

Anthracnose on Wintercreeper Euonymus is not Reduced with Polyethylene Sheeting or Sodium Hypochlorite¹

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Abstract

Wintercreeper euonymus (*Euonymus fortunei* (Turcz.) Hand.-Mazz. 'Emerald 'n Gold') plants were grown in containers on gravel beds that were covered or not covered with black polyethylene sheeting. Half of the beds in each bed treatment were sprayed with a 0.6% sodium hypochlorite (10% bleach) solution immediately after rating plants or performing needed cultural practices such as shearing and weeding (about monthly) to attempt to suppress anthracnose caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. Plants were rated for disease severity monthly from May through September at one site in 2005 and at two sites in 2006. Presence or absence of polyethylene sheeting or sodium hypochlorite did not affect disease ratings. Disease ratings increased curvilinearly as the growing season progressed. In vitro tests showed that sodium hypochlorite did not inhibit mycelia growth of *C. gloeosporioides* but eliminated conidial germination.

Index words: bleach, *Colletotrichum gloeosporioides*, *Euonymus fortunei*, cultural disease control.

Significance to the Nursery Industry

Nursery producers seek management practices that maximize plant quality while minimizing production costs. Wintercreeper euonymus is a desirable groundcover plant, but it is prone to anthracnose. Few fungicides are effective at controlling the causal organism on wintercreeper euonymus. Alternative production methods that interfere with disease development would be desirable. Likewise, knowledge of treatments that are ineffective at reducing disease symptoms can help minimize production costs by eliminating those activities from the management program. Wintercreeper euonymus is often grown in containers placed on gravel-surfaced production blocks. The gravel can harbor plant and substrate debris that serves as a source of inoculum for further infection. Finding ways to reduce the amount of plant and substrate debris in the growing area may reduce an inoculum source thereby reducing disease incidence. This research showed that growing wintercreeper euonymus on black plastic sheeting was not effective at reducing anthracnose symptoms compared to growing on gravel beds. Likewise, spraying with 0.6% sodium hypochlorite to reduce the introduction of inoculum during cultural practices did not provide any anthracnose control.

Introduction

Disease control during nursery production is necessary to maintain crop quality. Wintercreeper euonymus is sus-

ceptible to anthracnose caused by *C. gloeosporioides* (8). Symptoms of anthracnose on this small shrub include leaf and stem lesions, defoliation, and stem dieback. Anthracnose on wintercreeper euonymus has become an increasing concern to commercial nursery producers since fungicides such as mancozeb that once provided adequate control of disease symptoms (13, 14) are no longer as effective (3, 5, 10, 11, 19). Potential crop loss has led to increased efforts to alter management practices to provide optimal conditions for production of a high quality crop.

For plant diseases to develop, a host, a pathogen, and an environment favorable for pathogen development must be present (2). Reducing exposure of the plant host to the pathogen, and creating an unfavorable environment for pathogen development can be strategies for reducing diseases when fungicides are ineffective. Past research has shown that *C. gloeosporioides* requires moisture for spore formation, dispersal, germination, and penetration (13). Environmental factors contributing to growth and spread of *C. gloeosporioides* include leaf wetness (13, 14, 15), high night temperatures (16), high light intensity (17), and high humidity (4, 13, 14).

It is possible to alter the growing environment to make it less favorable for *C. gloeosporioides* development by growing wintercreeper euonymus in shade rather than in full sun (17). Overhead irrigation during early afternoon rather than in the early morning has decreased anthracnose presumably due to the decreased amount of time that moisture remains on the plant leaves (17).

Overhead irrigation is commonly used in nursery production because it is inexpensive to install and maintain compared to drip irrigation (6). Use of overhead irrigation may, however, contribute to disease spread through the splashing dispersal of *C. gloeosporioides* conidia that may be present in plant debris on production blocks. Davidson et al. (6) recommended that producers place containers on a prepared surface covered with a plastic covering or plastic sheet covered with crushed stone. Crushed stone, however, provides a surface that can trap plant debris and growing substrate, providing a potential inoculum source. Placing plastic over the stone results in a smoother surface that is less likely to trap debris, thus potentially reducing inoculum in the production area.

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Sanitation is important during production. Cleaning greenhouse benches, pots, and tools with disinfectants is recommended. Bleach diluted as 1 part bleach in 9 parts water is one recommended disinfectant (1) since it is effective at killing pathogens, relatively inexpensive, and readily available. Research testing the use of bleach as a disinfectant on ground covers after performing cultural practices on nursery crops produced outdoors is lacking.

Adopting management practices that reduce *C. gloeosporioides* inoculum or interfere with conidial dispersal should decrease disease incidence. The objective of this study was to determine the effect of growing containerized wintercreeper euonymus plants on gravel or polyethylene sheeting with or without periodic treatment of the ground cover (gravel or plastic) with 0.6% sodium hypochlorite (10% bleach solution) on anthracnose symptoms.

Materials and Methods

2005. The study was initiated on May 3 at Greenleaf Nursery Co., Park Hill, OK, and was terminated on September 26. Rooted cuttings of wintercreeper euonymus 'Emerald 'n Gold' were planted in #1 containers with substrate consisting of pine bark:sand (6:1, by vol) amended with 3 kg·m⁻³ (5 lb·yd⁻³) dolomitic lime, 890 g·m⁻³ (1.5 lb·yd⁻³) 0N-20P-0K (triple superphosphate), 263 g·m⁻³ (0.5 lb·yd⁻³) 0N-0P-49.8K (KCl), 742 g·m⁻³ (1.3 lb·yd⁻³) 45N-0P-0K (urea), 1.2 kg·m⁻³ (2 lb·yd⁻³) trace elements (Micromax, Scotts Co., Marysville, OH), and 5.3 kg·m⁻³ (9 lb·yd⁻³) 21N-2.2P-8.3K controlled release fertilizer (Osmocote 21-5-10, Scotts Co.). The plants were grown under 47% shade cloth. Photosynthetic photon flux (PPF) was measured periodically using an Integrating Quantum/Radiometer/Photometer (Model No. LI-188B, LI-COR, Inc., Lincoln, NE). Maximum PPF was about 1040 μmol·m⁻²·s⁻¹. The study was conducted on a production block on which wintercreeper euonymus crops have been produced for many years. The production block had a gravel surface, but growing substrate and plant debris had collected in the gravel providing a potential inoculum source on the block. Standard nursery practices at Greenleaf Nursery determined timing of cultural activities such as shearing. Plants were irrigated with overhead sprinklers daily and received about 2.5 cm (1 in) of water at each irrigation. A combination of trifluralin (α,α,α,-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine) and isoxaben (N-[3-(1-ethyl-1-methylpropyl)-5-isoxazolyl]-2,6-dimethoxybenzamide) (Snapshot 2.5 TG, Dow AgroSciences, Indianapolis) was applied June 2 at 11.2 g·m⁻² (100 lb·A⁻¹) for weed control. Plants were hand-weeded as necessary thereafter.

Plants were placed on either gravel beds or 0.15 mm (6 mil) thick black polyethylene sheeting (Covalence Specialty Materials Corp., Minneapolis, MN)-covered gravel beds. To reduce introduction of inoculum from outside of the plots, half of the beds in each bed treatment were sprayed monthly with 0.6% sodium hypochlorite that was pH adjusted to 7.0 with 1 M HCl using a hand sprayer immediately after ratings and performance of cultural practices was completed. No human entry into the plots occurred between sodium hypochlorite applications. Thus, treatments were a factorial combination of bed type and sodium hypochlorite treatment. To further reduce plot to plot inoculum spread, shoes of persons entering each plot were enclosed in sterile plastic covers that were replaced upon entry into each new treatment bed. Each treatment replication consisted of a block of plants ar-

ranged seven pots wide by seven pots long. The border plants were treated as guards and not rated for disease severity. Each bed extended about 61 cm (24 in) beyond the guard pots on all sides to minimize contamination from surrounding treatments, thus there was a buffer zone of 122 cm (48 in) between guard rows of adjacent plots. Treatment combinations were replicated four times with 25 pots (subsamples) per replication being rated for disease severity. Plants were rated for anthracnose symptoms when the study was initiated (May 3) and at four week intervals thereafter until the end of the growing season (September 26). Disease symptoms were visually rated by the same person on every rating date on a scale of 1 to 12 based on the Horsfall-Barratt plant disease assessment system (9) where: 1 = 0%, 2 = 1 to 3%, 3 = 4 to 6%, 4 = 7 to 12%, 5 = 13 to 25%, 6 = 26 to 50%, 7 = 51 to 74%, 8 = 75 to 86%, 9 = 87 to 93%, 10 = 94 to 96%, 11 = 97 to 99%, and 12 = 100% diseased tissue.

Plant debris was collected from the research area prior to the study and symptomatic leaves and stems were collected from plants periodically and cultured on potato dextrose agar to confirm the causal organism.

2006. The experiment was repeated as described above with the following exceptions. The study was conducted at two sites: Greenleaf Nursery Co., Park Hill, OK, and the Oklahoma State University Nursery Research Station, Stillwater, OK. Plants were placed under 73% shade cloth at the Greenleaf Nursery site and 40% shade at the Oklahoma State University site. Maximum PPF at Greenleaf Nursery was 582 μmol·m⁻²·s⁻¹ and at Oklahoma State University was 1350 μmol·m⁻²·s⁻¹. The study was initiated May 3 and terminated September 19 at Greenleaf Nursery and initiated May 7 and terminated September 18 at Oklahoma State University.

In vitro. Plugs measuring 0.05 cm (0.02 in) in diameter of actively growing, 2-day-old cultures of *C. gloeosporioides* from wintercreeper euonymus and *Sclerotium rolfsii* from peanut (*Arachis hypogaea* L.) were transferred to the center of a 10 cm (3.9 in) diameter petri dish containing potato dextrose agar. *Sclerotium rolfsii* Sacc. was used as a positive control. Filter paper disks [0.5 cm (0.2 in) diameter] were cut from Whatman #2 filter paper using a hole punch. One drop of 0.6% sodium hypochlorite (10% bleach) was placed on each filter paper disk. Excess sodium hypochlorite was removed by blotting the disks on paper towels. Four treated filter paper disks were placed at the four cardinal compass positions approximately 1 cm (0.4 in) from the plugs. Control treatments were prepared in separate petri dishes as described above except that one drop of distilled water was placed on the filter paper disks. Cultures were incubated at room temperature for three to four days to allow fungal growth toward the sodium hypochlorite- or distilled water-saturated filter paper. Each treatment was replicated four times.

In a second study, a suspension of 10⁵ to 10⁶ conidia·ml⁻¹ was prepared from two isolates of *C. gloeosporioides*. The conidial suspension was dropped onto filter paper disks saturated with 0.6% sodium hypochlorite. One hundred conidia were examined for germination. The control consisted of conidial suspension dropped on distilled water-saturated filter paper disks. Each treatment was replicated four times.

Statistics. A randomized complete block design (4 replicates) with repeated measures and subsampling was used

Table 1. Visual ratings of anthracnose symptoms on wintercreeper euonymus grown in containers on gravel covered or not covered with polyethylene sheeting and treated or not treated with 0.6% sodium hypochlorite (10% bleach) after performing cultural practices. Mean visual ratings for each treatment and date were based on 12 plots of 25 plants (four plots in each of two years and two locations during the second year).

Bed surface	Sodium hypochlorite	Visual rating ^z ± standard error					
		May 3 ^y	June 1	June 28	July 24	August 24	September 19
Gravel	No	1.8 ± 0.2	2.0 ± 0.2	3.0 ± 0.4	5.2 ± 0.5	6.7 ± 0.8	7.7 ± 0.8
	Yes	1.8 ± 0.2	2.0 ± 0.2	2.8 ± 0.3	5.1 ± 0.5	6.7 ± 0.8	7.5 ± 0.7
Plastic	No	1.9 ± 0.3	1.9 ± 0.2	2.9 ± 0.4	4.7 ± 0.4	6.3 ± 0.7	7.1 ± 0.7
	Yes	1.8 ± 0.2	1.8 ± 0.2	2.6 ± 0.3	4.6 ± 0.4	6.4 ± 0.6	7.0 ± 0.6

^zRatings were based on the Horsfall-Barrett rating scale (see text).

^yListed dates are approximate since data were collected during two growing seasons and at two sites during the second growing season.

for the field studies. The data were pooled across year and site and the variation due to these effects was accounted for in the model. Bed type and sodium hypochlorite treatments were fixed main effect factors, while date of rating was the factor creating the repeated measurements. Disease rating was the response. Data were analyzed using analysis of variance procedures with PC SAS Version 9.1 (PROC MIXED, SAS Institute, Inc., Cary, NC), and orthogonal contrasts were used to determine differences and polynomial trends among treatments and rating dates.

Results and Discussion

Field experiment. Neither bed treatment nor sodium hypochlorite treatment affected disease incidence at any time (Table 1). Disease incidence increased curvilinearly as the growing season progressed (Fig. 1). Between week 9 (about June 28) and week 13 (about July 26) disease symptoms appeared on more than 10% of plant tissue (disease ratings ≥ 4) making the plants non-salable in some markets. By week 17 (about August 23) more than 50% of plant tissue displayed anthracnose symptoms (disease ratings ≥ 6).

In vitro experiment. Sodium hypochlorite did not inhibit growth of *C. gloeosporioides* or *S. rolfii* mycelium compared to the control treatment. In contrast, no conidia of either isolate of *C. gloeosporioides* germinated on sodium hypochlorite-treated disks, whereas 55% of conidia germinated on disks to which distilled water was applied. Thus, presence of sodium hypochlorite did not affect mycelium growth, but completely inhibited conidial germination.

Legard (12) speculated that overwintering (or oversummering in winter production regions) of *Colletotrichum* species (including *C. gloeosporioides*) inoculum in soil or on plant debris can be an important source of inoculum for diseases in strawberry. In contrast, Ekefan et al. (7) found that *C. gloeosporioides* survived in sterile mineral soil for over 20 weeks, but microbial antagonisms apparently exist in nonsterile mineral soils that eliminate *C. gloeosporioides* in a few days. Our study was conducted in a location where wintercreeper euonymus has been grown for many years. Although plants were grown on gravel beds, wintercreeper euonymus plant debris containing *C. gloeosporioides* conidial masses was widely present in pore spaces and on the gravel surface providing an inoculum source.

LaMondia (11) suggested that the combination of overhead watering and container production on polyethylene sheeting

would increase splash dispersal of conidia, thereby increasing anthracnose incidence on wintercreeper euonymus. Our results did not confirm LaMondia's suggestion since disease incidence on container stock with and without underlying polyethylene sheeting was similar.

Sodium hypochlorite is used as a disinfectant for a variety of applications. It has broad antimicrobial activity, but generally viruses and vegetative bacteria are more susceptible to hypochlorites than endospore-forming bacteria, fungi and protozoa (18). Stability of the free available chlorine in solution and the efficacy of its antimicrobial activity are affected by several factors including chlorine concentration, presence and concentration of heavy metal ions, pH of the solution, temperature of the solution, presence of organic materials, and UV irradiation (18). Lack of disease control by sodium hypochlorite in this study was likely due to the causal organism and the environment. Sodium hypochlorite reduced conidial germination, but mycelia growth was unaffected. Because of the time between sodium hypochlorite

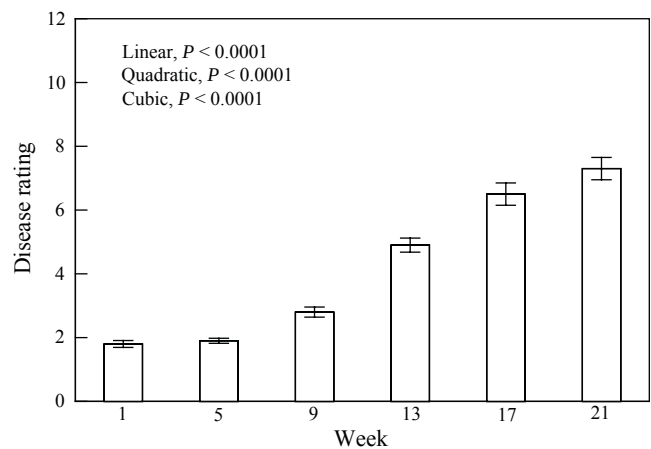


Fig. 1. Disease ratings of wintercreeper euonymus throughout the growing season from about May 3 to about September 19. Data were pooled over two years and two locations during the second year. Significant trends of main effect means are shown on the figure. Disease ratings were based on the Horsfall-Barratt rating scale (see text). Error bars represent standard errors. Mean visual ratings for each treatment were based on four treatments × four replications (16 plots) of 25 plants in each of two years and two locations during the second year (48 total plots).

applications and the fact that plants were not treated with the sodium hypochlorite to reduce conidial germination, the pathogen was able to initiate growth. The outdoor growing system is also not conducive to sodium hypochlorite activity since substances such as heavy metals (as essential plant nutrients in the substrate) and organic matter that destabilize sodium hypochlorite are readily present in the growing environment.

Ningen et al. (16) showed that anthracnose symptoms on wintercreeper euonymus declined with reduced night temperatures. Less disease at lower night temperatures may be attributed to less plant stress. Increased day and night temperatures as the growing season progressed in this study provided a better environment for *C. gloeosporioides* development. Increased temperatures also created a more stressful environment for wintercreeper euonymus and likely partially accounted for the seasonal increase in anthracnose symptoms.

Results of this study suggest that placing polyethylene sheeting under the containers and application of sodium hypochlorite to ground covers after cultural practices are performed failed to reduce the occurrence of anthracnose caused by *C. gloeosporioides* on wintercreeper euonymus. Altering other environmental conditions may be helpful in reducing anthracnose incidence on wintercreeper euonymus. Ningen et al. (17) showed that producing wintercreeper euonymus under shade coupled with afternoon irrigation significantly decreased anthracnose symptoms, resulting in a higher quality crop with fewer losses. Additionally, utilization of wintercreeper euonymus cultivars less susceptible to *C. gloeosporioides* would reduce anthracnose incidence in nursery and landscape plantings.

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