS

The field experiments were conducted in 1998 and 1999. Methods have been described in detail in chapter IV. Each plot measured 11 × 11 m and consisted of 11 rows spaced 1 m apart. Planting beds were 30 cm high. Rows ran in a west-east direction, at an angle of approximately 45^0 to the prevailing, southeasterly winds. Each plot was bordered on each

side by a strip of 4 to 10 m in which soybean or peanut was planted. Seeds of the watermelon cultivar Mickylee were planted on 18 June 1998, and on 14 July 1999, respectively. At the 3 to 4 leaf stage, seedlings were thinned to approximately 1 plant per meter of row.

Two treatments were assigned to a total of six plots that were arranged in a completely randomized design. In three plots, chlorothalonil (Bravo 720, 1.7 kg a.i./ha, ISK Biotech C., Menter, Ohio) was applied to plants three times once every 14 days. The fungicide was first applied when symptoms of anthracnose appeared. The area (0.5×0.5 m) around the point of inoculation in each fungicide-treated plot was not treated. No fungicide was applied to plants in the remaining plots. When male flowers of watermelon plants first appeared, 50 g of the inoculum, dry oat kernels colonized with *C. orbiculare*, were distributed around the crown of one watermelon plant at the southeast corner of each plot.

Three, 10-m transects that radiated from the point of inoculation were positioned in each plot (Fig. 4). The three transects, named D_x, D_{xy} and D_y, were oriented at angles of 0^o, 45^o, and 90^o, respectively, to the direction of the row. Quadrats (0.5 x 0.5 m) were positioned 0, 1, 3, 5, 7, and 9 m from the point of inoculation along each transect. In each quadrat, the number of defoliated leaves (N_d), the number of leaves with anthracnose lesions (N_s), and the number of leaves without anthracnose lesions (N_h) were counted separately. Disease was assessed at 3- to 7-day intervals for five times following the appearance of symptoms. Assessments were made 18, 25, 30, 37 and 44 days after inoculation in 1998, and 18, 25, 29, 36 and 39 days after inoculation in 1999. Proportion of diseased leaves (Z_q) in each quadrat was calculated by

$$Z_q = (N_d + N_s) / (N_d + N_s + N_h) \qquad (1)$$

The mean value of Z_q on average over three replicate plots for each distance on each transect was then Z.

To evaluate the hypothesis that direction affected the decrease in disease with distance from the point of inoculation at each time of disease assessment, for each fungicide treatment, discontinuous variation with direction in spatio dynamics was evaluated by

$$Z = 1/(1+ exp^{gS-h}) \qquad (2)$$

(12). In this model, S was the distance from inoculum (m); and h was the parameter for the overall logit-transformed disease incidence $\{ln [Z/(1-Z)]\}$ at $S = 0$. That is, $h = ln [Z_o/(1-Z_o)]$, with Z_o being the proportion of disease incidence at $S = 0$. Because the spread of anthracnose in each of the three directions in each plot had the same point source of the conidia ($S = 0$), a common upper limit was assumed for each of the three gradients. The value of g was described by

$$g = g_x D_x + g_{xy} D_{xy} + g_y D_y \qquad (3)$$

in which g_x, g_{xy} and g_y were the intrinsic rate of decrease of disease with distance (m^{-1}), respectively, with respect to the direction of D_x, D_{xy} and D_y. For an observation in the direction, D_x, D_{xy} or D_y, a value of 1 was assigned; otherwise, the value of D_x, D_{xy} or D_y was 0.

Discontinuous variation with direction in the spatio-temporal dynamics of watermelon anthracnose was evaluated for each fungicide treatment by

$$Z= 1/(1+ exp^{gS-r(T-t)}) \qquad (4)$$

(10), In this model, g and S were defined as in equation 3. In addition, T was the days after inoculation; r was the intrinsic rate (day^{-1}) of increase of disease with respect to

time; and t was the time required to reach an incidence of 0.5 at the point of inoculation ($S = 0$).

Velocity of disease spread (v in m/day) for each direction during an epidemic was calculated by

$$v = r / g \qquad (5)$$

(49).

Continuous variation with direction in spatio-temporal dynamics was evaluated for each fungicide treatment by

$$Z = 1/(1 + exp^{aSx + bSy - r(T-t)}). \qquad (6)$$

In equation 6, r, T, and t were defined as in equation 4; S_x was the distance (m) from the point of inoculation in the direction parallel to the row; S_y was the distance (m) from the point of inoculation in the direction perpendicular to the row; and a and b were the parameters for disease gradients with respect to S_x and S_y.

Parameters of each model were estimated by non-linear regression using the derivative-free option of the NLIN procedure of the Statistical Analysis System (SAS version 6.12, SAS Institute, Cary, NC). Overparameterization and fit of each model were evaluated by inspection of observed values and predicted values of Z plotted against independent variables, standardized residual errors plotted against predicted values, standard errors of parameter estimates, and the asymptotic correlation between parameter estimates (62).

RESULTS

Effects of direction and fungicide. When the spatial dynamics of watermelon anthracnose was evaluated for each fungicide treatment and on each date of disease assessment, the decrease in disease with distance differed among the three directions. Gradients were flatter in the direction parallel to the row (D_x) (Fig. 7A). Gradients were steeper in the direction perpendicular to the row (D_y) or in the intermediate direction (D_{xy}). At each date of disease assessments, estimates of the intrinsic rates of decrease of disease with respect to distance (g) were smaller along row, and were similar in two directions of across rows (D_{xy} and D_y) (Table VIII). Difference in g between along row and across rows tended to be small at the first and last assessment, and was greater on other dates.

The spatio-temporal dynamics of watermelon anthracnose also differed among directions for each fungicide treatment (Table IX). In each year, values of g were always smaller in the direction of along the row (D_x) than in the direction of across rows (either D_{xy} or D_y). Furthermore, the effect of fungicide on the spatio-temporal dynamics depended on direction (Table IX). Fungicide had a greater effect on the intrinsic rate of decrease of disease with distance in the direction of along row than in the direction of across rows. The magnitudes of reduction of estimates of g by fungicide were large along row. In contrast, the magnitudes of reduction were small cross rows. The effects were observed in each year (Table IX).

Effects of fungicide on velocity of disease spread (v) again varied with direction (Table IX). Fungicide application reduced the velocity of disease spread (v) along the row to a greater extent than fungicide application reduced the velocity across rows. Fungicide significantly reduced the intrinsic rate of increase of disease with respect to

time (r) (Table IX). There was no evidence that r varied with direction (results not shown). Effects of fungicide on the time required to reach an incidence of 0.5 (t) were small (Table IX).

Descriptive three-dimensional model. When continuous variation with direction in the spatio-temporal dynamics of watermelon anthracnose was evaluated for each fungicide treatment, the intrinsic rate of decrease of disease with respect to distance (g) changed with direction (Table X). The response of the intrinsic rate to direction was smallest when the direction was parallel to the row, as indicated by small estimates of parameter a. As the direction approached the perpendicular, the magnitude of the response decreased, which was indicated by large estimates of a.

The descriptive three-dimensional model (equation 6) adequately described continuous variation with direction in the spread of anthracnose with time and with space from a point source for each fungicide treatment in each year (Table X). Standard errors of the estimates were small. Estimates of parameters were not strongly correlated with each other. Residual plots for the model were judged to be acceptable for each case. The predicted disease incidence adequately fit the actual observed data (Fig. 1 A and B).

DISCUSSION

This study is important because the effects of direction on fungicide-mediated reduction in the rate of spread of a foliar disease have been characterized explicitly for the first time. Fungicide application affected the rate of spread of watermelon anthracnose, but this effect varied with direction. That is, the application of a fungicide

inhibited the spread of disease in two ways. Firstly, the study confirmed that fungicide application decreased the intrinsic rate of increase of disease with respect to time. Secondly, this study confirmed that fungicide application increased the intrinsic rate of decrease of disease with respect to distance. More importantly, fungicide application had the greatest effect on the intrinsic rate of decline in disease level in the direction that was parallel to rows of plants. In the direction that was perpendicular to rows, the effect of fungicide was small.

Ignoring effects of direction could affect accurate estimation of the rate of spread. Compared with the previous study in which incidence was averaged over all three directions from the point source (see chapter IV), the averaging approach apparently overestimated the intrinsic rate of decrease of disease with respect to distance along rows. In contrast, the approach significantly underestimated the intrinsic rate across rows. These disadvantages have been clearly indicated by the results of a previous study (see chapter IV) and the current study. Unfortunately, averaging all directions is the common approach in most previous studies of spread (7, 18, 51). This may also explain the reason that the intrinsic rate of decrease of disease with distance was less sensitive to fungicide application than the intrinsic rate of increase of disease with time in the previous study (see chapter IV).

Difference in the spread of anthracnose within row and across rows appears to be influenced by movement of surface water and prevailing wind. The surface water formed during storms or a rain facilitates movement of the conidia along rows compared with cross rows. High bed ridges formed in our field plots confined movement of the water. The prevailing wind may assist the dispersal of the conidia by splashing rain. For this

study, effects of the lack of watermelon foliage overlap among rows were negligible because uniform canopies had already formed in plots during the experimental periods. Surface water and prevailing wind as the factors that influence the spread of disease have been observed on many crops (4, 18, 23, 24, 30, 50, 55).

A descriptive three-dimensional model (equation 6) that was developed in this study is an extended version of the spatio-temporal logistic model proposed by Jeger (33). In the model, one-dimensional spatial term is extended into two-dimensional spatial linear terms. This model worked well under two different epidemics for each of two years (Table X). The strength of this model is that it is simpler, has more clearly biological significance in parameters, and provides more descriptive information about disease progress in a field compared with previously reported two-dimensional spatial models (28, 42, 56).

The results presented here have important implications for studies of effects of specific factors on the spatio-temporal dynamics of disease. Special cautions should be given to studies in which the direction of spread has been ignored. For some studies in which spread of disease along a single row of plants or the mean level of disease on average over two or more directions is investigated, the rate of spread of disease could be either overestimated or underestimated. More work is still needed to evaluate the spread of anthracnose in commercial watermelon fields in which the spacing between rows is larger than our study system.

Table VIII. Parameter estimates of a spatial logistic model [a] that characterized qualitative effects of direction on the rate of decrease in the incidence of watermelon anthracnose from a point source for each of two fungicide treatments on each of five assessments

Fungicide application[b]	Disease assessment[c]	1998				1999			
		g_x (m^{-1})[a]	g_{xy} (m^{-1})	g_y (m^{-1})	h	g_x (m^{-1})	g_{xy} (m^{-1})	g_y (m^{-1})	h
-	1	0.145 ± 0.035[d]	0.133 ± 0.034	0.164 ± 0.038	-0.95 ± 0.12	0.546 ± 0.063	0.618 ± 0.074	0.608 ± 0.072	0.55 ± 0.13
	2	0.017 ± 0.012	0.056 ± 0.014	0.069 ± 0.014	-0.94 ± 0.06	0.387 ± 0.054	0.619 ± 0.091	0.567 ± 0.082	1.40 ± 0.22
	3	0.143 ± 0.063	0.489 ± 0.068	0.452 ± 0.063	2.58 ± 0.35	0.272 ± 0.034	0.350 ± 0.039	0.347 ± 0.038	1.73 ± 0.18
	4	0.186 ± 0.052	0.421 ± 0.035	0.455 ± 0.035	4.35 ± 0.28	0.252 ± 0.039	0.331 ± 0.037	0.306 ± 0.037	3.52 ± 0.27
	5	...[e]	0.561 ± 0.073	0.449 ± 0.076	7.68 ± 0.64	0.149 ± 0.054	0.269 ± 0.043	0.311 ± 0.042	3.72 ± 0.32
+	1	0.160 ± 0.041	0.133 ± 0.037	0.157 ± 0.040	-0.90 ± 0.13	0.762 ± 0.113	...	0.880 ± 0.137	0.76 ± 0.19
	2	0.046 ± 0.015	0.075 ± 0.016	0.122 ± 0.019	-0.76 ± 0.07	0.757 ± 0.158	0.944 ± 0.209	0.808 ± 0.170	1.52 ± 0.34
	3	0.199 ± 0.039	0.530 ± 0.053	0.461 ± 0.047	2.41 ± 0.24	0.388 ± 0.078	0.491 ± 0.101	0.458 ± 0.093	1.33 ± 0.30
	4	0.281 ± 0.039	0.358 ± 0.039	0.327 ± 0.039	2.86 ± 0.26	0.271 ± 0.051	0.299 ± 0.052	0.338 ± 0.055	1.90 ± 0.28
	5	0.235 ± 0.027	0.266 ± 0.026	0.325 ± 0.025	3.95 ± 0.20	0.250 ± 0.051	0.310 ± 0.053	0.277 ± 0.052	2.12 ± 0.30

[a] In the model, $Z = 1 / (1 + exp^{g S - h})$, Z was the mean incidence of disease on average over three replicate plots for each distance in each of three direction on each date of disease assessment; S was the distance from the point source (m); h was the parameter for the overall logit-transformed incidence $\{ln [Z/(1-Z)]\}$ at $S = 0$; and g was the intrinsic rate of decrease of disease with respect to distance. As described in text, the parameter g was described by $g = g_x D_x + g_{xy} D_{xy} + g_y D_y$ in which Dx was the spread direction parallel to the row inoculated with the point source; D_{xy} was the spread direction at a 45° angle to the row; Dy was the spread direction perpendicular to the row; g_x, g_{xy} and g_y represented the parameters for the intrinsic rate of disease of disease with respect to distance, respectively, in the direction of D_x, D_{xy} and D_y. For an observation of the direction, D_x, D_{xy}, or D_y, had a value of 1; otherwise, the value of D_x, D_{xy}, or D_y was 0.

[b] Plots applied with chlorothalonil (+) or none (-) at 2-week intervals .

[c] Disease assessment 1 through 5 were made, respectively, 18, 25, 30, 37, and 44 days after inoculation in 1998, and 18, 25, 29, 36, and 39 days after inoculation in 1999.

[d] Estimate ± standard error.

[e] Not estimated because the values of Z at all distances from the point source were 1 or 0.

Table IX. Parameter estimates of a spatio-temporal logistic model [x] that characterized qulitative effects of direction on the rate of decrease in the incidence of watermelon anthracnose from a point source for each of two fungicide treatments in each of 2 years

Year	Fungicide application[b]	g_x (m^{-1}) [a]	g_{xy} (m^{-1})	g_y (m^{-1})	r (day^{-1})	t (day)	v_x (m/day)[c]	v_{xy} (m/day)	v_y (m/day)
1998	-	0.095 ± 0.036 [d]	0.311 ± 0.044	0.313 ± 0.045	0.292 ± 0.024	24.6 ± 0.6	3.07	0.94	0.93
	+	0.147 ± 0.032	0.304 ± 0.037	0.294 ± 0.036	0.193 ± 0.014	23.4 ± 0.8	1.31	0.63	0.66
1999	-	0.314 ± 0.028	0.393 ± 0.030	0.390 ± 0.030	0.232 ± 0.012	19.9 ± 0.5	0.74	0.59	0.59
	+	0.345 ± 0.033	0.402 ± 0.036	0.389 ± 0.035	0.150 ± 0.012	21.0 ± 0.9	0.43	0.37	0.39

[a] In the model, $Z = 1 / (1 + exp^{gS - r(T-t)})$, Z was the mean incidence of disease on average over three replicate plots for each distance in each of three directions on each date of disease assessment; T was the number of days after inoculation; S was the distance(m) from the point source; r was the intrinsic rate of disease increase with T; t was the parameter for the time required to reach an incidence of 0.5 at the point of inoculation (i.e. $S = 0$); and g was the intrinsic rate of decrease of disease with respect to distance. As described in text, g was described by $g = g_x D_x + g_{xy} D_{xy} + g_y D_y$ in which Dx represented the spread direction parallel to the row with the point source; D_{xy} represented the spread direction at a 45° angle to the row; Dy represented the spread direction perpendicular to the row; and g_x, g_{xy} and g_y was the intrinsic rate of decrease of disease with respect to distance, respectively, in the direction of D_x, D_{xy} and D_y. For an observation of the direction, D_x, D_{xy}, or D_y, had a value of 1; otherwise, the value of D_x, D_{xy}, or D_y was 0.

[b] Plots applied with chlorothalonil (+) or none (-) at 2-week intervals .

[c] v_x, v_{xy} and v_y were, respectively, estimated by the form of e / g_x, e/g_{xy}, and e / g_y for velocity of spread (v) in the direction of D_x, D_{xy}, and D_y.

[d] Estimate ± standard error.

Table X. Parameter estimates and correlation between parameter estimates for a descriptive three-dimensional model [a] that described continuous variation with direction in the rate of decrease of the incidence of watermelon anthracnose from a point source over time for each of two fungicide treatments

Year	Fungicide Application[b]	Parameter estimates[a]				Correlation between parameter estimates[c]					
		a (m^{-1})	*b* (m^{-1})	*r* (day^{-1})	*t* (days)	*a* vs. *b*	*a* vs. *r*	*a* vs. *t*	*b* vs. *r*	*b* vs. *t*	*r* vs. *t*
1998	-	0.104 ± 0.031[d]	0.324 ± 0.039	0.292 ± 0.023	24.5 ± 0.6	0.59	0.23	-0.67	0.59	-0.52	0.06
	+	0.134 ± 0.027	0.291 ± 0.032	0.194 ± 0.014	23.6 ± 0.7	0.41	0.29	-0.64	0.56	-0.53	0.08
1999	-	0.250 ± 0.026	0.326 ± 0.028	0.229 ± 0.014	20.6 ± 0.5	0.53	0.50	-0.53	0.61	-0.47	0.14
	+	0.297 ± 0.032	0.343 ± 0.034	0.148 ± 0.013	21.6 ± 1.0	0.56	0.46	-0.48	0.49	-0.45	0.31

[a] The model has the form, $Z = 1/(1 + exp^{aSx+ Sy - r(T- t)})$, in which Z was the mean incidence of disease (measured as a proportion) on average over three replicate plots at the location (S_x, S_y) at time T; S_x was the distance (m) from the point of inoculation in the direction parallel to the row; S_y was the distance (m) from the point of inoculation in the direction perpendicular to the row; T was the number of days after inoculation; r was the intrinsic rate of increase of disease with T; t was the parameter for the time required to reach an incidence of 0.5 at the point inoculation (i.e. at $S_x = S_y = 0$); and a and b were parameters that together characterized the intrinsic rate of decrease in disease with respect to distance in each direction.

[b] Plots applied with chlorothalonil (+) or none (-) at the 2-week intervals.

[c] Associated correlation matrices of the parameter estimates.

[d] Estimate ± standard error.

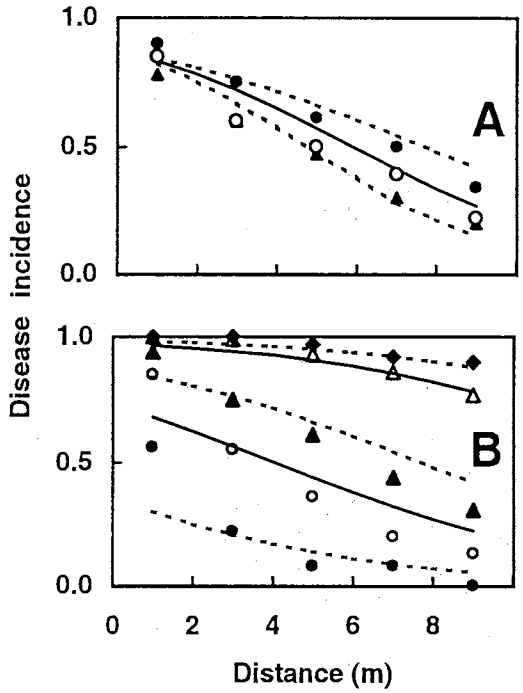

Fig. 7 Examples of the incidence of anthracnose, together with estimated curves of the descriptive three-dimensional model, in relation to distance froma point source. **A**, Comparison of the spread of disease along row (*Dx* - close circles with dashed line), and cross rows (*Dy* - open circles with solid line, or *Dxy* - close triangles with dashed line) at the third assessment; **B**, Disease gradients along row (*Dx*) at each of 5 assessments times, time 1 (close circles with dashed line), 2 (open circles with solid line), 3 (close triangle with dashed line), 4 (open triangle with solid line), and 5 (close diamond with dashed line), both in plots that were not treated with chlorothalonil in 1999. Each point was the mean of three replicate plots.

CHAPTER VI

Summary

1. Anthracnose incidence significantly decreased with the increase of total dose (*D*) of chlorothalonil applied during a cropping season and the number (*N*) of applications. The rate of the response to *D* was increased by increasing *N*. Effects of *D* and N on variation in incidence were characterized by a new model.

2. Incidence increased with increasing time interval from the day of inoculation when chlorothalonil, benomyl, and a mixture of chlorothalonil and benomyl were applied prior to inoculation; difference in disease in plots treated with chlorothalonil and benomyl were small. However, differences in plots treated with chlorothalonil and benomyl were great when they were applied after inoculation; disease increased more quickly for chlorothalonil than for benomyl. No difference in disease in plots treated with benomyl and the mixture was observed.

3. An analytic method was developed to evaluate effects of fungicide on spatio-temporal dynamics. Effects of chlorothalonil on the intrinsic temporal rates of disease progress were great, but the effects on the intrinsic rates of decrease of disease with respect to distance were small. The fungicide adequately decreased velocities of the spread (v) of anthracnose from the point source.

4. Chlorothalonil applications had a greater effect on the intrinsic rate of decrease of disease with respect to distance along rows of plants than across rows. A new

three-dimensional model was developed to describe the spread of anthracnose in two dimensions in a field over time.

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