Application Rates and Spray Intervals of Ergosterol-Biosynthesis Inhibitor Fungicides for Control of Entomosporium Leaf Spot of Photinia

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ABSTRACT

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The ergosterol-biosynthesis inhibitor (EBI) fungicides myclobutanil, tebuconazole, diniconazole, and flusilazole were compared with chlorothalonil and triforine for the control of Entomosporium leaf spot on photinia. Applied weekly, myclobutanil (0.15 g a.i./L) was equal to or better than chlorothalonil (1.35 g a.i./L) for control of leaf spot, and these two fungicides were superior to triforine. Tebuconazole and diniconazole provided good disease control but reduced growth of plants. Application of myclobutanil at 2-wk intervals was efficacious in controlling disease. All of the fungicides tested controlled Entomosporium leaf spot, but only myclobutanil caused no phytotoxicity to photinia.

Additional keywords: red tip

Entomosporium leaf spot of photinia, incited by *Entomosporium mespili* (DC.) Sacc. (= *E. maculatum* Lév., teleomorph *Diplocarpon maculatum* (Atk.) Jorstad), is an important disease of photinia and other members of the subfamily Pomoideae of the Rosaceae (10). Photinia, including *Photinia serrulata* Lindl., *P. glabra* (Thunb.) Maxim. (15), and *Photinia* × *fraseri* cv. Birmingham, are popular landscape plants in the southeastern United States and are extremely susceptible to *E. mespili*. Symptoms of this disease are reddish purple to black spots (approximately 1 cm in diameter) on leaves. Severe disease pressure can cause almost complete defoliation of the plant.

Preventative fungicide treatments are needed in Alabama nurseries to produce marketable plants. Photinia in the landscape must also be protected with fungicides to prevent extensive defoliation and to maintain attractive plants. The Alabama Cooperative Extension Service recommends fungicide applications from budbreak until foliage maturity, on a 7- to 14-day schedule with a 7-day interval recommended during wet weather (4). Protection of plants is particularly critical during periodic growth flushes to prevent infection of expanding leaves by *E. mespili* (1).

Chlorothalonil has proved effective in previous trials against leaf spot of photinia (3,8) and has been widely used by the nursery industry for disease control. Other fungicides registered for leaf spot control on *Photinia* × *fraseri* include mancozeb, triforine, and triadimefon. Of these, triforine and triadimefon are ergosterol-biosynthesis inhibitors (EBI) fungicides that provide leaf spot control equal to that provided by chlorothalonil (3). The objective of these trials was to evaluate the efficacy of the EBI fungicides tebuconazole, myclobutanil, flusilazole, and diniconazole over a range of application rates and treatment schedules for control of leaf spot on photinia. Preliminary reports have been published (5-7,9).

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MATERIALS AND METHODS

During 1987-1989, *Photinia* × *fraseri* cv. Birmingham liners were potted in a pine bark and rice hull compost (3:1, v/v) amended with 3.6 kg of dolomitic limestone, 1.2 kg of gypsum, 0.9 kg of Micromax (Sierra Chemical, Milpitas, CA), and 8.3 kg of N-P-K (8-3.2-6.6) fertilizer per cubic meter of potting medium. Medium averaged pH 6 at the start of the trials. Potted plants were placed outside on oyster shell beds in full sun and spaced on 30.5-cm centers. Plants were watered daily with overhead sprinklers and were pruned periodically. Fungicides were applied to runoff on leaf surfaces with a hand-held CO_2 sprayer. A randomized complete block design with four replicates of three plants per treatment was used. Inoculum was maintained by randomly placing diseased photinia plants among the blocks of test plants. Blocks of plants were rerandomized after each fungicide application. Foliage symptoms were rated visually on specified dates using the Barratt-Horsfall rating system of 1-12, where 1 = 0%, 2 = 0-3%, 3 = 3-6%, 4 = 6-12%, 5 = 12-25%, 6 = 25-50%, 7 = 50-75%, 8 = 75-87%, 9 = 87-94%, 10 = 94-97%, 11 = 97-100%, and 12 = 100% of leaves exhibiting symptoms. Defoliation was not taken into account. Canopy height and width (centimeters) of each plant were recorded for calculation of a growth index (GI) according to common horticultural practice: GI = height + width + width/3 (11).

Fungicide comparisons. During 1987 through 1989, fungicides were compared for control of Entomosporium leaf spot on photinia. Commercially available fungicides were applied at recommended rates while others were applied at rates previously observed to be efficacious (A. K. Hagan, *personal observation*). Treatments each year included: 1) an untreated control; 2) two fungicides commonly used commercially, chlorothalonil (Daconil 2787 4.17F, 1.35 g a.i./L) and triforine (Triforine 1.6E, 0.18 g a.i./L); 3) tebuconazole (Lynx 25W, 0.04 g a.i./L); and 4) myclobutanil (Systhane or Eagle 40W, 0.15 g a.i./L). In 1987, the fungicide flusilazole (Nustar 3.3E, 0.08 g a.i./L) was also included for comparison. All fungicides were applied on a 7-day spray schedule: in 1987 from 15 March to 15 June (14 sprays), in 1988 from 7 April to 28 August (11 sprays), and in 1989 from 9 May to 26 July (12 sprays). Penetrator 3 adjuvant was tank-mixed with tebuconazole, diniconazole, and myclobutanil at 0.06 ml/L of spray volume (0.25%, v/v) as recommended by the manufacturer. Disease ratings were made on 21 May and 22 June 1987; on 11 May, 3 June, 7 July, 14 August, and 19 September 1988; and on 14 June, 26 July, 23 August, and 20 September 1989. Plant dimensions were recorded on 6 September in 1987 and 1988 and on 31 July in 1989, as described above.

Application rate comparisons. Differential rates of fungicides were compared in 1987 and 1988 by applying 0.25, 0.5, 1, and/or 2 times previously stated rates of fungicides. In both years, treatments included myclobutanil applications at 0.075, 0.15, and 0.30 g a.i./L and tebuconazole at 0.02, 0.04, and 0.08 g a.i./L. In 1987, additional treatments were flusilazole applications at 0.04, 0.08, and 0.16 g a.i./L; fungicides were applied weekly. Additional treatments in 1988 were tebuconazole at 0.01 g a.i./L and diniconazole (Spotless 25W) applications at 0.08, 0.15, 0.30, and 0.60 g a.i./L. In 1988, fungicides were applied at 2-wk intervals. Disease ratings and plant dimensions were recorded on the same dates as those presented above.

Application interval comparisons. In studies conducted in 1988 and 1989, differential intervals among fungicide applications were compared for leaf spot control. In 1988, myclobutanil 40W (0.15 g a.i./L) and chlorothalonil 4.17F (1.35 g a.i./L) applications were made on a 1-, 2-, and 4-wk schedule. In 1989, myclobutanil was applied at 0.08, 0.15, and 0.30 g a.i./L on a 1-, 2-, and 4-wk schedule. Fungicide applications were made from 7 April to 28 August 1988 (21 weekly applications) and between 9 May and 26 July 1989 (12 weekly applications). Myclobutanil was tank-mixed with Penetrator 3 adjuvant (0.25%, v/v). Disease ratings and plant dimensions were recorded as previously described on the dates noted above.

Data analysis. Areas under the disease progress curves (AUDPC) were calculated from disease ratings and corrected for 0% disease severity: $AUDPC = \Sigma[(1/2)(y_{i+1} + y_i)(t_{i+1} - t_i)] - h$, where y = the disease rating at time t, i = the day of the assessment from the first date of assessment, and h = correction factor for disease rating '1' being 0% disease (2). AUDPC values were used as comparative measures of disease throughout the season in plots within years. Analysis of variance (ANOVA) was used to determine differences among fungicide treatments in each year of each study. Treatment means were compared with Fisher's protected least significant difference (14). Except where indicated, treatment differences were determined to be significant at the 0.05 level.

In studies involving quantitative factors (fungicide rates and application intervals), initial use of ANOVA was followed by regression analysis (12). Linear regression was used to compare effects on AUDPC and GI due to fungicidal rates or intervals between applications (12). Significance of the model coefficient ($P < 0.10$) was used as a criterion for model acceptance and to show that there were differences among factor levels. Residuals of models were evaluated for model appropriateness. Coefficients of determination (r^2) estimated the proportion of variation in the dependent variable (AUDPC or GI) explained by the independent variable.

RESULTS

Fungicide comparisons. Intensity of Entomosporium leaf spot was severe each year as indicated by disease ratings made in June and AUDPC values on nontreated control plants (Table 1). Weekly applications of all fungicides significantly reduced disease severity compared with the untreated control. However, differences in disease control were observed among fungicides. In 1987 and 1989, chlorothalonil, myclobutanil, and tebuconazole provided significantly ($P < 0.05$) better protection than triforine (Table 1). Little or no leaf spotting was observed with applications of each of these fungicides, as indicated by low disease ratings and AUDPC = 0. Flusilazole was as effective as the better fungicides in 1987. In 1988, triforine and myclobutanil were significantly better than the other fungicides in minimizing AUDPC, e.g., AUDPC < 100 (Table 1). Despite significant reductions in disease intensity compared with the untreated control, triforine was less effective than the other fungicides in two of three years, and light but significant leaf spotting was observed on all triforine-treated plants.

Plants treated weekly with triforine, chlorothalonil, tebuconazole, or flusilazole in 1987 had GI < 50. These GIs were significantly lower ($P < 0.05$) than those of plants treated with myclobutanil (GI = 58.1) or untreated (GI = 55.7) (Table 2). Plants treated with tebuconazole and flusilazole were significantly smaller than untreated plants. In 1988, photinia treated weekly with triforine and chlorothalonil had significantly lower GIs than those of other plants (Table 2). In 1989, plants treated with tebuconazole had lower GIs than untreated plants or plants treated with chlorothalonil (Table 2).

Application rate comparisons. Analysis of variance of 1987 data indicated a significant effect only with the interaction term (fungicide*application rate). Further analyses indicated that differential rates of application of each of the fungicides (myclobutanil, tebuconazole, and flusilazole) did not affect AUDPC. However, significant differences in GI were noted due to both fungicide and application rate. Linear regression showed a significant decrease in GI of plants treated with increasing rates of both tebuconazole ($P < 0.05$) and flusilazole ($P < 0.10$) (*data not shown*). Application rates of 0.04 g a.i./L or more of either of these fungicides, according to regression models, resulted in plants with GI < 44. However, myclobutanil, applied at differential rates, had no effect on GIs of photinia.

In 1988, ANOVA indicated that differential application rates had no effect on AUDPC, but AUDPC was significantly affected by fungicide. Subsequent regression analyses indicated that increasing rates of tebuconazole only, applied at 2-wk intervals, significantly decreased AUDPC (Fig. 1), whereas application rates of myclobutanil and diniconazole did not affect AUDPC. Analysis of variance also indicated a significant effect on GI due to application rate, fungicide, and their interaction. Increasing application rates of each of these fungicides significantly ($P < 0.01$) decreased GI (Fig. 2). Parameters of the model describing the effect of increasing rates of tebuconazole on GI were similar to those of the model from 1987 data. Photinia plants treated with diniconazole, at all application rates, were dwarfed compared with all other plants, as indicated by the low intercept (27.6) of the regression line (Fig. 2).

Application interval comparisons. In the second study in 1988, ANOVA indicated that fungicides, application intervals, and the interaction term (fungicide*interval) had significant effects on AUDPC and GI. Increasing application

Table 1. June disease ratings[x] and areas under disease progress curves (AUDPC) for fungicides applied weekly for control of Entomosporium leaf spot on photinia, 1987–1989

	1987		1988		1989	
Fungicide (g a.i./L)	Disease	AUDPC	Disease	AUDPC	Disease	AUDPC
Untreated control	7.6 a[y]	523.5 a	4.6 a	603.4 a	4.3 a	423.0 a
Triforine (0.18)	1.8 b	124.9 b	1.0 b	20.6 c	2.3 b	192.0 b
Chlorothalonil (1.35)	1.2 c	28.9 c	1.3 b	40.1 bc	1.0 c	0.0 c
Myclobutanil (0.15)	1.0 c	7.5 c	1.0 b	13.5 c	1.0 c	0.0 c
Tebuconazole (0.04)	1.0 c	0.0 c	1.1 b	64.5 b	1.0 c	0.0 c
Flusilazole (0.07)	1.0 c	0.0 c	ND[z]	ND	ND	ND

[x] Disease rating was based on the Barratt-Horsfall system, where 1 = no diseased leaves and 12 = 100% leaves diseased.
[y] Letters in each column, when different, indicate significant ($P < 0.05$) differences among treatments, based on Fisher's protected least significant difference.
[z] Not done (treatments were not applied).

Table 2. Growth indices[x] for photinia treated with weekly applications of fungicides for control of Entomosporium leaf spot, 1987–1989

Fungicide (g a.i./L)	1987	1988	1989
Untreated control	55.7 ab[y]	60.9 a	48.3 a
Triforine (0.18)	49.9 bc	40.7 c	52.9 ab
Chlorothalonil (1.35)	49.6 bc	39.0 c	52.2 a
Myclobutanil (0.15)	58.1 a	57.8 ab	51.8 ab
Tebuconazole (0.04)	37.2 d	51.4 b	36.4 b
Flusilazole (0.07)	43.6 cd	ND[z]	ND

[x] Growth index = height + width + width/3.
[y] Letters in each column, when different, indicate significant ($P < 0.05$) differences among treatments, based on Fisher's protected least significant difference.
[z] Not done (treatments were not applied).

intervals of each fungicide from 1 wk to 4 wk significantly ($P < 0.01$) increased AUDPC in 1988 (Fig. 3A). Models indicated that AUDPC = 100 with 1.2-, 1.9-, and 2.7-wk intervals between applications of tebuconazole, chlorothalonil, and myclobutanil, respectively. Both the intercept and the regression coefficient of the line for myclobutanil, with AUDPC as a dependent variable, were lower than with the other two fungicides. The linear regression model describing the effect of myclobutanil application intervals on GI was not significant, which explained the interaction term of the ANOVA. However, increased time intervals between tebuconazole and chlorothalonil applications significantly increased GIs ($P < 0.10$) of treated plants (Fig. 3B).

Rates and intervals of myclobutanil applications were evaluated in 1989. Rates and intervals between applications of myclobutanil and the interaction of rate*interval were significant on both AUDPC and GI. Growth index was negatively related to fungicide rates ($P < 0.01$) only with weekly applications of myclobutanil (*data not shown*). The model predicted GI > 62 with weekly applications of the highest rate (0.30 g a.i./L) of myclobutanil. In addition, AUDPC was negatively correlated with myclobutanil rate ($P = 0.0160$) only with 1- and 2-wk intervals between applications (*data not shown*). Models estimated little or no disease development with 0.08 g a.i./L (lowest rate) of myclobutanil with either 1- or 2-wk intervals between applications.

DISCUSSION

Chlorothalonil and triforine are currently registered for use on photinia to control Entomosporium leaf spot. These fungicides were tested against newer EBI fungicides that may become available for use on ornamental plants. In 1987, studies included myclobutanil, tebuconazole, and flusilazole. The efficacy for disease control of each of these was comparable to or better than that of chlorothalonil and triforine (Table 1). However, weekly applications of tebuconazole and flusilazole for over 2 mo reduced the GI of photinia plants (Table 2), and flusilazole was not included in subsequent tests.

Reduced GIs of photinia may be due to shortened internodes and result in heavier leaf display. In some situations, this may be desirable, especially when accompanied by decreased disease problems. However, at least one of the fungicides, diniconazole, did have an adverse effect on plants. Plants treated with increasing rates of diniconazole (Fig. 2) were detrimentally stunted. In addition, changes in foliage color and flowering habits occurred when photinia plants were treated with fungicides. These changes could have an effect on the marketability of the plants and most likely resulted from the numerous (>10) fungicide applications that were necessary because of extensive periods of favorable environmental conditions for disease that prevail in Alabama.

In 1987 and 1988, differential rates of fungicides were compared to ascertain that the application rates used were appropriate. In both years, application rates had no effect on efficacy of disease control by myclobutanil, tebuconazole, or diniconazole. However, plants treated with increasing rates of these fungicides had decreasing GIs, with the latter two fungicides causing obviously adverse effects on plant growth.

Weekly applications of fungicides may be undesirable, not only because of the cost of labor and materials but also because of the potential for environmental harm. In 1988 and 1989, the efficacy for control of Entomosporium leaf spot of photinia was compared among 1-, 2- and 4-wk intervals between applications of tebuconazole, myclobutanil, and chlorothalonil. Disease control decreased and GI increased as application intervals increased for all fungicides (Fig. 3). Regression models, however, estimated that 2-wk intervals between applications of myclobutanil resulted in AUDPCs equal to or lower than those resulting with weekly applications of chlorothalonil or tebuconazole (Fig. 3A). In 1989, even the lowest rate of myclobutanil, applied on a 2-wk schedule, provided almost complete disease control. In addition, increasing intervals between myclobutanil applications did not have an effect on GI. In 1989, even high application rates of myclobutanil had no effect on GI with 2- or 4-wk application intervals.

E. mespili appears to survive winter months in the southeastern United States as mycelium in leaves (13). Leaves with

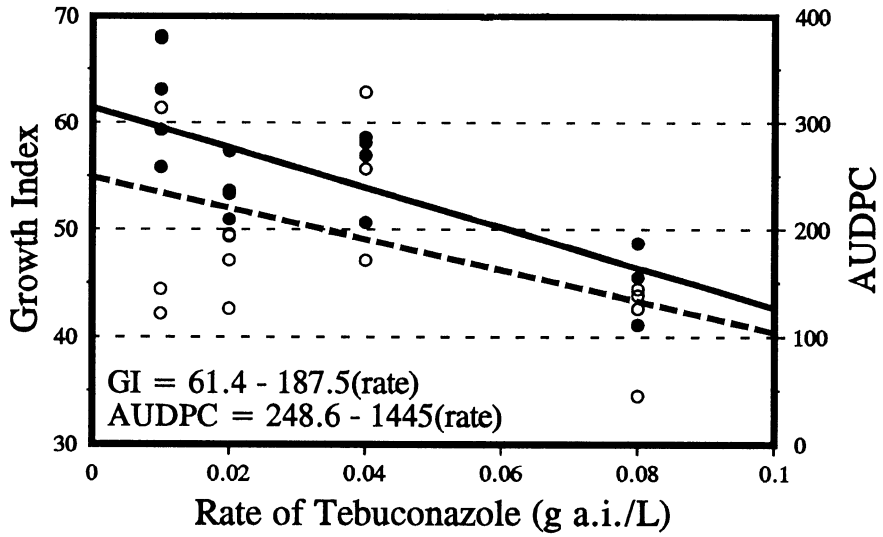

Fig. 1. Growth index (solid line, ●) and AUDPC (dashed line, ○) from plants treated in 1988 with differential rates of tebuconazole. Coefficients of determination (r^2) and significance of regression model (P) were 0.59 and 0.0005, respectively, for growth index and 0.20 and 0.0839, respectively, for AUDPC.

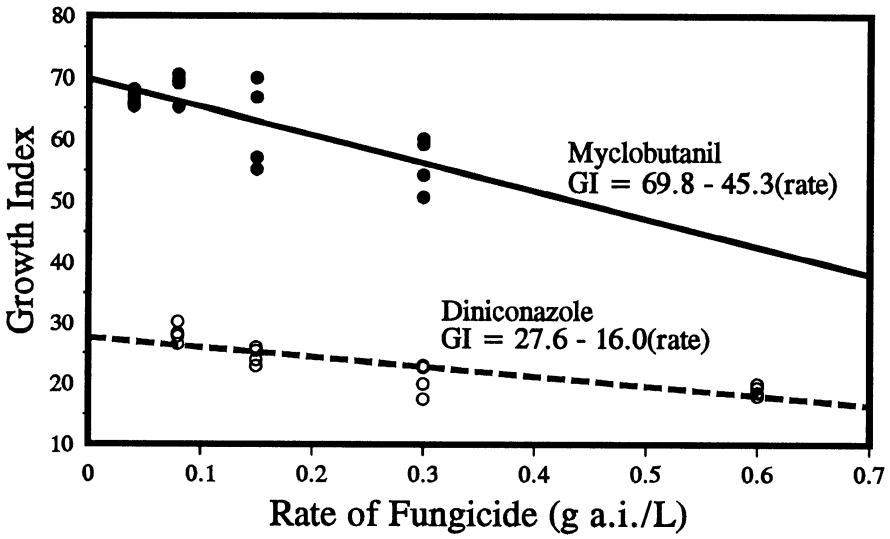

Fig. 2. Growth index of photinia plants treated in 1988 with differential rates of myclobutanil (solid line, ●) and diniconazole (dashed line, ○). Coefficients of determination (r^2) and significance of regression models (P) were 0.56 and 0.0009, respectively, for plants treated with myclobutanil and 0.70 and 0.0001, respectively, for plants treated with diniconazole.

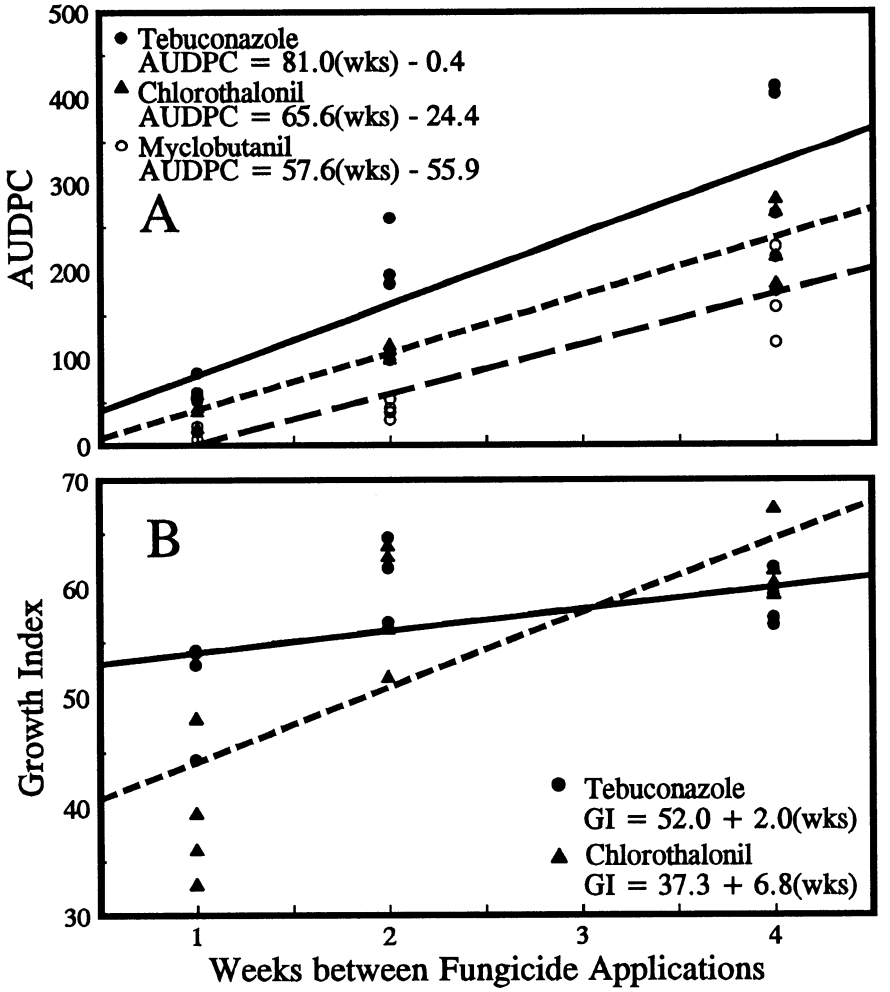

Fig. 3. Effects of 1-, 2-, and 4-wk intervals between fungicide applications on **(A)** AUDPCs and **(B)** growth indices of photinia plants. Coefficients of determination (r^2) and significance of regression models (P) describing effects on AUDPC were >0.68 and <0.01, respectively, for plants treated with myclobutanil, tebuconazole, and diniconazole. Coefficients of determination (r^2) and significance of regression models (P) describing effects on growth index were 0.25 and 0.0975, respectively, for plants treated with tebuconazole and 0.58 and 0.0040, respectively, for plants treated with chlorothalonil.

lesions can be found throughout the year, but conidia are not usually noticed until early April (K. L. Bowen, *personal observation*). In Alabama, wet spring conditions and sporadic rainfall throughout the summer, along with night temperatures below 28 C, allow disease development for up to 5 mo. Thus, more than 12 applications of fungicides may be required when applications are made on a weekly schedule. Fungicides that provide adequate disease control when applied at intervals greater than 1 wk may be preferable. In these studies, lower rates of myclobutanil at 2-wk intervals appeared to be efficacious for controlling Entomosporium leaf spot of photinia without affecting plant growth. This fungicide would provide growers with a viable alternative to chlorothalonil and triforine if registered for use on photinia for control of Entomosporium leaf spot.

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