Management of Euonymus Anthracnose and Fungicide Resistance in *Colletotrichum gloeosporioides* by Alternating or Mixing Fungicides[1]

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Abstract

Fungicidal management of anthracnose leaf spot of euonymus (*Euonymus fortunei* 'Emerald 'n Gold' or 'Emerald Gaiety'), caused by *Colletotrichum gloeosporioides*, was examined. Fungicide resistance was present in the pathogen population. Weekly applications to foliage for 12 weeks to the same plants in 1997 and 1998 of: thiophanate-methyl alone; or thiophanate-methyl, chlorothalonil, ethylene-bis-dithiocarbamate and copper applied sequentially; or mixtures of thiophanate-methyl plus chlorothalonil alternated weekly with thiophanate-methyl plus ethylene-bis-dithiocarbamate were applied. Applications of the same treatments, azoxystrobin, or each of the above fungicides alone were applied at biweekly intervals in 1999. Unsprayed plants served as controls. Percent defoliation was lowest for fungicide mixtures (thiophanate-methyl plus chlorothalonil alternated with thiophanate-methyl plus ethylene-bis-dithiocarbamate) and was highest for unsprayed controls and plants treated with thiophanate-methyl alone. The average number of lesions per 100 leaves was lower for mixed and sequential fungicide programs than for untreated or thiophanate-methyl alone spray programs. Emerald 'n Gold had more lesions per leaf than Emerald Gaiety, but the level of defoliation was greater for Emerald Gaiety. Fungicide-resistant *C. gloeosporioides* isolates, which were recovered on media amended with thiophanate-methyl or with chlorothalonil, were in lowest frequency from plants treated with the mixed fungicide program. Management of euonymus anthracnose and fungicide resistance in *C. gloeosporioides* may be achieved by applying fungicide mixtures with different modes of action.

Index words: azoxystrobin, benzimidazoles, chlorothalonil, copper hydroxide, Daconil, Dithane, Domain, ethylene-bis-dithiocarbamate, *Euonymus fortunei*, iprodione, Heritage, Kocide, resistance management.

Fungicides used in this study: Daconil 2787 (chlorothalonil); Dithane (ethylene-bis-dithiocarbamate); Domain FL (thiophanate-methyl); Heritage 50W (azoxystrobin); Kocide 101 (copper hydroxide) and Zyban (ethylene-bis-dithiocarbamate and thiophanate-methyl).

Significance to the Nursery Industry

Anthracnose of *Euonymus fortunei*, characterized by extensive leaf spotting, stem lesions, and premature leaf drop, has been difficult to control under nursery conditions. This may be due, in part, to fungicide resistance in the causal fungus, *Colletotrichum gloeosporioides*. The results of these experiments indicate that adequate anthracnose control may be achieved by the timely application of fungicides with different modes of action in mixtures or applied in sequence. The use of fungicides in this manner may also slow the development of resistance to thiophanate-methyl in the pathogen population. The combination of sanitation, environmental modification, cutting propagation from uninfected stock, and the use of more efficacious fungicides in mixtures or in sequence may all contribute to the development of effective management programs for euonymus anthracnose.

Introduction

Anthracnose leaf spot and stem blight of *Euonymus fortunei* (Turcz.) Hand.-Mazz., caused by *Colletotrichum gloeosporioides* (Penz.) Penz.&Sacc. in Penz., was first described in 1980 (13). At that time, maneb, mancozeb, and chlorothalonil completely protected plants from anthracnose. Benomyl significantly reduced but did not completely eliminate infection of euonymus by *C. gloeosporioides* (approximately 10% infection compared to the untreated control) (13).

Despite the repeated application of fungicides, the severity of euonymus anthracnose has steadily increased in commercial nurseries over the last ten years and may be associated with reduced sensitivity to benzimidazole and chlorothalonil fungicides *in vitro* (11). Future disease management programs need to contain a strategy to manage fungicide resistance. Two such strategies may include alternating fungicides with different modes of action or mixing fungicides with different modes of action (2).

To determine if these strategies could affect both disease management and fungicide resistance, we tested (i) whether disease incidence could be reduced by the use of fungicides applied in mixtures or in sequence, and (ii) the proportion of fungicide-insensitive euonymus anthracnose isolates that were affected by these different management programs.

Materials and Methods

Euonymus fortunei (cultivars 'Emerald 'n Gold' and 'Emerald Gaiety') plants naturally infected with *Colletotrichum gloeosporioides* were obtained from a commercial nursery and placed on an herbicide-treated area at the Connecticut Agricultural Experiment Station Valley Laboratory in Windsor, Connecticut. Plants were grown in 7.5 liter pots and watered daily with overhead irrigation (approximately 0.7 cm or 0.25 in per day). Plants were fertilized with 12 gm Osmocote (16:6:10) per pot on May 5, 1998, and June 7, 1999.

In 1997 and 1998, twenty plants of each cultivar were each treated weekly for 12 weeks to runoff with one of four fungi-

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cide programs: 1) an untreated control; 2) thiophanate-methyl alone (Domain FL, 865 µg ai/ml (ppm ai), The Scotts Company, Marysville, OH); 3) a sequence consisting of thiophanate-methyl, (865 µg ai/ml), chlorothalonil (Daconil 2787, 900 µg ai/ml, ISK Biosciences Corp., Mentor, OH), ethylene-bis-dithiocarbamate (EBDC) (Dithane T/O, 1,350 µg ai/ml, Rohm and Haas Co., Philadelphia, PA) and copper hydroxide (Kocide 101, 750 µg ai/ml, Griffin Corp., Valdosta, GA); or 4) a mixture of thiophanate-methyl (865 µg ai/ml) plus chlorothalonil (900 µg ai/ml) alternated with a mixture of thiophanate-methyl plus EBDC (Zyban, 231 µg ai/ml thiophanate-methyl and 923 µg ai/ml EBDC, The Scotts Company, Marysville, OH). The sequence of treatments was repeated 3 times, and the mixture treatments were repeated 6 times each for a total of 12 applications.

In 1997, fungicide applications were made on June 13, 20, and 27; July 3, 11, 18, and 25; and August 1, 8, 15, 22, and 29. Plants were rated for percent defoliation, and 100 leaves per treatment per cultivar were arbitrarily sampled to count the number of lesions per leaf on August 7 and September 9, 1997. Euonymus plants were over-wintered in a plastic-covered hoop house from December 1997 to April 1998.

In 1998, fungicide applications were made on June 11, 18, and 26; July 2, 10, 17, 24, and 31; and August 7, 14, 21, and 28. Plants were rated for percent defoliation, and 100 leaves per treatment were arbitrarily sampled to count the number of lesions per leaf on July 1 and September 8, 1998.

Fungicide applications were made on June 7 and 21; July 7, and 19; August 2, 16, and 30; and September 13, 1999. Fungicide treatments consisted of: 1) an untreated control, 2) chlorothalonil alone, 3) thiophanate-methyl alone, 4) EBDC alone, 5) azoxystrobin alone (Heritage 50W 300 µg ai/ml, Zeneca Ag Products, Wilmington, DE), 6) a sequence consisting of chlorothalonil, thiophanate-methyl, azoxystrobin, and ethylene-bis-dithiocarbamate (EBDC), or 7) a mixture of thiophanate-methyl plus EBDC alternated with a mixture of thiophanate-methyl plus chlorothalonil alternated with a mixture of azoxystrobin plus chlorothalonil. There were eight plants per cultivar per treatment. Plants were rated for percent defoliation and 100 leaves per treatment were arbitrarily sampled to count the number of lesions per leaf on October 14, 1999.

Changes in fungicide sensitivity to selected fungicides were determined by changes in fungal recovery on fungicide amended media. On September 9, 1997; September 22, 1998; and December 2, 1999, 0.2 mm square anthracnose lesions were excised from leaves, surface sterilized in 0.5 % NaOCl for 45 seconds, and placed on half-strength PDA or media amended with 1,000 µg ai/ml chlorothalonil or thiophanate-methyl. Lesions from which *C. gloeosporioides* grew at least 1 cm (0.4 inch) into surrounding media were rated as fungicide-resistant. Isolations were attempted from 84 lesions per treatment per fungicide in 1997 and 1999 and 126 lesions per treatment per fungicide in 1998. All data were analyzed by analysis of variance, and means were separated by linear contrasts.

Results and Discussion

By mid summer 1997, typical symptoms of anthracnose were seen on both cultivars of euonymus. Fungicide treatments significantly reduced the number of leaf lesions and percent defoliation in 1997 (Table 1). Thiophanate-methyl alone reduced leaf lesion counts by the first evaluation date in August; however, additional applications of thiophanate-methyl did not further reduce either the number of leaf lesions or defoliation. The four fungicide sequence treatment and the fungicide tank mixture combinations decreased the

Table 1. Number of anthracnose lesions and percent defoliation of *Euonymus fortunei* treated weekly with a single fungicide, a sequence of fungicides or a mixture of fungicides, 1997.

	Number of leaf lesions[x]		Percent defoliation[w]	
Treatment	August 7	Sept 9	August 7	Sept 9
Untreated	5.0	14.4	34.7	55.8
Thiophanate-methyl	3.4	11.7	38.4	52.9
Fungicide sequence[z]	1.5	2.8	12.9	17.2
Fungicide mixture[y]	0.7	1.3	8.9	9.0
ANOVA	Significance[v]			
Treatment	0.0001	0.0001	0.0001	0.0001
Cultivar	0.0001	0.007	NS	0.0001
Treatment × Cultivar	0.0001	NS	NS	NS
Linear contrasts of the means				
Fungicide vs. Control	0.0001	0.0001	0.001	0.0001
Thiophanate vs. Control	0.004	NS	NS	NS
Mixture vs. Sequence	NS	NS	NS	NS

[z]Sequence consisting of thiophanate-methyl, chlorothalonil, ethylene-bis-dithiocarbamate (EBDC), and copper hydroxide.

[y]Mixtures consisting of thiophanate-methyl plus chlorothalonil alternated with a mixture of thiophanate-methyl plus EBDC.

[x]Number of lesions per leaf on 100 leaves per cultivar per treatment.

[w]Percent defoliation of each plant determined by the average of two independent estimates.

[v]Nonsignificant (NS) or significant Analysis of Variance (ANOVA) at P value.

Table 2. Number of anthracnose lesions and percent defoliation of *Euonymus fortunei* treated weekly with a single fungicide, a sequence of fungicides or a mixture of fungicides, 1998.

	Number of leaf lesions[x]		Percent defoliation[w]	
Treatment	July 1	Sept 8	July 1	Sept 8
Untreated	10.0	11.7	33.5	67.0
Thiophanate-methyl	12.8	11.3	58.0	75.2
Fungicide sequence[z]	10.0	8.6	42.3	56.5
Fungicide mixture[y]	1.6	2.3	16.9	21.6
ANOVA	Significance[v]			
Treatment	0.0001	0.0001	0.0001	0.0001
Cultivar	0.0001	NS	0.0001	0.0001
Treatment × Cultivar	0.02	0.02	NS	NS
Linear contrasts of the means				
Fungicide vs. Control	NS	0.0001	NS	0.03
Thiophanate vs. Control	NS	NS	0.004	NS
Mixture vs. Sequence	0.0001	0.0001	0.002	0.0001

[z]Sequence consisting of thiophanate-methyl, chlorothalonil, ethylene-bis-dithiocarbamate (EBDC), and copper hydroxide.

[y]Mixtures consisting of thiophanate-methyl plus chlorothalonil alternated with a mixture of thiophanate-methyl plus EBDC.

[x]Number of lesions per leaf on 100 leaves per cultivar per treatment.

[w]Percent defoliation of each plant determined by the average of two independent estimates.

[v]Nonsignificant (NS) or significant Analysis of Variance (ANOVA) at P value.

Table 3. Effects of fungicide management program on percent recovery of *Colletotrichum gloeosporioides* on PDA media amended with 1,000 ppm of selected fungicides, 1997 and 1998.

Treatment	Unamended	Thiophanate-methyl	Chlorothalonil
Untreated	81.0	77.2	51.7
Thiophanate-methyl	76.9	69.7	38.2
Fungicide sequence[z]	79.3	68.5	67.6
Fungicide mixture[y]	70.5	40.4	24.2
1997	94.9	69.3	45.8
1998	64.9	60.4	45.1
ANOVA		Significance[x]	
Treatment	NS	0.0001	0.0001
Year	0.0001	0.04	NS
Treatment × Year	NS	NS	NS
Linear contrasts of the means			
Fungicide vs. Control	NS	0.001	NS
Thiophanate vs. Control	NS	NS	NS
Mixture vs. Sequence	NS	0.0001	0.0001

[z]Sequence consisting of thiophanate-methyl, chlorothalonil, ethylene-bis-dithiocarbamate (EBDC), and copper hydroxide.

[y]Mixtures consisting of thiophanate-methyl plus chlorothalonil alternated with a mixture of thiophanate-methyl plus EBDC.

[x]Nonsignificant (NS) or significant Analysis of Variance (ANOVA) at P value.

number of leaf lesions and the percent defoliation due to anthracnose.

The overwinter mortality of euonymus was 12.0% for the untreated plants, 18.8% for the thiophanate-methyl treated plants, 2.9% for the fungicide sequence treatment, and 2.7% for the fungicide mixture-treated plants. Due to warm, wet weather in May 1998, new anthracnose leaf lesions appeared prior to the initiation of the spray program. As a result, disease severity was higher earlier in 1998 than in 1997 (Table 2). In 1998, applications of thiophanate-methyl alone did not reduce the level of anthracnose when compared to the unsprayed control. The application of fungicide tank mixtures resulted in significantly lower numbers of leaf lesions and less defoliation when compared to the fungicide sequence program. In both 1997 and 1998, Emerald 'n Gold averaged 7.7 lesions per leaf, while Emerald Gaiety averaged 5.3. However, percent defoliation was higher for Emerald Gaiety than for Emerald 'n Gold (47.7 and 24.9%, respectively).

C. gloeosporioides was recovered from 65 to 95 % of surface sterilized leaf lesions on half-strength PDA (Table 3). Fungicide-resistant isolates were recovered from all fungicide treatments. Euonymus plants that had been treated with mixtures of thiophanate-methyl and either EBDC or chlorothalonil had reduced recovery of thiophanate-methyl-, and chlorothalonil-resistant isolates compared to the other fungicide treatments.

In 1999, the level of anthracnose-induced defoliation was higher on the thiophanate-methyl and untreated plants than for all other treatments, which were not different from each other (Table 4). The number of anthracnose lesions per leaf was lower on plants treated with the fungicide tank mixtures and fungicide sequence program than on plants treated with all other treatments. The highest numbers of lesions occurred on the thiophanate-methyl and chlorothalonil treated plants, which were not different from the untreated controls.

When euonymus anthracnose was first described in 1980, this disease was effectively controlled by chlorothalonil, benzimidazole, or EBDC fungicides (13). However, since that time, the disease has become increasingly difficult to control with fungicides. Control failures may be due to a long term reliance on fungicide applications to control disease while maintaining conducive environmental conditions and propagating from diseased plant material.

C. gloeosporioides has recently been demonstrated to be insensitive to a number of fungicides used to control anthracnose on euonymus (11). Our *in vitro* results (11) indicated that EBDC was the most efficacious single active ingredient fungicide evaluated of the fungicides recommended for management of euonymus anthracnose (5). Chlorothalonil was very effective in preventing spore germination at concentrations as low as 1 µg ai/ml, but effects on hyphal growth *in vitro* were much less (11). Chlorothalonil may be most effective as a protectant fungicide against conidial germination on plant surfaces. Once conidia have germinated and the fungus has infected host tissues, this fungicide may be ineffective in inhibiting further growth or reducing survival. However, different fungicides applied in mixtures or in sequence were more effective than repeated application of a single fungicide. Fungicide mixtures were more effective than fungicides applied in sequence in 1998, with similar trends in 1997 and 1999. The use of thiophanate-methyl plus EBDC fungicides and thiophanate-methyl plus chlorothalonil resulted in superior disease control and reduced the recovery of fungicide-insensitive *C. gloeosporioides* isolates compared to alternating thiophanate-methyl, chlorothalonil, EBDC, and copper hydroxide fungicides.

Fungicide-insensitive isolates of *C. gloeosporioides* were present in the naturally infected euonymus from a commer-

Table 4. Number of anthracnose lesions, percent defoliation and percent thiophanate-methyl resistant isolates from *Euonymus fortunei* treated weekly with single fungicides, a sequence of fungicides or a mixture of fungicides, 1999.

Treatment	Number of lesions[x]	Percent defoliation[w]	Percent T-M resistant[v]
Untreated	6.1	20.3	61.9
Thiophanate-methyl	8.7	46.0	47.6
EBDC	6.7	11.3	47.6
Chlorothalonil	8.1	14.3	40.5
Azoxystrobin	7.7	13.7	50.0
Fungicide sequence[z]	3.9	9.7	42.9
Fungicide mixture[y]	2.3	9.0	31.0
ANOVA		Significance[u]	
Treatment	0.0001	0.0001	0.04
Cultivar	0.0001	NS	NS
Treatment × Cultivar	0.004	NS	NS
Linear contrasts of the means			
Fungicide vs. Control	0.0001	0.001	0.001
Thiophanate vs. Control	NS	NS	NS
Mixture vs. Sequence	NS	NS	NS

[z]Sequence consisting of thiophanate-methyl, chlorothalonil, ethylene-bis-dithiocarbamate (EBDC), and copper hydroxide.

[y]Mixtures consisting of thiophanate-methyl plus chlorothalonil alternated with a mixture of thiophanate-methyl plus EBDC.

[x]Number of lesions per leaf on 100 leaves per cultivar per treatment.

[w]Percent defoliation of each plant determined by the average of two independent estimates.

[v]Percent lesions with *C. gloeosporioides* isolates recovered on media amended with 1000 µg ai/ml thiophanate methyl

[u]Nonsignificant (NS) or significant Analysis of Variance (ANOVA) at P value.

cial nursery used in these experiments. Large numbers of leaf lesions and severe defoliation were correlated with increased recovery of fungicide-insensitive isolates, lending further support to the hypothesis that the development of fungicide insensitivity was associated with recent control failures in commercial nurseries. All fungicide insensitivity in these experiments was naturally occurring. Disease severity and fungicide insensitivity increased over the two years of the experiment under conducive environmental conditions.

Principles for the management of both disease and fungicide insensitivity include reducing fungicide exposure, use of resistant cultivars, sanitation, and alternating or mixing fungicides with different modes of action (2).

Reducing fungicide exposure can be difficult when the infection period is long and multiple fungicide applications are required to produce a marketable crop. Euonymus anthracnose can occur early when conditions are warm and wet, as early as May in Massachusetts, and infection periods may continue into at least September (13). We have observed severe levels of disease in hoop houses in late winter in Connecticut.

Plant resistance to euonymus anthracnose has not been identified. While there were some differences in severity, all cultivars tested in 1980 were susceptible under controlled conditions (13). In the present study, Emerald 'n Gold had increased disease severity compared to Emerald Gaiety, consistent with previous observations (13). However, we observed increased defoliation in Emerald Gaiety compared to Emerald 'n Gold. Mahoney and Tattar (13) did not mention defoliation as a symptom of anthracnose on euonymus. While symptomatic leaves were not the only ones shed, leaves with large numbers of anthracnose lesions were most commonly lost. Symptoms of anthracnose on azalea, also caused by *Colletotrichum*, included both leaf spot and defoliation (18).

Sanitation or crop hygiene is an important component in future disease and fungicide insensitivity management programs. The removal of diseased tissues and modification of environmental conditions such as amount and frequency of overhead irrigation should help reduce the reliance on fungicides for anthracnose control.

Alternating or mixing fungicides with different modes of action has been proposed as a disease and fungicide resistance strategy (2). Mathematical models have indicated that these techniques may delay but not prevent fungicide insensitivity by reducing the growth rate of both sensitive and insensitive isolates of target pathogens or by a reduction in the growth of fungicide-insensitive isolates relative to sensitive isolates (4, 8, 17).

Results of field experiments in other disease systems designed to address this issue have been inconsistent (19). Alternating applications of benomyl and triphenyl tin has delayed the appearance of benomyl-insensitivity in *Cercospora beticola* (7), but the use of captan in mixtures with benomyl or iprodione for one year did not prevent an increase in the incidence of benomyl or iprodione insensitivity in *Botrytis cinerea* on grapes (14). Mixtures of vinclozolin and chlorothalonil increased control over either fungicide applied singly, but did not delay the increase in the percentage of conidia resistant to vinclozolin (21). However, mixtures of fungicides were demonstrated to delay or reduce the development of fungicide insensitivity in turfgrass, tomato, potato, grape, or strawberry (6, 10, 15, 16). In comparison studies, mixtures were superior to alternating fungicides in control of fungicide resistance in cereal powdery mildews (1, 3, 9, 12). Alternatively, the use of fungicide mixtures at approximately half rates was not as effective as alternating full rates for reducing the development of triadimenol insensitivity (20).

If the effects on fungicide resistance are similar for mixtures or fungicides applied in sequence, the use of mixtures may have some advantages. Fungicide mixtures that complement each other in terms of spectrum and mode of action (19) may achieve more effective disease control. Also, mixtures may be packaged together, making application easy. Additionally, mixtures and unrelated fungicides may be applied in sequence over time. In any case, our results indicate that once present, thiophanate-methyl resistance may be fairly stable in the *C. gloeosporioides* population, requiring long-term management of fungicide resistance.

The combination of sanitation, environmental modification, propagation from uninfected stock, and the use of more efficacious fungicides in mixtures or in sequence may all contribute to the development of effective management programs for euonymus anthracnose.

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