Influence of Physiological Age of Pima Cotton on the Need for Fungicide Treatment to Suppress Alternaria Leaf Spot

D. Shtienberg, Y. Kremer, and A. Dinoor

First author, Department of Plant Pathology, Agricultural Research Organization, The Volcani Center, P.O. Box 6, Bet-Dagan 50250, Israel; second and third authors, Department of Plant Pathology and Microbiology, Faculty of Agriculture, P.O. Box 12, Rehovot 76100, Israel.

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ABSTRACT

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Field and controlled-environment trials were conducted to develop concepts for integrating age-related resistance and fungicides in the management of *Alternaria macrospora,* the causal agent of Alternaria leaf spot of Pima cotton. The influence of physiological age of the crop and of the individual leaves on infection incidence and on the rate of lesion expansion was examined on leaves sampled from the field. Both components increased with the physiological age of individual leaves but were not affected by the age of the crop. The effects of age-related resistance on the need for fungicide treatment were examined in two trials in 1990 and 1991. The efficacy of maneb applied in a 10- or 14-day schedule relative to weekly maneb applications decreased with crop age, while relative control efficacy of tebuconazole applied in a 10-, 14-, 17- or 21-day schedule did not change. It was concluded that the type of fungicide and the frequency of application should be determined by the age-related response of the host plant to the pathogen.

Additional keywords: control, epidemiology, IPM.

Alternaria macrospora A. Zimmerm. in cotton (*Gossypium barbadense* L., cv. Pima) occurs in most cotton-growing areas of the world (4) but is only severe in a few areas. In Israel, where yield losses of up to 40% have been reported from the disease (2,17,21,22), Pima cotton fields are sprayed with fungicides five to 10 times during a typical growing season. Until recently, only protectant fungicides were commercially available for suppression of the disease, but the new systemic fungicides tebuconazole (Folicur) and difenoconazole (Score) may provide improved disease control. For incorporation of these new fungicides in the management of Alternaria leaf spot, specific guidelines need to be developed.

In some Alternaria diseases, such as early blight of potato and tomato, the plants are relatively resistant to disease when young but become increasingly susceptible with age (12–14,23). In cotton, susceptibility to *A. macrospora* is highest in seedlings, decreases in young plants, and then increases again with age to a second peak in senescent plants (1,16,24). The plants are relatively resistant to infection between the cotyledon stage and the initiation of flowering, after which there is a marked increase in their susceptibility (20). Consequently, sprays applied prior to flowering did not contribute substantially to the suppression of Alternaria leaf spot, whereas spray applications after flowering reduced disease development significantly (21).

The effects of genotype and physiological age on components of resistance have been investigated in the case of early blight in potatoes (10,11,13) and may serve as a model for Alternaria leaf spot of cotton. For example, infection frequency of potato early blight decreased with height of the canopy but increased over time as the crop aged (11); leaf position and crop physiological age affected the latent period and lesion expansion rate. The lower the leaf position and the older the plant, the shorter the latent period and the greater the lesion expansion rate (10,11,13). Much less is known about the effects of crop physiological age on the components of *A. macrospora* in cotton. Studies conducted so far have shown that disease severity (a parameter incorporating both infection frequency and lesion expansion rate) (1,16) and spore production increase with crop age (15).

Integration of several control measures should reduce the need for frequent applications of fungicides. If host response to Alternaria leaf spot and/or fungicide efficacy changes with time, then it might be possible to integrate these measures to develop a more efficient disease-management program. The objectives of this study were to quantify changes in infection incidence and lesion expansion rate with the increasing age of the crop and of individual leaves and to characterize changes in the efficacy of fungicidal control with crop age in order to develop concepts for integrating age-related resistance and fungicides in the management of Alternaria leaf spot of Pima cotton. A preliminary report of parts of this work has been published (7).

MATERIALS AND METHODS

Changes in infection incidence and lesion expansion rate with crop age. Plants removed from the field in 1990 and 1991 were used to determine host growth over time. Five replicate 1-m rows of plants (approximately 50 plants in total) were arbitrarily selected and cut from an untreated plot adjacent to the site of the field trials described below. Sampling was carried out at 10- to 14-day intervals, starting soon after emergence (early April) and ending just before chemical defoliation (mid-September). In 1990, the nodes on the main stem only were counted; in 1991, the nodes on the main stem as well as those on monopodial branches were counted. After leaves to be used for further analysis were subsampled as described below, all leaves were removed. These leaves were used to estimate plant biomass. In 1990, the dry weight of leaves was determined after oven drying at 72 C for 48 h; in 1991, the total leaves per plant were counted. The relationship between the number of nodes on the main stem (dependent variable) and the physiological age of the crop at each sampling date (independent variable) was quantified by means of linear regression analysis for the period of 0–100 physiological days (P-days) after emergence. A similar analysis was performed in 1991 for the monopodial branches. The regression equation was then used to calculate the time of leaf emergence, and the physiological age of individual leaves could thus be estimated on each sampling date.

The temperature-dependent physiological age of the crop and of individual leaves from emergence to each sampling date was calculated according to Marani (8) as follows: 1) maximal temperatures above 30 C were taken as 30 C; 2) average day and night temperatures (T_d and T_n, respectively) were calculated from maximal and minimal temperatures (T_{max} and T_{min}, respectively) with the empirical factors of 0.77 and 0.19 as

$$T_d = T_{min} + 0.77 \times (T_{max} - T_{min}) \quad (1)$$

$$T_n = T_{min} + 0.19 \times (T_{max} - T_{min}) ; \quad (2)$$

3) degree-days above the threshold of 12 C were calculated separately for day and night and weighted according to day and night lengths; and 4) the sum of degree-days was divided by 14 (the difference between the optimal temperature for cotton growth, 26 C, and the lower threshold temperature, 12 C), thus making a P-day equivalent to one day with a constant temperature of 26 C. Records of daily maximum and minimum temperatures were taken from a hygrothermograph placed in a weather shelter located within the plant canopy at a height of 50 cm.

Changes in infection incidence and in lesion expansion rate with respect to the age of the crop and of individual leaves were evaluated under controlled-environment conditions on leaves sampled from the field. Leaves from alternating nodes on the main stem of two plants were sampled in both trials, and in 1991 leaves from monopodial branches were also sampled. Leaves were cut into square segments (2 × 2 cm); care was taken not to include segments with any visible disease symptoms. For each group of leaves (identified according to their origin on the plant), five leaf segments were placed in a 9-cm petri plate on distilled water agar containing benzimidazole (0.05 g/L). Four of the leaf segments in each plate were inoculated. One 2.5-μl drop containing approximately 50 conidia of *A. macrospora* was placed on the center of each of the four segments. The fifth leaf segment was not inoculated and served as a control. The isolate of *A. macrospora* used in all trials was derived from infected cotton plants in a commercial field and maintained in petri plates on a medium containing 5% V8 juice and 2% agar. There were four replicates (petri plates) of each of the sampled leaves at every sampling date (a total of 16 leaf segments per sampled leaf). Plates were placed in an incubator at 25 C with a 12-h photoperiod of fluorescent light. After 6–8 days, lesion number and size (across two diameters) were recorded. These measurements served as a basis for determination of infection incidence and lesion expansion rate. Infection frequency was calculated as the percentage of leaf segments that showed lesions. The natural logarithm of the final lesion area was divided by the number of days in the interval (i.e., 6–8 days) to calculate the lesion expansion rate for each lesion, as previously described (6).

Changes in efficacy of fungicidal control with crop age. Trials were conducted in two commercial fields located in the coastal plain of Israel. Seed of the susceptible Pima cultivar S-5 was sown to a depth of 2–4 cm on 28 March, 1990, and 3 April, 1991 (10 seeds per meter of row with 1 m spacing between rows). The previous crop was also Pima cotton. The crop was drip-irrigated once every 3–5 days, with a total irrigation of 5,000–5,200 m^3 per hectare per season. Experimental plots in each trial were arranged in a randomized complete block design with four replicates per treatment. Each experimental plot was 18 × 25–35 m. Fungicides (90–100 L of water per hectare) were applied by means of a tractor-mounted boom sprayer with Cone-Jet X3 nozzles at a pressure of 350 kPa. Sprays did not contain spreader, sticker, or adjuvant.

Treatments consisted of two fungicides applied at various intervals. The fungicides were maneb (Manebgan, 50% FC, Agan Ltd., Israel) and tebuconazole (Folicur, 25% EC, Bayer AG, Germany). The choice of these two fungicides was based on previous trials in which no substantial differences were revealed in control efficacy between plants sprayed with maneb or a number of other protectant fungicides and plants sprayed with tebuconazole or difenoconazole (18,21,22). Applications were initiated according to the recommended threshold (i.e., when an average of one lesion per 10-m row of plants was detected on true leaves).

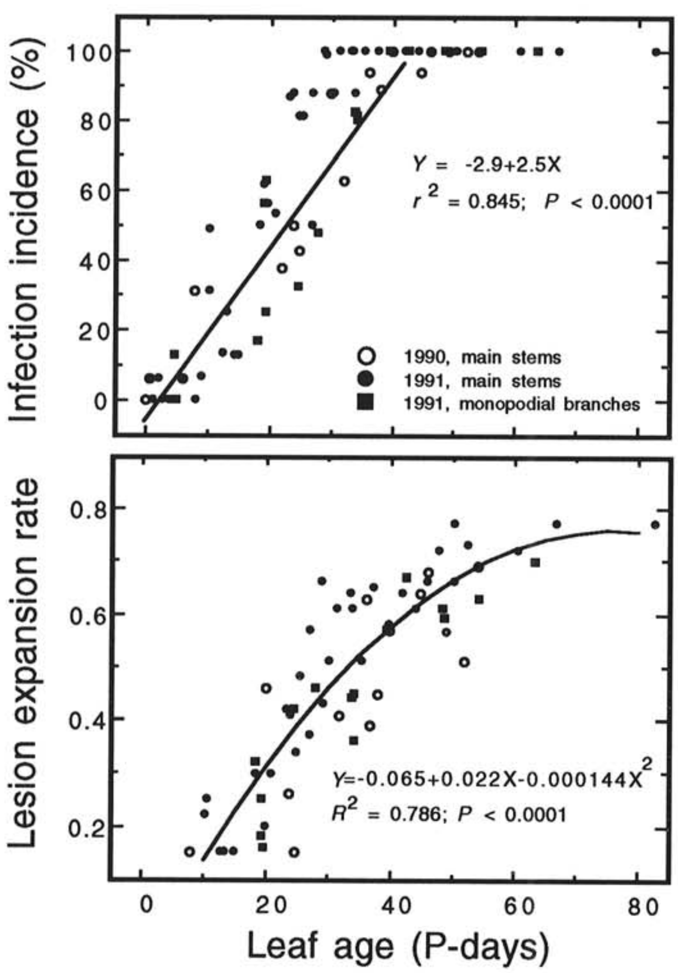

Fig. 1. Effects of the physiological age of individual leaves on infection frequency and on lesion expansion rate of *Alternaria macrospora*. Curve fitting excludes infection frequency values of 100%. P-days = physiological days.

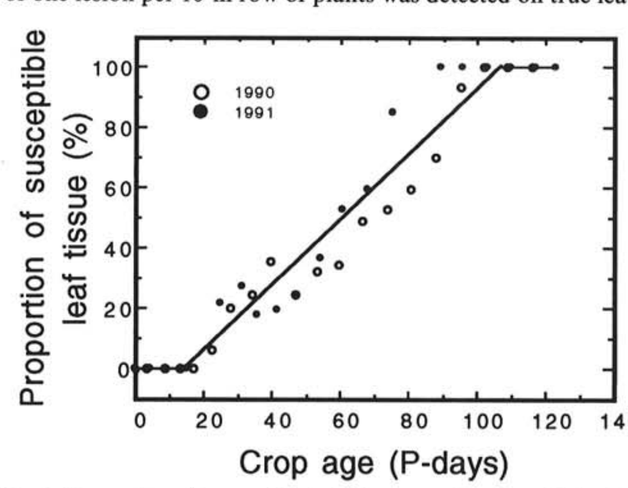

Fig. 2. Proportion of susceptible leaf tissue as a function of the physiological age of the crop. At each sampling date, the fraction of plant biomass at or above the age of 20.9 physiological days (P-days) (equivalent to 50% infection frequency) was considered susceptible leaf tissue.

This occurred on 3 June, 1990 (41 P-days after emergence) and 7 July, 1991 (60 P-days after emergence). Subsequent applications in the various treatments were maneb (2.0 kg a.i./ha) applied in 7-, 10-, or 14-day schedules (13, 8, or 6 sprays in total, respectively) and tebuconazole (0.25 kg a.i./ha) applied in 10-, 14-, or 17-day schedules in 1990 (7, 6, or 4 sprays in total) and in 14-, 17-, or 21-day schedules in 1991 (5, 4, or 4 sprays in total). Plots not treated with fungicides served as controls.

Disease-induced defoliation was assessed visually every 5–10 days from early June until mid-September each year on 10 arbitrarily 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 (<30–50 cm), middle (30–50 to 70–90 cm) and upper (>70–90 cm) levels of the canopy. (Ranges are given because the height of the crop varied slightly among trials). Disease severity was determined with the aid of a disease assessment key (5). Leaf abscission sites on the main stems and branches were easily distinguished, and leaf shedding was determined with the aid of a defoliation assessment key (22). These assessments served as the basis for estimates of the 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 [22]). Disease in each experimental plot was assessed independently by two individuals, and the average scores were recorded. For some analyses, the area under the defoliation progress curve (AUDPC) as calculated by Shaner and Finney (19) was used. The period over which AUDPC was calculated was the first disease assessment until the end of the growing season. AUDPC units are proportion days.

Efficacy of fungicidal control achieved in the different treatments was determined in relation to that achieved by weekly applications of maneb. Defoliation records assessed in the maneb 7-day plots (D_{M7}) and in the untreated plots (D_U) were used to evaluate the control efficacy achieved by spraying of maneb in a 7-day schedule (C_{M7}).

$$C_{M7} = 1 - D_{M7}/D_U \qquad (3)$$

Thus, higher control efficacy is associated with more effective disease suppression. Similarly, the control efficacy achieved by the spraying of maneb or tebuconazole in each of the other spraying-interval treatments (C_T) was calculated in relation to defoliation in the untreated plots. The control efficacy in each of the treatments was then related to that achieved in the maneb 7-day treatment (relative control efficacy, R_T) as follows:

$$R_T = C_T/C_{M7} \times 100\,. \qquad (4)$$

RESULTS

The influence of the physiological age of the crop and of individual leaves on infection incidence and lesion expansion rate

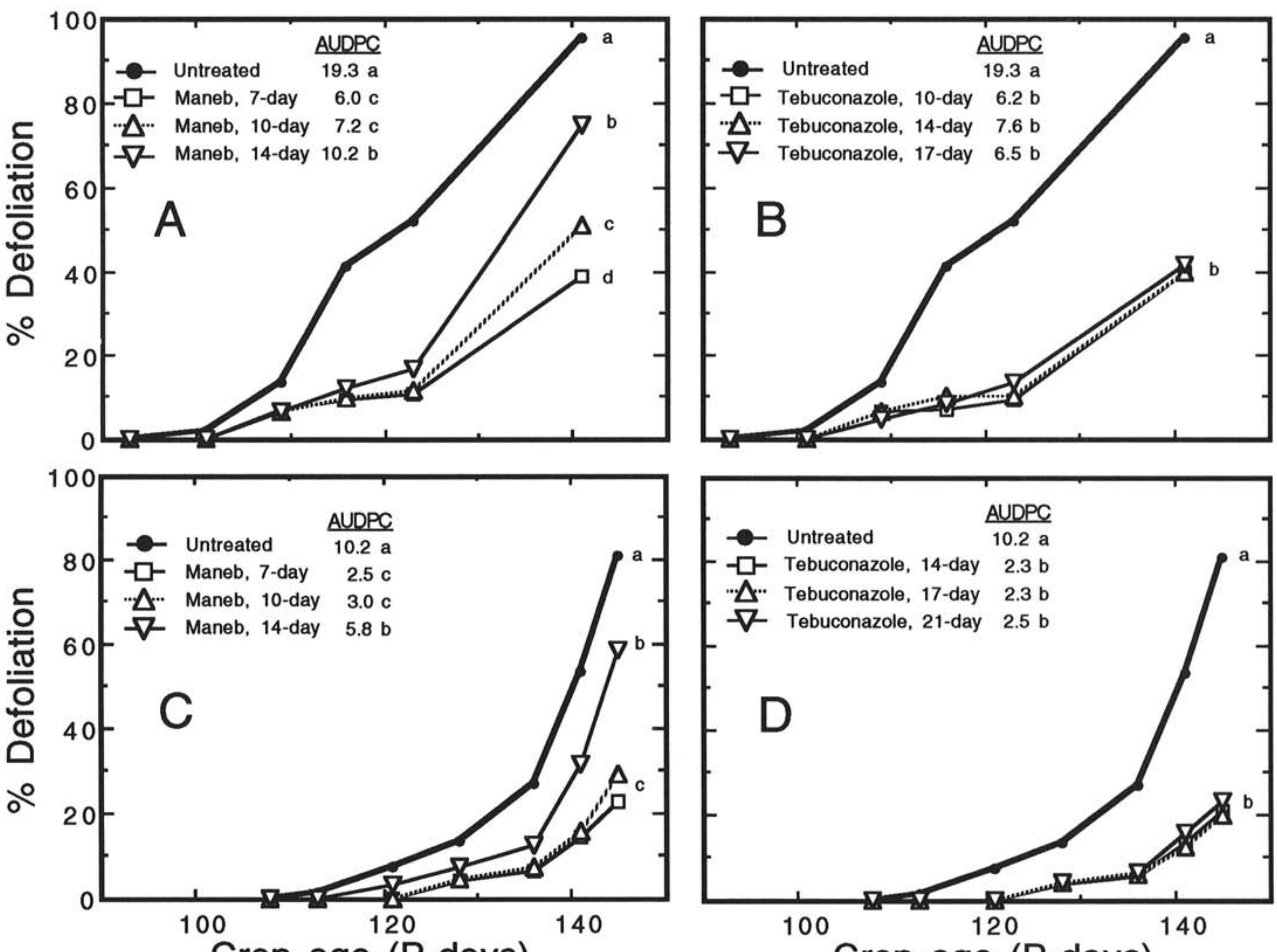

Fig. 3. Effects of fungicide and spraying intervals on Alternaria leaf spot in Pima cotton in **A and B,** 1990 and **C and D,** 1991. **A and C,** Maneb was applied at a rate of 2.0 kg a.i./ha; **B and D,** tebuconazole was applied at a rate of 0.250 kg a.i./ha. P-days = physiological days; AUDPC = area under the defoliation progress curve. AUDPC units are proportion days. Points on the graph (at the last observation date) and AUDPC values followed by different letters differ significantly ($P < 0.05$) according to Duncan's multiple range test.

was examined by means of a multiple regression analysis where the dependent variables were infection incidence or lesion expansion rate and the independent variables were the age of the crop and the age of individual leaves. Since insignificant differences in infection incidence and in lesion expansion rate were found among leaves sampled from main stems in 1990 and 1991 and among leaves sampled from main stems and monopodial branches in 1991, all records were pooled. Of the variance explained by the regression equations (R^2 of 84.7% for infection incidence and 74.6% for lesion expansion rate), the partial regression coefficient for the age of the crop was insignificant ($P = 0.3$), whereas the partial regression coefficient for the age of individual leaves was highly significant ($P < 0.0001$).

Values of infection incidence and lesion expansion rate increased with the increasing age of individual leaves (Fig. 1). The relationship between the age of individual leaves and the infection incidence was used to calculate the value of I_{50} (i.e., the time taken to reach an infection incidence of 50% of the maximum). On the basis of 1990 and 1991 data, the value of I_{50} was 20.9 P-days. At any sampling date, the fraction of plant biomass at or above the age of 20.9 P-days was considered susceptible leaf tissue. The proportion of susceptible leaf tissue was calculated for each sampling date in both seasons. This proportion increased linearly with the physiological age of the crop, reaching 100% (i.e., all leaf tissue susceptible according to the definition) at 108 P-days after emergence (Fig. 2).

Defoliation induced by Alternaria leaf spot developed slowly at first (Fig. 3). Defoliation in the untreated plots started to increase approximately 100–110 P-days after emergence and by the end of the season had reached 95% in 1990 and 81% in 1991 (Fig. 3). The percentage of defoliation in all fungicide-treated plots was significantly lower compared to the percentage of defoliation in untreated plots throughout the entire growing season. In maneb-treated plots, the effects of the different spray intervals on defoliation were insignificant initially, but the differences increased in proportion to the frequency of maneb applications (Fig. 3). With tebuconazole, defoliation levels were similar for all treatments and were equivalent to the defoliation level in the treatment in which maneb was applied weekly. Similar trends were observed when disease intensity was expressed in terms of AUDPC values (Fig. 3). No other foliar diseases were observed in either trial throughout the entire growing seasons.

Relative to the efficacy of the 7-day maneb treatment, efficacy of 10- and 14-day maneb treatments decreased with crop age (Fig. 4A). The decline commenced earlier and was more pronounced in the 14-day treatment than in the 10-day treatment. In contrast, relative control efficacy was close to 100% throughout the entire growing seasons for all tebuconazole treatments (Fig. 4B).

DISCUSSION

The literature contains contradictory reports on the relative influence of crop age and the age of individual leaves on potato and cotton response to Alternaria. While some authors (e.g., 6,10,11) claim that the age of the crop is less important, others (e.g., 13,16) have found it to be a major factor. In the present study, both infection incidence and lesion expansion rate increased with the age of individual leaves (Fig. 3) while the effect of crop age was negligible. Growth of cotton is managed by careful adjustment of fertilization and irrigation levels (9). As the crop approaches the reproductive phase (45–50 P-days after emergence), the production of new leaves gradually diminishes. Approximately 100 P-days after emergence, the initiation of new leaves ceases almost entirely. We believe that this higher susceptibility of the crop to leaf spot (Fig. 2) results from the aging of the leaves attached to the plants.

The systemic fungicide tebuconazole was more effective than the protectant fungicide maneb in the suppression of Alternaria leaf spot. Its superior efficacy was manifested by a longer period of effectiveness. The disease suppression achieved by the application of tebuconazole in a 17- (in 1990) or 21-day (in 1991) schedule was equivalent to that achieved by maneb applied weekly (Figs. 3 and 4). The activity of systemic fungicides within the leaf tissue may cause them to be less subjected to weathering than are protectant fungicides (3). The effects of maneb on *A. macrospora* were related to changes in crop resistance with age. Applications of the fungicide at times when the crop was relatively resistant were more effective than applications when the crop was susceptible.

Results of this and previous studies (21,22,25) have enabled us to develop concepts for the integration of age-related resistance and fungicide in the management of Alternaria leaf spot in order to reduce fungicide use. Applications of fungicides are not needed in immature cotton plants because they are relatively resistant. Accordingly, sprayings should be initiated only when host response to Alternaria shifts toward increased susceptibility, i.e., upon flowering (21). The frequency of subsequent sprayings should be determined by further changes in the age-related resistance and the efficacy of the fungicide. Consequently, protectant fungicides should be applied initially at relatively long intervals, which will shorten as the crop ages. Toward the end of the season, more effective control by means of a systemic fungicide is recommended. This approach was examined recently for the Alternaria-cotton and Alternaria-potato pathosystems with appreciable success (Shtienberg, *unpublished*). Elements of these concepts have been utilized, in part, in the management of potato early blight in Wisconsin (25) and may be implemented in other pathosystems resulting in improved disease control and reduced pesticide use.

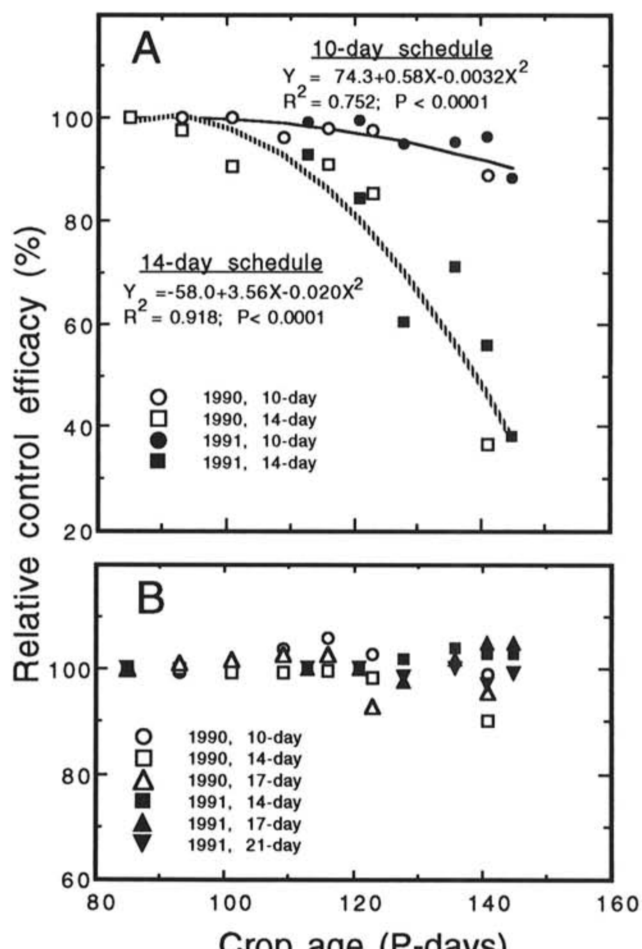

Fig. 4. Changes in relative control efficacy with crop age for **A,** maneb and **B,** tebuconazole applied at different spraying intervals. Values of relative control efficacy indicate the level of disease suppression achieved by the different treatments relative to that of maneb applied weekly. Maneb was applied at a rate of 2.0 kg a.i./ha and tebuconazole at 0.250 kg a.i./ha. P-days = physiological days.

LITERATURE CITED

1. Bashi, E., Rotem, J., Pinnschmidt, H., and Kranz, J. 1983. Influence of controlled environment and age on development of Alternaria macrospora and on shedding of leaves in cotton. Phytopathology 73:1145-1147.
2. Bashi, E., Sachs, Y., and Rotem, J. 1983. Relationship between disease and yield in cotton fields affected by *Alternaria macrospora*. Phytoparasitica 11:89-97.
3. Edgington, L. V., Martin, R. A., Bruin, G. C., and Parsons, I. M. 1980. Systemic fungicides: A perspective after 10 years. Plant Dis. 64:19-23.
4. Ellis, M. B., and Holiday, B. P. 1970. *Alternaria macrospora*. No. 246 in: Descriptions of Pathogenic Fungi and Bacteria. Commonw. Mycol. Instit., Kew, England.
5. Ephrath, J. E., Shtienberg, D., Dreishpoun, J., Dinoor, A., and Marani, A. 1989. *Alternaria alternata* in cotton (*Gossypium hirsutum*) cv. Acala: Effects on gas exchange, yield components and yield accumulation. Neth. J. Plant Pathol. 95:157-166.
6. Johnson, K. B., and Teng, P. S. 1990. Coupling a disease progress model for early blight to a model of potato growth. Phytopathology 80:416-425.
7. Kremer, Y., Shtienberg, D., and Dinoor, A. 1992. Alternaria leaf spot in Pima cotton: Development of a model for timing sprays during the growing season. (Abstr.) Phytoparasitica 20:237.
8. Marani, A. 1979. Growth rate of cotton bolls and their components. Field Crops Res. 2:169-175.
9. Munro, M. J. 1987. Cotton. Longman Scientific & Technical, New York.
10. Pelletier, J. R., and Fry, W. E. 1989. Characterization of resistance to early blight in three potato cultivars: Incubation period, lesion expansion rate, and spore production. Phytopathology 79:511-517.
11. Pelletier, J. R., and Fry, W. E. 1990. Characterization of resistance to early blight in three potato cultivars: Receptivity. Phytopathology 80:361-366.
12. Pound, G. S. 1951. Effect of air temperature on incidence and development of the early blight disease of tomato. Phytopathology 41:127-135.
13. Pscheidt, J. W. 1985. Epidemiology and control of potato early blight, caused by *Alternaria solani*. Ph.D. thesis. University of Wisconsin, Madison.
14. Rotem, J. 1981. Fungal diseases of potato and tomato in the Negev desert. Plant Dis. 65:315-318.
15. Rotem, J., Blickle, W., and Kranz, J. 1989. Effect of environment and host on sporulation of *Alternaria macrospora* in cotton. Phytopathology 79:263-266.
16. Rotem, J., Drepper, W., Bashi, E., and Kranz, J. 1990. The effect of age, nutrition and soil moisture on predisposition of cotton plants to infection by *Alternaria macrospora*. Phytopathol. Mediterr. 29:19-24.
17. Rotem, J., Eidt, J., Wendt, U., and Kranz, J. 1988. Relative effects of *Alternaria alternata* and *A. macrospora* on cotton crops in Israel. Plant Pathol. 37:16-19.
18. Sachs, Y. 1992. A trial to control *Alternaria* in Pima cotton. (Abstr.) Phytoparasitica 20:238.
19. Shaner, G., and Finney, R. E. 1977. The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. Phytopathology 67:1051-1056.
20. Shtienberg, D. 1991. Effects of leaf susceptibility and fungicide seed treatment on leaf spot of Pima cotton caused by *Alternaria macrospora*. Plant Pathol. 40:415-421.
21. 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.
22. Shtienberg, D., and Dreishpoun, J. 1991. Suppression of Alternaria leaf spot in Pima cotton by systemic fungicides. Crop Prot. 10:381-385.
23. Sinden, S. L., Goth, R. W., and O'Brien, M. J. 1973. Effect of potato alkaloids on the growth of *Alternaria solani* and their possible role as resistance factors in potatoes. Phytopathology 63:303-307.
24. Spross-Blickle, B., Rotem, J., Lerl, M., and Kranz, J. 1989. The relationship between infection of the cotyledons of *Gossypium barbadense* and *G. hirsutum* with *Alternaria macrospora* and cotyledon abscission. Physiol. Mol. Plant Pathol. 35:293-299.
25. Stevenson, W. R., Pscheidt, J. W., Thielman, D. G., and Shields, E. J. 1986. PDM (Potato Disease Management): A Computer Tool for Potato Disease Management. Version 1.1. University of Wisconsin, Madison.