Development and Evaluation of Guidelines for the Initiation of Chemical Control of Alternaria Leaf Spot in Pima Cotton in Israel

D. SHTIENBERG, Department of Plant Pathology and Microbiology, Faculty of Agriculture, P.O. Box 12, Rehovot 76100, Israel

ABSTRACT

Shtienberg, D. 1992. Development and evaluation of guidelines for the initiation of chemical control of Alternaria leaf spot in Pima cotton in Israel. Plant Dis. 76:1164-1168.

The optimal time to initiate spraying for management of *Alternaria macrospora* in Pima cotton (*Gossypium barbadense*) was determined in two field trials in 1990. Sprays applied prior to flowering did not contribute substantially to overall disease suppression irrespective of disease intensity at the time of spraying. Two field trials conducted in 1991 revealed that when disease was present in a field a spraying program initiated on flowering was preferable to one initiated according to the current action threshold (i.e., when an average of one lesion per 10-m row of plants was detected on true leaves), because it required fewer applications. Reduction in the number of applications did not affect control efficiency or decrease yield. Accordingly, the following guidelines are proposed: fungicides should not be applied prior to flowering, irrespective of disease intensity. Starting at the time of flowering, the crop should be inspected periodically and sprayed when disease is present in the field as determined by the action threshold level. By following these guidelines, growers can expect to achieve a saving of one to three spray applications with no substantial increase in disease development.

Additional keywords: epidemiology, yield loss

Alternaria leaf spot (caused by *Alternaria macrospora* A. Zimmerm.) in Pima cotton (*Gossypium barbadense* L.) occurs in most cotton-growing areas of the world (3). Although the disease is not considered to be a major hazard in the cotton industry worldwide, untreated crops in Israel have been found to yield 20–40% less than fungicide-treated crops (2,15,19). These yield losses are attributed mainly to excessive leaf shedding by diseased plants (2).

The pathogen is able to survive on infested crop debris, on alternative hosts, or within seed (6,14). Overwintering inoculum (i.e., spores and mycelium) infects the cotyledons, which are considerably more susceptible than the true leaves, and supports the early stages of the epidemic (1,17,20). At the beginning of the season, the host is relatively resistant to infection (7,17). In addition, night temperatures in Israel at that time are relatively low (10–18 C), and the canopy is not yet dense enough to promote the formation of a microclimate favorable for disease development (13). Once flowering begins, the host becomes progressively more susceptible to infection, while higher temperatures help create a microclimate more conducive for disease development. Accordingly, the yield loss potential of Alternaria leaf spot increases as the crop ages.

Although several measures are utilized to reduce the intensity of Alternaria leaf spot (e.g., crop rotation, avoiding overhead irrigation), fungicides are the main tool for disease management. Growers in Israel are currently advised to inspect the crop periodically and to initiate spraying when disease incidence on cotyledons exceeds 20%. Further spray applications are recommended when an action threshold is reached, i.e., when one lesion per 10-m row of plants (approximately 0.01 lesions per plant) is detected on true leaves. During a regular growing season, a Pima cotton field is normally sprayed with fungicides five to 10 times. However, these recommenda-

Present address of the author: Department of Plant Pathology, ARO, The Volcani Center, P.O. Box 6, Bet-Dagan 50250, Israel.

Contribution no. 3455-E, 1992 series, from the Agricultural Research Organization, The Volcani Center.

Accepted for publication 27 May 1992.

tions are not based on experimental results (Y. Sachs, *personal communication*).

Numerous trials have demonstrated that early spraying has little or no effect on overall disease suppression of *A. solani* in potatoes (5,12,18). If early spraying is similarly ineffective in the suppression of Alternaria leaf spot in cotton, it might be worthwhile to delay the initiation of chemical control and hence reduce the number of applications. The purpose of this study was to develop more effective guidelines for the initiation of chemical control of Alternaria leaf spot in Pima cotton in Israel and to evaluate these guidelines relative to current recommendations.

MATERIALS AND METHODS

Cultural practices. The effects of spraying schedules initiated at various times were examined in two field trials in 1990 (trials 1 and 2). The results of these trials were used to develop guidelines for the initiation of chemical control of Alternaria leaf spot. The efficiency of disease management achieved by spraying according to developed guidelines was compared with that achieved by following current recommendations in two field trials in 1991 (trials 3 and 4). Experiments were conducted in commercial fields located on the coastal (trials 1 and 3) or inland (trials 2 and 4) plains of Israel. These regions differ in soil type and also to some extent in microclimate. Cultivar S-5 was sown in trials 1 and 3, and cv. F-177 was sown in trials 2 and 4; both cultivars are susceptible to *A. macrospora*. The previous crop in all experimental fields was also Pima cotton. Seed was sown to a depth of 2–4 cm in the last week of March 1990 or the first week of April 1991 (10 seeds/m, with 1-m spacing between rows). The crop was drip-irrigated once every 3–5 days, with a total irrigation of 5,000–5,200 m^3/ha per season. In mid-October 1991, yield (fiber and seeds) was collected by hand from a randomly chosen sample area of 2 m^2 in the central part of each experimental plot. Yield was expressed as the amount of fiber and seeds (in metric tons per hectare) harvested.

The crop in the experimental sites was inspected periodically, and its phenological growth stage was recorded. A temperature-dependent physiological age, from emergence to each sampling date, was calculated according to Marani (9) as follows: maximum temperatures above 30 C were taken as 30 C. Average day and night temperatures (T_d and T_n) were calculated from maximum and minimum temperatures (T_{max} and T_{min}) by use of the empirical factors 0.77 and 0.19, as follows: $T_d = T_{min} + 0.77 \times (T_{max} - T_{min})$; $T_n = T_{min} + 0.19 \times (T_{max} - T_{min})$. Day-degrees above the threshold of 12 C were calculated separately for day and night and weighted according to day and night lengths. The sum of day-degrees was divided by 14, thus making a physiological-day (P-day) equivalent to one day with a constant temperature of 26 C. Records of daily maximum and minimum temperatures were taken from regional stations of the Israeli weather forecast service, which were located within 10 km of each experimental site.

Experimental design. Experimental plots in each trial were arranged in a complete block design with four replicates per treatment. The size of each experimental plot varied according to the mode of fungicide application; it was 4 × 12 m in trials 1 and 2, 18 × 25 m in trial 3 and 18 × 35 m in trial 4. Sprays were applied with a motorized back-sprayer at a pressure of 275 kPa in 180–200 L of water per hectare (trials 1 and 2) or with a tractor-mounted boom sprayer at a pressure of 350 kPa in 90–100 L of water per hectare (trials 3 and 4). Sprays did not contain a spreader, sticker, or adjuvant of any type.

Treatments consisted of different fungicides applied on different starting dates. Two systemic fungicides were used; tebuconazole (Folicur, 25% EC, Bayer AG, Germany) was applied at a rate of 0.25 kg a.i./ha in trials 1, 3, and 4, and difenoconazole (Score, 25% EC, Ciba-Geigy Ltd., Switzerland) was applied at a rate of 0.125 kg a.i./ha in trials 2 and 4. The protectant fungicide maneb (Manebgan, 50% FC, Agan Ltd., Israel) was applied at a rate of 2 kg a.i./ha in all trials.

Disease assessment. Disease was assessed visually every 7–14 days from mid-April until mid-September each year. From mid-April to middle or late June, the number of lesions in two samples (each consisting of a randomly chosen 2-m row of plants) was counted for each of the experimental plots. On the basis of these estimates, disease intensity (i.e., the number of lesions per 10-m row of plants or per plant) was calculated. From early July to mid-September, disease-induced defoliation was assessed on 10 randomly chosen plants in each experimental plot as follows: the disease severity of attached leaves and the proportion of shed leaves were assessed separately for the lower (<40 cm), middle (41–80 cm), and upper (>81 cm) levels of the canopy. Disease severity was determined with the aid of a disease assessment key (4). Leaf shedding was determined with the aid of a defoliation assessment key (19) after counting the easily distinguished sites of abscission on the stem and branches. These assessments served as the basis for estimates of defoliation induced by Alternaria leaf spot (calculated as a weighted sum of estimates of the disease severity of attached leaves and the proportion of shed leaves [19]). To minimize subjectivity errors, disease in each experimental plot was assessed independently by two individuals, and the average scores were recorded. For some analyses, the area under the disease progress curve (AUDPC), as calculated by Shaner and Finney (16), was used as an estimate of disease intensity. AUDPC units are proportion days.

Development of guidelines for the initiation of chemical control. In trials 1 and 2, the optimal time of spraying initiation was determined as follows: in one treatment spraying was initiated 1

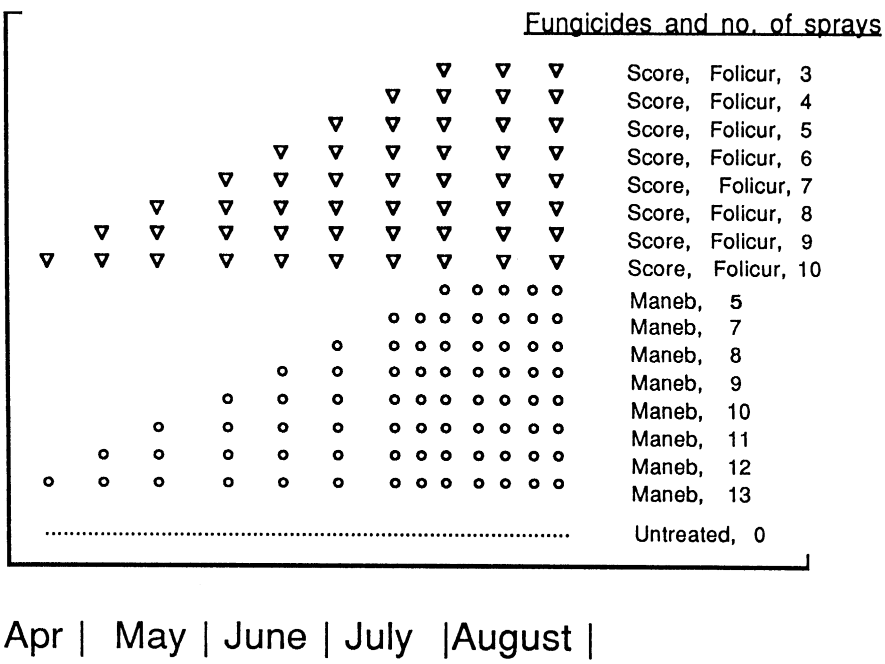

Fig. 1. Fungicides, times of application, and total number of sprays applied in two field trials conducted in 1990 (Score = difenoconazole, Folicur = tebuconazole).

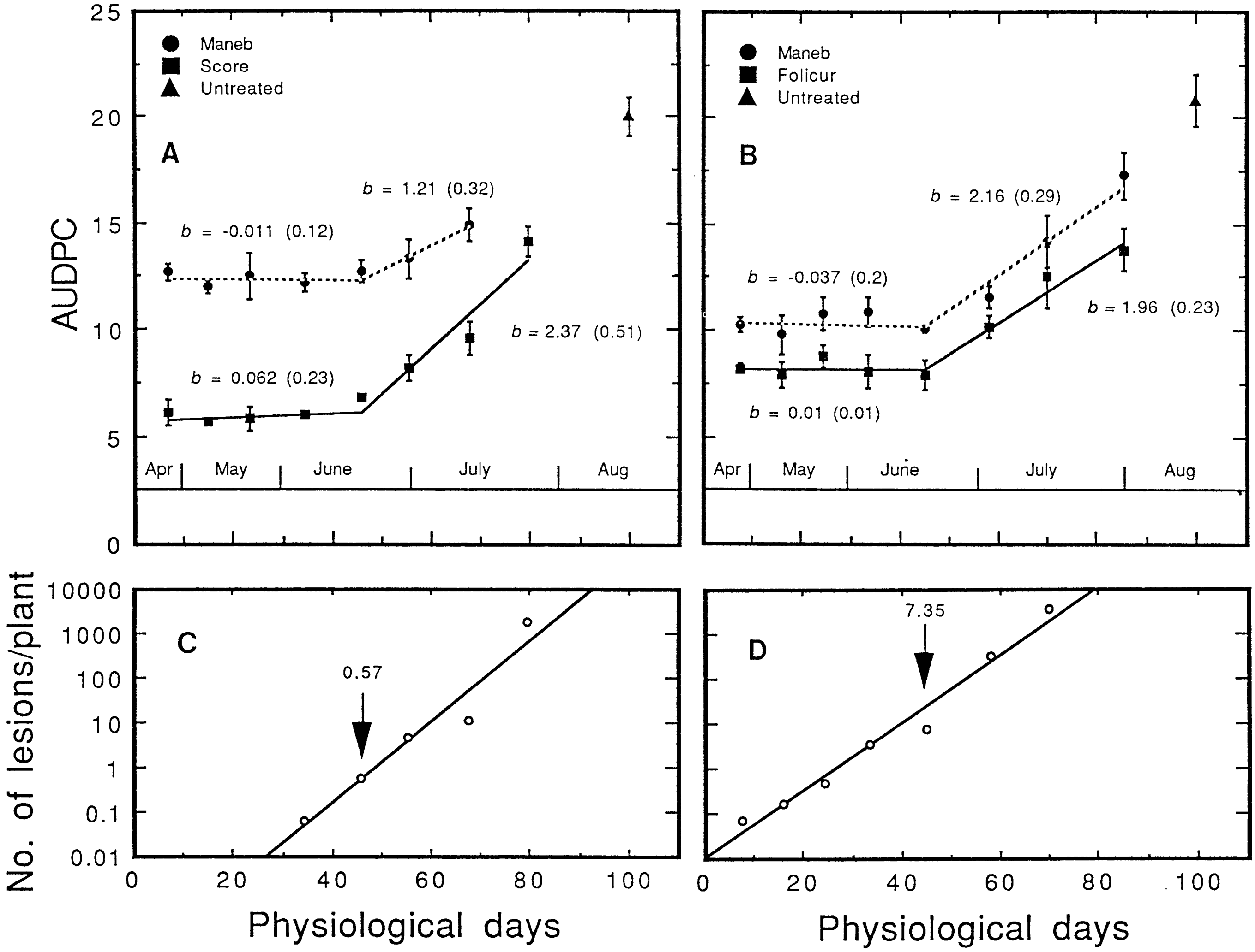

Fig. 2. Effect of the time of spraying initiation on Alternaria leaf spot in Pima cotton in trial 1 **(A)** and 2 **(B)**. Disease intensity is expressed in terms of area under the disease progress curve (AUDPC) units. The regression equation was determined by means of a piecewise linear regression analysis (**A**, $R^2 = 0.893$, $P < 0.0001$; **B**, $R^2 = 0.958$, $P < 0.0001$). Numbers close to regression lines indicate the values of the corresponding slopes ($b \pm$ SE). Bars indicate the standard error for each data point. Disease intensity (number of lesions per plant) recorded at the time of spraying initiation for each treatment in trial 1 **(C)** and 2 **(D)**. Arrows indicate the time of occurrence of the change in slope of the regression equation. The adjacent numbers indicate disease intensity at that time. (Score = difenoconazole, Folicur = tebuconazole.)

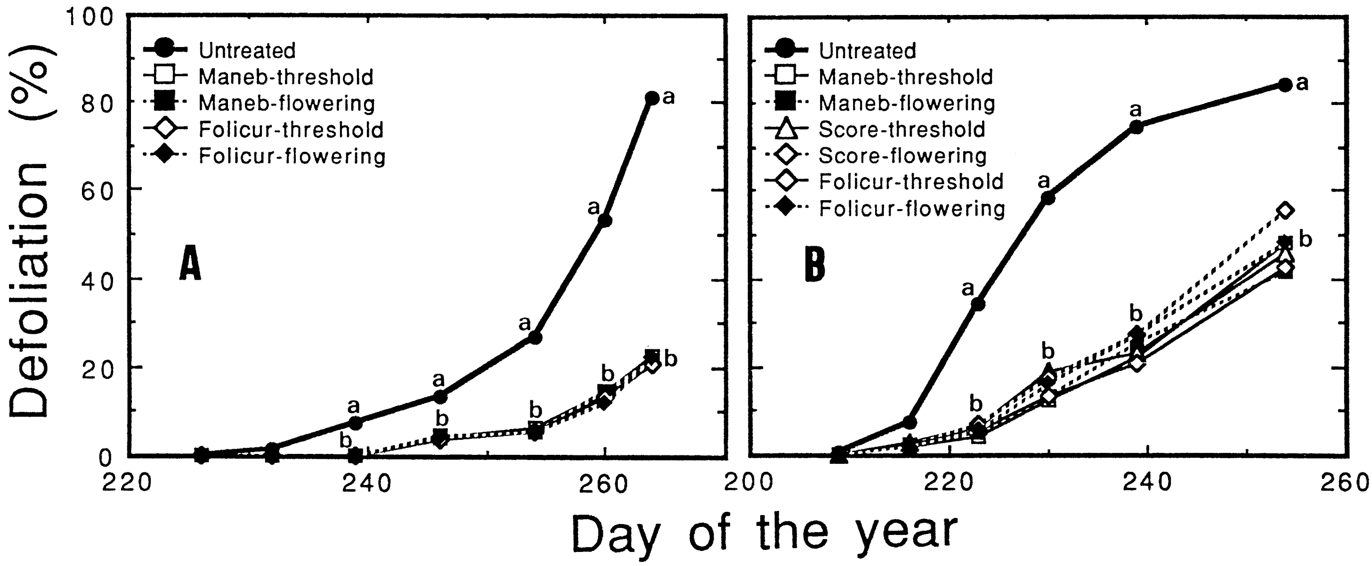

Fig. 3. Effects of fungicides and time of spray initiation on Alternaria leaf spot in Pima cotton in trials 3 **(A)** and 4 **(B)**. Sprays were initiated on the basis of the current action threshold (when an average of one lesion per 10-m row of plants was detected on true leaves) or flowering (when 50% of the plants contained at least one open flower). Tebuconazole (Folicur) and difenoconazole (Score) were applied at biweekly intervals and maneb at weekly intervals. Values on the graph (for each disease assessment date) followed by different letters differ significantly ($P < 0.05$) as determined by Fisher's protected least significant difference test.

wk after emergence. Subsequent treatments were initiated in successive plots at biweekly intervals until late June, giving a total of eight treatments (Fig. 1). This scheme was used for the systemic and protectant fungicides. Following application of the first spray in each treatment, the systemic fungicide (tebuconazole or difenoconazole) was applied at biweekly intervals; the protectant fungicide (maneb) was applied at biweekly intervals until mid-June, and thereafter at weekly intervals. The last spray in all treatments was applied in the last week of August (approximately 3 wk before chemical defoliation). Plots not treated with fungicides (controls) served as a reference basis in both trials (Fig. 1).

Piecewise linear regression was used to derive a model describing the effect of spray initiation time on the resulting epidemic. The independent variable in the analysis was the date of spray initiation (expressed in terms of weeks after emergence), and the dependent variable was the severity of the epidemic (in terms of AUDPC units). Models consisting of two pieces were fitted to the data of trials 1 and 2. Candidate dates for the onset of changes in the slope of the regression equation were selected by examination of plots of the raw data. The best-fitting dates were chosen according to the residual sum of squares of the candidate models (11). Analyses were performed with aid of the computer software package MINITAB (10).

Several parameters were examined in trials 1 and 2 in an attempt to identify those criteria that would establish a biological indicator for the initiation of fungicide applications. Parameters were evaluated for their utility for use by farmers. Considered parameters included disease intensity, calender date, physiological age, and phenological growth stage.

Comparison of methods for determining the initiation of chemical control. Two sets of guidelines for spray initiation were examined in trials 3 and 4. Sprays were initiated on flowering (i.e., when 50% of the plants contained at least one open flower) or according to the current action threshold (when an average of one lesion per 10-m row of plants was detected on true leaves). Following application of the first spray, tebuconazole or difenoconazole was applied at biweekly intervals, and maneb was applied at weekly intervals until early September. In each trial, plots not treated with fungicides served as controls.

RESULTS

Development of guidelines for the initiation of chemical control. In trials 1 and 2, disease symptoms appeared soon after emergence. Three weeks after emergence, 16.3% of the cotyledons in trial 1 and 42.5% in trial 2 were infected. At this stage no evidence of infection was observed on true leaves. During the first two months of growth, signs of infection on true leaves were scattered, and progress of the epidemic was manifested mainly by an increase in the number of infected plants. It was only after flowering that disease severity increased and infected leaves showed premature abscission.

The optimal time for the initiation of spraying was determined from the piecewise linear regression as the point at which the slope of the regression equation changed. In both trials, this change occurred on the fifth date of spray initiation. The value of the first part of the slope of the regression equation (between the first and fifth dates of spray initiation) was not significantly different from zero ($P = 0.05$), indicating that the first four sprays did not contribute substantially to overall disease suppression (Fig. 2A and B). On the other hand, the value of the second part of the slope (between the fifth and eighth dates of spraying initiation) was significantly greater than zero ($P < 0.05$), indicating that the effectiveness of disease suppression was reduced progressively in line with the delay in time of spray initiation (Fig. 2A and B). Although the systemic fungicides were more effective in disease suppression than the protectant fungicide (as indicated by their lower AUDPC values), the optimal time of spray initiation was similar for both types of fungicides in both trials.

In trials 1 and 2, the optimal time for spray initiation was on the fifth date. However, since that date was determined on the basis of experimental considerations only (Fig. 1), it was not meaningful in biological terms. Accordingly, the next step was to express that date in more general terms to establish a biological indicator for the initiation of chemical control. Disease intensity differed significantly among the two trials and was therefore a poor indicator of the optimal time for spray initiation; on the fifth date of spray initiation there were 0.57 lesions per plant in trial 1 and 7.35 lesions per plant in trial 2. However, some parameters in the two trials were comparable: the calendar date was June 18 in trial 1 and June 16 in trial 2; in both trials the crop was at the growth stage of flowering; and the physiological age of the crop was 43.2 P-days in trial 1 and 45.3 P-days in trial 2. Of these parameters, the phenological growth stage (i.e., flowering) was the one chosen for use in further experiments as an indicator of the optimal time to initiate spraying.

Comparison of methods for determining the initiation of chemical control. In trials 3 and 4, spraying was initiated according to the current action threshold as well as on the basis of flowering. In trial 3, disease developed relatively late, and the action threshold level was reached on July 7, whereas the crop had already started to flower on June 30. In trial 4, these events occurred on June 23 and June 30, respectively. Disease intensity at the time of flowering was 0.5 lesions per 10-m row of plants in trial 3 and 107.5 lesions per 10-m row of plants in trial 4. The physiological age of the crop at the time of flowering was 48.3 P-days in trial 3 and 52.1 P-days in trial 4.

Over the entire season defoliation in plots treated with fungicides was significantly lower ($P < 0.05$) than in untreated plots. On the other hand, differences in defoliation between the

Table 1. Effects of fungicides and criteria used for spray initiation on the development of Alternaria leaf spot in Pima cotton and on yield in two field trials conducted in 1991

Fungicide[w]	Criteria for spray initiation[x]	No. of sprays	Final defoliation (%)	AUDPC[y]	Yield (t/ha)
Trial 3					
Untreated	...	0	81.1 a[z]	7.8 a	3.46 b
Maneb	Threshold	9	21.8 b	1.9 b	4.65 a
Maneb	Flowering	10	22.7 b	1.9 b	4.66 a
Tebuconazole	Threshold	5	22.3 b	1.7 b	4.38 a
Tebuconazole	Flowering	5	20.7 b	1.8 b	4.09 a
Trial 4					
Untreated	...	0	84.3 a	22.8 a	2.08 b
Maneb	Threshold	10	48.3 b	7.7 b	3.10 a
Maneb	Flowering	9	41.7 b	7.7 b	3.10 a
Difenoconazole	Threshold	6	45.8 b	8.4 b	3.20 a
Difenoconazole	Flowering	5	55.4 b	9.5 b	2.51 ab
Tebuconazole	Threshold	6	42.5 b	7.3 b	3.11 a
Tebuconazole	Flowering	5	48.3 b	8.7 b	3.08 a

[w] Tebuconazole was applied at a rate of 0.25 kg a.i./ha, difenoconazole at a rate of 0.125 kg a.i./ha, and maneb at a rate of 2 kg a.i./ha.

[x] Spraying was initiated on the basis of the current action threshold (when an average of one lesion per 10-m row of plants was detected on true leaves) or flowering (when 50% of the plants contained at least one open flower).

[y] Area under the disease progress curve. AUDPC units are proportion days.

[z] Numbers followed by different letters in each column (within each trial) differ significantly ($P < 0.05$) as determined by Fisher's protected least significant difference test.

various fungicide treatments were insignificant (Fig. 3, Table 1). In general, yields in plots treated with fungicides were significantly higher than in untreated plots, but the differences in yield between plots treated with different fungicides were insignificant (Table 1).

DISCUSSION

Mumford and Norton (8) distinguished between four models as a basis for decision making in pest management: the threshold model, the marginal analysis model, the decision theory model, and the behavior model. The threshold model was designed to replace predetermined scheduling of spraying times with a flexible approach (22) determined by given states of the crop, disease, and weather (21). For cotton, Israeli growers currently base the decision to initiate spraying only on the state of the disease. The present study shows that disease as a sole criterion is a poor indicator of the optimal time to initiate spraying. This conclusion is based on the fact that at the optimal time for spray initiation the disease intensity in trial 2 was 13 times greater than in trial 1. On the other hand, the calendar date, phenological growth stage, and physiological age of the crop were good indicators of the optimal time to initiate spraying. The calendar date was rejected as a criterion because it was not meaningful in biological terms. The physiological age of the crop, which integrates the effects of temperature on crop development, has been used successfully as an indicator of the optimal time to initiate chemical control of early blight in potatoes (5,12). Since the phenological growth stage of the crop is closely related to its physiological age (9), and because flowering is a simple, highly visible indicator, flowering was the parameter chosen to indicate that spraying should be initiated.

Our results showed that when disease was present in the field, sprays applied according to the current action threshold, but prior to flowering, did not contribute substantially to disease management (Fig. 2). In contrast, a spraying program at the time of flowering was preferable because fewer applications were needed to achieve a similar degree of control (Fig. 3) and without affecting yield (Table 1).

The results obtained in trial 3 indicated that the criteria for initiation of spraying should not be restricted to flowering alone. At the time of flowering, disease incidence was very low, and spraying was not necessary (Table 1). The decision to initiate spraying should therefore be based on the state of both the crop and disease. In Israel, weather need not be taken into account, since fluctuations in the weather are infrequent in the summer, and their effects on *A. macrospora* development are relatively marginal (J. Rotem, *personal communication*).

In view of our results, we propose the following guidelines for initiation of chemical control of Alternaria leaf spot in Pima cotton in Israel. Fungicides should not be applied prior to flowering, irrespective of disease intensity. Starting at the time of flowering, the crop should be inspected periodically and sprayed when disease is present in the field as determined by the action threshold level. By following these guidelines, growers can expect to achieve a saving of one to three spray applications with no substantial increase in disease development.

ACKNOWLEDGMENTS

This research was supported by the Valzi-Pikovski Foundation, the Israeli Cotton Council, Lidor Ltd., and by C.T.Z. Ltd. I thank A. Dinoor for his support and advice, J. Rotem for review, S. Smith for editing this manuscript, and Y. Dreishpoun, Y. Kremer, A. Yaniv, Y. Baum, and R. Epshtain for assisting in the field trials.

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