NURSERY PRODUCTION OF *EUONYMUS FORTUNEI*: CHEMICAL AND CULTURAL PRACTICES FOR CONTROLLING ANTHRACNOSE

By

CHERYL RENEE' BOYER

Bachelor of Landscape Architecture

Oklahoma State University

Stillwater, Oklahoma

2003

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
May 2005

NURSERY PRODUCTION OF *EUONYMUS FORTUNEI*:

CHEMICAL AND CULTURAL PRACTICES FOR

CONTROLLING ANTHRACNOSE

Thesis approved:

Janet C. Cole

Thesis Advisor

Michael W. Smith

Kenneth E. Conway

A. Gordon Emslie

Dean of the Graduate College

ACKNOWLEDGEMENTS

I have always wanted to follow in my parent's footsteps and attend graduate school, but I didn't think it would be a reality for me as an undergraduate landscape architecture student—there just wasn't much of a reason to pursue a postgraduate education in a field of practice. It's easy to say that things have a way of working themselves out, but I have witnessed God's plan for my life take shape and happen in these past few years. I can say without hesitation that graduate school has been a journey of faith for me. For all the days I just didn't feel like working on this document and the days that I was so stressed out that I seemed paralyzed to accomplish anything, I give thanks to my heavenly Father for calming me, reassuring me, and nudging me onwards.

This has also been a journey of growth for me, both personally and as a young professional. I have Dr. Janet Cole to thank for much of that development. Dr. Cole invested a great deal of time, energy, faith, and friendship in me. I am eternally grateful that she counseled me as an undergraduate and took me on as her graduate student. The time I have spent as her apprentice is invaluable and I cannot imagine having had this experience with anyone else. I think Stevie Ningen said it best in her thesis that Dr. Cole made her feel like a success even when she felt like a failure. I feel like a success because of Dr. Cole. Thank you from the bottom of my heart, I am forever in your debt.

The other members of my committee, Dr. Ken Conway and Dr. Mike Smith, imparted valuable lessons to me about being a graduate student as well as my future in

academia. I thank them for their time and patience with me as I learned how to be a scientist. Rhiannon Battles and Autumn Nolting took my nursery survey (which didn't have time to make it into this document) and ran with it! They managed everything for me at one of the busiest times in my life and I thank them for their effort and for caring about me. Thanks also to Donna Dollins, Margaret Struble, Harsha Desilva and the other staff members in the Horticulture and Landscape Architecture department for being cheerful, helping me when I needed it and chatting with me when I was procrastinating. Thanks to Drs. Lou Anella and Doug Needham for letting me help them with their classes, I appreciate the responsibility they entrusted to me. Thanks also to William Cole for letting me have so much of his wife's time and for giving me a hard time every chance he got!

The Oklahoma State University Horticulture and Landscape Architecture department has been my academic home for the past seven years. I thank everyone involved in the department for supporting me in my academic pursuits. The Oklahoma Garden Clubs and National Garden Clubs supported me financially with generous scholarships for which I am incredibly grateful. The following groups also contributed to my education with scholarships: The Tulsa Area Iris Society, The African Violet Society of America, McAlester Scottish Rite, Marjorie H. Andrews, and the OSU Graduate College. Greenleaf Nursery Company supplied a portion of my assistantship and the HORT/LA department also supplied opportunities for me to be a teaching assistant. Thank you so much! Without your assistance, this time would have been much more difficult.

My Mom and Dad have inspired me, encouraged me, and loved me through everything. They taught me how to work hard, have integrity and character, and dream big! I thank them for all their hugs, smiles, and the occasional tank of gas. I like living so close to them and I will miss them as we move on to the next place God has for us to go.

My precious husband, Rusty, has been a blessing in my life for some time now. He has supported me through everything I have wanted to do. He has put his own education on hold in order to help me attain my dreams. He is a calming force in my life and I appreciate all the times he listened to me talk about my day and offered a shoulder to cry on when I was frustrated. Thanks for keeping me in reality and making me take some time to have fun.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION—ANTHRACNOSE ON *EUONYMUS FORTUNEI*

Introduction

Anthracnose of *Euonymus fortunei* (Turcz.) Hand.-Mazz. is an increasing concern of commercial nursery producers over the past few decades (Mahoney & Tattar, 1980a, 1980b; LaMondia, 2001a, 2001b; Ningen et al., 2004, 2005). *Euonymus fortunei* is a consistent profit earner (Fig. 1.1) that is highly susceptible during production to anthracnose caused by the fungus *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. (Farr et al., 1989) (Fig. 1.2). Potential crop loss and thus earning loss for the nursery industry has increased efforts to find methods of management that are both financially beneficial to the grower and provide superior crop returns.

Euonymus fortunei

Euonymus fortunei is a fast-growing, woody, evergreen species that is best suited for growth in hardiness zones 5 to 9 (Whitcomb, 1996). It has small, opposite leaves, generally about 2.5 cm long with an elliptical shape. *Euonymus fortunei* has many cultivars, 53 according to Dirr (1998). Most cultivars have a unique pattern of variegation and growth as well as a range of susceptibility to anthracnose. Some cultivars are groundcovers reaching 10 to 30 cm in height. Other cultivars are low growing mounds reaching around 90 cm in height and still others are true clinging vines sometimes reaching up to 21 m in height (Dirr, 1998). In addition to anthracnose, *E. fortunei* is also

susceptible to crown gall (*Agrobacterium tumefaciens*) (Smith & Townsend) Conn., leaf spots (*Cercospora euonymi* Ell.), powdery mildew (*Oidium euonymi-japonici*) (Arc.) Sacc., aphids (*Aphis clerodendri* Matsumura), and scales (*Unaspis euonymi*) (Comstock) (Dirr, 1998). Anthracnose on *E. fortunei* occurs primarily during production and has not been problematic in the landscape. This may be due, in part, to a cooler root zone and thus less environmental stress once the plant is installed in the landscape. Ningen et al. (2005) found that increased shade intensity reduced the severity of anthracnose on *E. fortunei* during production. It was theorized that lower disease ratings with higher shade intensities could be partially attributed to lower ambient air temperatures in the shade than in the sun. Plants in full sun suffered the effects of high ambient temperatures around the root zone that was detrimental to plant growth. Shading may have reduced heat stress by decreasing root zone temperatures of the plants in the study. Ningen (2004) found that anthracnose disease severity also decreased with lower night temperatures.

Cultivars

Research has focused on three cultivars of *E. fortunei*. 'Emerald 'n Gold' (Fig. 1.3) is a small shrub characterized by small green leaves with yellow variegation. 'Emerald Gaiety' (Fig. 1.4) has a similar growth pattern to 'Emerald 'n Gold,' but leaves are green with white margins. 'Canadale Gold' (Fig. 1.5) is a slightly larger shrub than 'Emerald 'n Gold' and 'Emerald Gaiety' and 'Canadale Gold' has larger green and yellow leaves than the other two cultivars. Several studies have shown that 'Emerald 'n Gold' and 'Emerald Gaiety' are susceptible to anthracnose, but 'Emerald Gaiety' was less susceptible than 'Emerald 'n Gold' (LaMondia, 2001a; Mahoney and Tattar, 1980a,

1980b; Ningen, 2003). Ningen (2003) tested ‘Canadale Gold’ and found it to be similar to ‘Emerald ’n Gold’ in susceptibility.

Mahoney and Tattar (1980a, 1980b) also tested the cultivars ‘Argenteomarginata’, ‘Sheridan Gold,’ and *E. fortunei* var. *radicans* (Miq.) Rehd. and found that all cultivars were susceptible to anthracnose under laboratory conditions, but ‘Emerald ’n Gold’ and *E. fortunei* var. *radicans* were the most susceptible. Ningen (2003) also tested ‘Emerald Surprise’ and found it to be similar to ‘Emerald ’n Gold’ in susceptibility. In all trials ‘Emerald Gaiety’ was the least susceptible of cultivars tested to anthracnose (Mahoney and Tattar, 1980a, 1980b; Ningen, 2003, 2004, 2005) except LaMondia (2001a) noted that ‘Emerald ’n Gold’ had more lesions per leaf, but ‘Emerald Gaiety’ had more defoliation.

Anthracnose

Anthracnose is a term often applied to common disease symptoms such as lesions and stem dieback. It is a widespread parasitic disease that can be caused by a number of different organisms, most of which are closely related. The term anthracnose comes from a Greek word meaning carbuncle, and refers to the boil-like lesions that typically appear on host plants. In general, symptoms include small to large, dark brown spots or slightly sunken lesions on the leaves, stems, flowers or fruit. These lesions may spread to twigs where stem cankers and dieback may develop.

The term anthracnose is also used for diseases of other plants such as dogwood, sycamore, and ash anthracnose that are caused by organisms other than *Colletotrichum gloeosporioides*, but create symptoms similar to those associated with anthracnose on *E.*

fortunei. For example, dogwood anthracnose is caused by *Discula destructiva* Redlin, *sp. nov*. (Redlin, 1991).

Anthracnose in *Euonymus*

Anthracnose on *Euonymus fortunei* was first identified in a Massachusetts nursery by Mahoney and Tattar (1980a, 1980b) and later reported in Florida (Chase, 1983) and Oklahoma (Koelsch, 1993; Ningen et al., 2003, 2004, 2005). It is caused by the fungus *Colletotrichum gloeosporioides* and is characterized by small, light tan, concentric circles with orange/pink conidia eventually appearing near the outer edge of the lesion (Mahoney and Tattar, 1980a, 1980b; LaMondia, 2001a, 2001b; Ningen et al., 2004). Lesions (Fig. 1.6) occur on both upper and lower leaf surfaces and the necrotic centers may eventually drop out of the leaf leaving a shot-hole appearance (Mahoney and Tattar, 1980a, 1980b; LaMondia, 2001a, 2001b; Ningen, 2003). Leaf abscission, stem dieback and defoliation (Fig. 1.7, 1.8) are also symptoms of anthracnose on *E. fortunei* (Mahoney and Tattar, 1980a, 1980b; LaMondia, 2001a, 2001b; Ningen et al., 2004, 2005).

Colletotrichum gloeosporioides

Colletotrichum gloeosporioides is a fungus. A fungus is an organism characterized as being eukaryotic, spore-producing, lacking chlorophyll and living on absorptive nutrition. Most fungi are formed of filamentous, branched threads called hyphae, and the entire mass of connected threads that make up the vegetative body of a fungus is a mycelium (Alexopoulous et al., 1996). Reproduction of *C. gloeosporioides* occurs asexually through the separation of minute fragments of the mycelium into spores. The spores are formed in acervuli (erumpent, cushionlike masses of hyphae bearing conidiophores). Acervuli may have stiff marginal bristles (setae) that are sometimes

difficult to see, but are the distinguishing characteristic of *C. gloeosporioides* (Fig. 1.9). Conidia (slime-spores), held together by a gelatinous coating, appear pinkish to bright orange in mass and are quite distinguishable. They are not wind-borne, but can be disseminated by wind-splashed rain. After landing on a suitable host, the conidium sends out a short germ tube that, on contact with the epidermis, enlarges at the tip into a brown thick-walled appressorium. From this, a peglike infection hypha penetrates the cuticle (Horst, 1990; Abang et al., 2003). *Colletotrichum gloeosporioides* is the imperfect stage of *Glomerella cingulata* (Stoneman Spauld. and H. Schrenk) (Barnett and Hunter, 1998). *Glomerella cingulata* does not have a recognized role in anthracnose epidemics although it has been isolated from host plants (Abang et al., 2003).

Fungal organisms generally thrive in wet conditions. *Colletotrichum gloeosporioides* requires moisture for spore formation, dispersal, germination and penetration (Mahoney and Tattar, 1980a). Mahoney and Tattar (1980b) showed that leaf lesions were more numerous after 24 hours or more of leaf wetness than with shorter periods of leaf wetness. Since *C. gloeosporioides* is dependent on periods of precipitation and high relative humidity for disease establishment it is notable that infections correlate with wet weather and always occur on recently emerged growth (Mahoney and Tattar, 1980a). This may indicate that older leaves have some level of resistance to the infection (Abang et al., 2003). Factors contributing to the growth and spread of *C. gloeosporioides* include leaf wetness (Mahoney and Tattar, 1980a, 1980b; Ningen, 2003), high night temperatures (Ningen et al., 2004), low shade intensity during production (Ningen et al., 2005), and high humidity (Mahoney and Tattar, 1980a, 1980b; Chakraborty et al., 1990). *Colletotrichum gloeosporioides* is capable of over-wintering on the diseased tissue of

infected plants (Mahoney and Tattar, 1980a, 1980b; Abang, 2003) and new infections can occur throughout the growing season (Mahoney & Tattar, 1980a). It is a common secondary parasite in previously weakened plant tissue (Mahoney & Tattar, 1980a).

Colletotrichum gloeosporioides causes anthracnose and other diseases on many plants including azalea (hybrids of *Rhododendron* spp.) anthracnose (Stathis and Plakidas, 1958), scented geranium (*Pelargonium graveolens* L'Her. Ex Ait.) leaf blight (Kalra et al., 1988), hydrangea (*Hydrangea hortensia* H.) leaf spot (Kumari and Nair, 1981), sedum (*Sedum morganianum* Walth., S. *pachyphylllum* Rose, and *Sempervivum tectorum* L.) leaf rot (Chase, 1983), white leadtree (*Leucaena leucocephala* (Lam) de Wit.) leaf lesions (Mohanan, 1988), common periwinkle (*Vinca minor* L.) leaf and stem lesions (Koelsch, 1993; Koelsch et al., 1995a, 1995b), South African eucalyptus (*Eucalyptus* spp.) die back (Smith and Wingfield, 1998), Philippine mango (*Mangifera indica* L.) anthracnose (Estrada et al., 2000; Subramanian, 1995) Australian & Mexican tropical legume (*Stylosanthes* spp.) anthracnose (He et al., 1998; Munaut et al., 2001), water yam (*Dioscorea alata* L.) leaf necrosis and stem dieback (Abang et al., 2002, 2003), mulberry (*Morus* spp.) leaf black spot disease (Kumar et al., 2001), pepper (*Capsicum annuum* L. and *Capsicum frutescens* L.) fruit anthracnose (Manandahar et al., 1995; Oh et al., 1999), mallow (*Lavatera* sp.) anthracnose (Mortensen, 1991), strawberry (*Fragaria* X *ananassa* Duchesne) anthracnose, crown rot and wilt (Bonde et al., 1991), and avocado (*Persea* sp.) and tomato (*Lycopersicon esculentum* Mill.) fruit anthracnose (Cooper et al., 1998; Kim et al., 2000).

A significant number of studies have been conducted to investigate anthracnose caused by *C. gloeosporioides* on water yam. Much of this information is directly

applicable to the study of anthracnose in *E. fortunei* because Abang et al. (2003) found that genetic differentiation among pathogen populations from different *Dioscorea* spp., mango, and citrus was low, suggesting that the same *C. gloeosporioides* population attacks both yam and non-yam hosts. Abang et al. (2003) found that the most important sources of *C. gloeosporioides* inoculum are infested crop debris, infected parent plants, and alternative hosts. Ekefan et al. (2000b) showed that survival of *C. gloeosporioides* in the soil is unlikely. Microbial antagonism may be responsible for the apparent death in soil. Interaction studies showed that *Aspergillus* spp. inhibited the radial growth and sporulation of *C. gloeosporioides* on artificial media, and reduced its survival in sterile soil by 100% after 10 days of incubation (Ekefan et al., 2000b). A selective medium consisting of pencycuron (50 mg/l), tolcofos-methyl (10 mg/l), streptomycin sulphate (100 mg/l), chlortetracycline (100 mg/l) and chloramphenicol (100 mg/l) in potato dextrose agar (PDA) basal medium was created to help isolate and determine viability of *C. gloeosporioides* (Ekefan et al., 2000a).

Control

Several studies tested methods to control anthracnose caused by *C. gloeosporioides* on *E. fortunei* with little success. Various fungicides were tested, and some decreased anthracnose symptoms. Other studies tested altered growing environments on anthracnose symptoms.

Chemical Control: Fungicides

Fungicides can be classified by their mode of action. Contact fungicides are only effective in controlling disease symptoms if they come into direct contact with the fungus. Thus, contact fungicides act only on the plant surface and provide coverage for

up to 14 days. In contrast, systemic fungicides are translocated through the plant and last up to 28 days (Agrios, 1997). Numerous fungicides have been tested for controlling anthracnose.

Fungicides in the field

Evidence of increased fungicide resistance has been noted since anthracnose on *E. fortunei* was first described. At that time, maneb (manganese ethylene bis dithiocarbamate), mancozeb (manganese ethylene bis dithiocarbamate complex with zinc salt) and chlorothalonil (tetrachloroisophthalonitrile) provided complete control of anthracnose symptoms on *E. fortunei* (Mahoney and Tattar, 1980a, 1980b), but later studies showed less control with these fungicides (LaMondia, 2001a, 2001b; Cole et al., 2005; Ningen, 2003). Other fungicides including thiophanate-methyl (dimethyl [1,2-phenylene-dis(iminocarbonothioyl)] bis[carbamate]; dimethyl 4,4'-*o*-phenylenebis[3-thioallophanate]) (LaMondia, 2001a, 2001b; Ningen, 2003), copper hydroxide (LaMondia, 2001a, 2001b; Cole et al., 2005), azoxystrobin (methyl (*E*)-2-{2-[6-(2-cyanophenoxy) pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate) (LaMondia, 2001a; Cole et al., 2005), myclobutanil ((*RS*)-2-(4-chlorophenyl)-2-(1*H*-1,2,4-triazol-1-ylmethyl) hexanenitrile) (Cole et al., 2005), trifloxystrobin (methyl (*E*)-methoxyimino-{(*E*)-α-[1-(α,α,α-trifluoro-*m*-tolyl)ethylideneaminooxy]-*o*-tolyl}acetate) (Cole et al., 2005; Ningen, 2003), propiconazole (1-[[2(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]1-H-1,2,4-triazole) (Ningen 2003) and iprodione (3-(3,5-dichlorophenyl)-N-(1-methlyethyl)-2,4,-dioxo-1-imidazolidinecarboxamide) (LaMondia, 2001b) were tested with little success in controlling anthracnose symptoms. LaMondia (2001a, 2001b) found that resistance to multiple fungicides was common and suggested that use of several different

fungicides in a tank mixture or rotation provided better control of *C. gloeosporioides* than the use of a single fungicide when chemicals with different modes of action were used. Systemic fungicides include thiophanate-methyl, propiconazole, myclobutanil, azoxystrobin and iprodione (Hutson and Miyamoto, 1998). Contact fungicides include chlorothalonil, maneb (Sijpesteijn, 1982), mancozeb (Copping and Hewitt, 1998) and trifloxystrobin (Bartlett et al., 2001). Copper sulfate pentahydrate has been advertised as "fully systemic" (Phyton Corp., 2004) however, it has conventionally been considered a contact fungicide (i.e. Bordeaux mixture) (Copping and Hewitt, 1998). Copper is an essential nutrient with minimal mobility (Marschner, 2003). Therefore, copper sulfate pentahydrate and previously tested copper hydroxide are classified as contact fungicides in these studies. Cole et al. (2005) tested numerous fungicides in the field including mancozeb, copper hydroxide, trifloxystrobin, chlorothalonil, myclobutanil, and azoxystrobin and found that chlorothalonil and mancozeb were the most efficacious of the fungicides tested. Ningen (2003) tested chlorothalonil, trifloxystrobin, mancozeb, propiconazole, and thiopahanate-methyl alone, in rotations, and in mixtures and found that fungicide rotations and mixtures containing mancozeb alone and/or chlorothalonil provided the greatest control of *C. gloeosporioides*. However, no fungicide tested provided complete control.

Fungicides in vitro

Poison agar tests have been developed to test the effect of fungicides on mycelial growth of fungi that cause diseases. LaMondia (2001b) developed the first baseline sensitivity of fungicides against *C. gloeosporioides* on *E. fortunei*. Fungicides tested included thiophanate-methyl, chlorothalonil, iprodione (3-(3,5-dichlorophenyl)-N-(1-

methylethyl)-2,4,-dioxo-1-imidazolidinecarboxamide), copper hydroxide, and maneb. LaMondia (2001b), Cole et al. (2005) and Koelsch et al. (1995a) found that fungicides that performed well in the field (contact fungicides) did not necessarily inhibit mycelial growth of *C. gloeosporioides* in poison agar tests while systemic fungicides showed a greater amount of inhibition in vitro than contact fungicides.

Cultural Control

In many cases, certain cultural practices can adequately control fungal diseases (Agrios, 1997). Optimal growing conditions for *C. gloeosporioides* on *E. fortunei* have been identified thus it is possible to create a less favorable environment for *C. gloeosporioides* while maintaining favorable conditions for growth of *E. fortunei*.

Pathogen spread has been linked to ineffective cultural practices such as unclean pruning tools that transfer *C. gloeosporioides* spores from infected stock plants to cuttings (Mahoney and Tattar, 1980b). Maintaining disease-free parent plants is essential. Proper sanitation during pruning and propagation as well as proper plant spacing to encourage air movement and thus quick drying of leaves and adequate light and moisture penetration is essential (Agrios, 1997). Mahoney and Tattar (1980a) found that propagation techniques in the nursery accentuated the spread of anthracnose throughout the site. This resulted when cuttings were taken from infected mother plants. A subsequent increase in inoculum occurred throughout the nursery as the infected young plants were lined out or placed in container areas. LaMondia (2001b) suggested that propagation of diseased cuttings or cuttings with latent infections may favor selection for fungicide resistance in a particular isolate over several generations of plants. Removing and destroying infected leaves and shoots, crop debris, and infected plants throughout the

growing season and winter helped reduce the incidence of anthracnose on *E. fortunei* (Mahoney & Tattar, 1980a; LaMondia, 2001a; Agrios, 1997). Ningen (2003) found that shearing rendered the disease less noticeable by removing infected plant parts.

Ningen et al. (2004) showed that *E. fortunei* plants grown with a 19.3 °C night temperature had fewer disease symptoms than plants grown at 28.6 °C night temperature. This confirms data presented by Mahoney and Tattar (1980b) that found the optimum temperature range for vegetative growth and spore germination of *C. gloeosporioides* was 25-30 °C. Ningen et al. (2004) recommended producing plants in propagation and growing structures where temperature control is possible so that high night temperatures can be avoided. Ningen et al. (2005) also found that anthracnose damage decreased as shade intensity increased on production blocks. Lower disease ratings with higher shade intensities may be partially attributed to lower ambient air temperatures in the shade than in the sun. Shading may reduce heat stress by decreasing root zone temperatures of the plants (Ningen et al., 2005).

Overhead irrigation is commonly used in nursery production because it is inexpensive to install and maintain compared to other irrigation methods (Davidson et al., 2000). Use of overhead irrigation may, however, contribute to disease spread through the splashing of spores. Use of drip irrigation and wider plant spacing may lower disease incidence, but it was not cost effective for commercial producers. LaMondia (2001b) suggested that the combination of overhead watering and production on plastic would increase splash dispersal and result in high disease incidence. Ningen et al. (2005) found that disease incidence was lower on afternoon overhead irrigated plants than on morning overhead irrigated plants. This was attributed to higher air temperatures and wind speeds

during the afternoon that would dry the foliage more quickly than during the morning, making environmental conditions for disease development less favorable. Also, the cooling effect of afternoon irrigation may reduce plant stress thus reducing disease susceptibility (Ningen et al., 2005). Overhead irrigation, when used, should be cycled during the middle of the day to facilitate rapid leaf drying (Mahoney and Tattar 1980a, 1980b; Ningen et al., 2005).

As early as 1909 crop rotation was suggested as a means of control for anthracnose (Duggar, 1909). Since *C. gloeosporioides* does not survive in the soil for more than a couple of weeks (Abang, 2002; Ekefan, 2000b) crop rotation may help manage the spread of *C. gloeosporioides* to some extent. Rotating plants to areas that have not had diseased *E. fortunei* plants or crop debris on them for several weeks could provide some control of the disease, but labor requirements would make this practice costly. Selecting and producing anthracnose-resistant cultivars can also help control the spread of *C. gloeosporioides* on *E. fortunei* (Agrios, 1997; Davidson, 2000).

Objectives of Research

The objectives of this research were to 1) determine the effect of selected fungicides on *C. gloeosporioides* on *E. fortunei* in production and in vitro, and 2) determine the effect of growing plants on plastic or gravel beds with or without periodic sodium hypochlorite application on incidence of *C. gloeosporioides* on *E. fortunei*.

Literature Cited

Abang, M.M., S. Winter, K.R. Green, P. Hoffman, H.D. Mignouna, and G.A. Wolf. 2002. Molecular identification of *Colletotrichum gloeosporioides* causing yam anthracnose in Nigeria. Plant Pathol. 51:63-71.

Abang, M.M., S. Winter, H.D. Mignouna, K.R. Green, and R. Asiedu. 2003. Molecular taxonomic, epidemiological and population genetic approaches to understanding yam anthracnose disease. African J. Biotechnol. 2:486-496.

Agrios, G.N. 1997. Plant Pathology. 4th ed. Academic Press, New York.

Alexopoulos, C.J., C.W. Mims, M. Blackwell. 1996. Introductory mycology. 4th ed. Wiley, New York.

Barnett, H.L. and B.B. Hunter. 1998. Illustrated genera of imperfect fungi. 4th ed. Burgess Publishing Co., Minneapolis.

Bartlet, D.W., J.M. Clough, C.R.A. Godfrey, J.R. Goodwin, A.A. Hall, S.P. Heaney, and S.J. Maund. 2001. Understanding the strobilurin fungicides. Pesticide Outlook, Royal Soc. Chem. 2001(4):143-148

Bonde, M.R., G.L. Peterson, and J.L. Maas, 1991. Isozyme comparisons for identification of *Colletotrichum* species pathogenic to strawberry. Amer. Phytopathol. Soc. 81:1523-1527.

Chakraborty, S., D. Ratcliff, and F.J. McKay. 1990. Anthracnose of *Stylosanthes scabra*: Effect of leaf surface wetness on disease severity. Plant Dis., 74:379-384.

Chase, A.R. 1983. Two foliar diseases of *Euonymus* spp. Foliage Dig. 6(1):4.

Cole, J.T., J.C. Cole, and K.E. Conway. 2005. Effectiveness of selected fungicides applied with or without surfactant in controlling anthracnose on three cultivars of *Euonymus fortunei*. J. Appl. Hort. 7(1):In Press

Cooper, W., M. Bouzayen, A. Hamilton, C. Barry, S. Rossall, and D. Grierson. 1998. Use of transgenic plants to study the role of ethylene and polygalacturonase during infection of tomato fruit by *Colletotrichum gloeosporioides*. Plant Pathol. 47:308.

Copping, L.G. and H.G. Hewitt. 1998. Chemistry and mode of action of crop protection agents. Royal Soc. Chem., Cambridge, U.K.

Davidson, H., R. Mecklenburg, and C. Peterson. 2000. Nursery management administration and culture. 4th ed. Prentice-Hall, Upper Saddle River, N.J.

Dirr, M.A. 1998. Manual of woody landscape plants. Their identification, ornamental characteristics, culture, propagation and uses. 5th ed. Stipes Publishing, Champaign, Ill.

Duggar, B.M. 1909. Fungous diseases of plants. Ginn and Company, Boston.

Ekefan, E.J., S.A. Simons, A.O. Nwankiti, and J.C. Peters. 2000a. Semi-selective medium for isolation of *Colletotrichum gloeosporioides* from soil. Expt. Agric. 36:313-321.

Ekefan, E.J., S.A. Simons, and A.O. Nwankiti. 2000b. Survival of *Colletotrichum gloeosporioides* (causal agent of yam anthracnose) in soil. Trop. Sci. 40:163-168.

Estrada, A.B., J.C. Dodd, and P. Jeffries. 2000. Effect of humidity and temperature on conidial germination and appressorium development of two Philippine isolates of the mango anthracnose pathogen *Colletotrichum gloeosporioides*. Plant Pathol. 49:608-618.

Farr, D.F., G.F. Bills, G.P. Chamuris, and A.Y. Rossman. 1989. Fungi on plants and plant products in the United States. Amer. Phytopathol. Soc. Press, St. Paul, Minn.

He, C., A.G. Rusu, A.M. Poplawski, J.A.G. Irwin, and J.M. Manners. 1998. Transfer of a supernumerary chromosome between vegetatively incompatible biotypes of the fungus *Colletotrichum gloeosporioides*. Genetics 150:1459-1466.

Horst, R.K. 1990. Westcott's plant disease handbook. 5th ed. Van Nostrand Reinhold, New York.

Hutson, D. and J. Miyamoto. 1998. Fungicidal activity: Chemical and biological approaches to plant protection. Wiley, West Sussex, England.

Kalra, A., T.N. Parameswaran, and N.S. Ravindra. 1988. A leaf blight of scented geranium caused by *Colletotrichum gloeosporioides* Penz. Current Sci. 57:1136-1137.

Kim, Y., Z. Liu, D. Li, and P.E. Kolattukudy. 2000. Two novel genes induced by hard-surface contact of *Colletotrichum gloeosporioides* conidia. Bacteriology 182:4688.

Koelsch. M.C. 1993. Etiology and control of diseases of *Vinca minor* L. during nursery production. M.S. Thesis, Dept. of Hort. and Landscape Architecture, Oklahoma State Univ., Stillwater.

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

Koelsch, M.C., J.C. Cole, and S.L. von Broembsen. 1995b. First report of leaf spots and stem lesions on common periwinkle caused by *Colletotrichum gloeosporioides*. Plant Dis. 79:83.

Kumar, V., V.P. Gupta, A.M. Babu, R.K. Mishra, V. Thiagarajan, and R.K. Datta. 2001. Surface ultrastructural studies on penetration and infection process of *Colletotrichum gloeosporioides* on mulberry leaf causing black spot disease. J. Phytopathology 149:629-633.

Kumari, P.S. and M.C. Nair. 1981. Post-infectional changes in total carbohydrates and phenolics in the various parts of the leaf spot incited by *Colletotrichum gloeosporioides* on *Hydrangea hortensia*. Indian Phytopathol. 34:470-471.

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 the *Euonymus* anthracnose pathogen, *Colletotrichum gloeosporioides*, to selected fungicides. J. Environ. Hort. 19:47-50.

Mahoney, M.J. and T. A. Tattar. 1980a. Causal organism for spot anthracnose disease identified. Amer. Nurseryman 151(13): 77-78.

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

Manandhar, J.B., G.L. Hartman, and T.C. Wang. 1995. Semiselective medium for *Colletotrichum gloeosporioides* and occurrence of three *Colletotrichum* spp. on pepper plants. Plant Dis. 79:376-379.

Marschner, H. 2003. Mineral nutrition of higher plants. 2nd ed. Academic Press, Amsterdam.

Mohanan, C., 1988. *Colletotrichum* foliar infections on *Leucaena leucocephala* in Kerala, India. Current Sci. 57:1299-1300.

Mortensen, K., 1991. *Colletotrichum gloeosporioides* causing anthracnose of *Lavatera* sp. Can. Plant Dis. Survey 71:155-159.

Munaut, F., N. Hamaide, and H. Maraite. 2001. Molecular and morphological characterization of *Colletotrichum gloeosporioides* from native Mexican *Stylosanthes* species. Plant Pathol. 50:383.

Ningen, S.S. 2003. Chemical and cultural controls of anthracnose on *Euonymus fortunei*. M.S. Thesis, Dept. of Hort. and Landscape Architecture, Oklahoma State Univ., Stillwater.

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

Ningen, S.S., J.C. Cole, M.W. Smith, D.E. Dunn, and K.E. Conway. 2005. Increased shade intensity and afternoon irrigation decrease anthracnose severity on three *Euonymus fortunei* cultivars. HortScience 40:111-113.

Oh, B.J., K.D. Kim, and Y.S. Kim. 1999. Effect of cuticular wax layers of green and red pepper fruits on infection by *Colletotrichum gloeosporioides*. J. Phytopathol. 147:547-552.

Phyton Corp. 2004. Phyton news: Mode of action. Phyton Corp., Edina, Minn. Oct. 2004 Nursery Edition.

Redlin, S. 1991. *Discula destructive* sp. Nov., cause of dogwood anthracnose. Mycologia 83:633-642.

Smith, H. and M.J. Wingfield. 1998. Eucalyptus die-back in South Africa associated with *Colletotrichum gloeosporioides*. South African J. Bot. 64:226.

Sijpesteijn, A.K. 1982. Mechanism of action of fungicides, p. 32-45. In: Dekker, J. and S. G. Georgeopoulos (eds.). Fungicide resistance in crop protection. Ctr. Agri. Publishing and Documentation, Wageningen, Neth.

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

Subramanian, J. 1995. Selection and characterization of resistance in mango (*Mangifera indica* L.) embryogenic cultures to the phytotoxin produced by *Colletotrichum gloeosporioides* Penz (anthracnose). Ph.D. Dissertation. Environ. Hort. Dept., Univ. of Florida, Gainesville.

Whitcomb, C.E. 1996. Know it & grow it III. A guide to the identification and use of landscape plants. Lacebark Publications, Stillwater, Okla.

Fig. 1.1. Healthy crop of *Euonymus fortunei* ‘Emerald ’n Gold’ at Greenleaf Nursery Company, Park Hill, Okla.

Fig. 1.2. Crop loss of *Euonymus fortunei* ‘Emerald ’n Gold’ as a result of anthracnose caused by *Colletotrichum gloeosporioides*. Photos courtesy of Stephanie Ningen.

Fig. 1.3. *Euonymus fortunei* ‘Emerald ’n Gold’.

Fig. 1.4. *Euonymus fortunei* ‘Emerald Gaiety’.

Fig. 1.5. *Euonymus fortunei* ‘Canadale Gold’.

Fig. 1.6. Anthracnose leaf lesions on *Euonymus fortunei* caused by *Colletotrichum gloeosporioides*.

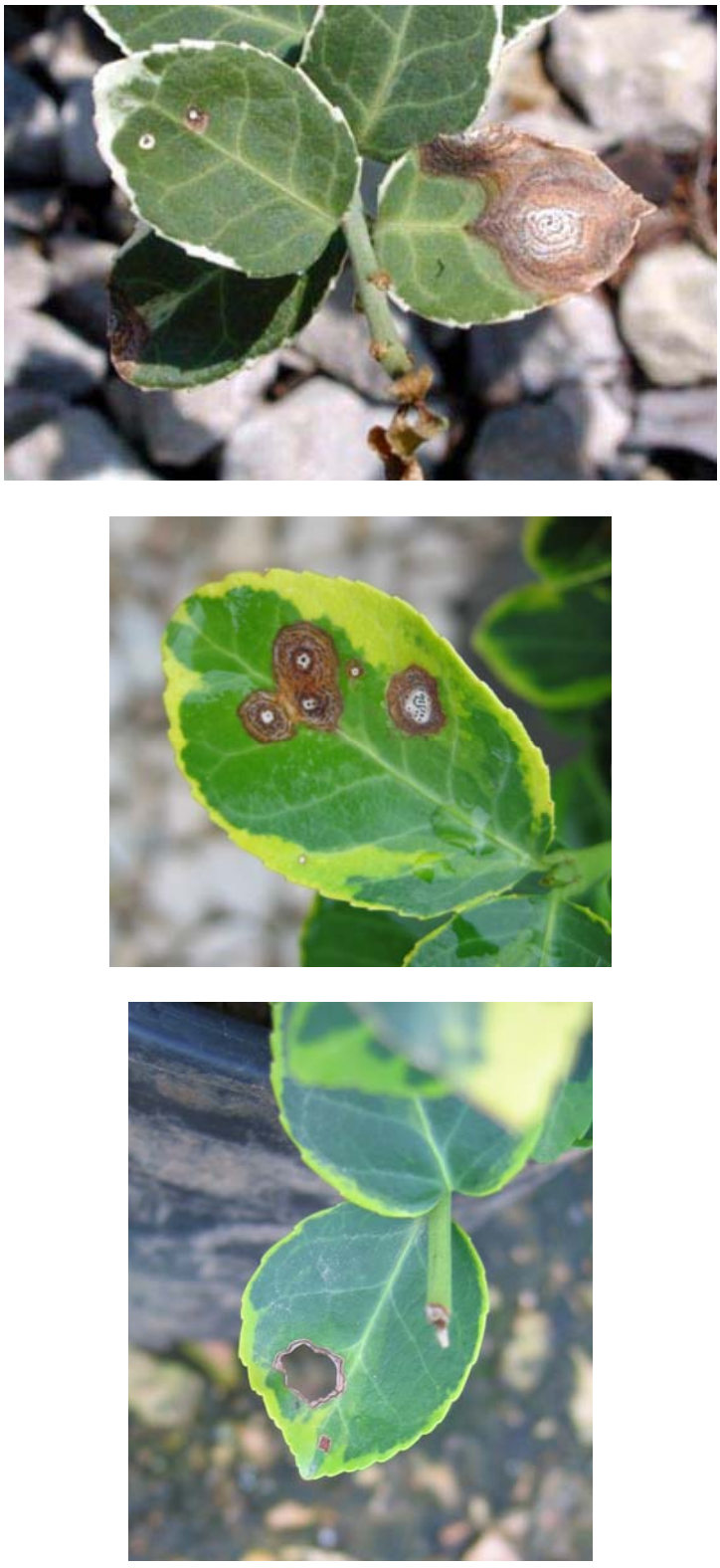
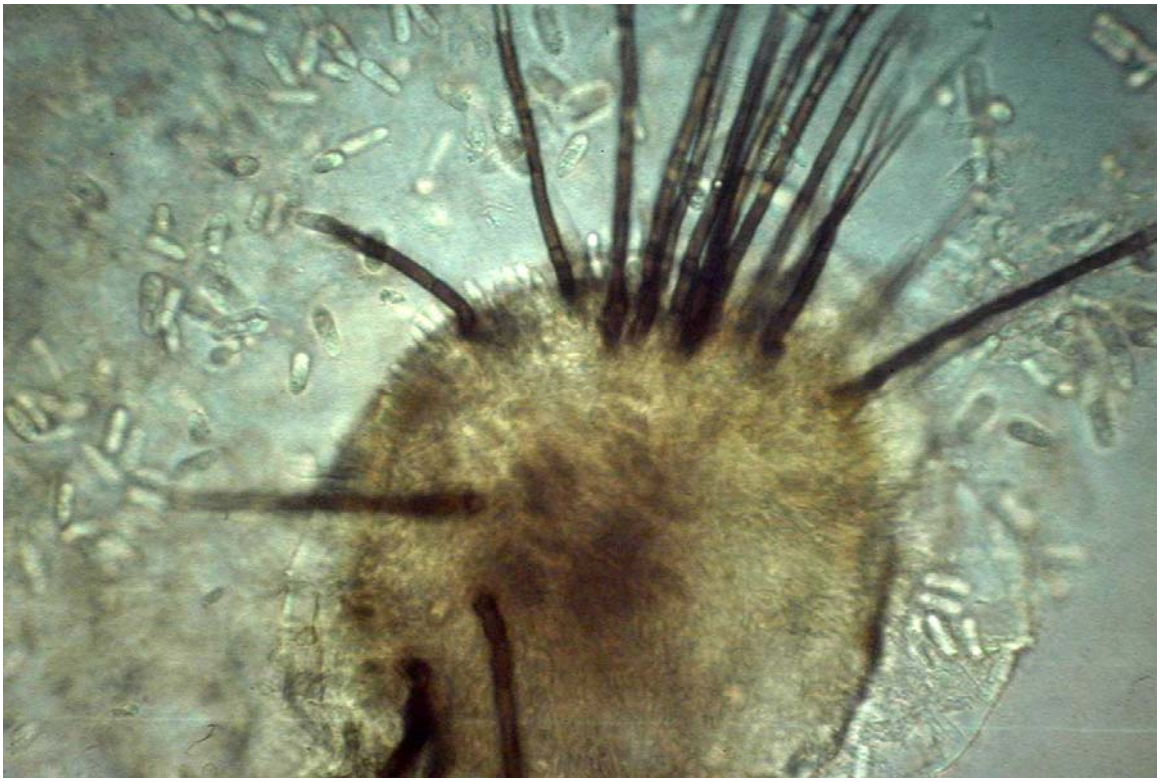


Fig. 1.7. Anthracnose stem lesion on *Euonymus fortunei* ‘Emerald ’n Gold’ caused by *Colletotrichum gloeosporioides*.

Fig. 1.8. Anthracnose defoliation and stem dieback on *Euonymus fortunei* 'Emerald 'n Gold' caused by *Colletotrichum gloeosporioides*.

Fig. 1.9. *Colletotrichum gloeosporioides* from *Euonymus fortunei*. a) acervuli and setae, b) conidia. Photos courtesy of Stephanie Ningen.

a.



b.

CHAPTER 2

GROWTH INHIBITION OF *COLLETOTRICHUM GLOEOSPORIOIDES* EXPOSED TO THREE FUNGICIDES AT VARIOUS CONCENTRATIONS IN VITRO

Cheryl R. Boyer and Janet C. Cole
Department of Horticulture and Landscape Architecture
Oklahoma State University, Stillwater, OK 74078

Kenneth E. Conway
Department of Entomology and Plant Pathology
Oklahoma State University, Stillwater, OK 74078

Additional index words: *Euonymus fortunei*, anthracnose, mancozeb, copper sulfate pentahydrate, trifloxystrobin.

ABSTRACT. Inhibition of mycelial growth of two isolates of *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. from *Euonymus fortunei* (Turcz.) Hand.-Mazz. by trifloxystrobin, mancozeb or copper sulfate pentahydrate was tested in vitro at 0, 1, 3.2, 10, 31.6, 100, 316, and 1000 mg a.i.·L^{-1}. Mycelial growth inhibition of both isolates increased curvilinearly as mancozeb or copper sulfate pentahydrate log$_{10}$ concentration increased. Mycelial growth inhibition increased curvilinearly for isolate 415 but linearly for isolate 423 as trifloxystrobin log$_{10}$ concentration increased. Trifloxystrobin and copper sulfate pentahydrate caused less than 50% inhibition of mycelial growth at all rates tested, but effective concentration to provide 50% inhibition (EC$_{50}$) of mancozeb on isolate 415 was 645.7 mg a.i.·L^{-1} and

on isolate 423 was 602.6 mg a.i.·L^{-1}. Chemical names used: A combination of manganese ethylene bis dithiocarbamate complex with zinc salt (mancozeb), copper sulfate pentahydrate, methyl (*E*)-methoxyimino-{(*E*)-α-[1-(α,α,α-trifluoro-*m*-tolyl)ethylideneaminooxy]-*o*-tolyl}acetate (trifloxystrobin).

Colletotrichum gloeosporioides is a fungus that causes anthracnose on *Euonymus fortunei* (Farr et al., 1989). It also causes diseases on many other species such as anthracnose of water yam (*Dioscorea alata* L.) (Abang et al., 2002, 2003), common periwinkle (*Vinca minor* L.) (Koelsch, 1993; Koelsch et al., 1995a, 1995b), azalea (*Rhododendron* spp.) (Stathis and Plakidas, 1958), and strawberry (*Fragaria* X *ananassa* Duchesne) (Bonde et al., 1991). Anthracnose was first reported on *E. fortunei* by Mahoney and Tattar (1980a, 1980b) in Massachusetts nurseries, later by Chase (1983) in Florida and then by Koelsch (1993) and Ningen et al. (2004, 2005) in Oklahoma.

Euonymus fortunei is a popular landscape shrub that retains its foliage throughout the year and adds color to the landscape with its often variegated foliage. Fifty-three cultivars have been described (Dirr, 1998; Whitcomb, 1996). Each cultivar is prized for its particular pattern of growth and foliage variegation including green, white, yellow, gold, orange, pink, red, maroon and purple. Many nursery producers depend on *E. fortunei* for consistent profits due to the number of cultivars available and their popularity with consumers.

Euonymus fortunei, however, is susceptible to anthracnose during production and in the landscape. Symptoms of anthracnose include leaf and stem lesions, stem dieback, and defoliation. Anthracnose is difficult to control, and control measures have proven

costly for nurseries. For example, one Oklahoma nursery estimates that as much as $200,000 has been spent annually on fungicides for anthracnose control on *E. fortunei* (D. Dunn, personal communication). Because of the high costs of disease control, *E. fortunei* has been eliminated from production by some nurseries.

Evidence of increased fungicide resistance has been noted since anthracnose on *E. fortunei* was first described. At that time, maneb (manganese ethylene bis dithiocarbamate), mancozeb (manganese ethylene bis dithiocarbamate complex with zinc salt) and chlorothalonil (tetrachloroisophthalonitrile) provided complete control of anthracnose symptoms on *E. fortunei* (Mahoney and Tattar, 1980a, 1980b), but later studies showed less control with these fungicides (LaMondia, 2001a, 2001b; Cole et al., 2005; Ningen, 2003). Other fungicides including thiophanate-methyl (dimethyl [1,2-phenylene-dis(iminocarbonothioyl)] bis[carbamate]; dimethyl 4,4'-*o*-phenylenebis[3-thioallophanate]) (LaMondia, 2001a, 2001b; Ningen, 2003), copper hydroxide (LaMondia, 2001a, 2001b; Cole et al., 2005), azoxystrobin (methyl (*E*)-2-{2-[6-(2-cyanophenoxy) pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate) (LaMondia, 2001a; Cole et al., 2005), myclobutanil ((*RS*)-2-(4-chlorophenyl)-2-(1*H*-1,2,4-triazol-1-ylmethyl) hexanenitrile) (Cole et al., 2005), trifloxystrobin (methyl (*E*)-methoxyimino-{(*E*)-α-[1-(α,α,α-trifluoro-*m*-tolyl)ethylideneaminooxy]-*o*-tolyl}acetate) (Cole et al., 2005; Ningen, 2003), propiconazole (1-[[2(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]1-H-1,2,4-triazole) (Ningen 2003) and iprodione (3-(3,5-dichlorophenyl)-N-(1-methlyethyl)-2,4,-dioxo-1-imidazolidinecarboxamide) (LaMondia, 2001b) were tested with little success in controlling anthracnose symptoms. LaMondia (2001a, 2001b) found that resistance to multiple fungicides was common and suggested that use of several different

fungicides in a tank mixture or rotation provided better control of *C. gloeosporioides* than the use of a single fungicide when chemicals with different modes of action were used. Systemic fungicides include thiophanate-methyl, propiconazole, myclobutanil, azoxystrobin and iprodione (Hutson and Miyamoto, 1998). Contact fungicides include chlorothalonil, maneb (Sijpesteijn, 1982), mancozeb (Copping and Hewitt, 1998) and trifloxystrobin (Bartlett et al., 2001). Copper sulfate pentahydrate has been advertised as "fully systemic" (Phyton Corp., 2004) however, it has conventionally been considered a contact fungicide (i.e. Bordeaux mixture) (Copping and Hewitt, 1998). Copper is an essential nutrient with minimal mobility (Marschner, 2003). Therefore, copper sulfate pentahydrate and previously tested copper hydroxide are classified as contact fungicides in these studies. Cole et al. (2005) tested numerous fungicides in the field including mancozeb, copper hydroxide, trifloxystrobin, chlorothalonil, myclobutanil, and azoxystrobin and found that chlorothalonil and mancozeb were the most efficacious of the fungicides tested. Ningen (2003) tested chlorothalonil, trifloxystrobin, mancozeb, propiconazole, and thiopahanate-methyl alone, in rotations, and in mixtures and found that fungicide rotations and mixtures containing mancozeb alone and/or chlorothalonil provided the greatest control of *C. gloeosporioides*. However, no fungicide tested provided complete control.

Mancozeb, trifloxystrobin and copper sulfate pentahydrate are labeled for control of *C. gloeosporioides* on *E. fortunei*, but these fungicides have not provided acceptable control in nurseries of *C. gloeosporioides* on *E. fortunei* in recent years.

The objective of this study was to determine the effectiveness of three fungicides on mycelial growth inhibition of *C. gloeosporioides* in vitro. Mancozeb (Protect, Cleary

Chemical Corp., Dayton, N.J.), trifloxystrobin (Compass, Olympic Horticultural Products, Bradenton, Fla.) and copper sulfate pentahydrate (Phyton-27, Source Tech Bio, Edina, Minn.) were the fungicides tested.

Materials and Methods

Leaf and stem samples with lesions were collected from *E. fortunei* and cultured to confirm the presence of *C. gloeosporioid*es. Two distinct isolates were selected for fungicide tests. The isolates differed in colony color and growth rate in vitro. Isolate 415 had white mycelium and a fast growth rate and isolate 423 had dark grey mycelium and a slower growth rate. Koch's postulates were tested and confirmed for both isolates.

A solution containing 3.9 g potato dextrose agar (PDA) and 90 mL water was prepared in each of seven 250 mL Erlenmeyer flasks. Flasks were sealed with aluminum foil and autoclaved for 20 min. After autoclaving flasks were placed in a waterbath (General Signal, Blue Island, Ill.) at 49 ºC for ten minutes. Ten mL of stock solution at the appropriate concentration was added to each Erlenmeyer flask to create final concentrations of 1, 3.2, 10, 31.6, 100, 316, and 1000 mg a.i.$\cdot L^{-1}$of fungicide. These concentrations were chosen because they provide equal intervals on a $\log_{10}$ scale. Separate solutions were prepared for each fungicide. Solutions from each fungicide and concentration were poured into each of five 9-cm diameter plastic petri dishes (20 mL per dish) for each of the *C. gloeosporioides* isolates.

A 0.7 cm diameter plug taken from the edge (active growth) of a mature *C. gloeosporioides* culture was placed upside down in the center of each dish. Each dish was sealed with parafilm (Pechiney Plastic Packaging, Chicago, Ill.) to prevent contamination and placed in a dark growth chamber (Percival model #I-35LL, Boone, Iowa) at 25 ºC.

Mycelial growth was measured daily for 6 days after inoculation by measuring the diameter at the widest point and perpendicular to the widest point then averaging the two measurements. Growth inhibition was calculated using the following equation:

% inhibition = [(isolate growth on non-amended media – isolate growth on fungicide-amended media)/ isolate growth on non-amended media] x 100.

Both isolates and all three fungicides were tested at the same time. The experiment was repeated three times. The growth chamber contained three shelves so dishes were randomized within fungicide and isolate by shelf. The fungicides were rotated on the shelves with each repetition so that all fungicides were tested on all shelves by the end of the study. Treatments were replicated 15 times (5 plates per isolate and fungicide and 3 repetitions). Regression analysis was conducted using PROC REG in SAS (SAS Institute, Cary, N.C.) with inhibition as the response to $\log_{10}$ concentration. This analysis was performed for all combinations of fungicide and isolate. Initially a quadratic model was used, but in the event that the quadratic term was not statistically significant at $\alpha = 0.05$, this model was abandoned for a linear one. For any given model, the EC_{50} was calculated.

Results and Discussion

A quadratic relationship existed between fungicide concentration and mycelial growth inhibition for isolate 415 with each fungicide tested (Fig. 2.1). Similar results occurred for isolate 423 with mancozeb and copper sulfate pentahydrate, but mycelial growth inhibition increased linearly as trifloxystrobin concentration increased with isolate 423. Mycelial growth inhibition was less than 50% at all concentrations of

trifloxystrobin and copper sulfate pentahydrate tested; whereas, EC_{50} of mancozeb on isolate 415 was 645.7 mg a.i.·L^{-1} and on isolate 423 was 602.6 mg a.i.·L^{-1}.

LaMondia (2001b) and Cole et al. (2005) found that copper hydroxide did not affect mycelial growth of *C. gloeosporioides* in vitro. Results of this study are similar in that inhibition of mycelial growth of both isolates by the copper-based fungicide copper sulfate pentahydrate was minimal (Fig. 2.1). Trifloxystrobin also minimally inhibited mycelial growth of both isolates in this study, but Cole et al. (2005) found some mycelial inhibition with trifloxystrobin. In the current study mancozeb inhibited mycelial growth, but only at high rates; whereas, LaMondia (2001b) saw little inhibition of mycelial growth with maneb, a similar fungicide. The rate of mancozeb that provided 50% inhibition of mycelial growth was less (645.7 and 602.6 mg a.i.·L^{-1} for isolate 415 and 423, respectively) than the recommended field application rate of 1.2 to 1.8 g a.i.·L^{-1}.

Differences in fungicide mode of action have been used to explain their affect on mycelial growth inhibition of *C. gloeosporioides* in vitro. LaMondia (2001b) speculated that systemic fungicides prevented mycelial growth but not conidial germination while contact fungicides prevented conidial germination but not mycelial growth. In the current study all fungicides tested were contact fungicides. However, different responses were observed for each fungicide. Mancozeb inhibited mycelial growth more than trifloxystrobin or copper sulfate pentahydrate while copper sulfate pentahydrate provided minimal mycelial inhibition. This minimal response may indicate that *C. gloeosporioides* has developed resistance to copper-based fungicides.

Of the fungicides tested in this study, mancozeb reduces mycelial growth of *C. gloeosporioides* more than trifloxystrobin or copper sulfate pentahydrate.

Literature Cited

Abang, M.M., S. Winter, K.R. Green, P. Hoffman, H.D. Mignouna, and G.A. Wolf. 2002. Molecular identification of *Colletotrichum gloeosporioides* causing yam anthracnose in Nigeria. Plant Pathol. 51:63-71.

Abang, M.M., S. Winter, H.D. Mignouna, K.R. Green, and R. Asiedu. 2003. Molecular taxonomic, epidemiological and population genetic approaches to understanding yam anthracnose disease. African J. Biotechnol. 2:486-496.

Bartlet, D.W., J.M. Clough, C.R.A. Godfrey, J.R. Goodwin, A.A. Hall, S.P. Heaney, and S.J. Maund. 2001. Understanding the strobilurin fungicides. Pesticide Outlook, Royal Soc. Chem. 2001(4):143-148.

Bonde, M.R., G.L. Peterson, and J.L. Maas, 1991. Isozyme comparisons for identification of *Colletotrichum* species pathogenic to strawberry. Amer. Phytopathol. Soc. 81:1523-1527.

Chase, A.R. 1983. Two foliar diseases of *Euonymus* spp. Foliage Dig. 6(1):4.

Copping, L.G. and H.G. Hewitt. 1998. Chemistry and mode of action of crop protection agents. Royal Soc. Chem., Cambridge, U.K.

Cole, J.T., J.C. Cole, and K.E. Conway. 2005. Effectiveness of selected fungicides applied with or without surfactant in controlling anthracnose on three cultivars of *Euonymus fortunei*. J. Appl. Hort. 7(1):In Press.

Dirr, M.A. 1998. Manual of woody landscape plants. Their identification, ornamental characteristics, culture, propagation and uses. 5th ed. Stipes Publishing, Champaign, Ill.

Farr, D.F., G.F. Bills, G.P. Chamuris, and A.Y. Rossman. 1989. Fungi on plants and plant products in the United States. Amer. Phytopathol. Soc. Press, St. Paul, Minn.

Hutson, D. and J. Miyamoto. 1998. Fungicidal activity: Chemical and biological approaches to plant protection. Wiley, West Sussex, England.

Koelsch. M.C. 1993. Etiology and control of diseases of *Vinca minor* L. during nursery production. M.S. Thesis, Dept. of Hort. and Landscape Architecture, Oklahoma State Univ., Stillwater.

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

Koelsch, M.C., J.C. Cole, and S.L. von Broembsen. 1995b. First report of leaf spots and stem lesions on common periwinkle caused by *Colletotrichum gloeosporioides*. Plant Dis. 79:83.

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 the *Euonymus* anthracnose pathogen, *Colletotrichum gloeosporioides*, to selected fungicides. J. Environ. Hort. 19:47-50.

Mahoney, M.J. and T. A. Tattar. 1980a. Causal organism for spot anthracnose disease identified. Amer. Nurseryman 151(13): 77-78.

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

Marschner, H. 2003. Mineral nutrition of higher plants. 2nd ed. Academic Press, Amsterdam.

Ningen, S.S. 2003. Chemical and cultural controls of anthracnose on *Euonymus fortunei*. M.S. Thesis, Dept. of Hort. and Landscape Architecture, Oklahoma State Univ., Stillwater.

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

Ningen, S.S., J.C. Cole, M.W. Smith, D.E. Dunn, and K.E. Conway. 2005. Increased shade intensity and afternoon irrigation decrease anthracnose severity on three *Euonymus fortunei* cultivars. HortScience 40:111-113.

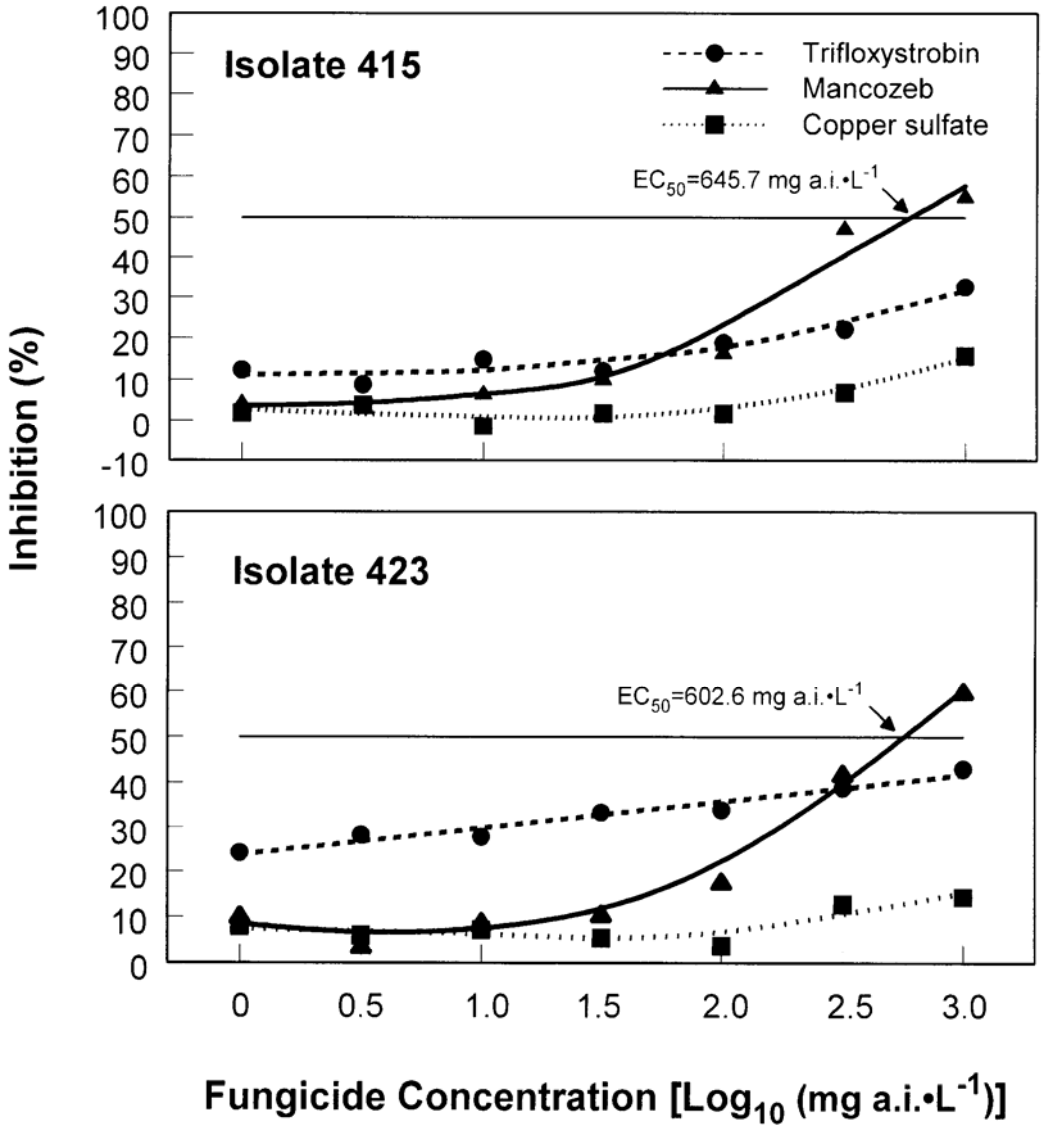
Phyton Corp. 2004. Phyton news: mode of action. Phyton Corp., Edina, Minn. Oct. 2004 Nursery Edition.

Sijpesteijn, A.K. 1982. Mechanism of action of fungicides, p. 32-45. In: Dekker, J. and S. G. Georgeopoulos (eds.). Fungicide resistance in crop protection. Ctr. Agri. Publishing and Documentation, Wageningen, Neth.

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

Whitcomb, C.E. 1996. Know it & grow it III. A guide to the identification and use of landscape plants. Lacebark Publications, Stillwater, Okla.

Fig. 2.1. Mycelial growth inhibition after 6 days at 25 ºC of two isolates of *Colletotrichum gloeosporioides* from *Euonymus fortunei* grown on potato dextrose agar amended with various concentrations of trifloxystrobin, mancozeb, or copper sulfate pentahydrate. The horizontal line indicates 50% inhibition. n=105. Isolate 415, trifloxystrobin ($y = 13.13 - 5.67x + 3.97x^2$, $r^2 = 0.54$***); mancozeb ($y = 6.61 – 11.49x + 9.61x^2$, $r^2 = 0.48$**); copper sulfate pentahydrate ($y = 6.33 – 11.02x + 4.64x^2$, $r^2 = 0.21$**). Isolate 423, trifloxystrobin ($y = 22.57 – 6.537x$, $r^2 = 0.49$***); mancozeb ($y = 14.23 – 20.92x + 12.14x^2$, $r^2 = 0.38$***); copper sulfate pentahydrate ($y = 10.36 – 8.24x + 3.26x^2$, $r^2 = 0.11$*). Significant at $P \leq 0.05$ (*), 0.01 (**), or 0.001 (***).



CHAPTER 3

EFFECTIVENESS OF COPPER SULFATE PENTAHYDRATE, MANCOZEB, AND HYDROGEN DIOXIDE IN CONTROLLING ANTHRACNOSE ON *EUONYMUS FORTUNEI*

Cheryl R. Boyer and Janet C. Cole
Department of Horticulture and Landscape Architecture
Oklahoma State University, Stillwater, OK 74078

Additional index words: Wintercreeper euonymus, *Colletotrichum gloeosporioides*, fungicides.

ABSTRACT. Plants of *Euonymus fortunei* (Turcz.) Hand.-Mazz. 'Emerald Gaiety', 'Emerald 'n Gold' and 'Canadale Gold' were sprayed to runoff weekly with one of three fungicide treatments in 2003 or one of four fungicide treatments in 2004 or water (control) to determine fungicide effectiveness in controlling anthracnose caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. Copper sulfate pentahydrate was applied at 0.4 or 0.6 g a.i.·L^{-1}, or mancozeb was applied at 1.8 g a.i.·L^{-1} in 2003 and 2004 while hydrogen dioxide was applied at 1.1 mL a.i.·L^{-1} in 2004. Plants were rated for disease incidence monthly. Cultivars differed at every rating date at both sites in both years with 'Emerald 'n Gold' generally having the highest disease ratings. 'Canadale Gold' typically had the lowest disease ratings while 'Emerald Gaiety' was intermediate in disease ratings. In 2003, no differences in disease rating occurred among fungicide treatments, but in 2004, plants treated

with mancozeb had the lowest disease ratings. Copper sulfate pentahydrate at 0.6 g a.i.·L^{-1} also reduced disease ratings, but neither copper sulfate pentahydrate at 0.4 g a.i.·L^{-1} nor hydrogen dioxide decreased disease ratings compared to water controls. Chemical names used: A combination of manganese ethylene bis dithiocarbamate complex with zinc salt (mancozeb), copper sulfate pentahydrate, methyl (*E*)-methoxyimino-{(*E*)-α-[1-(α,α,α-trifluoro-*m*-tolyl)ethylideneaminooxy]-*o*-tolyl}acetate (trifloxystrobin), hydrogen dioxide.

Anthracnose of *E. fortunei* has become an increasing concern for commercial nursery producers over the past few decades (Mahoney & Tattar, 1980a, 1980b; LaMondia, 2001a, 2001b; Ningen et al., 2004, 2005). An important nursery crop, *E. fortunei* is a consistent profit earner that is highly susceptible during production to anthracnose caused by the fungus *C. gloeosporioides* (Farr et al., 1989). Potential crop and thus earning loss for the nursery industry has lead to increased efforts to find methods of management that are both financially beneficial to the grower and provide superior crop returns.

Euonymus fortunei is a fast-growing, woody, evergreen species that is best suited for growth in hardiness zones 5 to 9 (Whitcomb, 1996). Generally a small shrub in form, *E. fortunei* has been reported to have as many as 53 cultivars with varying growth habits, leaf variation and susceptibility to anthracnose (Dirr, 1998).

Research has focused on three cultivars of *E. fortunei*. 'Emerald 'n Gold' is a small shrub characterized by small green leaves with yellow variegation. 'Emerald Gaiety' has a similar growth pattern to 'Emerald 'n Gold,' but leaves are green with

white margins. ‘Canadale Gold’ is a slightly larger shrub than ‘Emerald ’n Gold’ and ‘Emerald Gaiety’ and ‘Canadale Gold’ has larger green and yellow leaves than the other two cultivars. Several studies have shown that ‘Emerald ’n Gold’ and ‘Emerald Gaiety’ are susceptible to anthracnose, but ‘Emerald Gaiety’ was less susceptible than ‘Emerald ’n Gold’ (LaMondia, 2001a; Mahoney and Tattar, 1980a, 1980b; Ningen, 2003). Ningen (2003) tested ‘Canadale Gold’ and found it to be similar to ‘Emerald ’n Gold’ in susceptibility.

Mahoney and Tattar (1980a, 1980b) also tested the cultivars ‘Argenteomarginata’, ‘Sheridan Gold,’ and *E. fortunei* var. *radicans* (Miq.) Rehd. and found that all cultivars were susceptible to anthracnose under laboratory conditions, but ‘Emerald ’n Gold’ and *E. fortunei* var. *radicans* were the most susceptible. Ningen (2003) also tested ‘Emerald Surprise’ and found it to be similar to ‘Emerald ’n Gold’ in susceptibility. In all trials ‘Emerald Gaiety’ was the least susceptible of cultivars tested to anthracnose (Mahoney and Tattar, 1980a, 1980b; Ningen, 2003, 2004, 2005) except LaMondia (2001a) noted that ‘Emerald ’n Gold’ had more lesions per leaf, but ‘Emerald Gaiety’ had more defoliation.

Anthracnose on *Euonymus fortunei* was first identified in a Massachusetts nursery by Mahoney and Tattar (1980a, 1980b) and later noted in Florida (Chase, 1983) and Oklahoma (Koelsch, 1993). It is caused by the fungus *C. gloeosporioides* and is characterized by small, light tan, concentric circles with orange to pink conidia eventually appearing near the outer edge of the lesion (Mahoney and Tattar, 1980a, 1980b; LaMondia, 2001a, 2001b; Ningen et al., 2004). Lesions can occur on both upper and lower leaf surfaces and the necrotic centers may eventually drop out of the leaf

leaving a shot-hole appearance (Mahoney and Tattar, 1980a, 1980b; LaMondia, 2001a, 2001b; Ningen, 2003). Leaf abscission, stem dieback and defoliation are also symptoms of anthracnose on *E. fortunei* (Mahoney and Tattar, 1980a, 1980b; LaMondia, 2001a, 2001b; Ningen et al., 2004, 2005).

Evidence of increased fungicide resistance has been noted since anthracnose on *E. fortunei* was first described. At that time, maneb (manganese ethylene bis dithiocarbamate), mancozeb (manganese ethylene bis dithiocarbamate complex with zinc salt) and chlorothalonil (tetrachloroisophthalonitrile) provided complete control of anthracnose symptoms on *E. fortunei* (Mahoney and Tattar, 1980a, 1980b), but later studies showed less control with these fungicides (LaMondia, 2001a, 2001b; Cole et al., 2005; Ningen, 2003). Other fungicides including thiophanate-methyl (dimethyl [1,2-phenylene-dis(iminocarbonothioyl)] bis[carbamate]; dimethyl 4,4'-*o*-phenylenebis[3-thioallophanate]) (LaMondia, 2001a, 2001b; Ningen, 2003), copper hydroxide (LaMondia, 2001a, 2001b; Cole et al., 2005), azoxystrobin (methyl (*E*)-2-{2-[6-(2-cyanophenoxy) pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate) (LaMondia, 2001a; Cole et al., 2005), myclobutanil ((*RS*)-2-(4-chlorophenyl)-2-(1*H*-1,2,4-triazol-1-ylmethyl) hexanenitrile) (Cole et al., 2005), trifloxystrobin (methyl (*E*)-methoxyimino-{(*E*)-α-[1-(α,α,α-trifluoro-*m*-tolyl)ethylideneaminooxy]-*o*-tolyl}acetate) (Cole et al., 2005; Ningen, 2003), propiconazole (1-[[2(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]1-H-1,2,4-triazole) (Ningen 2003) and iprodione (3-(3,5-dichlorophenyl)-N-(1-methlyethyl)-2,4,-dioxo-1-imidazolidinecarboxamide) (LaMondia, 2001b) were tested with little success in controlling anthracnose symptoms. LaMondia (2001a, 2001b) found that resistance to multiple fungicides was common and suggested that use of several different

fungicides in a tank mixture or rotation provided better control of *C. gloeosporioides* than the use of a single fungicide when chemicals with different modes of action were used. Systemic fungicides include thiophanate-methyl, propiconazole, myclobutanil, azoxystrobin and iprodione (Hutson and Miyamoto, 1998). Contact fungicides include chlorothalonil, maneb (Sijpesteijn, 1982), mancozeb (Copping and Hewitt, 1998) and trifloxystrobin (Bartlett et al., 2001). Copper sulfate pentahydrate has been advertised as "fully systemic" (Phyton Corp., 2004) however, it has conventionally been considered a contact fungicide (i.e. Bordeaux mixture) (Copping and Hewitt, 1998). Copper is an essential nutrient with minimal mobility (Marschner, 2003). Therefore, copper sulfate pentahydrate and previously tested copper hydroxide are classified as contact fungicides in these studies. Cole et al. (2005) tested numerous fungicides in the field including mancozeb, copper hydroxide, trifloxystrobin, chlorothalonil, myclobutanil, and azoxystrobin and found that chlorothalonil and mancozeb were the most efficacious of the fungicides tested. Ningen (2003) tested chlorothalonil, trifloxystrobin, mancozeb, propiconazole, and thiopahanate-methyl alone, in rotations, and in mixtures and found that fungicide rotations and mixtures containing mancozeb alone and/or chlorothalonil provided the greatest control of *C. gloeosporioides*. However, no fungicide tested provided complete control.

Mancozeb, trifloxystrobin and copper sulfate pentahydrate are currently labeled for control of *C. gloeosporioides* on *E. fortunei*, but these fungicides have not provided acceptable control of *C. gloeosporioides* on *E. fortunei* in recent years.

The objective of this study was to determine the effectiveness of three fungicides in controlling anthracnose symptoms on *E. fortunei* plants in production. Mancozeb, copper sulfate pentahydrate and hydrogen dioxide were the fungicides tested.

Materials and Methods

2003. The study was initiated on 12 May at the Oklahoma State University Nursery Research Station, Stillwater, Okla., on 17 May at Greenleaf Nursery Co., Park Hill, Okla. and was terminated on 16 Sept. at Park Hill and 1 Oct. in Stillwater (Fig. 3.1). Rooted cuttings of *E. fortunei* (Greenleaf Nursery Co., Park Hill, Okla.) 'Emerald 'n Gold,' 'Emerald Gaiety,' and 'Canadale Gold' were planted in 3.8 L containers with media consisting of 6 pine bark : 1 sand amended with 3 $kg \cdot m^{-3}$ dolomitic lime, 889 $g \cdot m^{-3}$ 0N-20P-0K (triple superphosphate), 259 $g \cdot m^{-3}$ 0N-0P-49.8K (KCl), 111 $g \cdot m^{-3}$ trace elements (Frit 504 HF, Frit (U.K.) Ltd., Cambridge, U.K.), 889 $g \cdot m^{-3}$ $FeSO_4$, 741 $g \cdot m^{-3}$ 46N-0P-0K (urea), and 1.2 $kg \cdot m^{-3}$ Fe_2O_3 (GU-49, Master Builders, Inc., Cleveland, Ohio) and placed under 47% shade. Photosynthetic photon flux readings were determined periodically using an Integrating Quantum/Radiometer/Photometer (Model No. LI-188B, LI-COR, Inc., Lincoln, Nebr.). Maximum photosynthetic photon flux (PPF) was about 1073 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at plant height at both sites. Cuttings were randomly taken from presumably anthracnose-infected mother plants in the field. High disease incidence in the field eliminated the need to further inoculate. Plants at both sites were irrigated with about 1.25 cm of water daily with overhead sprinkler 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 on 2 June at Park Hill and 9 June at Stillwater at

11.2 g/m^2 for weed control. Plants were hand-weeded as necessary thereafter. Standard nursery practices at Greenleaf Nursery determined timing of cultural activities such as shearing.

Plants were sprayed to runoff (approximately 89 mL/m^2) weekly with the following fungicide treatments: copper sulfate pentahydrate (Phyton-27, Phyton Corp., Edina, Minn.) at 0.4 g a.i.·L^{-1}, copper sulfate pentahydrate at 0.6 g a.i.·L^{-1}, mancozeb (Protect, Cleary Chemical Corp., Dayton, N.J.) at 1.8 g a.i.·L^{-1} or water (control). Plants were rated for anthracnose symptoms when the study was initiated (12 May) and at four week intervals thereafter until the end of the growing season. Rating dates at Park Hill were 17 May, 19 June, 23 July, 22 August, and 16 September. Rating dates at Stillwater were 12 May, 10 June, 8 July, 4 August, 30 August, and 1 October. Disease ratings were on a scale of 1 to 12 based on the Horsfall-Barratt plant disease assessment system where: 1 = no diseased tissue, 2 = 1-3% diseased tissue, 3 = 4-6% diseased tissue, 4 = 7-12% diseased tissue, 5 = 13-25% diseased tissue, 6 = 26-50% diseased tissue, 7 = 26-50% disease free tissue, 8 = 13-25% disease free tissue, 9 = 7-12% disease free tissue, 10 = 4-6% disease free tissue, 11 = 1-3% disease free tissue, 12 = no disease free tissue (Horsfall and Barratt, 1945). The Horsfall-Barratt rating system is based on the Weber-Fechner law that states the visual acuity is proportional to the logarithm of the intensity of the stimulus (the stimuli are the diseased and healthy tissue) (Hebert, 1982).

The experimental design was a split-plot with fungicide as the main plot (4 treatments) and cultivar as the subplot (3 cultivars). There were 20 replications of each treatment. Data were analyzed using PROC GLM (SAS Institute, Inc., Cary, N.C.).

Means of significant interactions and main effects were separated using a protected least significant difference (LSD) procedure.

Daily average high and low temperatures were documented (Table 3.1) using a data logger (Watchdog 425, Spectrum Technologies, Plainfield, Ill.). Symptomatic leaves and stems were collected from plants throughout the growing season and cultured on potato dextrose agar to confirm the causal organism.

2004. The study described above was repeated with the addition of hydrogen dioxide (ZeroTol, BioSafe Systems, Glastonbury, Conn.) at 1.1 mL a.i.·L^{-1} as a fungicide treatment. The study was initiated on 17 May at Stillwater and on 19 May at Park Hill and was terminated on 4 Oct. at Park Hill and 8 Oct. in Stillwater. Rating dates at Park Hill were 19 May, 16 June, 13 July, 3 August, 8 September and 4 October. Rating dates at Stillwater were 17 May, 14 June, 14 July, 9 August, 10 September, and 8 October. A combination of trifluralin and isoxaben was applied on 3 June at Park Hill and 7 June at Stillwater for weed control. Maximum PPF was 1210 μmol·m^{-2}·s^{-1} at plant height at Park Hill and 1217 μmol·m^{-2}·s^{-1} at Stillwater.

Results

2003. No interaction between fungicide and cultivar occurred at either site for disease ratings (data not presented). No differences in disease ratings occurred among fungicide treatments at either site (data not presented). Cultivars differed at every rating date (Table 3.2). On 19 June at Park Hill all cultivars differed in disease ratings with ‘Emerald Gaiety’ having the highest ratings while ‘Canadale Gold’ had the lowest ratings. On 23 July, 22 August and 16 September ‘Emerald ’n Gold’ had the highest

disease ratings while ‘Canadale Gold’ and ‘Emerald Gaiety’ had lower ratings, but did not differ from each other.

On 10 June at Stillwater, ‘Emerald ’n Gold’ and ‘Canadale Gold’ had similar disease ratings but ‘Emerald Gaiety’ had higher disease ratings (Table 3.3). On 8 July ‘Emerald ’n Gold’ and ‘Emerald Gaiety’ had similar ratings while ‘Canadale Gold’ had lower ratings. On 4 August, 30 August, and 1 October ‘Emerald ’n Gold’ had the highest disease ratings while ‘Canadale Gold’ had the lowest ratings.

2004. No fungicide by cultivar interaction existed for disease ratings on 16 June, 13 July, 3 August, or 4 October at Park Hill (data not presented). On 16 June at Park Hill ‘Emerald Gaiety’ had the highest disease ratings while ratings of ‘Emerald Gaiety’ and ‘Canadale Gold’ did not differ (Table 3.4). On 13 July and 3 August ‘Emerald Gaiety’ had the highest disease ratings while ‘Canadale Gold’ had the lowest ratings. On 4 October ‘Emerald ’n Gold’ and ‘Emerald Gaiety’ had similar disease ratings but ‘Canadale Gold’ had lower ratings.

On 3 August and 4 October at Park Hill plants treated with mancozeb had lower disease ratings than any other fungicide treatments (Table 3.4).

On 8 September at Park Hill cultivar interacted with fungicide treatment for disease ratings (Table 3.5). No differences in disease ratings occurred among cultivars within any fungicide treatment. ‘Canadale Gold’ plants that were sprayed with mancozeb had lower disease ratings than ‘Emerald ’n Gold’ or ‘Emerald Gaiety’ sprayed with water, either rate of copper sulfate pentahydrate, or hydrogen dioxide.

At Stillwater, no fungicide by cultivar interaction occurred for disease ratings on 14 June, 14 July, 10 September, or 8 October (data not presented). On 14 June at

Stillwater ‘Emerald ’n Gold’ and ‘Canadale Gold’ had similar disease ratings while ‘Emerald Gaiety’ had lower ratings (Table 3.6). On 14 July ‘Emerald ’n Gold’ had the highest ratings while ‘Emerald Gaiety’ had the lowest ratings. Ratings of ‘Canadale Gold’ did not differ from those of the other cultivars on 14 July. On 10 September and 8 October ‘Emerald ’n Gold’ had the highest disease ratings while ‘Canadale Gold’ and ‘Emerald Gaiety’ had similar ratings.

On 14 July at Stillwater plants treated with mancozeb had lower disease ratings than those treated with water, copper sulfate pentahydrate at 0.4 g a.i.·L^{-1} or hydrogen dioxide, but ratings of plants treated with mancozeb did not differ from those of plants sprayed with copper sulfate at 0.6 g a.i.·L^{-1} (Table 3.6). Disease ratings did not differ between plants receiving copper sulfate pentahydrate at 0.4 g a.i.·L^{-1} and those receiving copper sulfate pentahydrate at 0.6 g a.i.·L^{-1}, nor did disease ratings differ between plants receiving water and hydrogen dioxide on 14 July. On 10 September disease ratings differed among all treatments with mancozeb having the lowest disease ratings while water had the highest. On 8 October plants sprayed with mancozeb had the lowest disease ratings while those treated with water or hydrogen dioxide had the highest disease ratings. Disease ratings of plants treated with copper sulfate pentahydrate at 0.6 g a.i.·L^{-1} or 0.4 g a.i.·L^{-1} were similar and higher than those treated with mancozeb but lower than those treated with water or hydrogen dioxide.

On 9 August at Stillwater a fungicide by cultivar interaction occurred for disease ratings (Table 3.5). ‘Emerald ’n Gold’ sprayed with water or hydrogen dioxide had higher disease ratings than ‘Canadale Gold’ but ‘Emerald Gaiety’ did not differ from the other cultivars. In contrast, ‘Emerald ’n Gold’ treated with mancozeb had higher ratings

than ‘Emerald Gaiety’, but ratings of ‘Canadale Gold’ did not differ from either other cultivar. No differences in disease ratings occurred among the cultivars with either copper sulfate pentahydrate treatment. ‘Emerald Gaiety’ treated with mancozeb had lower disease ratings than ‘Emerald ’n Gold’ treated with water, copper sulfate pentahydrate at either rate, or hydrogen dioxide. ‘Emerald Gaiety’ sprayed with mancozeb also had lower disease ratings than ‘Canadale Gold’ sprayed with water, 0.4 g a.i.·L^{-1} copper sulfate pentahydrate, or hydrogen dioxide.

Discussion

Disease ratings of ‘Canadale Gold’ were among the lowest of the cultivars at both sites during both years. ‘Emerald ’n Gold’ generally had higher ratings than ‘Emerald Gaiety’ in 2003 at both sites and in 2004 at Stillwater, but ‘Emerald Gaiety’ generally had higher disease ratings than ‘Emerald ’n Gold’ at Park Hill in 2004. Previous studies have shown ‘Emerald ’n Gold’ to be more susceptible to anthracnose caused by *C. gloeosporioides* than ‘Emerald Gaiety’ (LaMondia, 2001a; Mahoney and Tattar, 1980a, 1980b; Ningen, 2003, 2005) or ‘Canadale Gold’ (Ningen, 2003, 2005).

While copper sulfate pentahydrate at 0.6 g a.i.·L^{-1} reduced disease ratings of *C. gloeosporioides* on *E. fortunei,* plants treated with mancozeb generally had lower disease ratings than plants treated with other fungicides. Similar results occurred in vitro. Mancozeb reduced mycelial growth of *C. gloeosporioides* isolated from infected *E. fortunei* plants more than copper sulfate pentahydrate or trifloxystrobin (Chapter 2). Differences in fungicide mode of action have been used to explain their affect on mycelial growth inhibition of *C. gloeosporioides* in vitro. LaMondia (2001b) speculated that systemic fungicides prevented mycelial growth but not conidial germination while

contact fungicides prevented conidial germination but not mycelial growth. In the current study all fungicides tested were contact fungicides. However, different responses were observed for each fungicide. Mancozeb inhibited mycelial growth more than trifloxystrobin or copper sulfate pentahydrate while copper sulfate pentahydrate provided minimal mycelial inhibition. This minimal response may indicate that *C. gloeosporioides* has developed resistance to copper-based fungicides.

While there were statistical differences among the fungicide treatments, the horticultural significance was negligible. A grower or consumer would probably not be able to tell the difference between a plant displaying a disease rating of 5.8 (mancozeb, Table 3.4; 25-40% diseased tissue) and a plant displaying a disease rating of 6.7 (control, Table 3.4; 50-70% diseased tissue). Therefore, this study confirms the results of LaMondia (2001a, 2001b), Cole et al. (2005), and Ningen (2003) since none of the fungicides provided acceptable control of anthracnose on *E. fortunei*.

Since fungicides provide minimal control of anthracnose on *E. fortunei*, it is reasonable to look to other means of managing the disease. Ningen et al. (2005) found that increased shade intensity coupled with afternoon irrigation on *E. fortunei* plants significantly reduced the incidence and severity of anthracnose on production blocks. Based on this information, one Oklahoma nursery, Greenleaf Nursery Company, Park Hill, Okla. has modified their pesticide schedules to eliminate treating E. fortunei except when symptoms arise (Albert Bond, personal communication). Instead, they increased the shade intensity on the crop and switched to afternoon irrigation. Because of these cultural practices Greenleaf Nursery Company was able to save an excess of $12,000 in labor and $25,000 to $30,000 in chemical purchases. They also experienced indirect

savings as well since they were able to make pesticide applications to other crops and no longer needed to make repeated applications to *E. fortunei*. We recommend using mancozeb as a fungicide treatment when symptoms of anthracnose appear on *E. fortunei*, however, with proper cultural practices minimal fungicidal applications should be required.

Literature Cited

Bartlet, D.W., J.M. Clough, C.R.A. Godfrey, J.R. Goodwin, A.A. Hall, S.P. Heaney, and S.J. Maund. 2001. Understanding the strobilurin fungicides. Pesticide Outlook, Royal Soc. Chem. 2001(4):143-148.

Chase, A.R. 1983. Two foliar diseases of *Euonymus* spp. Foliage Dig. 6(1):4.

Cole, J.T., J.C. Cole, and K.E. Conway. 2005. Effectiveness of selected fungicides applied with or without surfactant in controlling anthracnose on three cultivars of *Euonymus fortunei*. J. Appl. Hort. 7(1):In Press.

Copping, L.G. and H.G. Hewitt. 1998. Chemistry and mode of action of crop protection agents. Royal Soc. Chem., Cambridge, U.K.

Dirr, M.A. 1998. Manual of woody landscape plants. Their identification, ornamental characteristics, culture, propagation and uses. 5th ed. Stipes Publishing, Champaign, Ill.

Farr, D.F., G.F. Bills, G.P. Chamuris, and A.Y. Rossman. 1989. Fungi on plants and plant products in the United States. Amer. Phytopathol. Soc. Press, St. Paul, Minn.

Hebert, T.T. 1982. The rationale for the Horsfall-Barratt plant disease assessment system. Phytopathology 72:1269.

Horsfall, J.G. and R.W. Barratt. 1945. An improved grading system for measuring plant diseases. Phytopathology 35:655. (Abstr.).

Hutson, D. and J. Miyamoto. 1998. Fungicidal activity: Chemical and biological approaches to plant protection. Wiley, West Sussex, England.

Koelsch. M.C. 1993. Etiology and control of diseases of *Vinca minor* L. during nursery production. M.S. Thesis, Dept. of Hort. and Landscape Architecture, Oklahoma State Univ., Stillwater.

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 the *Euonymus* anthracnose pathogen, *Colletotrichum gloeosporioides*, to selected fungicides. J. Environ. Hort. 19:47-50.

Mahoney, M.J. and T.A. Tattar. 1980a. Causal organism for spot anthracnose disease identified. Amer. Nurseryman 151(13): 77-78.

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

Marschner, H. 2003. Mineral nutrition of higher plants. 2nd ed. Academic Press, Amsterdam.

Ningen, S.S. 2003. Chemical and cultural controls of anthracnose on *Euonymus fortunei*. M.S. Thesis, Dept. of Hort. and Landscape Architecture, Oklahoma State Univ., Stillwater.

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

Ningen, S.S., J.C. Cole, M.W. Smith, D.E. Dunn, and K.E. Conway. 2005. Increased shade intensity and afternoon irrigation decrease anthracnose severity on three *Euonymus fortunei* cultivars. HortScience 40:111-113.

Phyton Corp. 2004. Phyton news: Mode of action. Phyton Corp., Edina, Minn. Oct. 2004 Nursery Edition.

Sijpesteijn, A.K. 1982. Mechanism of action of fungicides, p. 32-45. In: Dekker, J. and S. G. Georgeopoulos (eds.). Fungicide resistance in crop protection. Ctr. Agri. Publishing and Documentation, Wageningen, Neth.

Whitcomb, C.E. 1996. Know it & grow it III. A guide to the identification and use of landscape plants. Lacebark Publications, Stillwater, Okla.

Table 3.1. Average daily high and low temperatures and hours of leaf wetness between rating dates for anthracnose damage on container-grown *Euonymus fortunei* in fungicide studies at Park Hill, Okla. and Stillwater, Okla. in 2003 and 2004.

Location[y]	Interval between rating dates	Average daily temperature (°C) ± s.e./s.d.[z]		Hours of leaf wetness
		High	Low	
		2003		
Tahlequah	18 May to 19 June	25.9 ± 3.5	14.2 ± 3.4	--
	20 June to 23 July	31.9 ± 2.9	20.4 ± 3.1	--
	24 July to 22 Aug.	34.4 ± 2.6	20.5 ± 2.0	--
	23 Aug. to Sept. 16	29.0 ± 4.9	18.5 ± 4.4	--
Stillwater	13 May to 10 June	26.1 ± 3.8	14.3 ± 3.0	--
	11 June to 8 July	31.3 ± 2.4	19.4 ± 3.4	--
	9 July to 4 Aug.	36.5 ± 3.0	23.3 ± 2.9	--
	5 Aug. to 30 Aug.	36.1 ± 3.2	21.5 ± 2.1	--
	31 Aug. to 1 Oct.	25.8 ± 4.6	14.0 ± 4.7	--
		2004		
Park Hill	20 May to 16 June	29.2 ± 2.1	20.1 ± 3.1	15.8 ± 9.8
	17 June to 13 July	30.5 ± 2.8	20.2 ± 1.9	5.8 ± 3.5
	14 July to 3 Aug.	30.9 ± 2.4	20.3 ± 2.4	5.2 ± 5.7
	4 Aug. to 8 Sept.	30.3 ± 2.4	18.8 ± 3.6	2.4 ± 1.9
	9 Sept. to 4 Oct.	29.9 ± 2.5	16.4 ± 3.4	3.0 ± 2.3
Stillwater	18 May to 14 June	33.0 ± 3.9	18.6 ± 3.8	3.5 ± 4.7
	15 June to 14 July	33.6 ± 4.0	19.0 ± 2.5	4.8 ± 3.3
	15 July to 9 Aug.	33.9 ± 5.6	19.2 ± 2.5	5.4 ± 4.4
	10 Aug. to 10 Sept.	33.1 ± 3.3	19.1 ± 4.4	5.6 ± 3.8
	11 Sept. to 8 Oct.	31.7 ± 3.7	14.2 ± 4.6	4.0 ± 4.0
		Historical Temperatures (1971-2000)		
Tulsa	13 May to 14 June	28.4 ± 0.3	17.1 ± 0.3	--
	15 June to 14 July	32.9 ± 0.2	21.6 ± 0.2	--
	15 July to 9 Aug.	34.9 ± 0.1	23.0 ± 0.1	--
	10 Aug. to 6 Sept.	33.2 ± 0.2	21.0 ± 0.2	--
	7 Sept. to 8 Oct.	27.8 ± 0.3	15.9 ± 0.3	--

[z] Average daily high and low temperatures and hours of leaf wetness were calculated by summing the daily high and low temperatures and hours of leaf wetness, respectively, through the dates shown and dividing by the number of days in the interval between rating dates. s.d. = standard deviation. s.e. = standard error (used for historical temperatures).

[y] Temperatures for 2003 and historical temperatures were from Oklahoma Mesonet data. Temperatures and leaf wetness data for 2004 were taken by dataloggers in the research plots at plant height. Historical temperatures were available from Tulsa, Okla. Stillwater is located 113 km west of Tulsa. Park Hill is located 116 km SE of Tulsa.

Table 3.2. Disease ratings of three cultivars of *Euonymus fortunei* in 2003 at Park Hill, Okla. n=80.

	Disease Rating[z]			
Cultivar	**19 June**	**23 July**	**22 Aug.**	**16 Sept.**
Emerald 'n Gold	2.1 b[y]	2.7 a	3.4 a	4.6 a
Canadale Gold	1.4 c	2.3 b	3.2 b	4.2 b
Emerald Gaiety	2.4 a	2.3 b	3.1 b	3.9 b

[z] Anthracnose disease ratings were based on the Horsfall and Barratt rating system (see text).

[y] Mean separation by Protected LSD $P \leq 0.05$. Means followed by the same letter are not significantly different.

Table 3.3. Disease ratings on three cultivars of *Euonymus fortunei* in 2003 at Stillwater, Okla. n=80.

	Disease Rating[z]				
Cultivar	**10 June**	**8 July**	**4 Aug.**	**30 Aug.**	**1 Oct.**
Emerald 'n Gold	1.4 b[y]	1.6 a	5.5 a	7.2 a	8.2 a
Canadale Gold	1.2 b	1.2 b	3.2 c	4.3 c	5.7 c
Emerald Gaiety	1.9 a	1.6 a	4.0 b	5.9 b	6.9 b

[z] Anthracnose disease ratings were based on the Horsfall and Barratt rating system (see text).

[y] Mean separation by Protected LSD $P \leq 0.05$. Means followed by the same letter are not significantly different.

Table 3.4. Effect of cultivar (pooled over fungicide treatment) and fungicide treatment (pooled over cultivars) on disease ratings of *Euonymus fortunei* at Park Hill, Okla. in 2004. n=100.

	Disease Rating[z]			
Treatment	**16 June**	**13 July**	**3 Aug.**	**4 Oct.**
	Cultivar Main Effect			
Emerald 'n Gold	2.3 b	2.5 b	4.1 b	6.5 a
Canadale Gold	2.1 b	2.1 c	3.8 c	6.3 b
Emerald Gaiety	2.7 a	2.7 a	4.4 a	6.5 a
	Fungicide Main Effect			
Water	2.4	2.6	4.4 a	6.7 a
Mancozeb	2.4	2.3	3.5 b	5.8 b
Copper sulfate pentahydrate—0.6 g a.i.·L^{-1}	2.6	2.5	4.1 a	6.4 a
Copper sulfate pentahydrate—0.4 g a.i.·L^{-1}	2.4	2.3	4.2 a	6.5 a
Hydrogen dioxide	2.2	2.3	4.3 a	6.6 a

[z] Anthracnose disease ratings were based on the Horsfall and Barratt rating system (see text).

[y] Mean separation by Protected LSD $P \leq 0.05$. Means followed by the same letter are not significantly different.

Table 3.5. Effect of fungicide and cultivar on *Euonymus fortunei* anthracnose disease ratings in 2004 at Park Hill, Okla. on 8 September and at Stillwater, Okla. on 9 August. n = 100.

		Disease Rating[z]	
Fungicide	**Cultivar**	**Park Hill**	**Stillwater**
Water	Emerald 'n Gold (EG)	4.2	9.7
	Canadale Gold (CG)	4.2	8.0
	Emerald Gaiety (GA)	5.0	9.1
Mancozeb	EG	3.8	6.4
	CG	3.2	5.2
	GA	3.7	4.7
Copper sulfate pentahydrate—0.6 g a.i.·L^{-1}	EG	4.4	7.0
	CG	3.7	6.0
	GA	4.3	5.8
Copper sulfate pentahydrate—0.4 g a.i.·L^{-1}	EG	4.1	7.4
	CG	4.1	6.6
	GA	4.6	6.7
Hydrogen dioxide	EG	4.3	9.4
	CG	4.1	7.2
	GA	4.5	8.5
$LSD_{0.05}$ for differences among cultivars for the same fungicide		0.9	1.4
$LSD_{0.05}$ for differences among cultivars for different fungicide treatments		0.9	1.9

[z] Anthracnose disease ratings were based on the Horsfall and Barratt rating system (see text).

Table 3.6. Effect of cultivar (pooled over fungicide treatment) and fungicide treatment (pooled over cultivars) on disease ratings of *Euonymus fortunei* at Stillwater, Okla. in 2004. n=100.

	Disease Rating[z]			
Treatment	**14 June**	**14 July**	**10 Sept.**	**8 Oct.**
	Cultivar Main Effect			
Emerald 'n Gold	2.4 a	4.3 a	8.0 a	7.9 a
Canadale Gold	2.3 a	4.1 ab	6.6 b	6.3 b
Emerald Gaiety	2.1 b	3.9 b	6.9 b	6.5 b
	Fungicide Main Effect			
Water	2.3	4.5 a	8.9 a	8.4 a
Mancozeb	2.0	3.5 c	5.4 e	5.4 c
Copper sulfate pentahydrate—0.6 g a.i.·L^{-1}	2.3	3.7 bc	6.3 d	6.1 b
Copper sulfate pentahydrate—0.4 g a.i.·L^{-1}	2.3	3.9 b	6.9 c	6.5 b
Hydrogen dioxide	2.4	4.8 a	8.4 b	8.0 a

[z] Anthracnose disease ratings were based on the Horsfall and Barratt rating system (see text).

[y] Mean separation by Protected LSD $P \leq 0.05$. Means followed by the same letter are not significantly different.

Fig. 3.1 Two research sites. a) Greenleaf Nursery Company, Park Hill, Okla. b) Oklahoma State University Nursery Research Station, Stillwater, Okla.

a.

b.

CHAPTER 4

ANTHRACNOSE SEVERITY ON *EUONYMUS FORTUNEI* GROWN ON PLASTIC OR GRAVEL WITH OR WITHOUT PERIODIC SODIUM HYPOCHLORITE APPLICATION

Cheryl R. Boyer and Janet C. Cole
Department of Horticulture and Landscape Architecture
Oklahoma State University, Stillwater, OK 74078

Additional index words: *Colletotrichum gloeosporioides*, wintercreeper euonymus.

ABSTRACT. Containerized *Euonymus fortunei* (Turcz.) Hand.-Mazz. 'Emerald 'n Gold' plants were grown either on gravel beds or black plastic-covered gravel beds. Half of the beds in each bed treatment were sprayed with a 0.6% sodium hypochlorite solution monthly to attempt to reduce the presence of *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. inoculum. Plants were rated monthly from May through October for disease severity. Presence or absence of sodium hypochlorite did not affect disease ratings at any time. Disease ratings of plants on plastic-covered beds were lower than those of plants on gravel beds. Disease ratings decreased linearly as the growing season progressed.

Disease control in the nursery industry is important not only for the health of the crop, but also for consumer confidence and nursery profit margins. Large monetary losses are incurred when a crop must be destroyed due to plant disease. *Euonymus fortunei* is

susceptible to anthracnose caused by *C. gloeosporioides* (Farr et al., 1989). Symptoms of anthracnose on this small shrub include leaf and stem lesions, defoliation and stem dieback. Anthracnose of *E. fortunei* has become an increasing concern to commercial nursery producers over the past few decades (Mahoney & Tattar, 1980a, 1980b; LaMondia, 2001a, 2001b; Ningen et al., 2004, 2005). Potential crop and thus earning loss for the nursery industry has lead to increased efforts to find management practices that are financially beneficial to the grower and yield a high quality crop.

Euonymus fortunei is a fast-growing, woody, evergreen species that is best suited for growth in hardiness zones 5 to 9 (Whitcomb, 1996). Many cultivars exist, 53 according to Dirr (1998). Each cultivar has a unique pattern of variegation and growth, and cultivars differ in susceptibility to anthracnose. Past research has indicated that *E. fortunei* 'Emerald 'n Gold' (characterized by small green leaves with yellow variegation) is more susceptible to anthracnose than 'Emerald Gaiety' (LaMondia, 2001a; Mahoney and Tattar, 1980a, 1980b; Ningen, 2003, 2004, 2005) 'Emerald Surprise' (Ningen, 2003), or 'Canadale Gold' (Ningen, 2003, 2004, 2005).

Anthracnose on *E. fortunei* was first identified in a Massachusetts nursery by Mahoney and Tattar (1980a, 1980b) and later noted in Florida (Chase, 1983) and Oklahoma (Koelsch, 1993). It is caused by the fungus *C. gloeosporioides* and is characterized by small, light tan, concentric circles with orange to pink conidia eventually appearing near the outer edge of the lesion (Mahoney and Tattar, 1980a, 1980b; LaMondia, 2001a, 2001b; Ningen et al., 2004). Lesions can occur on both upper and lower leaf surfaces and the necrotic centers may eventually drop out of the leaf

leaving a shot-hole appearance (Mahoney and Tattar, 1980a, 1980b; LaMondia, 2001a, 2001b; Ningen, 2003).

Colletotrichum gloeosporioides requires moisture for spore formation, dispersal, germination and penetration (Mahoney and Tattar, 1980a). Mahoney and Tattar (1980b) showed that leaf lesions were more numerous after 24 hours or more of leaf wetness than with shorter periods of leaf wetness. Development of *C. gloeosporioides* depends on the presence of moisture. Disease symptoms tend to occur during or after wet weather or new growth (Mahoney and Tattar, 1980a). Abang et. al, (2003) speculated that older leaves are somewhat resistant to infection. Factors contributing to the growth and spread of *C. gloeosporioides* include leaf wetness (Mahoney and Tattar, 1980a, 1980b; Ningen, 2003), high night temperatures (Ningen et al., 2004), low shade intensity during production (Ningen et al., 2005), and high humidity (Mahoney and Tattar, 1980a, 1980b; Chakraborty et al., 1990).

Overhead irrigation is commonly used in nursery production because it is inexpensive to install and maintain compared to other irrigation methods (Davidson et al., 2000). Use of overhead irrigation may, however, contribute to disease spread through the splashing of spores. LaMondia (2001b) suggested that the combination of overhead watering and production on plastic would increase splash dispersal and result in high disease incidence. Production on plastic could contaminate more parts of the plants with *C. gloeosporioides* inoculum because irrigation water could bounce higher and farther than on gravel beds where water is more likely to be absorbed into the soil quickly. Since *C. gloeosporioides* is spread by water dispersal it is reasonable to hypothesize that production on plastic could be detrimental to the health of the crop due to more inoculum

being spread throughout the crop. However, Ekefan et. al. (2000) found that *C. gloeosporioides* is unable to survive in the soil for more than a few weeks. It survives the winter on the infected tissue of dead plants (Mahoney and Tattar, 1980a, 1980b; Abang, 2003). We speculate that *C. gloeosporioides* should survive an even shorter amount of time on plastic than on soil as long as infected crop debris is removed promptly thus reducing overall inoculum levels and disease symptoms in the crop.

Ningen et al. (2005) found that disease incidence was lower on afternoon sprinkler irrigated plants than on morning sprinkler irrigated plants. This was attributed to higher air temperatures and wind speeds during the afternoon that would dry the foliage more quickly than during the morning, making environmental conditions for disease development less favorable. Also, the cooling effect of afternoon irrigation may have reduced plant stress thus reducing disease susceptibility (Ningen et al., 2005).

Several studies have tested various methods of controlling anthracnose caused by *C. gloeosporioides* on *E. fortunei* with little success (Mahoney and Tattar, 1980a, 1980b; LaMondia 2001a, 2001b; Ningen, 2003; Cole et al., 2005). Various fungicides have been tested, and some have decreased anthracnose symptoms. Other studies have tested the effect of altering the growing environment on anthracnose symptoms. Ningen et al. (2005) found that anthracnose damage decreased as shade intensity increased on production blocks. Lower disease ratings with higher shade intensities may be partially attributed to lower ambient air temperatures in the shade than in the sun. Shading may have reduced heat stress by decreasing root zone temperatures of the plants (Ningen et al., 2005). Ningen et al. (2004) also showed that *E. fortunei* plants grown with a 19.3 °C night temperature had fewer disease symptoms than plants grown at 28.6 °C night

temperature. This confirms data presented by Mahoney and Tattar (1980b) which found that the optimum temperature range for vegetative growth and spore germination of *C. gloeosporioides* was 25 to 30 °C.

In many cases, good cultural practices can adequately control fungal diseases. There are many advantages to using cultural controls over fungicidal controls such as reducing environmental concerns (water pollution, etc.) and production costs. One nursery in Oklahoma reported an annual savings of $37,000 to $42,000 in labor and chemical costs alone after switching *E. fortunei* production to higher shade intensities coupled with afternoon irrigation (Albert Bond, personal communication). The nursery was able to eliminate all but spot applications for the entire growing season. In addition to reducing multiple fungicide applications on *E. fortunei* the nursery experienced indirect savings because other crops were able to receive pesticide applications which improved overall quality.

Optimal growing conditions for *C. gloeosporioides* on *E. fortunei* have been identified thus it is possible to create a less favorable environment for *C. gloeosporioides* while maintaining favorable conditions of growth of *E. fortunei*. The objective of this study was to determine the effect of growing *E. fortunei* plants on plastic or gravel beds with or without periodic sanitation by sodium hypochlorite on incidence of *C. gloeosporioides*.

Materials and Methods

The study was initiated on 19 May 2004 at Greenleaf Nursery Co., Park Hill, Okla. and was terminated on 16 September 2004. Rooted cuttings of *E. fortunei* ‘Emerald ’n Gold’ taken from various locations on the nursery were planted in 3.8 L containers

with media consisting of 6 pine bark : 1 sand amended with 3 $kg \cdot m^{-3}$ dolomitic lime, 889 $g \cdot m^{-3}$ 0N-20P-0K (triple superphosphate), 259 $g \cdot m^{-3}$ 0N-0P-49.8K (KCl), 111 $g \cdot m^{-3}$ trace elements (Frit 504 HF, Frit (U.K.) Ltd., Cambridge, U.K.), 889 $g \cdot m^{-3}$ $FeSO_4$, 741 $g \cdot m^{-3}$ 46N-0P-0K (urea), and 1.2 $kg \cdot m^{-3}$ Fe_2O_3 (GU-49, Master Builders, Inc., Cleveland, Ohio) and placed under 47% shade. Plants were irrigated with about 1.25 cm of water daily by overhead sprinkler 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 on 2 June at 11.2 g/m^2 for weed control. Plants were hand-weeded as necessary thereafter. Standard nursery practices at Greenleaf Nursery determined timing of cultural activities such as shearing.

Treatments were placed on gravel beds or 0.2 mm thick black plastic-covered gravel beds. Half of the beds in each bed treatment were sprayed monthly with a 0.6% sodium hypochlorite solution using a hand sprayer following each disease rating (Fig. 4.1). Sterile plastic covers were placed around the feet of persons entering each block and were replaced for entrance into each new treatment bed to reduce introduction of *C. gloeosporioides* inoculum into the treatment area on worker shoes. Thus, treatments were a factorial combination of bed type and sodium hypochlorite treatment. Each bed extended about 30 cm beyond the pots on all sides to minimize contamination from surrounding treatments. Each treatment replication consisted of a block of plants arranged 10 pots wide by 10 pots long. The border plants were treated as guards and not rated for disease severity. Treatment combinations were replicated four times with sixty-four pots (sub samples) per replication being rated for disease severity. Plants were rated for

anthracnose symptoms when the study was initiated (19 May 2004) and at four week intervals thereafter until the end of the growing season (4 October 2004). Rating dates were 19 May, 16 June, 13 July, 3 August, 8 September and 4 October. Disease ratings were on a scale of 1 to 12 based on the Horsfall-Barratt plant disease assessment system where: 1 = no diseased tissue, 2 = 1-3% diseased tissue, 3 = 4-6% diseased tissue, 4 = 7-12% diseased tissue, 5 = 13-25% diseased tissue, 6 = 26-50% diseased tissue, 7 = 26-50% disease free tissue, 8 = 13-25% disease free tissue, 9 = 7-12% disease free tissue, 10 = 4-6% disease free tissue, 11 = 1-3% disease free tissue, 12 = no disease free tissue (Horsfall and Barratt, 1945). The Horsfall-Barratt rating system is based on the Weber-Fechner law that states the visual acuity is proportional to the logarithm of the intensity of the stimulus (the stimuli are the diseased and healthy tissue) (Hebert, 1982).

The factorial treatment combination with repeated measurements was arranged in a latin square design. Replication and cutting source were random factors corresponding to row and column in the latin square. Bed type and sanitation treatments were fixed effects while disease rating data was a repeated measure. Data were analyzed using PROC MIXED, and orthogonal contrasts were used to determine differences and trends among treatments and rating dates (SAS Institute, Inc., Cary, N.C.).

Daily average high and low temperatures were recorded (Table 4.1) with a data logger (Watchdog 425, Spectrum Technologies, Plainfield, Ill.). Photosynthetic photon flux was measured monthly using an Integrating Quantum/Radiometer/Photometer (Model No. LI-188B, LI-COR, Inc., Lincoln, Nebr.). Maximum photosynthetic photon flux (PPF) was 1210 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at plant height. Symptomatic leaves and stems were

collected from plants throughout the growing season and cultured on potato dextrose agar to confirm the causal organism.

Results and Discussion

No interaction existed between ground bed and sodium hypochlorite treatment at any rating date (data not presented). Application of sodium hypochlorite did not reduce disease incidence compared to no sodium hypochlorite application (data not presented). Disease ratings were lower ($P \leq 0.05$) with plastic (4.5) than with gravel (5.0). Disease incidence decreased linearly as the season progressed (Fig. 4.2).

While growing *E. fortunei* plants on plastic resulted in a statistical decrease in disease ratings compared to growing on gravel beds the horticultural significance was negligible. Temperatures in the production area were about 2.5, 4, and 3 °C lower than the 25-year average for the region in June, July, and August, respectively (Table 4.1). Average daily lows were also lower than the regional average during these months. This may have resulted in lower disease incidence in the crop than what has been observed in previous production years. Ningen et al. (2004) showed that lower night temperatures resulted in fewer disease symptoms on *E. fortunei* than higher night temperatures. Less disease symptoms with lower night temperatures may be attributed to less plant stress.

Mahoney and Tattar (1980b) showed that leaf lesions caused by *C. gloeosporioides* on *E. fortunei* were more numerous after 24 hours or more of leaf wetness than with shorter periods of leaf wetness. Since *C. gloeosporioides* is dependent on periods of precipitation and high relative humidity for disease establishment, infections typically correlate with wet weather and occur on recently emerged growth (Mahoney and Tattar, 1980a). In our study, leaves remained wet an average of 15.8 hours

per day during May (Table 4.1). The leaves remained wet for 24 hours on 14 of the days in May (data not presented). The average number of hours per day in which leaves remained wet was lower during later months and may have contributed to the lower disease incidence as the season progressed.

Results of this study contradict LaMondia's (2001b) assumption that the combination of overhead watering and production on plastic would increase splash (and thus spore) dispersal and result in higher disease incidence. Abang et al. (2002) and Ekefan et al. (2000) found that *C. gloeosporioides* does not survive in the soil for more than a couple of weeks suggesting that spores on plastic have an even shorter survival time than those in the soil. This reduction in inoculum of *C. gloeosporioides* on the bed type may explain why disease ratings on plastic were lower than those on gravel in our study.

While production on plastic resulted in a statistical decrease in *E. fortunei* disease ratings, further investigation will be required to determine if production on plastic is economically feasible and/or beneficial for commercial nursery producers. In the meantime, Ningen et al. (2005) showed that producing *E. fortunei* under higher shade intensities coupled with afternoon irrigation significantly decreases anthracnose disease symptoms resulting in a higher quality crop with fewer losses. Additionally, the selection of cultivars less susceptible to anthracnose can reduce the incidence of *C. gloeosporioides* on production blocks.

Literature Cited

Abang, M.M., S. Winter, K.R. Green, P. Hoffman, H.D. Mignouna, and G.A. Wolf. 2002. Molecular identification of *Colletotrichum gloeosporioides* causing yam anthracnose in Nigeria. Plant Pathol. 51:63-71.

Abang, M.M., S. Winter, H.D. Mignouna, K.R. Green, and R. Asiedu. 2003. Molecular taxonomic, epidemiological and population genetic approaches to understanding yam anthracnose disease. African J. Biotechnol. 2:486-496.

Chakraborty, S., D. Ratcliff, and F.J. McKay. 1990. Anthracnose of *Stylosanthes scabra*: Effect of leaf surface wetness on disease severity. Plant Dis. 74:379-384.

Chase, A.R. 1983. Two foliar diseases of *Euonymus* spp. Foliage Dig. 6(1):4.

Cole, J.T., J.C. Cole, and K.E. Conway. 2005. Effectiveness of selected fungicides applied with or without surfactant in controlling anthracnose on three cultivars of *Euonymus fortunei*. J. Appl. Hort. 7(1):In Press.

Davidson, H., R. Mecklenburg, and C. Peterson. 2000. Nursery management administration and culture. 4th ed. Prentice-Hall, Upper Saddle River, N.J.

Dirr, M.A. 1998. Manual of woody landscape plants. Their identification, ornamental characteristics, culture, propagation and uses. 5th ed. Stipes Publishing, Champaign, Ill.

Ekefan, E.J., S.A. Simons, and A.O. Nwankiti. 2000. Survival of *Colletotrichum gloeosporioides* (causal agent of yam anthracnose) in soil. Trop. Sci. 40:163-168.

Farr, D.F., G.F. Bills, G.P. Chamuris, and A.Y. Rossman. 1989. Fungi on plants and plant products in the United States. Amer. Phytopathol. Soc. Press, St. Paul, Minn.

Hebert, T.T. 1982. The rationale for the Horsfall-Barratt plant disease assessment system. Phytopathology 72:1269.

Horsfall, J.G. and R.W. Barratt. 1945. An improved grading system for measuring plant diseases. Phytopathology 35:655. (Abstr.).

Koelsch. M.C. 1993. Etiology and control of diseases of *Vinca minor* L. during nursery production. M.S. Thesis, Dept. of Hort. and Landscape Architecture, Oklahoma State Univ., Stillwater.

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 the *Euonymus* anthracnose pathogen, *Colletotrichum gloeosporioides*, to selected fungicides. J. Environ. Hort. 19:47-50.

Mahoney, M.J. and T. A. Tattar. 1980a. Causal organism for spot anthracnose disease identified. Amer. Nurseryman 151(13):77-78.

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

Ningen, S.S. 2003. Chemical and cultural controls of anthracnose on *Euonymus fortunei*. M.S. Thesis, Dept. of Hort. and Landscape Architecture, Oklahoma State Univ., Stillwater.

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

Ningen, S.S., J.C. Cole, M.W. Smith, D.E. Dunn, and K.E. Conway. 2005. Increased shade intensity and afternoon irrigation decrease anthracnose severity on three *Euonymus fortunei* cultivars. HortScience 40:111-113.

Whitcomb, C.E. 1996. Know it & grow it III. A guide to the identification and use of landscape plants. Lacebark Publications, Stillwater, Okla.

Table 4.1. Average daily high and low temperatures and hours of leaf wetness between rating dates for anthracnose damage on container-grown *Euonymus fortunei* at Park Hill, Okla. in 2004, and average daily high and low temperatures for the region for 1971 to 2000.

Rating date	Average daily temperature (°C) ± s.e./s.d.[z]		Hours of leaf wetness[z]
	High	Low	
	2004		
20 May to 16 June	29.2 ± 2.1	20.1 ± 3.1	15.8 ± 9.8
17 June to 13 July	30.5 ± 2.8	20.2 ± 1.9	5.8 ± 3.5
14 July to 3 Aug.	30.9 ± 2.4	20.3 ± 2.4	5.2 ± 5.7
4 Aug. to 8 Sept.	30.3 ± 2.4	18.8 ± 3.6	2.4 ± 1.9
9 Sept. to 4 Oct.	29.9 ± 2.5	16.4 ± 3.4	3.0 ± 2.3
	Historical Temperatures (1971-2000)		
20 May to 16 June	28.9 ± 0.3	17.7 ± 0.3	--
17 June to 13 July	32.9 ± 0.2	21.7 ± 0.2	--
14 July to 3 Aug.	34.8 ± 0.1	23.1 ± 0.1	--
4 Aug. to 8 Sept.	33.3 ± 0.2	21.2 ± 0.2	--
9 Sept. to 4 Oct.	27.9 ± 0.2	16.1 ± 0.3	--

[z] Temperatures and leaf wetness data were measured in the research plot at plant height under shade. Temperatures for historical data at Tulsa, Okla. were provided by Oklahoma Mesonet. Park Hill is located 116 km SE of Tulsa, Okla. Average daily high and low temperatures and hours of leaf wetness were calculated by summing the daily high and low temperatures and hours of leaf wetness, 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. s.d. = standard deviation. s.e. = standard error (used for historical temperatures).

Fig. 4.1. Research site at Greenleaf Nursery Company, Park Hill, Okla. showing bed types and treatments.

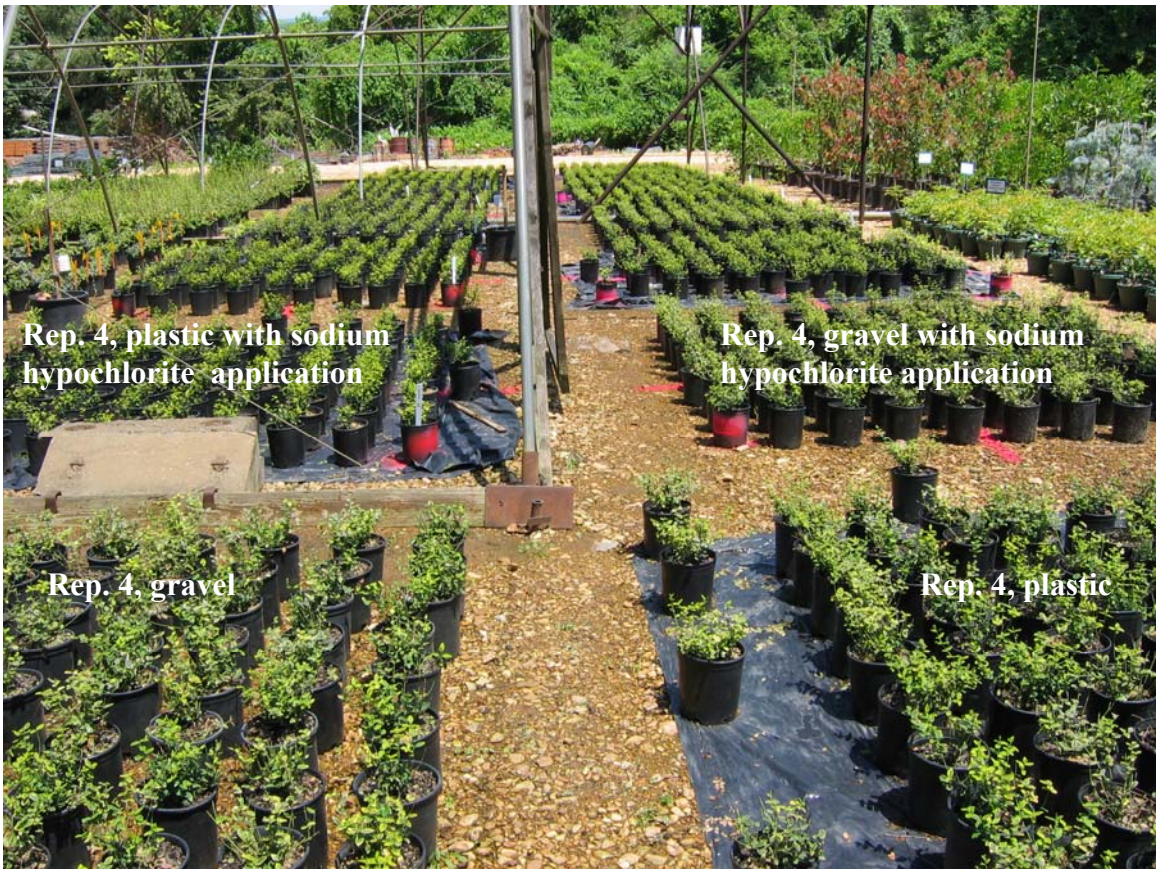
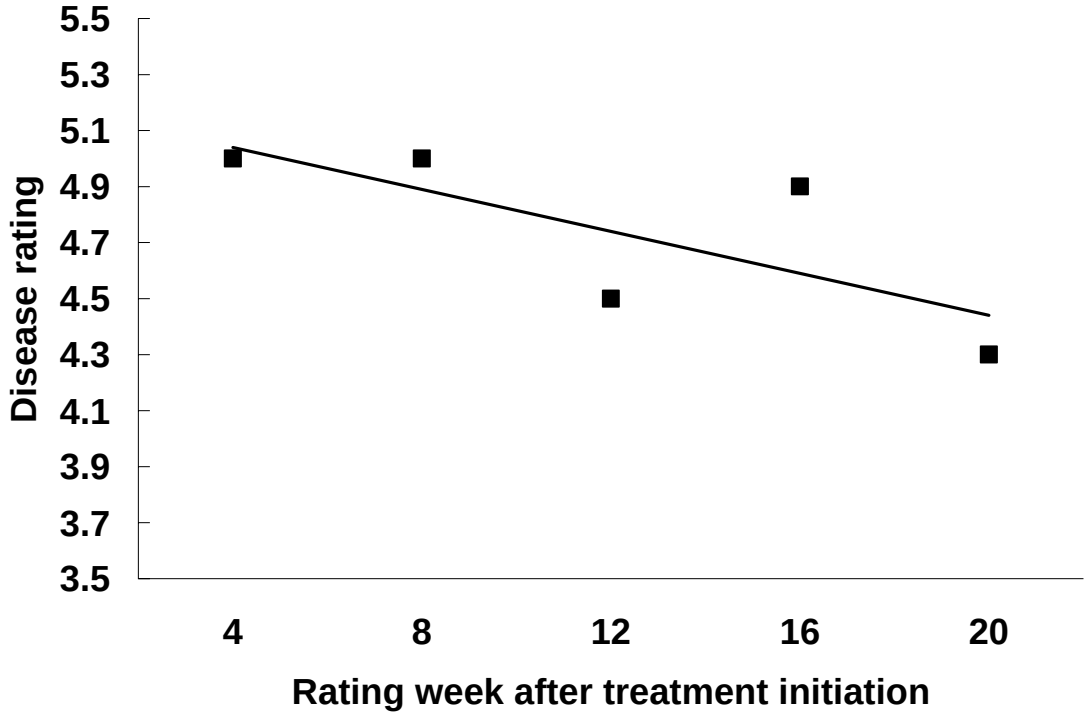


Fig. 4.2. Disease ratings of *Euonymus fortunei* during the 2004 growing season. Disease ratings are based on the Horsfall and Barratt rating scale (see text). n=4.



CHAPTER 5

SUMMARY

Euonymus fortunei (Turcz.) Hand.-Mazz. is an important horticultural crop in nursery production. It is a popular landscaping shrub with as many as 53 cultivars having been described. Unfortunately, it suffers during production from the symptoms of anthracnose caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. which include leaf and stem lesions, defoliation and stem dieback. Chemical and cultural controls that can help manage anthracnose on *E. fortunei* are advantageous to commercial nursery producers.

The objectives of this research were to 1) determine the effect of selected fungicides on *C. gloeosporioides* growing on *E. fortunei* in production and in vitro and 2) determine the effect of growing plants on plastic or gravel beds with or without periodic sodium hypochlorite application on incidence of *C. gloeosporioides* on *E. fortunei*.

Inhibition of mycelial growth of both *C. gloeosporioides* isolates by copper sulfate pentahydrate was minimal. Trifloxystrobin also provided minimal inhibition of mycelial growth of both isolates in this study. The rate of mancozeb that provided 50% inhibition of mycelial growth was less than the recommended field application rate suggesting that current label rates are appropriate for management of anthracnose symptoms on *E. fortunei.*

Of the fungicides tested in vitro, mancozeb reduced mycelial growth of *C. gloeosporioides* more than trifloxystrobin or copper sulfate pentahydrate which provided minimal mycelial inhibition.

In the fungicide study, cultivar differences existed at every rating date. Disease ratings of 'Canadale Gold' were among the lowest of the cultivars at both sites during both years. 'Emerald 'n Gold' generally had higher ratings than 'Emerald Gaiety' in 2003 at both sites and in 2004 at Stillwater, but 'Emerald Gaiety' generally had higher disease ratings than 'Emerald 'n Gold' at Park Hill in 2004.

Copper sulfate pentahydrate at 0.6 g a.i.·L^{-1} reduced disease ratings of *C. gloeosporioides* on *E. fortunei*; however, plants sprayed with mancozeb had lower disease ratings than those sprayed with copper sulfate pentahydrate.

In the cultural control study, no interaction existed between ground bed and sodium hypochlorite treatment at any rating date. Application of sodium hypochlorite did not reduce disease incidence compared to no sodium hypochlorite application. Disease incidence decreased linearly as the season progressed.

While growing *E. fortunei* plants on plastic resulted in a statistical decrease in disease ratings compared to growing on gravel beds the horticultural significance was negligible. Contrary to observations in past years, disease incidence decreased as the growing season progressed. Temperatures in the production area were about 2.5, 4, and 3°C lower than the 25-year average for the region in June, July, and August, respectively. Average daily lows were also lower than the regional average during these months. This may have resulted in lower disease incidence in the crop compared to that observed in

other years. Less disease symptoms with lower night temperatures may be attributed to less plant stress.

Colletotrichum gloeosporioides is dependent on periods of precipitation and high relative humidity for disease establishment. In this study, leaves remained wet an average of 15.8 hours per day during May and were wet for 24 hours on 14 of the days in May. The average number of hours per day in which leaves remained wet was lower during later months and may have contributed to the lower disease incidence as the season progressed.

This research shows that selection of cultivars less susceptible to anthracnose coupled with the use of mancozeb when symptoms are present will lower disease incidence in the crop. Production on plastic rather than gravel may also reduce disease incidence.

SELECTED BIBLIOGRAPHY

Abang, M.M., S. Winter, K.R. Green, P. Hoffman, H.D. Mignouna, and G.A. Wolf. 2002. Molecular identification of *Colletotrichum gloeosporioides* causing yam anthracnose in Nigeria. Plant Pathol. 51:63-71.

Abang, M.M., S. Winter, H.D. Mignouna, K.R. Green, and R. Asiedu. 2003. Molecular taxonomic, epidemiological and population genetic approaches to understanding yam anthracnose disease. African J. Biotechnol. 2:486-496.

Agrios, G.N. 1997. Plant Pathology. 4th ed. Academic Press, New York.

Alexopoulos, C.J., C.W. Mims, M. Blackwell. 1996. Introductory mycology. 4th ed. Wiley, New York.

Barnett, H.L. and Hunter, B.B. 1998. Illustrated genera of imperfect fungi. 4th ed. Burgess Publishing Co., Minneapolis.

Bartlet, D.W., J.M. Clough, C.R.A. Godfrey, J.R. Goodwin, A.A. Hall, S.P. Heaney, and S.J. Maund. 2001. Understanding the strobilurin fungicides. Pesticide Outlook, Royal Soc. Chem. 2001(4):143-148.

Bonde, M.R., G.L. Peterson, and J.L. Maas, 1991. Isozyme comparisons for identification of *Colletotrichum* species pathogenic to strawberry. Amer. Phytopathol. Soc. 81:1523-1527.

Chakraborty, S., D. Ratcliff, and F.J. McKay. 1990. Anthracnose of *Stylosanthes scabra*: Effect of leaf surface wetness on disease severity. Plant Dis. 74:379-384.

Chase, A.R. 1983. Two foliar diseases of *Euonymus* spp. Foliage Dig. 6(1):4.

Cole, J.T., J.C. Cole, and K.E. Conway. 2005. Effectiveness of selected fungicides applied with or without surfactant in controlling anthracnose on three cultivars of *Euonymus fortunei*. J. Appl. Hort. 7(1):In Press.

Cooper, W., M. Bouzayen, A. Hamilton, C. Barry, S. Rossall, and D. Grierson. 1998. Use of transgenic plants to study the role of ethylene and polygalacturonase during infection of tomato fruit by *Colletotrichum gloeosporioides*. Plant Pathol. 47:308.

Copping, L.G. and H.G. Hewitt. 1998. Chemistry and mode of action of crop protection agents. Royal Soc. of Chem., Cambridge, U.K.

Davidson, H., R. Mecklenburg, and C. Peterson. 2000. Nursery management administration and culture. 4th ed. Prentice-Hall, Upper Saddle River, N.J.

Dirr, M.A. 1998. Manual of woody landscape plants. Their identification, ornamental characteristics, culture, propagation and uses. 5th ed. Stipes Publishing, Champaign, Ill.

Duggar, B.M. 1909. Fungous diseases of plants. Ginn and Company, Boston.

Ekefan, E.J., S.A. Simons, A.O. Nwankiti, and J.C. Peters. 2000a. Semi-selective medium for isolation of *Colletotrichum gloeosporioides* from soil. Expt. Agric. 36:313-321.

Ekefan, E.J., S.A. Simons, and A.O. Nwankiti. 2000b. Survival of *Colletotrichum gloeosporioides* (causal agent of yam anthracnose) in soil. Trop. Sci. 40:163-168.

Estrada, A.B., J.C. Dodd, and P. Jeffries. 2000. Effect of humidity and temperature on conidial germination and appressorium development of two Philippine isolates of the mango anthracnose pathogen *Colletotrichum gloeosporioides*. Plant Pathol. 49:608-618.

Farr, D.F., G.F. Bills, G.P. Chamuris, and A.Y. Rossman. 1989. Fungi on plants and plant products in the United States. Amer. Phytopathol. Soc. Press, St. Paul, Minn.

He, C., A.G. Rusu, A.M. Poplawski, J.A.G. Irwin, and J.M. Manners. 1998. Transfer of a supernumerary chromosome between vegetatively incompatible biotypes of the fungus *Colletotrichum gloeosporioides*. Genetics 150:1459-1466.

Hebert, T.T. 1982. The rationale for the Horsfall-Barratt plant disease assessment system. Phytopathology 72:1269.

Horsfall, J.G. and R.W. Barratt. 1945. An improved grading system for measuring plant diseases. Phytopathology 35:655. (Abstr.).

Horst, R.K. 1990. Westcott's plant disease handbook. 5th ed. Van Nostrand Reinhold, New York.

Hutson, D. and J. Miyamoto. 1998. Fungicidal activity: Chemical and biological approaches to plant protection. Wiley, West Sussex, England.

Kalra, A., T.N. Parameswaran, and N.S. Ravindra. 1988. A leaf blight of scented geranium caused by *Colletotrichum gloeosporioides* Penz. Current Sci. 57:1136-1137.

Kim, Y., Z. Liu, D. Li, and P.E. Kolattukudy. 2000. Two novel genes induced by hard-surface contact of *Colletotrichum gloeosporioides* conidia. Bacteriology 182:4688.

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

Koelsch, M.C., J.C. Cole, and S.L. von Broembsen. 1995b. First report of leaf spots and stem lesions on common periwinkle caused by *Colletotrichum gloeosporioides*. Plant Dis. 79:83.

Koelsch. M.C. 1993 Etiology and control of diseases of *Vinca minor* L. during nursery production. M.S. Thesis, Dept. of Hort. and Landscape Architecture, Oklahoma State Univ., Stillwater.

Kumar, V., V.P. Gupta, A.M. Babu, R.K. Mishra, V. Thiagarajan, and R.K. Datta. 2001. Surface ultrastructural studies on penetration and infection process of *Colletotrichum gloeosporioides* on mulberry leaf causing black spot disease. J. Phytopathology 149:629-633.

Kumari, P.S. and M.C. Nair. 1981. Post-infectional changes in total carbohydrates and phenolics in the various parts of the leaf spot incited by *Colletotrichum gloeosporioides* on *Hydrangea hortensia*. Indian Phytopathol. 34:470-471.

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 the *Euonymus* anthracnose pathogen, *Colletotrichum gloeosporioides*, to selected fungicides. J. Environ. Hort. 19:47-50.

Marschner, H. 2003. Mineral nutrition of higher plants. 2nd ed. Academic Press, Amsterdam.

Mahoney, M.J. and T. A. Tattar. 1980a. Causal organism for spot anthracnose disease identified. Amer. Nurseryman 151(13): 77-78.

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

Manandhar, J.B., G.L. Hartman, and T.C. Wang. 1995. Semiselective medium for *Colletotrichum gloeosporioides* and occurrence of three *Colletotrichum* spp. on pepper plants. Plant Dis. 79:376-379.

Mohanan, C., 1988. *Colletotrichum* foliar infections on *Leucaena leucocephala* in Kerala, India. Current Sci., 57:1299-1300.

Mortensen, K., 1991. *Colletotrichum gloeosporioides* causing anthracnose of *Lavatera* sp. Can. Plant Dis. Survey 71:155-159.

Munaut, F., N. Hamaide, and H. Maraite. 2001. Molecular and morphological characterization of *Colletotrichum gloeosporioides* from native Mexican *Stylosanthes* species. Plant Pathol. 50:383.

Ningen, S.S. 2003. Chemical and cultural controls of anthracnose on *Euonymus fortunei*. M.S. Thesis, Dept. of Hort. and Landscape Architecture, Oklahoma State Univ., Stillwater.

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

Ningen, S.S., J.C. Cole, M.W. Smith, D.E. Dunn, and K.E. Conway. 2005. Increased shade intensity and afternoon irrigation decrease anthracnose severity on three *Euonymus fortunei* cultivars. HortScience 40:111-113.

Oh, B.J., K.D. Kim, and Y.S. Kim. 1999. Effect of cuticular wax layers of green and red pepper fruits on infection by *Colletotrichum gloeosporioides*. J. Phytopathol. 147:547-552.

Phyton Corp. 2004. Phyton news: Mode of action. Phyton Corp., Edina, Minn. Oct. 2004 Nursery Edition.

Redlin, S. 1991. *Discula destructive* sp. Nov., cause of dogwood anthracnose. Mycologia 83:633-642.

Sijpesteijn, A.K. 1982. Mechanism of action of fungicides, p. 32-45. In: Dekker, J. and S. G. Georgeopoulos (eds.). Fungicide resistance in crop protection. Ctr. Agri. Publishing and Documentation, Wageningen, Neth.

Smith, H. and M.J. Wingfield. 1998. Eucalyptus die-back in South Africa associated with *Colletotrichum gloeosporioides*. South African J. Bot. 64:226.

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

Subramanian, J. 1995. Selection and characterization of resistance in mango (*Mangifera indica* L.) embryogenic cultures to the phytotoxin produced by *Colletotrichum gloeosporioides* Penz (anthracnose). Ph.D. Dissertation. Environ. Hort. Dept., Univ. of Florida, Gainesville.

Whitcomb, C.E. 1996. Know it & grow it III. A guide to the identification and use of landscape plants. Lacebark Publications, Stillwater, Okla.

VITA

Cheryl R. Boyer

Candidate for the Degree of

Master of Science

Thesis: NURSERY PRODUCTION OF *EUONYMUS FORTUNEI*: CHEMICAL AND CULTURAL PRACTICES FOR CONTROLLING ANTHRACNOSE

Major Field: Horticulture

Biographical:

Personal Data: Born in Cushing, Okla., May 9, 1980, the daughter of Jimmy D. and Carolyn K. Mason. Married Russell P. Boyer on June 10, 2000.

Education: Graduated from Stillwater High School, Stillwater, Okla. in May 1998; received Bachelor of Landscape Architecture Degree from Oklahoma State University, Stillwater, Okla. in May of 2003. Completed the requirements for the Master of Science Degree in Horticulture at Oklahoma State University, Stillwater, Okla. in May of 2005.

Professional Experience: Employed by Oklahoma State University, Dept. of Horticulture and Landscape Architecture as a GPS (Global Positioning System) data technician mapping plant material at the OSU Botanical Gardens, 2000-2002; OSU HORT/LA graduate research and teaching assistant, 2003 to present.

Professional Memberships: American Society for Horticultural Science, Pi Alpha Xi, Sigma Xi.