Journal of Applied Horticulture, 7(1):16-19, January-June, 2005

Effectiveness of selected fungicides applied with or without surfactant in controlling anthracnose on three cultivars of *Euonymus fortunei*

James T. Cole[a], Janet C. Cole[b], and Kenneth E. Conway[c]

[a]Department of Horticulture, University of Arkansas, Fayetteville, AR 72701, USA,
[b]Department of Horticulture and Landscape Architecture, Oklahoma State University, Stillwater,OK 74078,USA, E-mail: janet.cole@okstate.edu. [c]Department of Entomology and Plant Pathology, Oklahoma State University, Stillwater, OK 74078, USA. E-mail: kenncon@okstate.edu.

Abstract

The effectiveness of six fungicides applied with and without the surfactant Hyper-Active™ in controlling anthracnose caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. on three cultivars of *Euonymus fortunei* (Turcz.) Hand.-Mazz. was evaluated at two locations, Stillwater, OK and Fayetteville, AR. The fungicides tested were mancozeb, copper hydroxide, trifloxystrobin, chlorothalonil, myclobutanil, and azoxystrobin. These fungicides were also incorporated into potato dextrose agar (PDA) to determine the effective concentration to obtain 50% inhibition (EC_{50}) of mycelial growth of *C. gloeosporioides*. In the field, chlorothalonil and mancozeb were the most efficacious of the fungicides tested. Presence or absence of the surfactant Hyper-Active™ in fungicide spray solutions did not affect control of anthracnose symptoms. Cultivars varied in susceptibility to anthracnose. At Fayetteville less anthracnose symptoms appeared on 'Emerald Gaiety' and 'Emerald Surprise' than on 'Emerald 'n Gold'; however, at Stillwater, disease incidence was similar on 'Emerald 'n Gold' and 'Emerald Surprise'. Mycelial growth in culture was most inhibited by the fungicides myclobutanil and trifloxystrobin.

Key words: Chemical control, *Colletotrichum gloeosporioides*, leaf spot, plant disease, stem lesion, *Euonymus fortunei*.

Introduction

Several *Euonymus fortunei* cultivars are used extensively in the landscape industry. Though not a large problem when planted in a landscape, anthracnose on *E. fortunei* has been a significant problem for nursery producers. Nursery losses include infection of rooted cuttings in overwintering plastic houses and defoliation of mature plants during the growing season. The losses for one producer were estimated as $400,000 in 2001 with a range from $200,000 to $500,000 annually (D. Dunn, Greenleaf Nursery, Park Hill, OK, personal communication). Nursery personnel consider 'Emerald Gaiety' and 'Emerald 'n Gold' to be the cultivars most susceptible to anthracnose, but other cultivars also have some disease susceptibility.

Mahoney and Tattar (1980) first isolated the causal organism on leaves and stems of *E. fortunei* plants in New England nurseries. Caused by *Colletotrichum gloeosporioides*, anthracnose symptoms include leaf spots and stem lesions that increase in size over time. During hot and humid conditions leaf spots enlarge to 0.6 to 1.3 cm in diameter and stems are infected followed by leaf abscission causing the plants to appear blighted.

Maneb (manganese ethylenebisdithiocarbamate), mancozeb (a coordination product of zinc ion and manganese ethylenebis-dithiocarbamate), and chlorothalonil (tetrachloroisophthalo-nitrile) were reported to completely protect plants from anthracnose in 1980 (Mahoney and Tattar, 1980). However, producers now report less control with these chemicals using standard fungicide spray schedules. Waller (1992) also reported that fungicide tolerance appeared when benzimidazoles were widely used to control *Colletotrichum* diseases in a number of crop species. Although many newer fungicides have been tested to control anthracnose symptoms (LaMondia, 2001a, 2001b), some recently released fungicides have not been evaluated for effectiveness in controlling *C. gloeosporioides*.

Use of surfactants can enhance pesticide effectiveness. Surfactants are used as activators in many herbicides to improve foliar absorption and ultimate biological activity (Kirkwood, 1993). Effectiveness of systemic fungicides may also be enhanced by using surfactants if foliar absorption is improved. Surfactants affect plant surfaces by solubilizing non-polar active ingredients, dissolving waxes, increasing cuticle penetration, increasing preferential sites of penetration, membrane permeability, and possible systemicity (Kirkwood, 1993).

Hyper-Active™ (Helena Chemical Co., Memphis, TN) is a nonionic spray adjuvant designed to specifically enhance fungicide activity by improving deposition, retention, wetting, and penetration of spray application mixtures (Anonymous, 1996). Improved spray coverage and penetration occurs in both plant and pest surfaces.

The purpose of this research was to determine the efficacy of several fungicides applied with and without Hyper-Active™ in controlling anthracnose in container production of *E. fortunei*. Three cultivars were tested to determine cultivar tolerance to *C. gloeosporioides*. Susceptibility of *C. gloeosporioides* to various fungicides *in vitro* was also investigated.

Materials and methods

Field studies: *Euonymus fortunei* 'Emerald Gaiety', 'Emerald 'n Gold' and 'Emerald Surprise' plants were commercially produced (Greenleaf Nursery Co., Park Hill, OK) in 15.2 cm diameter by 15.2 cm deep plastic pots in media consisting of 5 pine bark : 1 hardwood bark : 1 sand amended with 3 kg m^{-3} dolomitic lime, 890 g m^{-3} 0N-19.8P-0K (triple superphosphate), 255 g m^{-3} 0N-0P-49.8K (KCl), 111 g m^{-3} trace elements (Frit 504 HF, Frit (UK) Ltd., Cambridge, UK), 890 g m^{-3} $FeSO_4$, 742 g m^{-3} 46N-0P-0K (urea), 1.2 kg m^{-3} iron oxide (GU 49, Master Builders, Inc., Cleveland, OH), and 13.8 g a.i. m^{-3} chlorpyrifos (Chlorpyrifos 2.32% Granule, Micro Flo Co., Memphis, TN). Plants were planted in late June 1999 and moved to Fayetteville, AR and Stillwater, OK in early May 2000. Plants were placed under 30% shade (maximum photosynthetic photon flux of 1067 µmol $m^{-2}s^{-1}$ at plant height) at each test site. Plants were top-dressed with approximately 17 g pot^{-1} of 21N-2.2P-8.3K (Scotts Customblend 21-5-10, The Scotts Co., Marysville, OH) 8 May 2000. Overhead sprinkler irrigation applied 1.3 cm water each morning during the growing season. All plants were sheared periodically throughout the growing season based on normal shearing times at Greenleaf Nursery Co. Fungicide treatments began on 9 May 2000 at both sites. Fungicides were applied based on the longest labeled spray interval for each product. Fungicides and water control treatments were applied with and without the surfactant Hyper-Active™ (20% adjuvant). The surfactant rate was 1.25 ml l^{-1}. The following fungicide rates and spray intervals were used: (i) mancozeb (Rainshield NT, Rohm & Haas, Philadelphia, PA) at 1.4 g a.i. l^{-1} every 7 days, (ii) copper hydroxide (Kocide, Griffin L. L. C., Valdosta, GA) at 484 mg a.i. l^{-1} every 7 days, (iii) trifloxystrobin (benzeneacetic acid, (E,E)-alpha-(methoxyimino)-2-[[[[1-[3-trifluoromethyl) phenyl]ethylidene] amino] oxy] methyl], methyl ester, Compass, Bayer Corp., Kansas City, MO) at 150 mg a.i. l^{-1} every 14 days, (iv) chlorothalonil (Echo 720, Sipcam Agro, Roswell, GA) at 2.1 g a.i. l^{-1} every 14 days, (v) myclobutanil (a-butyl-a-(chlorophenyl)-1H-1,2,4 triazole-1-propanenitrile, Eagle, Rohm & Haas, Philadelphia, PA) at 180 mg a.i. l^{-1} every 28 days, or (vi) azoxystrobin (methyl (*E*)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate, Heritage, Syngenta, Richmond, CA) at 150 mg a.i. l^{-1} every 28 days. Foliage was sprayed to runoff in all treatments. Control plants were sprayed to runoff with tap water at 7-day intervals. Fungicide and water treatments were applied with a CO_2-pressurized backpack sprayer with an output of 670 ml min^{-1}.

Plants were visually rated using a modification of an assessment key (Key No. 2.2) for common leafspot of alfalfa (James, 1971) on a scale of 0 to 100 with 0 = no disease and 100 = defoliation at 4-week intervals beginning 9 May 2000. The Stillwater study was terminated on 28 Aug. 2000 while the Fayetteville study was terminated on 26 Sept. 2000.

A split plot design with six replications was used at each site. Fungicide and surfactant treatments were the main plot treatments while cultivar was the subplot treatment. A different person rated each site so data for each site were analyzed separately. All disease ratings were transformed with an arcsine transformation, then statistically analyzed using a General Linear Models Procedure (SAS Institute, Cary, NC). Means of significant main effects and interactions were separated using protected least significant difference (LSD) procedures.

Fungicidal activity *in vitro*: The effect of fungicides on the radial growth rate of *C. gloeosporioides* was compared as described by Kataria *et al.* (1991a,b) and Martin *et al.* (1984). Dilutions of fungicides were prepared by mixing appropriate amounts of each fungicide (based on active ingredient) in sterile water. Appropriate volumes of stock solution containing diluted fungicides were added to sterile, cool PDA (Difco Laboratories, Detroit, MI) to achieve concentrations of 3, 10, 30, 100, 300, and 1000 mg a.i. l^{-1} of the following active ingredients: i) copper hydroxide, ii) trifloxystrobin, iii) chlorothalonil, iv) myclobutanil, or v) azoxystrobin. A zero-concentration (nonamended PDA) treatment was prepared for each fungicide. Fungicide-amended PDA was dispensed aseptically into 9-cm diameter plastic petri dishes (20 ml per dish).

Mycelial plugs (7 mm in diameter) were cut from margins of actively growing cultures of *C. gloeosporioides* and inverted in the centers of fungicide-amended and nonamended PDA plates. Three replicate plates were prepared for each fungicide concentration. Plates were incubated at 25 °C in the dark. Colony diameters were measured in two directions (random and at right angles) and adjusted for the diameter of the plug. Measurements were taken each day for a 7-day period. Percent inhibition was determined using the formula: % inhibition = [(mean nonamended PDA - mean fungicide amended PDA) / mean nonamended PDA] X 100. Percentages were converted to probits and regression lines were prepared for all fungicide concentrations (Fig. 1).

Results

Field Study: Surfactant did not interact with fungicide or cultivar for disease ratings at either site or at any rating date (data not presented). Disease ratings were not affected by presence or absence of surfactant at either site or at any rating date (data not presented).

Disease pressure was lower throughout the growing season at Fayetteville than at Stillwater. The difference in disease severity between the two sites was likely caused by different stress levels. Plants at Fayetteville experienced lower daytime temperatures throughout the growing season than plants at Stillwater (Table 1). Ningen *et al.* (2004) noted less anthracnose symptoms caused by *C. gloeosporioides* on *E. fortunei* with lower night temperatures than with higher night temperatures. At Fayetteville, disease ratings were negligible until September when ratings were greater on 'Emerald 'n Gold' (1.4) than on 'Emerald Gaiety' (0.1) or 'Emerald Surprise' (0.1). No interaction existed between fungicide and cultivar in September.

At Stillwater, 'Emerald Gaiety' had more anthracnose symptoms (disease rating of 2.3) than 'Emerald 'n Gold' (1.5) or 'Emerald Surprise' (0.8) at the start of the study (9 May 2000). A similar trend occurred on 5 June 2000 and 3 July 2000 when the disease ratings on 'Emerald Gaiety' were greater (7.0 and 4.8, respectively) than on 'Emerald 'n Gold' (3.0 and 3.3, respectively) or 'Emerald Surprise' (1.7 and 2.6, respectively). Although statistical differences in disease ratings existed among cultivars on 9 May 2000, 5 June 2000, and 3 July 2000, horticulturally these differences were probably not noticeable since ratings averaged less than 5 for each cultivar on each rating date except 'Emerald Gaiety' on 5 June 2000 (disease rating of 7.0).

Table 1. Average daily high and low temperatures between rating dates for anthracnose on *Euonymus fortunei* at Fayetteville, AR and Stillwater, OK

Location	Rating date	zAverage daily temperature(°C)+SE	
		High	Low
Fayetteville	9 May 2000	—	—
	6 June 2000	26.2 + 0.8	15.6 + 1.0
	3 July 2000	27.1 + 0.5	17.9 + 0.6
	1 Aug. 2000	30.7 + 0.6	20.1 + 0.5
	29 Aug. 2000	35.0 + 0.4	22.0 + 0.4
	26 Sept. 2000	31.4 + 1.1	16.1 + 1.0
Stillwater	8 May 2000	—	—
	5 June 2000	34.2 + 1.4y	16.8 + 0.9y
	3 July 2000	34.8 + 0.7	19.0 + 0.8
	31 July 2000	38.9 + 0.7	20.6 + 0.4
	28 Aug. 2000	41.9 + 0.5	20.4 + 0.4

z Average daily high and low temperatures were calculated by summing the daily high and low temperatures, respectively, from the day after the previous rating date through the rating date shown and dividing by the number of days in the interval between rating dates. SE = Standard error.
y Average daily high and low for 5 June 2000 in Stillwater was calculated using 18 May 2000 as a starting date.

Fungicide interacted with cultivar for disease ratings at Stillwater on 31 July 2000 (Table 2). Disease ratings did not differ among cultivars receiving the water control treatment on 31 July 2000. ‘Emerald ’n Gold’ plants receiving azoxystrobin had higher disease ratings than ‘Emerald Surprise’ plants, but ‘Emerald Gaiety’ plants did not differ in disease ratings from either ‘Emerald’n Gold’ or ‘Emerald Surprise’ plants on 31 July 2000. In contrast, plants of ‘Emerald Gaiety’ and ‘Emerald ’n Gold’ receiving chlorothalonil, copper hydroxide, or mancozeb had higher disease ratings on 31 July 2000 than plants of ‘Emerald Surprise’ receiving the same fungicide treatment. ‘Emerald’n Gold’ plants that received myclobutanil had higher disease ratings than ‘Emerald Gaiety’ or ‘Emerald Surprise’ plants that received myclobutanil. ‘Emerald Gaiety’ plants treated with trifloxystrobin had higher disease ratings than ‘Emerald ’n Gold’ or ‘Emerald Surprise’ plants treated with trifloxystrobin. Although plants in all fungicide treatments regardless of cultivar generally had lower disease ratings than water controls on 31 July 2000, plants treated with chlorothalonil or mancozeb had the lowest disease ratings.

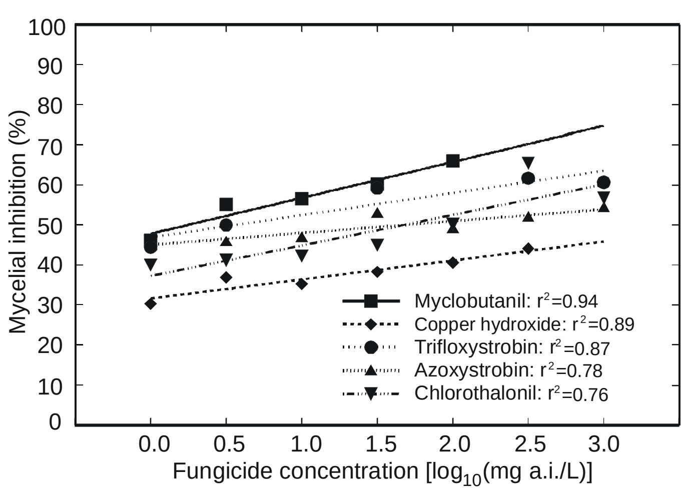

Fig. 1. Inhibition of *Colletotrichum gloeosporioides* grown *in vitro* with various concentrations of fungicides

Table 2. Disease ratings on 31 July 2000 and 28 Aug. 2000 from anthracnose caused by *Colletotrichum gloeosporioides* on three cultivars of *Euonymus fortunei* at Stillwater, OK treated with various fungicides. Data were pooled across surfactant treatments within each site (n=12)

Fungicide/cultivar	Disease ratingz	
	31 July 2000	28 Aug. 2000
Water		
Emerald Gaiety	71.2	95.8
Emerald ’n Gold	74.2	97.5
Emerald Surprise	62.9	95.0
Azoxystrobin		
Emerald Gaiety	64.2	94.2
Emerald ’n Gold	70.8	97.5
Emerald Surprise	53.3	97.5
Chlorothalonil		
Emerald Gaiety	55.0	65.8
Emerald ’n Gold	57.9	81.7
Emerald Surprise	34.6	48.3
Copper hydroxide		
Emerald Gaiety	70.8	80.8
Emerald ’n Gold	66.7	95.8
Emerald Surprise	47.9	73.3
Mancozeb		
Emerald Gaiety	62.1	71.7
Emerald ’n Gold	48.3	73.8
Emerald Surprise	29.6	45.8
Myclobutanil		
Emerald Gaiety	60.8	96.7
Emerald ’n Gold	84.2	100.0
Emerald Surprise	60.0	97.1
Trifloxystrobin		
Emerald Gaiety	69.2	90.8
Emerald ’n Gold	42.9	83.3
Emerald Surprise	31.2	60.8
LSD (P=0.05)		
Cultivar for the same fungicide treatment	16.3	15.1
Cultivar for different fungicide treatments	19.9	16.3

z Disease rating was on a scale of 0 to 100 with 0=no disease and 100 defoliation.

Fungicide interacted with cultivar for anthracnose disease ratings on 28 Aug. 2000 (Table 2). No difference among cultivars occurred on water control plants or those treated with azoxystrobin or myclobutanil. Of plants receiving chlorothalonil, ‘Emerald’n Gold’ had the highest disease ratings on 28 Aug. 2000 while the lowest disease ratings occurred on ‘Emerald Surprise’, and ‘Emerald Gaiety’ disease ratings were intermediate. ‘Emerald ’n Gold’ plants receiving copper hydroxide had higher disease ratings than copper hydroxide-treated ‘Emerald Surprise’ plants, while disease ratings of ‘Emerald Gaiety’ plants treated with copper hydroxide did not differ from either ‘Emerald ’n Gold’ or ‘Emerald Surprise’ plants. Plants of ‘Emerald Gaiety’ and ‘Emerald’n Gold’ treated with mancozeb or trifloxystrobin had higher disease ratings than ‘Emerald Surprise’ plants receiving the same fungicide treatment. Although plants in all fungicide treatments had unacceptably high disease ratings, disease ratings of plants receiving chlorothalonil or mancozeb were much lower than for plants of the same cultivar in the water control treatment.

Fungicidal activity *in vitro*: Of the fungicides tested, myclobutanil was the most inhibitory to fungal growth *in vitro* with an EC_{50} value of 1.7 mg l^{-1} (Fig. 1). Trifloxystrobin had an EC_{50} value of 5.6 mg l^{-1} while copper hydroxide and azoxystrobin had EC_{50} values greater than 1000 mg l^{-1}.

Discussion

LaMondia (2001b) found fungicide resistance in the *C. gloeosporioides* population in Connecticut. Percent defoliation was lowest in fungicide mixtures containing thiophanate methyl (dimethyl 4,4'-o-phenylenebis[3-thioallophanate]) plus cholorothalonil. Chlorothalonil alone was not as effective as when mixed with thiophanate methyl, even though it inhibited conidial germination. Our field evaluations identified two fungicides that were somewhat effective in controlling anthracnose on all *E. fortunei* cultivars tested: chlorothalonil and mancozeb. Fungicides, copper hydroxide and trifloxystrobin reduced anthracnose symptoms compared to water controls on some cultivars but not others, while fungicides, myclobutanil and azoxystrobin, were not effective in controlling anthracnose.

In contrast, in the agar-inhibition studies, myclobutanil was the most inhibitory of the fungicides tested (Fig. 1). Trifloxystrobin was intermediate in inhibition, and copper hydroxide and azoxystrobin showed little inhibition of mycelial growth at the rates tested. LaMondia (2001a,b) had similar conflicting results between his field data and agar plate data. He attributed the differences to two different modes of action. Some fungicides inhibited mycelial growth but not conidia germination. Those fungicides effective in the field inhibited conidial germination and prevented infection while those effective in the agar tests inhibited mycelial growth but might not prevent infection in the field. Chlorothalonil was the most effective in preventing spore germination at concentrations as low as 10 mg a.i. l^{-1} but mycelial growth of the pathogen was less sensitive to higher concentrations of that fungicide. He concluded that management programs should be developed to manage this disease, perhaps by mixing or alternating fungicides with different modes of action and nonchemical control tactics such as sanitation and environmental modifications. Results of research by Koelsch *et al.* (1995) support this conclusion since mixtures of thiophanate methyl and mancozeb were more effective at inhibiting growth *in vitro* of *C. gloeosporioides* isolated from *E. fortunei* than either fungicide alone.

No fungicide tested in this study provided acceptable control of anthracnose symptoms. Addition of the surfactant Hyper-Active™ to fungicide spray solutions did not affect performance of the fungicides compared to fungicide solutions without Hyper-Active™. Mixing fungicides that decrease anthracnose symptoms, such as chlorothalonil and mancozeb, may provide more satisfactory control of *C. gloeosporioides* during production of *E. fortunei* than applying those same fungicides alone. Similarly, rotations of fungicides that individually provide partial control of anthracnose symptoms may help producers better manage disease symptoms to provide quality plants to consumers. This research confirms previous observations that 'Emerald Surprise' is more tolerant of *C. gloeosporioides* than 'Emerald Gaiety' and 'Emerald 'n Gold'. Production of cultivars more tolerant of *C. gloeosporioides* such as 'Emerald Surprise' may help producers to better manage disease symptoms.

Acknowledgements

The authors appreciate the donation of fungicides from the various manufacturers. Partial funding and plant material was provided by Greenleaf Nursery Co., Park Hill, OK. We thank Peggy Reed, former technician, Department of Horticulture and Landscape Architecture, Oklahoma State Univ., for assistance with the laboratory portions of this research. This research was supported under projects ARK01883 and OKL02324. Approved for publication by the directors of the Arkansas and Oklahoma Agricultural Experiment Stations.

References

Anonymous, 1996. Hyper-Active™ [sic] adjuvant for insecticide, miticide, and fungicide sprays. Specimen Label. Helena Chemical Company, Memphis, TN.

James, W.C. 1971. *A Manual of Assessment Keys for Plant Diseases*. Can. Dept. Agr. Publ. No. 1458. Amer. Phytopathol. Soc., St. Paul, MN.

Kataria, H.R., U. Hugelshofer and U. Gisi, 1991a. Sensitivity of *Rhizoctonia* species to different fungicides. *Plant Pathol.,* 40: 203-211.

Kataria, H.R., P.R. Verma and U. Gisi, 1991b. Variability of the sensitivity of *Rhizoctonia solani* anastomosis groups to fungicides. *J. Phytopathol.,* 133: 121-133.

Kirkwood, R.C. 1993. Use and mode of action of adjuvants for herbicides: A review of some current work. *Pesticide Sci.,* 38: 93-102.

Koelsch, M.C., J.C. Cole and S.L. von Broembsen, 1995. Effectiveness of selected fungicides in controlling foliar diseases of common periwinkle (*Vinca minor* L.). *HortSci.,* 30: 554-557.

LaMondia, J.A., 2001a. Management of euonymus anthracnose and fungicide resistance in *Colletotrichum gloeosporioides* by alternating or mixing fungicides. *J. Environ. Hort.,* 19: 51-55.

LaMondia, J.A. 2001b. Resistance of euonymus anthracnose pathogen, *Colletotrichum gloeosporioides*, to selected fungicides. *J. Environ. Hort.,* 19: 47-50.

Mahoney, M.J. and T.A. Tattar, 1980. Identification, etiology and control of *Euonymus fortunei* anthracnose caused by *Colletotrichum gloeosporioides*. *Plant Dis.,* 64: 854-856.

Martin, S.B., L.T. Lucas and C.L. Campbell, 1984. Comparative sensitivity of *Rhizoctonia solani* and rhizoctonia-like fungi to selected fungicides *in vitro*. *Phytopathology,* 74: 778-781.

Ningen, S.S., J.C. Cole and K.E. Conway, 2004. Cultivar and night temperature affect severity of anthracnose on *Euonymus fortunei*. *HortSci.,* 39: 230-231.

Waller, J.M. 1992. *Colletotrichum* diseases of perennial and other cash crops. *In*: J.A. Bailey and M J. Jeger (eds.). *Colletotrichum*: *Biology, Pathology and Control.* CAB Intl. Wallingford, UK, p. 167-185.