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# Resistance of the Euonymus Anthracnose Pathogen, *Colletotrichum gloeosporioides*, to Selected Fungicides[1]

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## Abstract

Fifty-five isolates of *C. gloeosporioides* recovered from euonymus leaf spot and stem lesions from four nurseries in Connecticut were tested *in vitro* for fungicide resistance. Commercially formulated fungicides were added to potato dextrose agar at 0, 1, 10, 100, and 1,000 µg ai/ml, and the regression of normalized growth rate was used to determine the $EC_{50}$. Isolates exhibited a range of sensitivity to benzimidazoles, chlorothalonil, and iprodione. Resistance to multiple fungicides was common, and fungicide resistant isolates were recovered from all nurseries. Forty-four of forty-eight isolates were resistant to thiophanate-methyl; twenty-nine had $EC_{50}$ values over 1,000 µg ai/ml. Thirty-four isolates had $EC_{50}$ values greater than 1,000 µg ai/ml chlorothalonil; only eight were less than 100 µg ai/ml. Only two isolates of *C. gloeosporioides* were sensitive to 10 µg ai/ml iprodione. Over 91% of isolates had $EC_{50}$ values of less than 500 µg ai/ml for copper hydroxide, but the growth of four was not totally inhibited by concentrations of 1,000 µg ai/ml. All isolates tested had $EC_{50}$ values less than 500 µg ai/ml ethylene-bis-dithiocarbamate (EBDC), and 94.5% were sensitive to less than 100 µg ai/ml of this fungicide. Inhibition zones developed only around disks amended with 10,000 µg ai/ml EBDC. Conidia in contact with disks amended with 10,000 µg ai/ml EBDC for 48 hr did not germinate. After transfer to unamended media, fungal growth was inhibited after contact with 10,000 µg ai/ml thiophanate-methyl or iprodione, but not with copper hydroxide. Management programs for euonymus anthracnose need to be developed to control both the disease and the selection of fungicide resistant isolates of *C. gloeosporioides*.

**Index words:** benzimidazoles, Chipco 26019, chlorothalonil, copper hydroxide, Daconil, Dithane, Domain, ethylene-bis-dithiocarbamate, iprodione, Kocide.

**Fungicides used in this study:** Chipco 26019 (iprodione); Daconil 2787 (chlorothalonil); Dithane (ethylene-bis-dithiocarbamate); Domain FL (thiophanate-methyl); Kocide 101 (copper hydroxide).

### Significance to the Nursery Industry

The severity of leaf and stem anthracnose of *Euonymus fortunei* has steadily increased in recent years. These experiments demonstrate that the anthracnose pathogen, *Colletotrichum gloeosporioides*, is resistant to iprodione and benzimidazole fungicides. In addition, isolates vary in hyphal sensitivity to chlorothalonil. This information will be important to develop effective anthracnose management programs in euonymus and to avoid selection of fungicide-resistant isolates of the causal fungus, *C. gloeosporioides*.

### Introduction

Anthracnose is a common disease of a number of shrubs and trees (8). Disease control is commonly achieved with fungicide application (3). In 1980, *Colletotrichum gloeosporioides* (Penz.) Penz.&Sacc. in Penz. was first reported causing a leaf and stem anthracnose disease of *Euonymus fortunei* (Turcz.) Hand.-Mazz. in 1980 (5). At that time, several fungicides were evaluated for efficacy. Maneb, mancozeb, and chlorothalonil effectively protected plants from infection (5). When compared to the untreated control, benomyl significantly reduced but did not completely eliminate infection.

Despite repeated applications of copper fungicides, thiophanate-methyl, chlorothalonil and the EBDC fungicides, the severity of anthracnose on euonymus has steadily increased under commercial nursery conditions over the last decade. Occasional control failures with fungicides have occurred in Connecticut nurseries; however, it is not known whether these failures were the result of fungicide resistance or other factors such as weather, application problems, or poor timing of sprays in relation to infection.

The objectives of this research are (i) to determine if fungicide resistance exists in commercial nurseries and (ii) to determine the level of sensitivity of *C. gloeosporioides* to fungicides that may be used to manage anthracnose.

### Materials and Methods

Isolates of *C. gloeosporioides* were recovered from leaf and stem lesions on euonymus that were collected from four commercial nurseries in Connecticut. *C. gloeosporioides* was isolated from surface sterilized lesions (0.5% NaOCl for one minute) on 15% water agar. Single spored isolates were transferred to potato dextrose agar (PDA, Difco) and maintained on one-half strength PDA.

Up to 55 isolates were tested *in vitro* for fungicide sensitivity to benzimidazoles, benomyl (Benlate 50 WP, DuPont Ag. Products, Wilmington, DE), thiophanate-methyl (Cleary's 3336 WP, W. A. Cleary Chemical Corp., Somerset, NJ; or Domain FL, The Scotts Company, Marysville, OH), chlorothalonil (Daconil 2787, ISK Biosciences Corp., Mentor, OH), iprodione (Chipco 26019, Rhone-Poulenc, Research Triangle, NC), ethylene-bis-dithiocarbamate (EBDC) (Dithane T/O, Rohm and Haas Co., Philadelphia, PA) and copper hydroxide (Kocide 101, Griffin Corp., Valdosta, GA).

*Media technique*. Commercially formulated fungicides were added to molten, sterile, one-half strength PDA to create stock solutions of 10,000 µg ai/ml (ppm ai). Fungicide concentrations of 0; 1; 10; 100; and 1,000 µg ai/ml were pre-

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pared by dilution of the stock solution into additional sterile molten agar. Media were poured into 15 × 100 mm petri plates and a 2 $mm^2$ plug of mycelium cut from the edge of an actively growing culture on one-half strength PDA was inverted and placed in the center of each fungicide-amended plate. Each of 55 isolates was transferred to two plates of each of five concentrations of four fungicides. Plates were incubated in the dark at 20C (68F). Colony diameter was measured daily from day 3 to day 7, and mean growth rate (mm per day) was calculated. Growth rate was normalized as a percent of isolate growth on unamended media (0 µg ai/ml). Normalized growth rate was regressed against log µg ai/ml fungicide concentration to determine the $EC_{50}$ (µg ai/ml concentration that suppressed fungal growth to one half that of the fungus on unamended media) for each fungicide-isolate combination. Isolates were considered resistant to benzimidazoles and dicarboximides if $EC_{50}$ values were greater than 10 µg ai/ml. This study was conducted twice.

Approximately 450 conidia from each of twelve isolates was streaked over the surface of plates amended with 0, 1, 10, 100, and 1,000 µg ai/ml concentrations of chlorothalonil fungicide to compare conidial germination with hyphal growth. Germinated conidia were counted after 48 hours.

*Paper disk technique*. Conidia of *C. gloeosporioides* were washed from five- to seven-day-old petri dish cultures using sterile distilled water, filtered through sterile cheesecloth, and then $5.0 \times 10^5$ conidia were spread over the surface of solidified half-strength PDA in 9-cm-dia. culture dishes using a bent glass rod. Fungicides were serially diluted with sterile distilled water to concentrations of either 10,000, 100, 10, or 1 µg ai/ml. Sterile 1.0-cm-dia. analytical paper disks were dipped into appropriate suspensions of each fungicide, blotted, and placed onto the surface of the PDA dish seeded with conidia. Agar plates were incubated for 48 hours. Fungal inhibition zones were determined by measuring the distance from the edge of the disk to the edge of fungal growth. All treatments were replicated three times for each of the five fungal isolates selected. To determine if conidia survived after 48 hours contact with the disk, fungicide-amended disks and adhering conidia were lifted from the surface and momentarily touched to the surface of unamended PDA plates. Conidia transferred from the disks to PDA were incubated for 3 to 5 days, and resultant colony growth was evaluated. Colony growth exhibiting robust mycelium that covered the complete area of the disk was rated as (++), growth over an incomplete portion of the disk area was rated (+), and no growth was rated as (–).

## Results and Discussion

*C. gloeosporioides* isolates exhibited a wide range of sensitivity to benzimidazoles, chlorothalonil, and iprodione fungicides (Table 1). Resistance to more than one fungicide was common, and resistant isolates were recovered from all four nurseries. No trend of fungicide resistance was discernible between nurseries. Forty-four of forty-eight isolates were resistant to thiophanate-methyl; twenty-nine had $EC_{50}$ values greater than 1,000 µg ai/ml. Thirty-four of fifty-four isolates had $EC_{50}$ values greater than 1,000 µg ai/ml chlorothalonil, and only eight were sensitive to less than 100 µg ai/ml. Only two isolates were sensitive to less than 10 µg ai/ml iprodione. The majority of isolates had $EC_{50}$ values less than 500 µg ai/ml for copper hydroxide, although four were not sensitive to concentrations of 1,000 µg ai/ml. These isolates continued to grow on media amended with 1,000 µg ai/ml copper hydroxide, although their growth was thinner than that noted on unamended media. All of the isolates tested had $EC_{50}$ values less than 500 µg ai/ml EBDC and EBDC plus thiophanate-methyl fungicides (80 percent ai consists of EBDC). The majority of isolates were sensitive to less than 100 µg ai/ml EBDC.

Although conidial germination on fungicide-amended plates was stopped for all isolates tested by as little as 1 µg ai/ml chlorothalonil, hyphal growth of *C. gloeosporioides* was much less sensitive to higher concentrations of this fungicide.

Zones of inhibition did not develop around analytical paper disks amended with up to 10,000 µg ai/ml of either thiophanate-methyl, copper hydroxide, or iprodione (Table 2). Inhibition zones developed only around disks amended with 10,000 µg ai/ml EBDC. Conidia in contact with disks amended with 10,000 µg ai/ml EBDC for 48 hours prior to transfer to unamended PDA did not germinate. In contrast, growth was inhibited after contact with 10,000 µg ai/ml thiophanate-methyl or iprodione, but was not affected by copper hydroxide.

Fungicide resistance in *C. gloeosporioides* was suspected due to increasing difficulty in management of euonymus anthracnose and the apparent reduction of fungicide efficacy. The extent and level of fungicide resistance observed in this study, however, was unexpected and will affect future decisions concerning the management of euonymus anthracnose in commercial nurseries.

Recommended fungicide concentrations typically applied in commercial nursery settings (volume of 935 liters per ha, 100 gal per acre equivalent) are approximately 750 µg ai/ml copper (Kocide), 865 µg ai/ml thiophanate-methyl (Domain), 900 µg ai/ml chlorothalonil (Daconil), and 1,350 µg ai/ml EBDC (Dithane). A pre-formulated mix of thiophanate-methyl and EBDC (Zyban) which is also recommended, contains approximately 230 µg ai/ml thiophanate-methyl and 925 µg ai/ml EBDC. As concentrations of thiophanate-methyl, chlorothalonil, and copper hydroxide in the spray tank are substantially lower than the levels of insensitivity reported in the present study (e.g. 10,000 µg ai/ml), fungicide resistance may be a factor associated with recent difficulties in controlling euonymus anthracnose.

While no previous baseline sensitivity had been reported for these fungicides against *C. gloeosporioides* from euonymus, an earlier report indicated that the disease was readily controlled by chlorothalonil, benzimidazole, and EBDC (5). Attempts to assemble baseline fungicide-sensitive euonymus isolates of *C. gloeosporioides* from culture collections, researchers, or commercial nurseries were unsuccessful. Therefore, previous reports of fungicide efficacy in euonymus and, to a lesser extent, other *Colletotrichum* species on other crops were used to help interpret our data in relation to fungicide sensitivity in *C. gloeosporioides*. In 1980, benomyl resulted in approximately 90% control of anthracnose on euonymus. Chlorothalonil and EBDC fungicides completely protected euonymus leaves from infection by *C. gloeosporioides* (5). In addition, copper and EBDC fungicides were also reported to be effective in controlling an anthracnose leaf spot of azalea (9). Other *Colletotrichum* species, such as *C. acutatum* and *C. fragariae*, have previously been reported to be resistant to benzimidazoles (1, 4, 9). In contrast, *C.*

**Table 1. Fungicide sensitivity of isolates of *Colletotrichum gloeosporioides* recovered from *Euonymus fortunei* and grown on fungicide-amended agar for 7 days at 20C.**

| | Thiophanate-methyl | | Chlorothalonil | | Copper hydroxide | | EBDC | | Iprodione | | T-M + EBDC | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Isolate | $EC_{50}$[z] | SE[y] | $EC_{50}$ | SE | $EC_{50}$ | SE | $EC_{50}$ | SE | $EC_{50}$ | SE | $EC_{50}$ | SE |
| 1 | 170.1 | 6.9 | >1,000 | 6.7 | 190.6 | 28.9 | 33.3 | 27.3 | >1,000 | 3.1 | NT[w] | |
| 2 | 464.2 | 2.2 | 391.4 | 9.3 | 206.0 | 30.3 | 27.1 | 25.8 | 393.0 | 5.9 | NT | |
| 3 | NT | | >1,000 | 8.7 | NT | | 35.0 | 28.5 | 250.0 | 4.6 | NT | |
| 4 | NT | | 690.7 | 7.5 | NT | | 32.5 | 28.2 | 300.6 | 8.4 | NT | |
| 5 | >1,000 | 7.7 | 873.3 | 4.8 | 241.8 | 30.8 | 22.8 | 25.7 | 165.0 | 8.1 | NT | |
| 6 | >1,000 | 9.8 | 735.1 | 11.2 | 203.6 | 31.0 | 25.7 | 25.4 | 360.8 | 8.0 | NT | |
| 7 | NT | | >1,000 | 6.7 | NT | | 22.5 | 24.7 | 214.6 | 10.4 | NT | |
| 8 | NT | | >1,000 | 7.0 | NT | | 33.6 | 28.2 | 306.5 | 6.5 | NT | |
| 9 | >1,000 | 6.4 | 129.5 | 13.7 | >1,000 | 22.6 | 30.0 | 25.1 | 211.7 | 6.3 | NT | |
| 10 | >1,000 | 5.8 | 275.0 | 9.7 | 433.8 | 26.6 | 28.8 | 23.8 | 212.1 | 3.9 | NT | |
| 11 | NT | | 211.1 | 9.4 | NT | | 23.5 | 24.0 | 262.4 | 5.8 | NT | |
| 12 | NT | | 5.0 | —[x] | NT | | 22.8 | 22.8 | 202.4 | 10.4 | NT | |
| 13 | 132.6 | 9.6 | 870.4 | 32.7 | 170.3 | 27.5 | 100.2 | 22.4 | 502.4 | 23.9 | 56.2 | 16.2 |
| 14 | >1,000 | 8.8 | >1,000 | — | 455.1 | 24.0 | 26.3 | 22.7 | 309.9 | 19.3 | 56.8 | 16.7 |
| 15 | >1,000 | —[y] | 79.6 | — | >1,000 | 20.9 | 20.8 | 20.8 | >1,000 | 11.7 | 28.0 | 24.6 |
| 16 | 718.5 | 18.6 | >1,000 | — | >1,000 | 12.3 | 71.1 | 9.4 | 1.2 | 25.2 | 76.1 | 16.0 |
| 17 | >1,000 | — | >1,000 | 6.1 | 296.5 | 24.0 | 35.4 | 26.7 | 0.9 | 21.2 | 32.8 | 26.8 |
| 18 | NT | | NT | | 140.4 | 22.0 | 29.3 | 7.2 | NT | | NT | |
| 19 | >1,000 | — | >1,000 | — | 385.2 | 25.0 | 90.2 | 26.7 | 405.1 | 18.5 | 114.6 | 26.8 |
| 20 | >1,000 | 4.2 | 76.6 | — | 291.2 | 26.5 | 60.0 | 28.9 | 164.3 | 16.8 | 161.6 | 29.8 |
| 21 | >1,000 | 3.8 | 73.0 | — | 270.8 | 22.8 | 17.1 | 28.4 | 113.8 | 19.8 | 88.2 | 22.6 |
| 22 | >1,000 | — | 254.5 | — | 394.0 | 23.0 | 35.9 | 23.5 | 287.9 | 15.3 | 130.4 | 26.1 |
| 23 | >1,000 | — | >1,000 | — | 708.2 | 21.3 | 19.0 | 22.5 | 471.4 | 14.6 | 4.6 | 17.9 |
| 24 | >1,000 | — | 72.0 | — | >1,000 | 25.2 | 8.5 | 27.9 | 516.2 | 16.2 | 23.9 | 25.6 |
| 25 | >1,000 | 8.6 | 194.4 | — | 330.7 | 31.9 | 25.4 | 22.0 | 57.1 | 14.3 | 130.2 | 27.0 |
| 26 | >1,000 | — | >1,000 | 4.9 | 182.8 | 18.9 | 31.6 | 22.4 | 336.8 | 9.2 | 30.3 | 28.3 |
| 27 | 30.9 | 5.8 | >1,000 | — | 255.1 | 28.0 | 15.9 | 27.6 | 50.4 | 14.7 | NT | |
| 28 | >1,000 | — | 71.5 | — | 197.4 | 27.3 | 11.6 | 29.4 | 918.7 | 16.2 | 4.6 | 15.6 |
| 29 | 611.7 | 21.2 | 136.6 | — | 321.7 | 29.5 | 29.4 | 33.0 | >1,000 | 23.3 | 28.0 | 26.8 |
| 30 | >1,000 | 8.5 | >1,000 | — | 333.3 | 28.0 | 34.1 | 22.5 | 546.6 | 13.4 | 121.8 | 24.7 |
| 31 | >1,000 | — | >1,000 | — | 407.8 | 29.6 | 27.4 | 23.2 | 784.6 | 19.0 | 25.1 | 24.9 |
| 32 | >1,000 | 3.6 | 32.7 | — | 267.3 | 26.9 | 15.0 | 35.0 | 972.9 | 17.1 | 3.2 | 11.6 |
| 33 | >1,000 | — | 652.2 | 20.1 | 288.3 | 24.7 | 43.4 | 24.5 | 67.8 | 16.0 | 83.0 | 32.8 |
| 34 | >1,000 | — | >1,000 | 9.9 | 447.3 | 24.0 | 29.0 | 23.9 | 266.4 | 18.6 | 4.4 | 16.5 |
| 35 | 667.8 | 12.6 | >1,000 | 4.5 | 200.9 | 28.6 | 44.6 | 23.5 | 102.5 | 11.7 | 28.1 | 22.9 |
| 36 | 7.4 | 11.9 | >1,000 | 9.2 | 207.7 | 28.3 | 102.2 | 28.2 | 146.4 | 16.0 | 13.8 | 16.3 |
| 37 | 49.4 | 17.0 | >1,000 | — | 205.2 | 27.9 | 27.2 | 30.4 | 331.4 | 14.8 | 47.4 | 13.4 |
| 38 | >1,000 | 7.6 | >1,000 | 5.7 | 241.4 | 30.1 | 34.0 | 25.5 | 242.2 | — | 37.5 | 15.5 |
| 39 | 52.5 | 11.2 | >1,000 | — | 185.0 | 26.7 | 22.0 | 21.1 | 260.6 | 20.1 | 14.0 | 17.2 |
| 40 | 102.3 | 7.4 | >1,000 | 6.7 | 254.6 | 29.7 | 30.6 | 24.3 | 101.2 | 17.6 | 18.1 | 20.3 |
| 41 | 120.4 | 5.3 | >1,000 | — | 308.6 | 29.0 | 44.4 | 24.0 | 18.6 | 19.6 | 10.5 | 51.5 |
| 42 | 100.1 | 7.8 | >1,000 | 5.2 | 205.2 | 31.2 | 132.1 | 27.2 | 16.2 | 19.1 | 28.5 | 19.4 |
| 43 | 14.2 | 8.9 | >1,000 | 6.8 | 219.0 | 28.4 | 43.3 | 26.3 | 143.7 | 16.2 | 14.7 | 17.9 |
| 44 | >1,000 | 5.0 | >1,000 | 17.7 | 467.0 | 28.6 | 44.5 | 19.9 | 169.7 | 14.6 | 607.4 | 25.2 |
| 45 | >1,000 | — | >1,000 | 8.1 | 244.6 | 25.7 | 245.3 | 27.4 | 256.4 | 11.9 | 137.9 | 25.4 |
| 46 | >1,000 | — | >1,000 | — | 277.2 | 27.1 | 71.8 | 32.3 | 472.5 | 18.9 | 215.2 | 34.4 |
| 47 | >1,000 | 4.4 | 57.0 | — | 778.4 | 29.5 | 51.9 | 25.8 | 93.0 | 13.3 | 649.4 | 25.5 |
| 48 | 180.9 | 8.6 | >1,000 | — | 182.2 | 27.8 | 30.1 | 23.5 | 20.7 | 23.0 | 93.1 | 14.8 |
| 49 | 7.9 | 12.5 | >1,000 | — | 157.2 | 24.8 | 28.1 | 22.9 | 19.7 | 15.6 | 12.0 | 15.1 |
| 50 | 85.2 | 19.6 | >1,000 | — | 231.7 | 30.3 | 28.2 | 22.0 | 663.1 | 14.3 | 31.6 | 22.3 |
| 51 | >1,000 | — | >1,000 | 13.3 | 203.6 | 26.9 | 69.2 | 25.3 | 88.1 | 16.4 | NT | |
| 52 | 625.3 | 14.6 | >1,000 | 6.4 | 352.0 | 29.7 | 21.9 | 18.3 | 939.6 | 11.9 | 28.0 | 24.4 |
| 53 | >1,000 | — | >1,000 | 5.0 | 212.1 | 26.0 | 66.4 | 23.9 | 121.7 | 21.1 | NT | |
| 54 | 7.1 | 15.7 | >1,000 | — | 146.9 | 27.2 | 21.5 | 21.4 | 128.7 | 22.8 | 70.1 | 25.6 |
| 55 | >1,000 | 6.9 | >1,000 | 7.5 | 254.8 | 25.0 | 183.9 | 22.3 | 127.2 | 17.3 | NT | |

[z]$EC_{50}$ is the concentration of fungicide ai (µg/ml) that suppresses the growth rate to half that of the fungus on fungicide-free agar, calculated from the normalized growth rate and log µg/ml fungicide.

[y]Standard error.

[x]Regression not reported, no significant relationship, $EC_{50}$ determined directly from data.

[w]NT = not tested.

*gloeosporioides* isolates from citrus and papaya were sensitive to benzimidazole fungicides (1).

The relative sensitivity to chlorothalonil may vary considerably among pathogenic fungi. While chlorothalonil failed to suppress mycelial growth of *C. gloeosporioides* isolates in these experiments, it was effective against spore germination at only 1 µg ai/ml. Other researchers have demonstrated different isolate sensitivities to chlorothalonil. Burpee (2) noted that there were six-fold differences in $EC_{50}$ between certain isolates of *Sclerotinia homoeocarpa* when tested on

**Table 2. Fungicide sensitivity of isolates of *Colletotrichum gloeosporioides* recovered from *Euonymus fortunei* as determined by inhibition zones around fungicide-amended disks and subsequent growth of conidia in contact with fungicide-amended disks after transfer to unamended media.**

| Concentration (µg/ml) | Fungicide | | | |
|---|---|---|---|---|
| | Thiophanate-methyl | EBDC | Copper hydroxide | Iprodione |
| | Zone of Inhibition (mm)[z] | | | |
| 10,000 | 0.0 | 1.3 | 0.0 | 0.0 |
| 1,000 | 0.0 | 0.0 | 0.0 | 0.0 |
| 10 | 0.0 | 0.0 | 0.0 | 0.0 |
| 0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | Growth after transfer to unamended media[y] | | | |
| 10,000 | + | – | ++ | + |
| 1,000 | ++ | + | ++ | ++ |
| 10 | ++ | ++ | ++ | ++ |
| 0 | ++ | ++ | ++ | ++ |

[z]Inhibition zones were determined by measuring the distance from the edge of the disk to the edge of fungal growth. Data the mean of three replicates of each of five isolates.

[y]After 48 hr, conidia from fungicide-amended disks were momentarily touched to unamended PDA plates and incubated for 3 to 5 days. Strong fungal growth over the complete area of the disk was rated as (++), growth over an incomplete portion was rated (+), and no growth was rated as (–). Data are the mean of three replicates of each of five isolates.

chlorothalonil-amended media *in vitro*. However, the reduced sensitivity seen *in vitro* was not evident in field tests. Chlorothalonil was reported to inhibit spore germination of *C. acutatum* at concentrations of 100 to 500 µg ai/ml, but colony radial growth was reduced by only 37% at 500 µg ai/ml and 45% at 1000 µg ai/ml (4, 6). *Mycosphaerella fijiensis* ascospores were prevented from germination by a four-hour exposure to chlorothalonil (the $EC_{50}$ was estimated to be 0.03 µg ai/ml) (11). Zitter et al. (12) reported that as little as 0.01 µg ai/ml chlorothalonil reduced conidial germination of *Ulocladium cucurbitae in vitro*. Chlorothalonil may also reduce pseudothecia production and lesion expansion by *Mycosphaerella fijiensis*, presumably by arresting hyphal growth in the post-germination phase (10). In general, however, chlorothalonil may be most effective when used as a protectant fungicide to prevent conidial germination on plant surfaces. Once conidia of pathogens with insensitive hyphae such as *C. gloeosporioides* have germinated and infected tissues, this fungicide may be relatively ineffective in inhibiting further growth or reducing survival.

The nursery environment and the techniques used to propagate *Euonymus fortunei* may contribute to increased disease and fungicide resistance. Among these are the close proximity of many potted plants which require daily overhead watering, and production on plastic that can increase splash dispersal and result in high disease incidence. *C. gloeosporioides* overwinters as mycelia in latent lesions in leaves and stems (5). Euonymus is commonly propagated by cuttings taken from field-grown fungicide-treated nursery stock, and these cuttings may often have lesions. Propagation of diseased cuttings or cuttings with latent infections may favor selection for fungicide resistance in a particular isolate over several generations of plants.

The recognition that fungicide resistance is present in isolates of *C. gloeosporioides* from euonymus should result in reduced use of those fungicides in favor of other broad spectrum fungicides that continue to be effective. Future management programs for control of euonymus anthracnose need to be developed that will control the disease and minimize fungicide resistant isolates in a population, perhaps by the use of mixing or alternating fungicides with different modes of action. These management programs need to incorporate the effective use of efficacious fungicides with nonchemical control tactics such as sanitation and environmental modification.

## Literature Cited


1. Adaskaveg, J.E. and R.J. Hartin. 1973. Characterization of *Colletotrichum acutatum* isolates causing anthracnose of almond and peach in California. Phytopathology 87:979–987.

2. Burpee, L.L. 1997. Control of dollar spot of creeping bentgrass caused by an isolate of *Sclerotinia homoeocarpa* resistant to benzimidazole and demethylation-inhibitor fungicides. Plant Disease 81:1259–1263.

3. Childs, R.D., ed. 1997. The 1997 New England Management Recommendations for Insects, Diseases and Weeds of Shade Trees and Woody Ornamentals. University of Massachusetts: Amherst, MA.

4. LaMondia, J.A. 1993. In vitro evaluation of fungicides against *Colletotrichum acutatum* isolates from strawberry. Advances in Strawberry Res. 12:34–37.

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

6. McInnes, T.L., L.L. Black, and J.M. Gatti, Jr. 1992. Fungicides for control of strawberry anthracnose crown rot in summer nurseries. Advances in Strawberry Res. 11:12–16.

7. Moorman, G.W. and R.J. Lease. 1992. Benzimidazole- and dicarboximide-resistant *Botrytis cinerea* from Pennsylvania greenhouses. Plant Disease 76:477–480.

8. Sinclair, W.A., H.H. Lyon, and W.T. Johnson, eds. 1982. Diseases of Trees and Shrubs. Cornell University Press: Ithaca, NY.

9. Stathis, P.D. and A.G. Plakidas. 1958. Anthracnose of azaleas. Phytopathology 48:256–260.

10. Washington, J.R., J. Cruz, F. Lopez, and M. Fajardo. 1998a. Infection studies of *Mycosphaerella fijiensis* on banana and the control of black Sigatoka with chlorothalonil. Plant Disease 82:1185–1190.

11. Washington, J.R., J. Cruz, and M. Fajardo. 1998b. Detection of chlorothalonil in dew water following aerial spray application and its role in the control of black Sigatoka in banana. Plant Disease 82:1191–1198.

12. Zitter, T.A. and L.W. Hsu. 1992. Influence of temperature and fungicide on germination, growth, and virulence of *Ulocladium cucurbitae* on cucumber. Phytopathology 82:358–362.