Identification, Etiology, and Control of *Euonymus fortunei* Anthracnose Caused by *Colletotrichum gloeosporioides*

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ABSTRACT

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A leaf and stem spotting disease on field- and container-grown *Euonymus fortunei* 'Emerald 'n Gold' and 'Gaiety' was found at several nurseries in New England. *Colletotrichum gloeosporioides* was consistently isolated from diseased leaves and stems. Leaf and stem lesions were discrete, circular, dark brown, and 0.5–3.0 mm in diameter with light tan necrotic centers. Initial infection occurred during late May in Massachusetts and repeated infections occurred throughout the growing season. Under laboratory conditions, lesion development was most severe after 24 hr or more of leaf wetness, and the optimum temperature range for vegetative growth of the fungus and spore germination was 25–30 C. All *E. fortunei* cultivars tested under laboratory conditions were susceptible to infection, and maneb, mancozeb, and chlorothalonil completely protected leaves from lesion development.

In 1978 a leaf and stem spotting disease on field- and container-grown *Euonymus fortunei* 'Emerald 'n Gold' and 'Gaiety' was found in several New England nurseries. Discrete, circular, dark brown lesions 0.5–3.0 mm in diameter, with light tan necrotic centers, appeared on upper and lower leaf surfaces (Fig. 1). Leaf lesions initially appeared as light tan spots, but during the later stages of infection, reddish discoloration was noted in the tissue surrounding the lesions, and the necrotic lesion centers often dropped out, creating a "shot-hole" appearance.

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We have consistently isolated *Colletotrichum gloeosporioides* Penz. from diseased *E. fortunei* leaves and stems. Although this imperfect state of *Glomerella cingulata* causes disease on a wide range of plants (4) and is a common secondary parasite in previously weakened plant tissue, there have been no reports of a *Euonymus* disease caused by *C. gloeosporioides*. Heald and Wolf (2) encountered *C. griseum* on leaves and stems of *E. japonicus* Thunb. in Texas. The disease was also reported by Bain (1) in 1931 on *Euonymus* spp. in Alabama. In 1977 a disease complex involving a *Phytophthora* sp., a *Colletotrichum* sp., and poor cultural practices was reported by Peterson et al (3) in New Jersey. In that study, the *Colletotrichum* sp. was reported to be responsible for the leaf lesions.

The objectives of this study were to identify the causal organism and determine its etiology as related to control, determine the susceptibility of several *E. fortunei* cultivars to the disease, and evaluate the effectiveness of

several fungicides in controlling the disease.

MATERIALS AND METHODS

Isolation. Tissue sections containing lesions were excised from *E. fortunei* 'Emerald 'n Gold' leaves and surface-sterilized for 5 min in 0.5% sodium hypochlorite. After soaking, tissue sections were plated on 2% potato-dextrose agar (PDA) and potato carrot agar (PCA) at 20 g of potato and 20 g of carrot per liter.

Field observations. Twenty *E. fortunei* 'Emerald 'n Gold' plants in 1-L plastic containers were placed outdoors in Amherst, MA, on 22 May 1978. Leaves and stems contained numerous lesions from the previous growing season. New growth was examined at 3-day intervals for evidence of new infections. A rain gauge and a recording hygrothermograph were used to monitor precipitation, temperature, and relative humidity in the test plot.

Effect of leaf wetness. Rooted cuttings of *E. fortunei* 'Emerald 'n Gold' were grown in a 1:1:1 peat, sand, perlite mix in 10-cm plastic pots. Recently emerged leaves were inoculated with 2×10^6 spores of *C. gloeosporioides* per milliliter of distilled water. Spores were harvested from 12- to 14-day-old colonies grown on PCAL (PCA with 5% lactic acid added, 10 drops/ml agar). Spore suspensions were sprayed on upper leaf surfaces with a handheld atomizer after the upper leaf surfaces had been swabbed with sterile distilled water.

Immediately after inoculation, plants were placed in a mist/humidity chamber consisting of a 40 × 55 × 35 cm clear polyethylene chamber with a water-saturated 5-cm sand base. The mist source consisted of an electric cool-water humidifier (Northern Co., Waynesboro, MS) in the chamber. The chamber was situated in a darkened incubator at 27 ± 1 C, and mist was applied for 2.5 hr during each 24-hr period. At 6, 12, 18, 24, and 48 hr respectively, inoculated plants were removed and transferred to a greenhouse bench. Five days after inoculation, leaves were harvested, and lesions per leaf were counted with the aid of a dissecting microscope. Individual leaf areas were determined with a Li-Cor Portable Area Meter (Lambda Instruments Corp., Lincoln, NE) and lesions per square centimeter were calculated for each leaf. An average of 33 leaves from four plants was used in each treatment, and each treatment was replicated three times. Ten control leaves were sprayed with sterile distilled water and included in each treatment.

Susceptibility of *E. fortunei* cultivars. In the mist/humidity system of the leaf wetness experiment, recently emerged leaves of the *E. fortunei* cultivars 'Emerald 'n Gold,' 'Emerald Gaiety,' 'Argenteo Marginatus,' 'Sheridan Gold,' and 'Radicans' were swabbed with sterile distilled water and sprayed with a suspension of 2×10^6 spores/ml distilled water. The plants remained in the mist/humidity chamber for 24 hr in darkness at 27 C, after which they were removed to a greenhouse bench. Five days after inoculation, lesions/cm^2 of leaf area were determined. Each treatment had an average of 33 leaves (four plants), and each treatment was replicated twice. Ten control leaves were sprayed with sterile distilled water.

Fungicidal action. The fungicides sprayed on the upper surfaces of recently emerged *E. fortunei* 'Emerald 'n Gold' leaves after swabbing with sterile distilled water were maneb (Maneb WP) 1.7 g/L, benomyl (Benlate 50% WP) 1.3 g/L, mancozeb (Manzate 200 WP) 1.8 g/L, and chlorothalonil (Daconil 2787 75% WP) 2.7 g/L. Three drops of Triogen spreader-sticker were added to each liter of spray material except chlorothalonil.

After the fungicides had dried, 2×10^6 spores/ml of distilled water (harvested from 12- to 14-day-old colonies) were sprayed on the upper surfaces of the newly emerged leaves. Inoculated plants were incubated in the mist/humidity chamber for 24 hr, after which the plants were removed to a greenhouse bench. Five days after inoculation, lesions/cm^2 of leaf area were determined. An average of 34 leaves per treatment was used, and each treatment was replicated twice. Controls consisted of 13 leaves sprayed with 2×10^6 spores/ml of distilled water after swabbing.

RESULTS

Pathogen isolation and identification. The fungus isolates produced concentric rings of acervuli in response to alternating fluorescent light and darkness when grown on PCA. The dark setae within the acervuli were characteristic of a *Colletotrichum* sp., and more recently J.A. von Arx (*personal communication*) further identified the fungus as a form species of *C. gloeosporioides* Penz.

Field observations. Initial leaf lesions were detected on 1 June 1978 on recently emerged *Euonymus* leaves. *C. gloeosporioides* was isolated from infected tissue. Lesions were detected on newly emerged foliage several times during the growing season. In all cases in which new infections were detected, the relative humidity had been greater than 90% for 24 hr during the 4 days before symptom expression, and in all cases, some precipitation occurred during that 4-day period. During 1979, symptom expression initially occurred on 24 May after 72 hr of rain. During April 1979, *C. gloeosporioides* was isolated from a 1978 leaf lesion, and disease symptoms were produced on *E. fortunei* 'Emerald 'n Gold' leaves inoculated with conidia

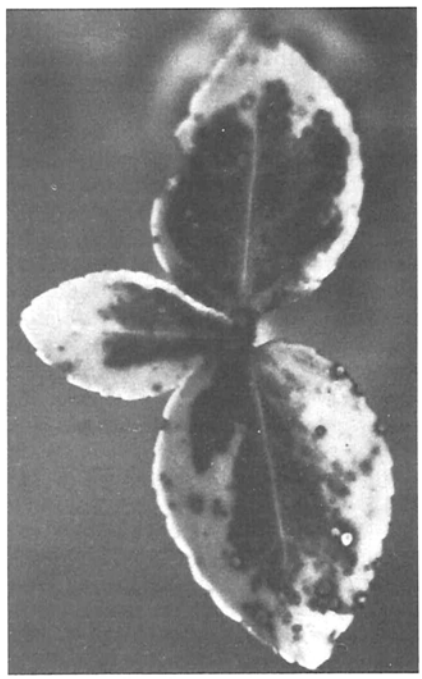

Fig. 1. Symptoms on *Euonymus fortunei* caused by *Colletotrichum gloeosporioides*. Note "shot-hole" appearance.

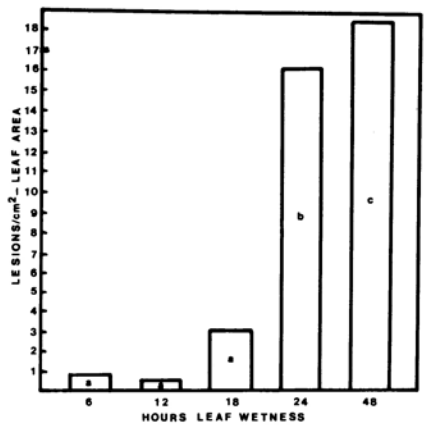

Fig. 2. Effect of leaf wetness on development of symptoms. Bars labeled by the same letter are not significantly different ($P \leq 0.05$).

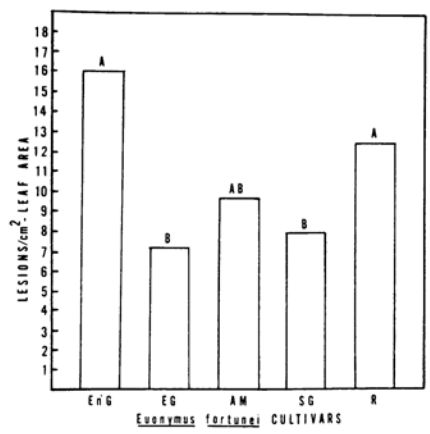

Fig. 3. Susceptibility of *Euonymus fortunei* cultivars to lesions of *Colletotrichum gloeosporioides*. En'G = Emerald 'n Gold, EG = Emerald Gaiety, AM = Argenteo Marginatus, SG = Sheridan Gold, R = Radicans. Bars labeled by the same letter are not significantly different ($P \leq 0.05$).

Table 1. Effect of protective fungicides on *Colletotrichum gloeosporioides* lesion development on *Euonymus fortunei* 'Emerald 'n Gold'[z]

Fungicide	Leaf lesions (no./cm^2)
Maneb	0.0 a
Manzate 200	0.0 a
Daconil 2787	0.0 a
Benlate	1.5 b
Control	13.3 c

[z] Values followed by the same letter are not significantly different, $P \leq 0.05$.

harvested from that isolate.

Effect of leaf wetness. There was a significant increase in symptom development between 18 and 24 hr of leaf wetness and between 24 and 48 hr of leaf wetness, but the 6, 12, and 18 hr leaf wetness regimes did not differ significantly (Fig. 2). In all trials, symptoms developed approximately 48 hr after inoculation, and in all cases symptoms were absent on older leaves.

Susceptibility of *E. fortunei* cultivars. At 24 hr of leaf wetness, symptom development of *E. fortunei* 'Emerald 'n Gold,' 'Emerald Gaiety,' 'Argenteo Marginatus,' 'Sheridan Gold,' and 'Radicans' was 16.0, 7.3, 9.7, 8.0, and 12.6 lesions/cm^2, respectively (Fig. 3).

Fungicidal action. Maneb, mancozeb, and chlorothalonil at recommended rates effectively protected recently emerged *E. fortunei* 'Emerald 'n Gold' leaves from lesion development. Benomyl reduced lesion development but did not completely protect the leaves. Symptoms developed only on benomyl-treated leaves, which had a mean of 1.5 lesions/cm^2 of leaf area (Table 1). All treatments differed significantly from the control (13.3 lesions/cm^2 of leaf area).

DISCUSSION

Our results indicate that the *C. gloeosporioides* responsible for the *Euonymus* disease is a typical anthracnose type disease organism. The fungus can overwinter in previously infected host tissue, most likely as vegetative mycelium, and it appears to depend on periods of precipitation and high relative humidity for spore dispersal, germination, and penetration.

Field observations show that the fungus is capable of repeated infections of new tissue throughout the growing season. These infections were correlated with wet weather conditions. Our results also show that initial infections take place during late May in Massachusetts. Because of severe infections during August, symptomfree foliage was unavailable for further observation, and the final 1978 infection period was not determined.

The optimum temperature range for vegetative growth and spore germination was 25–30 C. The formation of appresoria by germinating *C. gloeosporioides* conidia indicates that a direct cuticle penetration mechanism may be involved in the infection process.

Severity of *C. gloeosporioides* lesion development on *E. fortunei* 'Emerald 'n Gold' appears to be correlated with the period of leaf wetness. Under laboratory conditions, at least 24 hr of leaf wetness was required for maximum infection, at a high spore load (2×10^6/ml) and optimum temperature (27 C). The minimum leaf wetness period may be considerably longer under natural conditions.

E. fortunei anthracnose was observed only on the cultivars 'Emerald 'n Gold' and 'Gaiety' in the field. The results of our experiments show, however, that all cultivars tested were susceptible to lesion development under laboratory conditions and that 'Emerald 'n Gold' and 'Radicans' were most susceptible. Under optimum infection conditions in the laboratory, the pathogen may overcome any resistance present under natural conditions.

Maneb, mancozeb, and chlorothalonil completely protected *E. fortunei* 'Emerald 'n Gold' foliage from *C. gloeosporioides* lesion development. Because the disease is most prevalent during wet weather, control practices must be developed according to local weather conditions. Protective fungicide sprays should be applied during moist weather in May and June, beginning at budbreak, and whenever 24 hr or more of wet weather is forecast during the remainder of the growing season. Where overhead irrigation is used in *Euonymus* production, irrigation should be limited to midday applications to facilitate rapid drying of foliage.

Propagation techniques for *E. fortunei* may accentuate the spread of anthracnose throughout the nursery. Cuttings from infected mother plants result in infected propagation material, and a subsequent increase in *C. gloeosporioides* inoculum occurs throughout the nursery as the infected young plants are lined out or placed in container areas. To reduce the incidence of infected cuttings and to reduce the *C. gloeosporioides* inoculum load, infected leaves and shoots should be removed from plants and destroyed throughout the growing season as well as during winter. Special attention should be paid to keeping mother plants free of disease.

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