ETIOLOGY AND CONTROL OF FOLIAR DISEASES OF COMMON PERIWINKLE (*VINCA MINOR* L.)

By

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ETIOLOGY AND CONTROL OF FOLIAR

DISEASES OF COMMON PERIWINKLE

(*VINCA MINOR* L.)

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PREFACE

The purpose of these studies was to determine the major causal agent(s) of foliar diseases of common periwinkle (*Vinca minor* L.) during nursery production in Oklahoma and to investigate chemical and cultural control options. Disease symptoms included leaf spots, leaf anthracnose, and stem dieback. The pathogen most frequently observed was *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. in Penz., which was associated with leaf anthracnose and stem dieback. Fungicides tested provided little control; therefore, careful cultural practices, such as the use of irrigation methods that reduce foliage wetness or create less splashing and use of resistant cultivars were identified as potential methods of controlling foliar diseases.

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CHAPTER I

INTRODUCTION

Common periwinkle (*Vinca minor* L.) is known by several names: common periwinkle, lesser periwinkle, myrtle, and running myrtle, (Bailey and Bailey, 1976). To avoid confusion with the annual periwinkle (*Catharanthus roseus* L.), this perennial ornamental will be referred to as common periwinkle. This landscape plant is a member of the Apocynaceae family. It is native to the Old World (Bailey and Bailey, 1976) - primarily Europe and western Asia where it has been cultivated for thousands of years (Dirr, 1990). The leaves are dark green and opposite in arrangement. The flowers of common periwinkle (native species) are single with a lilac-blue corolla; however, flowers of cultivars may range from white to deep purple. It is propagated through cuttings or division (Bailey and Bailey, 1976).

There are numerous cultivars of common periwinkle. Plant variations include flower color, single or double flowers, and variegated foliage. The two types of plant material used throughout this research were common periwinkle (native species) and 'Bowles' periwinkle (*Vinca minor* L. 'Bowles'). Common periwinkle is harvested from the wild, and is therefore, genetically diverse. It has long, narrow deep green leaves and purple flowers. 'Bowles' has lighter green leaves that are more rounded. The flower color of 'Bowles' periwinkle is similar to that of the common periwinkle (native species) (Bailey and Bailey, 1976).

Common periwinkle is widely used as an ornamental ground cover in landscapes (Huner, et al, 1988). It tolerates dry sites, shade, and has been recommended for use in urban areas under stress (Corley, 1986). Common periwinkle tolerates soils with a pH between 5.0 and 6.5 (Hamilton, 1981). Once established in the landscape, common periwinkle can thrive; however, if diseased plants are introduced into the planting area and improper cultural practices such as excessive moisture, exposure to too much sunlight, or poor air movement around wet foliage are followed, disease symptoms rapidly spread and the plants will not survive.

The popularity of common periwinkle among homeowners and landscape personnel has increased in recent years due to its relatively low maintenance once established, and its shade tolerance. The production of common periwinkle in Oklahoma has however, decreased, resulting in its being eliminated from many nursery inventories (Mark Andrews, Greenleaf Nursery, Park Hill, OK and Gary Percefull, Arrowhead Growers, Tulsa, OK, personal communications). The primary reason for this decline is the incidence of foliar diseases. Disease symptoms include anthracnose, stem and leaf spots, and stem dieback. The diseases are associated with common periwinkle production, due to introduction of diseases from infected propagation material and production practices which encourage the spread of pathogens.

Many Oklahoma nurseries propagate their own common periwinkle plants; however, some nurseries import common periwinkle propagation material from out of state sources. It is estimated that there is a 10% to 60% loss of plants to disease depending upon the plant stage at the outbreak of disease symptoms (Mark Andrews, Greenleaf Nursery, Park Hill, OK and Gary Percefull, Arrowhead Growers, Tulsa, OK,

personal communication). This high loss rate and the need for careful cultural practices makes common periwinkle a high maintenance crop, resulting in increased labor costs compared to other ornamental ground cover crops. It is, therefore, not economically beneficial for most nurseries to propagate and maintain this crop year-round. Some wholesalers ship common periwinkle in for quick availability to retailers and landscapers. The short amount of time that the crop remains in the seller's inventory reduces the need for chemical disease control, since the plants are generally sold before disease symptoms appear, making the plants less saleable, and resulting in a profit loss.

There are numerous reports of pathogenic organisms attacking common periwinkle. A *Colletotrichum* Corda in Sturm. sp. has been recorded from anthracnose symptoms in Florida and North Carolina. *Phoma exigua* Desmaz. var. *inoxydabilis* Boerema & Vegh in Vegh et al. has been reported in California (Farr et al., 1989). Other organisms infecting common periwinkle include species of; *Botryosphaeria* Ces and De Not., *Phomopsis* (Sacc.) Bubak., *Diplodia* Fr. in Mont., *Macrophoma* (Sacc.) Berl. and Voglino, *Phytophthora* de Bary., *Alternaria* Ness, nom. cons., *Cladosporium* Link: Fr., and *Botrytis* P. Mich. ex Pers. (Farr, et al., 1989). *Phoma exigua* Desm. var. *exigua* Mass. has been isolated and its pathogenicity to common periwinkle has been determined (Paulson and Schoeneweiss, 1971). The pathogen *Phyllosticta vinceae-minoris* Bres. et Krieg. has also been documented to be the causal agent for leaf spots (Voros and Nagy, 1969). Many of these fungi are reported to be wound pathogens which enter the plants through natural or artificial (mechanically created) openings (Hawthorne and Otto, 1986).

Phoma exigua Desm. Boerema and Howeler is difficult to correctly identify. *Phoma* Sacc., nom. cons. has many species and *P. exigua* has several varieties. *Phoma*

sp. causes leaf spot in holly (*Ilex aquifolium* L.) (Mishra and Dickinson, 1981), and in Koreanspice viburnum (*Viburnum carlesii* Hemsl.) (Gullino, 1983). *Phoma* spores have been shown to survive in hibiscus (*Hibiscus rosa-sinensis* L.) flowers (Rao and Manoharachary, 1985). In addition to common periwinkle, *P. exigua* var. *exigua* (Paulson and Schoeneweiss, 1971) also attacks hollyhock (*Alcea rosea* L.) and numerous vegetable crops (Morgan-Jones and Burch, 1988).

Colletotrichum gloeosporioides (Penz.) Penz. and Sacc. in Penz. (the imperfect stage of *Glomerella cingulata* (Stoneman) Spauld. and H. Schrenk.) also infects ornamental plants, causing foliar lesions. Much research on dieback of camellia cultivars (*Camellia* L.) caused by this fungus has been documented (Baxter, 1991; Baxter et al., 1988; and Can et al., 1978). *C. gloeosporioides* has also been identified as the causal agent of anthracnose or leaf spot on *Euonymus fortunei* (Turcz.) Hand.-Mazz. 'Emerald n' Gold' and 'Gaiety' (Mahoney and Tattar, 1980). Other diseases on ornamental plants caused by *C. gloeosporioides* include: 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. pachyphyllum* Rose, and *Sempervivum tectorum* L.) leaf rot (Chase, 1983), and *Leucaena leucocephala* (Lam) de Wit. leaf lesions (Mohanan, 1988). In addition, this organism is known to cause rotting in a variety of fruits such as avocados and kiwifruit (Muirhead et al., 1982; Hawthorne and Otto, 1986).

COLLETOTRICHUM GLOEOSPORIOIDES

C. gloeosporioides produce conidia in a fruiting structure called an acervulus contained in a gelatinous matrix which aids in dispersing spores by water splashing during

irrigation practices (Louis and Cooke, 1985). The acervuli are orange in color. The matrix is a protective coating that prevents damage to the spores from detrimental temperatures, short-wave radiation, desiccation, and pre-mature germination (Chung and Wilcoxson, 1969; Griffiths, and Peverett, 1980; Louis and Cooke, 1983; Nicholson and Moraes, 1980). Harrower (1976) and Griffiths and Peverett (1980) assumed that this matrix allows germination during or soon after spore dissemination after proper dilution of the matrix substance has occurred. Therefore, disease severity and spore germination of *C. gloeosporioides*, *C. lagenarium* (Passer.) Ell. & Halsted, and *P. medicaginis* Malbr. & Roum. are greatly reduced when the protective matrix has been removed (Williams and Allison, 1952; Allison, 1962; Renfro, and Wilcoxson, 1963; Chung and Wilcoxson, 1969).

Trevorrow et al. (1988) noted that within 72 hours after inoculation, 58% of conidia of *C. gloeosporioides* germinated. Fungal adaptations necessitated wet leaf surfaces for a specific time period for conidia suspension, dispersal, and infection (Fitt and McCartney, 1985). This period of leaf wetness is the foundation for many forecasting models for numerous plant pathogens that have this type of matrix (Louis and Cooke, 1985; Royle and Butler, 1986; Jones, 1986).

The matrix of many fungi including *C. gloeosporioides* f. sp. *jussiaea* Earle contains crowded spores in which the number of spores germinated is greatly reduced or eliminated by the crowded conditions (Allen, 1976; Cochrane, 1958). This is common with many fungi and is referred to as autoinhibition or self-inhibition that controls the proper distribution of fungal species (Allen, 1976; Cochrane, 1958; Gottleib, 1973; Lax et al., 1985; and Sussman and Halvorson, 1966). *Colletotrichum* is a Deuteromycete that has endogenous self-inhibitors (Allen, 1976) as do uredospores of rusts. Numerous

studies have determined that crowded spores have endogenous self-inhibitors that block germination (Allen, 1955, 1972; Bell and Daly, 1962; Macko et al., 1972; Musumeci et al., 1974). Lax et al. (1985) discovered that the reduction in *C. gloeosporioides* spore germination was not associated with pH or other substances removed by dispersal. Lax et al. (1985) also isolated the inhibitor which had a high biological activity. This inhibitor had the potential for utilization in the development of a model for fungistatic compounds, since it does inhibit one *Fusarium* sp. and many *Colletotrichum* spp. (Lax et al., 1985).

High humidity and temperature, and a specific amount of darkness following initial inoculation affect *C. gloeosporioides* disease incidence and severity. Chakraborty et al. (1990) reported that infection of *C. gloeosporioides* on *Stylosanthes scabra* Vog. was optimal when plants were exposed to 16 h of leaf wetness, a temperature between 20° and 30°C, and high relative humidity for the first 12 hours after inoculation. Each lesion that appeared represented a single infection (Chakraborty et al., 1990).

Abraham et al. (1988) isolated, identified, and confirmed the pathogenicity of *C. gloeosporioides* in *Artocarpus incisa*. Prolonged disease symptoms resulted in twig die-back. In addition to the infection on directly inoculated twigs, infection that initially began on the leaf lamina, spread to the petiole, then throughout the plant (Abraham et al., 1988).

Much work on *G. cingulata* infecting cultivars of camellia (*Camellia* sp. L.) has been documented (Baxter, 1991; Baxter, et al., 1988; Dickens and Cook, 1989). Specific environmental conditions which favor twig blight and dieback have been identified in various cultivars. Can et al. (1978) associated overhead sprinklers with the spread of

spores by splashing water from plant part to plant part and plant to plant. Resistant cultivars of camellias have been documented (Baxter, 1991).

No research has been conducted on the physiological host-pathogen relationship between *Colletotrichum* spp. and common periwinkle. The relationship of the fungal pathogen *C. lindenuthianum* (Sacc. et Mgn) Bir et Cav. has been investigated on french beans (*Phaseolus vulgaris* L.) (Benhamou et al., 1991; O'Connell et al., 1985). They reported that initially primary mycelium hyphae invaded plant cells, as the fungus colonized an area of plant tissue. Once this was established, the hyphae penetrated the cell walls and intercellular spaces. Endo PG was found to allow the fungus to enter affected cell walls. The primary walls became swollen and eventually shredded tissue resulted. Secondary mycelium grew from infected cells which lead to disintegration of cell walls. Subsequently, necrosis of plant tissue appeared and death resulted.

Increased nitrogen levels have been associated with thinner cell walls which allow easier fungal infection in camellias (Can et al., 1978). Benhamou et al. (1991) reported the production rate of cell wall degrading enzymes was determined by the nutritional environment of the pathogen. Pectic compounds appear to aid in the host's defense system against fungal infection. Cervone et al. (1987) discovered PGIP (proteins) inhibited some PG (enzyme) activity. The pH within host cells influence the PGIP inhibition.

Chemical control of *C. gloeosporioides* on common periwinkle is difficult to achieve, since most fungicides are not labeled for use on common periwinkle. Government regulations and increasing costs of research necessary to obtain labeling for minor crop use have discouraged chemical companies from obtaining such labels.

Cultural practices can be associated with the continued occurrence of infection and spread of *C. gloeosporioides* on common periwinkle. Plants are typically produced in a minimum amount of space under shade with overhead irrigation. These cultural practices provide optimum conditions for fungal spores to germinate, disseminate, and infect the plants. The increased moisture and shade requirements for optimum plant production, also provide higher humidity and lower light intensity for the clear spores to proliferate.

FUNGICIDAL CONTROL OF FOLIAR DISEASES

There are pros and cons for the use of systemic and protectant fungicides. Protectant fungicides must be applied prior to disease occurrence and several applications are required. Systemic fungicides may eliminate the disease pathogen due to plant absorption and translocation (Edgington et al., 1980). Several studies have demonstrated that the plant cuticle is associated with absorption of substances and rate of absorption is related to plant metabolic activity (Barrier and Loomis, 1957; Sargent and Blackman, 1962).

Thiophanate methyl. Thiophanate methyl (dimethyl [(1,2-phenylene)-bis (iminocarbonothioyl)] bis [carbamate])--also known as dimethyl 4,4'-o-phenylenebis [3-thioallophanate] is a benzimidazole. This compound has several commercial names and rates for application to agronomic crops as well as ornamentals. There has been much controversy over reports that many benzimidazoles do not effectively control certain resistant fungi. However, these reports do not apply to thiophanate methyl (Selling et al., 1970; Soeda et al., 1972; Vonk and Sijpesteijin, 1971).

Lyons and Lyda (1987) stated that the fungitoxicity of thiophanate methyl is due to carbendazim (a product of hydrolysis). Buchenauer et al. (1973) reported carbendazim

was the product of thiophanate-methyl a few days after an application on cotton due to exposure to sunlight. The study of Lyons and Lyda (1987) restated the findings of Soeda et al. (1972) and Buchenauer et al. (1973) in which carbendazim residues were detected in and on all plant parts for a long time period after foliar application. The research of Lyons and Lyda (1987) consisted of the application and detection of carbendazim of soybeans. It was determined with young and actively growing plants, that carbendazim does not decompose (remained in plant at fungitoxic rates for 2-3 weeks) and there was a minimal amount of dispersal of the fungicide throughout the plant as it matured. Senescent plants contained a small amount of the residue, since there was less active uptake. The overall recommendation from this study was to apply the fungicide to cover the entire plant (Lyons and Lyda, 1987). Muirhead et al. (1982) determined that carbendazim did not effectively control the post-harvest anthracnose disease caused by *G. cingulata* Stonem. Spauld. and Schrenk var. *minor* Wr. of avocado (*Persea americana* Mill.).

Propiconazole. Propiconazole (1-[2-(2',4'-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl-methyl]-1*H*-1,2,4-triazole) is a member of the triazoles, a large group of compounds that inhibit C-14 demethylase (Hancock and Weete, 1985). This compound is the propyl analog of etaconazole and is an inhibitor of the synthesis of brassicasterol by *Taphrina deformans* (Weete et al., 1983). It prevents fungal sterol formation. Basidiomycetes, Ascomycetes, and Deuteromycetes have this mechanism.

Propiconazole is a systemic fungicide used for many major cereal diseases (Owen and Donzel, 1986), leaf spot on peanuts (*Arachis hypogea* L.) (Hancock and Weete, 1984), pecan scab, and wheat rusts in which the sterol-biosynthesis is inhibited (Brown

et al., 1986; Latham and Hammond, 1983). Plants treated with propiconazole had smaller lesions and a 93% to 97% reduction in conidia produced from lesions of northern leaf blight as compared to the control plants (Bowen and Pedersen, 1988). Bowen and Pedersen (1988) stated that the incidence of northern leaf blight was lower on plants treated with propiconazole than on mancozeb treated plants.

Bowen and Pedersen (1988) also noted increased inhibition of colony growth of *Exserohilum turcicum* (Pass.) K.J. Leonard & E.G. Suggs as propiconazole rates increased. However, Siegel (1981) used *in vitro* tests to determine that there was no inhibition of conidial germination with propiconazole, which is a characteristic of sterol-biosynthesis inhibiting compounds (Siegel, 1981).

Mancozeb. The fungicide mancozeb has been used for control of diseases caused by *Colletotrichum* spp. on alfalfa (Broscious and Kirby, 1988). In addition, this pesticide has been tested as a protectant for northern leaf blight (*E. turcicum*) and was determined to be effective when applied after disease symptoms appeared (Bowen and Pedersen, 1988). Lesions from plants treated with mancozeb had a 50% to 72% reduction of conidia compared to the control plants (Bowen and Pedersen, 1988). Dickens (1990) conducted a fungicide trial for the control of white rust (*Puccinia horiana* Henn.) with propiconazole on chrysanthemum (*Chrysanthemum morifolium* L). Mancozeb and triforine were compared and showed significant control, but were similar in reduction of infection when sprayed once as a protectant. Propiconazole had similar results when used as a protectant, by reducing infection and, therefore, the effectiveness of this fungicide was not greater than mancozeb and triforine when used as protectants (Dickens, 1990).

Triforine. The fungicide triforine is a colorless, odorless, crystalline substance that disintegrates at 155°C and has a rodent LD_{50} over 6000 mg/kg. Bourke et al. (1977) recorded triforine residues in McIntosh, Cortland and Delicious apples (0.03 to 0.08 ppm), blueberries (*Vaccinium* spp. L) (0.17 ppm) and prunes (*Prunus* spp. L.), peaches (*Prunus* spp. L), and grapes (*Vitis* spp. L.) (averaging 0.06). Triforine has been used to control powdery mildew (*Podesphaera leucotricha* (Ell. & Everh.) Salm. on apples (*Malus* spp. L) (Cimanowski and Szkolnik, 1985).

Chlorothalonil. Chlorothalonil has been used to control numerous diseases on ornamentals, turf, and vegetables. The conidia and sclerotia of tomato anthracnose (*C. coccodes* (Wallr.) S.J. Hughes) were shown *in vitro* to be sensitive to chlorothalonil (Dillard, 1988). The distribution of inoculum determines the effectiveness of *C. lagenarium* (Passer.) Ell. & Halsted on cucumbers (*Cucumis sativa* L.) (Thompson and Jenkins, 1985). Barnard (1984) tested chlorothalonil and copper hydroxide for the most effective control against stem canker (*Cylindrocladium scoparium* Morg.) on Eucalyptus seedlings (Barnard, 1984). No phytotoxicity was apparent with copper hydroxide, but it was not as effective as chlorothalonil in controlling stem canker (Barnard, 1984).

Integrated pest management (IPM) utilizes a combination of cultural practices influenced by environmental conditions and chemical treatments for pest control. Cultural practices that influence the reduction of fungal disease occurrence and severity on plant material include the use of resistant cultivars, irrigation techniques to reduce spore dissemination, and disease-free plant material, providing proper light for plant material, sanitation of tools and growing media, and early detection of disease symptoms. These can reduce plant stress for improved plant health and better ability of the plant to resist

the attack of pathogenic organisms. These practices can reduce pesticide use due to less disease; hence, less fungicide use. The reduction in use of pesticides with IPM may enable nursery and greenhouse growers to reduce chemical costs and adverse effects on the environment. Fungicides can be used in an IPM program when utilized with other disease management techniques (Broscious and Kirby, 1988).

Continued research on fungicide control of pathogens which infect common periwinkle and cultural practices which may contribute to pathogen spread could solve problems currently faced by Oklahoma producers. This would facilitate an increase in common periwinkle production in Oklahoma to potentially supply local demands as well as those of surrounding states for saleable plants. The objectives of these studies were: 1) to isolate and identify pathogen(s) that create foliar diseases on common periwinkle, 2) to determine optimum conditions for infection by these pathogens, 3) to evaluate the effectiveness of several fungicides in controlling foliar diseases of common periwinkle, 4) to evaluate cultivar differences in susceptibility to fungal pathogen(s), and 5) to determine methods of pathogen dissemination among cultivars.

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CHAPTER II

ETIOLOGY OF FOLIAR DISEASES OF COMMON PERIWINKLE (*VINCA MINOR* L.) OCCURRING DURING NURSERY PRODUCTION

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ABSTRACT

The production of common periwinkle (*Vinca minor* L.), a shade tolerant ground cover, has declined in recent years due to foliar diseases. Two fungal pathogens, *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. in Penz. and *Phoma exigua* Desmaz. var. *inoxydabilis* Boerema & Vegh in Vegh et al. were consistently observed on infected plant material from Oklahoma nurseries. Symptoms caused by *C. gloeosporioides* included severe anthracnose and stem dieback, while *P. exigua* var. *inoxydabilis* caused leaf and stem lesions. The pathogenicity of two isolates of *C. gloeosporioides* and one isolate of *P. exigua* var. *inoxydabilis* was evaluated on common periwinkle and 'Bowles' periwinkle. Specific conditions required for infection were determined for *C. gloeosporioides*. Infection was greatest at $\pm$28°C with high humidity and a dark initiation period.

Keywords: anthracnose, *Colletotrichum gloeosporioides*, infection, leaf spots, lesser periwinkle, myrtle, *Phoma exigua* var. *inoxydabilis*, running myrtle.

Abbreviations: K1 = *Colletotrichum gloeosporioides* isolated from 'Bowles' periwinkle, K2 = *Colletotrichum gloeosporioides* isolated from 'Emerald Gaiety' evergreen wintercreeper, K3 = unidentified fungus isolated from 'Bowles' periwinkle, K4 = *Phoma exigua* var. *inoxydabilis*, K5 = *Botryosphaeria* sp., MEA = malt extract agar, PDA = potato-dextrose agar, PPF = photosynthetic photon flux, SDH_2O = sterilize distilled deionized water.

INTRODUCTION

Common periwinkle (*Vinca minor* L.) is also known by several names; myrtle, running myrtle, and lesser periwinkle (Bailey and Bailey, 1976). This landscape plant is a member of the Apocynaceae family. Common periwinkle is widely used as an ornamental ground cover in landscapes (Huner, et al., 1988). It tolerates poor soils, dry sites, shade, and has been recommended for use in urban areas under stress (Corley, 1986).

The popularity of common periwinkle among homeowners and landscape personnel has increased in recent years due to its relatively low maintenance once established and its shade tolerance. The production of common periwinkle in Oklahoma has, however, decreased, resulting in its being eliminated from many nursery inventories. It is estimated that there can be a 10% to 60% loss of plants to disease depending upon the plant stage at the outbreak of disease symptoms (Mark Andrews, Greenleaf Nursery, Park Hill, OK and Gary Percefull, Arrowhead Growers, Tulsa, OK, personal communication). The primary reason for this decline is the incidence of foliar diseases. Disease symptoms include anthracnose, stem and leaf spots, and stem dieback.

The objectives of these studies were to 1) isolate and identify the pathogen(s) that create foliar diseases on common periwinkle and 'Bowles' periwinkle (*Vinca minor* L. 'Bowles'), and 2) to determine optimum conditions necessary for infection.

MATERIALS AND METHODS

Nursery growers with disease problems were surveyed to obtain infected samples. Several cultivars were examined. The cultivars most frequently produced among growers were common periwinkle and 'Bowles' periwinkle. Growers indicated that the common periwinkle was more susceptible to diseases than 'Bowles' periwinkle; therefore, both the common periwinkle and 'Bowles' periwinkle were used throughout this research. Plants maintained for experiments were sprayed periodically when symptoms appeared with the fungicides propiconazole (Banner, Ciba-Geigy, Greensboro, NC) at 0.93 ml $liter^{-1}$, thiophanate methyl (Domain, Grace-Sierra, Milpitas, CA) at 1.6 ml $liter^{-1}$, and thiophanate methyl/mancozeb (Zyban, Mallinckrodt, Inc., St. Louis, MO) at 1.8 g $liter^{-1}$.

Plant Material. Liners of 'Bowles' periwinkle plants (10 cm) were transplanted in 3.8 liter plastic pots and 9.5 cm x 9.5 cm x 8 cm deep plastic pots containing a growing medium consisting of sand : Fafard #2 (Conrad Fafard Inc., Springfield, MA) (1:1 by volume) amended with 4.1 kg m^{-3} 24N-2P-6.7K slow release fertilizer (Osmocote, Grace-Sierra, Milpitas, CA), and 0.7 kg m^{-3} micronutrients (Micromax, Grace-Sierra). Rooted cuttings of common periwinkle were purchased and remained in original 5 cm by 5 cm by 6.25 cm deep containers with a medium consisting of perlite : vermiculite : peat (2:1:1 by volume) amended with 2.3 kg m^{-3} dolomite. Plants were maintained in a polyethylene greenhouse with minimum/maximum temperatures of 15/30°C under long days provided by a 2 h night interruption with incandescent lighting. All plants were fertilized with 20N-4.3P-16.6K (Peters 20-10-20 Peat-Lite Special, Grace-Sierra) and soluble trace elements (S.T.E.M., Grace-Sierra) every three weeks. Plants were watered by hand.

Isolation and Identification. Isolations were made from small circular leaf spots, leaf spots with concentric rings and stem lesions. Tissues were plated onto malt extract agar (MEA) consisting of 15 g bacto agar, 10 g malt extract, and 1 liter distilled deionized water autoclaved at 120°C for 20 minutes in 9 cm plastic petri dishes, and were incubated at 27°C. Leaf and stem pieces were moist incubated at room temperature by placing samples in plastic bags on moistened paper towels and allowing sporulation to occur.

Several fungal organisms were identified. These fungi included; *Alternaria* Ness, nom. cons. species, *Botryosphaeria* Ces and De Not. species (K5), *Cladosporium* Link: Fr. species, *Phoma exigua* Desmaz. var. *inoxydabilis* Boerema & Vegh in Vegh et al. (K4), and *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. in Penz. (the imperfect stage of *Glomerella cingulata* (Stoneman) Spauld. and H. Schrenk.) (K1). Using the technique described by Boerema (1976), *P. exigua* var. *inoxydabilis* (K4) was isolated from small leaf spots. Plant tissue samples most frequently contained leaf and stem spots, anthracnose areas, and stem dieback. These symptoms were caused by the agent *C. gloeosporioides*. The other pathogens were not as frequently isolated.

Growth Media Study. Several media were compared for optimum spore production and mycelial growth. Media included MEA, potato dextrose agar (PDA consisting of 39 g potato dextrose agar and 1 liter of distilled deionized water autoclaved at 120°C for 20 minutes), vinca potato dextrose agar (150 ml 'Bowles' periwinkle fragmented in a Waring blender, 39 g potato dextrose agar, 900 ml distilled deionized water autoclaved for 30 minutes) (Paulson and Schoeneweiss, 1971), and V-8 agar (200 ml V-8 juice with 2 g of $CaCO_3$ diluted with 800 ml distilled deionized water and mixed on a stirring plate for 20 minutes, 15 g agar was added and the media was autoclaved at 120°C for 30 minutes)

(Ribeiro, 1978). Fungal pieces were placed in petri dishes of these media, wrapped in aluminum foil, and the resulting mycelium was allowed to grow to the edge of petri dishes in an incubator at 27°C. PDA was the best medium for both growth and sporulation and was used for further culturing. Cultures were, however, maintained on MEA in small bottles.

Temperature Optimum Growth Study. Mycelial growth rates of two fungal isolates of *C. gloeosporioides* isolated from common periwinkle (K1) and 'Emerald Gaiety' evergreen wintercreeper (*Euonymus fortunei* (Turcz.) Hand.-Mazz. 'Emerald Gaiety') (K2) and *P. exigua* var. *inoxydabilis* (K4) from common periwinkle, were determined on PDA over a range of temperatures (18, 21, 24, 27, and 30°C). Radial growth was measured every other day until growth reached the edge of the plate. This study was replicated three times within each trial and repeated twice. All three fungi grew fastest at 27°C and this temperature was used to culture them in further studies.

Spore Production and Inoculation Techniques. Fungi for spore production were placed on PDA, wrapped in aluminum foil, and incubated at 27°C. Acervuli appeared within 6 days and all cultures were harvested by 14 days. Spores were removed by rinsing cultures with sterilized distilled deionized water (SDH_2O). A hemacytometer (Baxter Healthcare Corporation, McGaw Park, IL) was used to estimate spore concentrations. Spore concentrations for the first studies were higher (10^6 ml^{-1}) than in later experiments (10^4 ml^{-1}). Spore suspensions and SDH_2O were misted with a hand mist sprayer onto plant leaves or shoots until runoff. Control treatments consisted of SDH_2O misted onto plant material.

Greenhouse Inoculation Study. The objective of this study was to determine the pathogenicity of *C. gloeosporioides* isolated from 'Bowles' periwinkle (K1), an unidentified potential pathogenic fungus (K3), *P. exigua* var. *inoxydabilis* (K4), and *Botryosphaeria* sp. (K5) on common periwinkle and 'Bowles' periwinkle on wounded and non-wounded runners within a greenhouse environment. Nine plants of each cultivar were inoculated with spores of one of the four fungal isolates or with SDH_2O (as control plants). The 'Bowles' periwinkle plants had numerous full shoots cascading over pot edges. Common periwinkle plants were smaller and had one to four shoots for each pot. Spore production and inoculation techniques were as described above.

Two shoots from each plant were wounded with a dissecting needle and labeled using colored twist-ties. A minimum of five internodes and 10 leaves per shoot were wounded for this treatment. Two non-wounded shoots of each plant were similarly identified as an additional treatment. After inoculation, the plants were placed on a mist bench covered with plastic in a greenhouse. The mist sprayed for 12 seconds every 12 minutes. Barriers were used to separate each cultivar and fungal treatment during the mist period to avoid contamination. The plants remained in the mist area for 45 h and were then removed and placed onto benches in a greenhouse on a complete randomized design with a 15 cm spacing between 'Bowles' periwinkle plants and 8 cm spacing between common periwinkle plants. Plants were hand watered as necessary and fertilized with 20N-4.3P-16.6K water soluble fertilizer (Peters 20-10-20 Peat Lite Special, Grace-Sierra) at 22.5 mg N $liter^{-1}$ and micronutrients (S.T.E.M., Grace-Sierra) at 0.06 g $liter^{-1}$ once. The number of lesions on marked shoots was counted weekly for eight weeks. Upon termination of the study wounded and other symptomatic plant parts were removed.

These infected plant parts were then incubated in a moist chamber for further examination to confirm that symptoms resulted from the inoculated fungal isolate.

Shoot Inoculation Study. The objective of this study was to determine pathogenicity of K1, K3, K4, and K5 within the controlled environment of a growth chamber. The chamber was maintained at 22°C for the first 18 days and 29.4°C, thereafter. Rooted cuttings of common periwinkle in 5 cm by 5 cm by 6.25 cm deep plastic pots and 'Bowles' periwinkle in 9.5 cm by 9.5 cm by 8 cm deep plastic pots were used for this study. Shoots of six plants each of common periwinkle and 'Bowles' periwinkle were inoculated with K1, K3, K4, or K5 spores or SDH_2O (controls) misted onto shoots.

Two shoots were chosen from each plant to be inoculated according to a method modified from Paulson and Schoeneweiss (1971). Shoots were placed in petri dishes filled with Redi-earth (W.R. Grace and Company, Cambridge, MA) sterilized at 120°C for 20 minutes and dishes were fastened with masking tape while shoots were still attached to the parent plant. Dishes were cut on opposite sides to allow the shoots to pass through the dishes without bruising plant tissue. The shoots and growing medium within the dishes were then inoculated with spore suspensions of each respective fungal isolate. After inoculation, plants received 24 h of darkness. Thereafter, the day/night cycle consisted of 12 h light with a PPF of 126 µmol m^{-2} s^{-1} and 12 h of darkness. Plants were watered as needed in the pots with tap water (approximately every second day). The medium within the petri dishes was watered as needed with SDH_2O (approximately every fourth day). Plants were fertilized once with water soluble fertilizer as described in Greenhouse Inoculation Study above. The spore count of the fungal suspensions were as follows: K1 - 9.1 x 10^5 ml^{-1}, K3 - 2.6 x 10^5 ml^{-1}, K4 - 5.5 x 10^5 ml^{-1}, K5 - 0.9 x 10^5 ml^{-1}.

Termination of the study consisted of removing inoculated shoots from the plants after 43 days. These shoots were moist incubated and examined after 15 days. Microscopic examination was used to confirm the pathogenicity of the fungal isolates by viewing the fruiting structures and spores with a light compound microscope (Nikon Labophot, Japan).

Growth Chamber Studies. Growth chambers were maintained at 22°C and 28°C. Light treatments compared in growth chamber studies included: full light treatments (120 to 151 µmol m^{-2} s^{-1}), shaded light using 50% shade cloth (22 to 39 µmol m^{-2} s^{-1}), dark initiated treatments (16 to 18 h of dark after inoculation of plant tissue with light reading of 120-151 µmol m^{-2} s^{-1}, thereafter), and dark treatments (petri dishes were covered with foil). Day/night cycles consisted of 14/10 hours of each. The humidity and mist cycles varied depending upon the study conducted.

Plant material was removed from stock plants maintained in greenhouses located at the Oklahoma State University Nursery Research Station, Stillwater, OK. For all Detached Shoot Studies, 'Bowles' periwinkle shoots ranged in length from 25 to 30 cm. Five shoots each were inoculated and placed in 250 ml flasks filled with SDH_2O. There were two replicates of each treatment for every study. Shoots were rated daily. For Detached Shoot Study I, the number of lesions per replicate was recorded. The percentage of leaves infected per shoot was determined for Detached Shoot Studies II-IV. Leaves for all studies were rated daily to observe the progress and time required for optimum infection.

Leaves of common periwinkle and 'Bowles' periwinkle were examined in all the Detached Leaf Studies. Five leaves of each cultivar depending upon treatment were

placed in petri dishes lined with sterilized paper towels and moistened with SDH_2O. The leaves were inoculated with the respective isolates and then placed in petri dishes which were sealed with parafilm before exposing to the appropriate light treatments and incubation conditions. There were two replicates of each treatment for every leaf study. Light, shade, and dark initiated treatments were wrapped in clear plastic bags and dark treatments were wrapped in foil. Each leaf was rated daily for each replicate within each treatment then replicates were averaged within treatments. Leaves were rated on a scale as follows: 1 = no disease; 2 = lesions observed; 3 = solid lesions on less than 50% of tissue; 4 = solid lesions on more than 50% of tissue; and, 5 = total necrosis of leaf tissue. A 95% confidence interval was calculated for each treatment in each growth chamber experiment.

Detached Leaf Study I. The objective of this study was to compare the pathogenicity of K1 and K2 isolates of *C. gloeosporioides* with three light treatments on common periwinkle and 'Bowles' periwinkle leaves. The study was conducted within a growth chamber programmed at 28°C. Five leaves equal in maturity and size from each cultivar were placed in 9 cm petri dishes and inoculated as described above. The three light treatments consisted of full, shaded, and dark. The spore count for K1 was 2.46×10^5 ml^{-1} and for K2 was 1.46×10^5 ml^{-1}. All plates were placed in the growth chamber following inoculation with the appropriate isolate. Termination of this study occurred after 14 days. This experiment was repeated using K1 and K2 suspensions containing spore counts of 5.35×10^6 ml^{-1} and 2.64×10^4 ml^{-1}, respectively, and terminated described above after 16 days.

Detached Leaf Study II. Leaves were inoculated as in Detached Leaf Study I with K1 and K2 suspensions with spore counts of 1.3 x 10^4 ml^{-1} and 1.34 x 10^4 ml^{-1}, respectively. The same three light treatments were provided along with a fourth treatment. This additional treatment was placed in a second growth chamber, also maintained at 28°C, and consisted of 16 h of darkness after inoculation, then the same day/night cycle as other two light treatments. This study was terminated as described above after 10 days.

Detached Leaf Study III. In this study, only dark initiated (a period of 18 h of darkness after inoculation prior to the programmed day/night cycle) incubation was evaluated. The spore count for the K1 suspension was 1.29 x 10^4 ml^{-1} and for K2 was 1.17 x 10^4 ml^{-1}. The study was terminated as described above after 10 days.

Detached Shoot Study I. Specific infection requirements of the two isolates of K1 and K2 of *C. gloeosporioides* on 'Bowles' periwinkle were determined throughout a series of studies. In this first study, several variables were evaluated for spore inoculation and growth. The inoculation period was for 45 h and included the following treatments: 1) inoculated shoots were placed on a mist bench at 16.5°C, 60 µmol m^{-2} s^{-1}, mist intervals of 12 seconds of mist every 12 minutes during day cycle, 2) shoots placed in full light, 3) shoots placed in shaded light (50% shade cloth), and 4) shoots placed in darkness (within a cabinet). Shoots in treatments 2, 3, and 4 were placed in clear plastic bags during the inoculation period. Following the inoculation period, treatments were placed either in the low (22°C) or high (28°C) temperature growth chambers. The spore count for K1 was 2.56 x 10^5 ml^{-1}. The isolate K2 was not used for this first study. The study was terminated with a final rating after 13 days.

Detached Shoot Study II. In a second study, the two light treatments, shade and full, were selected for further observations. The objective of this study was to compare infection by K1 and K2 within a growth chamber under full light and shaded light at 28°C. After inoculation, shoots were placed directly into the chamber. The spore count for K1 was 2.46×10^5 ml^{-1} and for K2 was 1.46×10^5 ml^{-1}. The growth chamber was programmed at 70% relative humidity. To insure programmed humidity and a minimum amount of leaf wetness, mist was sprayed for 3 minutes every half hour throughout the day cycle and four times for 5 minutes during the night cycle. This experiment was terminated after 14 days with a final rating.

Detached Shoot Study III. The objective of a third study was to investigate infection rate with increased moisture levels. The adjustments incurred included: relative humidity was increased from 70% to 90%, mist intervals were increased to 2 minutes of mist every 13 minutes throughout the day cycle while mist during the night cycle was maintained as in Detached Shoot Study II. The spore count for the K1 suspension was 5.35×10^6 ml^{-1} and for the K2 suspension was 2.69×10^4 ml^{-1}. This study was terminated after 14 days with a final rating.

Detached Shoot Study IV. A fourth study using increased moisture levels with three light treatments was conducted with the K1 and K2 isolates. Two growth chambers with temperatures of 28°C, greater then 90% relative humidity, and misting frequencies of 3 minutes of mist every 13 minutes throughout the day cycle were used. The mist cycle during the night cycle was maintained in both chambers as described in Detached Shoot Study II. The light treatments compared were full, shade, and dark initiated (17 h of darkness immediately following inoculation prior to day/night cycle initiation). Spore

count for the K1 suspension was 1.3 x 10^4 ml^{-1} and for K2 was 1.34 x 10^4 ml^{-1}. The study was terminated with a final rating after 8 days.

Detached Shoot Study V. This final study was to confirm the previous findings for the full and dark initiated treatments only. Methods were followed as with previous studies using the K1 isolate. The K2 isolate was not used since it had a lower infection rate than K1. The spore count for K1 was 1.29 x 10^4 ml^{-1} and was terminated after 8 days.

RESULTS

Greenhouse Inoculation Study. Few symptoms occurred during this study (data not shown). Some wounded runners developed necrosis within 1 to 2 weeks after initiation of the experiment; however, this could have been due to physical wounding damage, rather than due to disease infection. Pathogenicity of K1 and K4 were observed, although infections were few. The low numbers of infected plants probably were due to ineffective inoculation methods. Splashing of water from hand watering may have influenced the spread of pathogens from non-inoculated plants. Selected wounded and non-wounded shoots were removed from plants at the end of the study and were moist incubated to confirm infection with the inoculated pathogens.

Shoot Inoculation Study. No symptoms were observed within the first 18 days with a temperature of 18°C at plant height; however, symptoms were more prevalent after the temperature was increased to 29°C. The rate of fertilizer applied severely burned the foliage exposed to growth chamber air.

Upon termination of this study, plant parts from the petri dishes were examined for disease symptoms and acervuli. Symptoms of plant parts inoculated with *C. gloeosporioides* (K1) included anthracnose on leaves and stems. Necrotic leaves and

stems contained *C. gloeosporioides* acervuli. The fungus *Botryosphaeria* sp. (K5) was associated with tan spots encircled with black areas. *P. exigua* var. *inoxydabilis* (K4) fruiting structures were found in black sunken or raised "pimples" on necrotic leaf tips. *C. gloeosporioides* was occasionally observed on other symptomatic plant material that had not been initially inoculated with this fungal pathogen. The isolate K3 did not prove to be a true pathogen.

Detached Leaf Study I. Disease severity was greatest in the dark (Figure 2.1 and 2.2). The K1 isolate caused a higher disease severity than the K2 isolate regardless of light intensity. These trends were similar both times that this experiment was conducted. Formation of acervuli in both the K1 and K2 isolates occurred in all treatments. Leaves of control plants may have been wounded during harvest which would account for some leaves receiving a rating.

Detached Leaf Study II. The common periwinkle inoculated with K1 and receiving the dark initiation treatment had the greatest disease severity of all treatments (Figure 2.3). The K1 isolate had a higher rating of infection than the K2 isolate within each cultivar in all light treatments.

Detached Leaf Study III. The results from this study confirmed that dark initiation resulted in high levels of infection with both isolates as found in the previous study.

Detached Shoot Study I. This study examined the effect of light levels and temperature on disease incidence after inoculation. The conditions for optimum infection of *C. gloeosporioides* after inoculation on detached shoots from 'Bowles' periwinkle were similar for both stems (Figure 2.4) and leaves (Figure 2.5). The full light treatment had the highest disease incidence. The shade treatment also showed a high level of infection

after inoculation. Some spots appeared on shoots and leaves; however, no disease organism was associated with these spots. Infection was low when plants were inoculated and then placed on a mist bench in a greenhouse, probably due to a water temperature of 16°C and lengthy and frequent mist cycles.

Detached Shoot Study II. The full light treatment again had the higher disease incidence, but in this study the K2 isolate caused the higher disease incidence (Figure 2.6). Spots on leaves were primarily located on leaf margins and associated with wounded areas. Mist was used to increase leaf wetness for spore germination on the leaf surface; however, leaves were dry at times.

Detached Shoot III. Disease incidence was higher with the full light treatment and the K2 isolate had a higher incidence (Figure 2.7) as was observed in the previous study. However, symptom development was less than expected compared to leaf studies run at the same time.

Detached Shoot Study IV. The dark initiated treatment had the highest disease incidence (Figure 2.8). The full and shade treatments did not achieve satisfactory infection levels.

Detached Shoot Study V. Symptoms were observed on the dark initiated inoculated shoots within 24 h after application of the programmed day/night cycle. Small spots grew to large lesions within 4 days, after inoculation, at which time the study was terminated (data not shown).

DISCUSSION

Foliar diseases of common periwinkle have affected the numbers produced by nursery and greenhouse growers. The diseases are difficult to control once introduced due

to the intense cultural practices required during production. Symptoms include small leaf spots, leaf anthracnose, and stem dieback.

The two pathogens confirmed in these studies to be causal agents of diseases of common periwinkle and 'Bowles' periwinkle, *C. gloeosporioides* and *P. exigua* var. *inoxydabilis*, were found on most samples from surveyed growers, with *C. gloeosporioides* being the most prevalent. *Colletotrichum* Corda in Sturm. spp. have been reported on common periwinkle in Florida and North Carolina. *P. exigua* var. *inoxydabilis* has been found on common periwinkle in California (Farr et al., 1989). Other pathogens observed in this study that have been reported on common periwinkle were *Alternaria* sp., *Botryosphaeria* sp., *Cladosporium* sp., and *Phomopsis* (Sacc.) Bubak. Other fungal pathogens reported to occur on common periwinkle include: *Botrytis* P. Mich. ex Pers., *Diplodia* Fr. in Mont., *Macrophoma* (Sacc.) Berl. and Voglino, and *Phytophthora* de Bary. (Farr, et al., 1989). *Phoma exigua* Desm. var. *exigua* Mass. has been isolated and its pathogenicity to common periwinkle has been determined (Paulson and Schoeneweiss, 1971). The pathogen *Phyllosticta vinceae-minoris* Bres. et Krieg. has also been documented to be the causal agent for leaf spots (Voros and Nagy, 1969).

Pathogenicity tests confirmed *C. gloeosporioides* and *P. exigua* var. *inoxydabilis* to infect leaves and stems with or without wounds. Hawthorne and Otto (1986) reported that many fungi found on common periwinkle are wound pathogens which enter the plants through natural or artificial (mechanically created) openings.

The K1 isolate caused a higher disease severity more frequently than the K2 isolate. These two isolates were used throughout the infection studies to determine pathogenicity on common periwinkle. *C. gloeosporioides* is known to cause leaf spots

or anthracnose on many other ornamentals. A few plants include: numerous camellia cultivars (*Camellia* L.) (the imperfect stage of *Glomerella cingulata* (Stoneman) Spauld. and H. Schrenk.) (Baxter, 1991; Baxter et al, 1988), 'Emerald Gaiety' evergreen wintercreeper (*Euonymus fortunei* (Turcz.) Hand.-Mann. 'Emerald n' Gold' and 'Gaiety') (Mahoney and Tattar, 1980a and 1980b), 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. pachyphyllum* Rose, *Sempervivum tectorum* L.) leaf rot (Chase, 1983), and *Leucaena leucocephala* (Lam) de Wit. leaf lesions (Mohanan, 1988).

P. exigua var. *inoxydabilis* was in samples from nurseries but was not studied as extensively as *C. gloeosporioides*. It was a pathogen of both common periwinkle and 'Bowles' periwinkle. *Phoma exigua* Desm. Boerema and Howeler is difficult to correctly identify. *Phoma* Sacc., nom. cons. has many species and *Phoma exigua* has several varieties. The technique described by Boerema (1976) was used to identify *P. exigua* var. *inoxydabilis*. *Phoma* sp. infects holly (*Ilex aquifolium* L.) (Mishra and Dickinson, 1981) and causes leaf spots on Koreanspice viburnum (*Viburnum carlesii* Hemsl.) (Gullino, 1983). In addition to its ability to cause disease of *P. exigua* var. *exigua* on common periwinkle (Paulson and Schoeneweiss, 1971), this fungus also attacks hollyhock (*Alcea rosea* L.) and numerous vegetable crops (Morgan-Jones and Burch, 1988). *P. exigua* var. *exigua* was not found on any samples.

Leaf studies determined that common periwinkle had a higher disease incidence when infected with either isolate of *C. gloeosporioides* than 'Bowles' periwinkle. This indicated that it might be beneficial to evaluate the relative susceptibility of existing

cultivars to *C. gloeosporioides* to look for suitable resistance levels that could be useful both in nursery production and landscape settings.

Disease severity that resulted from inoculations was greater in later experiments than in earlier ones. The spore concentration of initial studies was higher than in these later studies. For many fungi, including *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. in Penz. f. sp. *jussiae* Earle, crowded spores greatly reduces or eliminates the number of spores germinated under such high concentrations (Allen, 1976; Cochrane, 1958). This autoinhibition or self-inhibition apparently controls the proper distribution of fungal species (Lax et al., 1985; Allen, 1976; Cochrane, 1958; Gottleib, 1973; Sussman and Halvorson, 1966). *Colletotrichum* is a Deuteromycete that has endogenous self-inhibitors (Allen, 1976), as do uredospores of rusts. Numerous other studies have determined that crowded spores have endogenous self-inhibitors that block germination (Allen, 1955, 1972; Bell and Daly, 1962; Macko et al., 1972; Musumeci et al., 1974). Lax et al. (1985) discovered that the reduction in *C. gloeosporioides* spore germination was not associated with pH or other substances removed by dispersal. Lax et al. (1985) also isolated the inhibitor which had a high biological activity. This inhibitor had the potential for utilization in the development of a model for fungistatic compounds, since it does inhibit one *Fusarium* sp. and many *Colletotrichum* species (Lax et al., 1985).

Infection occurred within 48 h after inoculation with a temperature of 28°C, 16 to 18 h darkness after inoculation, suitable leaf wetness with a high humidity level (>90%) and short, frequent mist cycles. Fungal adaptations necessitate wet leaf surfaces for a specific time period for conidia suspension, dispersal, and infection (Fitt and McCartney, 1985). Chakraborty et al. (1990) reported that infection of *C. gloeosporioides* on

Stylosanthes scabra was optimal when plants were exposed to 16 h of leaf wetness, a temperature between 20° and 30°C, and high relative humidity for the first 12 h after inoculation. Each lesion that appeared represented a single infection (Chakraborty et al., 1990). McRae and Auld (1988) and Manandhar et al. (1988) determined *C. orbiculare* (Berk. & Mont.) Arx required a period of darkness for appressorium formation on *C. truncatum* (Schwein.) (Andrus & W. D. Moore). Trevorrow et al. (1988) noted that within 72 h, 58% of conidia germinated after inoculation. This period of leaf wetness is the foundation for many forecasting models for numerous plant pathogens that have this type of matrix (Louis and Cooke, 1985; Royle and Butler, 1986; Jones, 1986).

Production of common periwinkle occurs under humid and mild conditions. Frequent watering intervals are necessary for increased growth. *C. gloeosporioides* produces clear conidia in light orange color acervuli contained in a gelatinous substance (Louis and Cooke, 1985). The matrix aids in dispersal of spores by water splashing during irrigation (Louis and Cooke, 1985), and serves as a protective coating that prevents damage to the spores from detrimental temperatures, short-wave radiation, desiccation, and pre-mature germination (Chung and Wilcoxson, 1969; Griffiths, and Peverett, 1980; Louis and Cooke, 1983; Nicholson and Moraes, 1980). Harrower (1976) and Griffiths and Peverett (1980) assumed that this matrix allows germination during or soon after spore dissemination after proper dilution of the matrix substance has occurred. Therefore, disease severity and spore germination of *C. gloeosporioides*, *C. lagenarium* (Passer.) Ell. & Halsted, and *P. medicaginis* Malbr. & Roum. are greatly reduced when the protective matrix has been removed (Williams and Allison, 1952; Allison, 1962; Renfro, and Wilcoxson, 1963; Chung and Wilcoxson, 1969).

Alteration of cultural practices may help reduce disease, but this is not an easy task within a production environment. Resistant cultivars may aid in reduction of disease. Fungicides can effectively be used in an Integrated Pest Management (IPM) program when utilized with other disease management techniques (Broscious and Kirby, 1988). The reduction in use of pesticides with IPM may enable nursery and greenhouse growers to reduce chemical costs and to protect the environment.

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Figure 2.1. Disease severity in full light, shaded light and dark using two isolates of *C. gloeosporioides* (K1 and K2) on common periwinkle and 'Bowles' periwinkle leaves. Rating scale: 0 = no lesions, 1 = leaf spots, 2 = <50% necrosis on leaf tissue, 3 = >50% necrosis on leaf tissue, and 4 = total necrosis of leaf tissue. Legend key: K1 = *C. gloeosporioides* isolate from 'Bowles' periwinkle, K2 = *C. gloeosporioides* isolate from 'Emerald Gaiety' evergreen wintercreeper, Control = no inoculum, Full = petri dishes placed under uninhibited lighting, Shade = petri dishes placed under 50% shade cloth, and Dark = petri dishes wrapped in aluminum foil. Vertical bars indicate 95% confidence intervals.

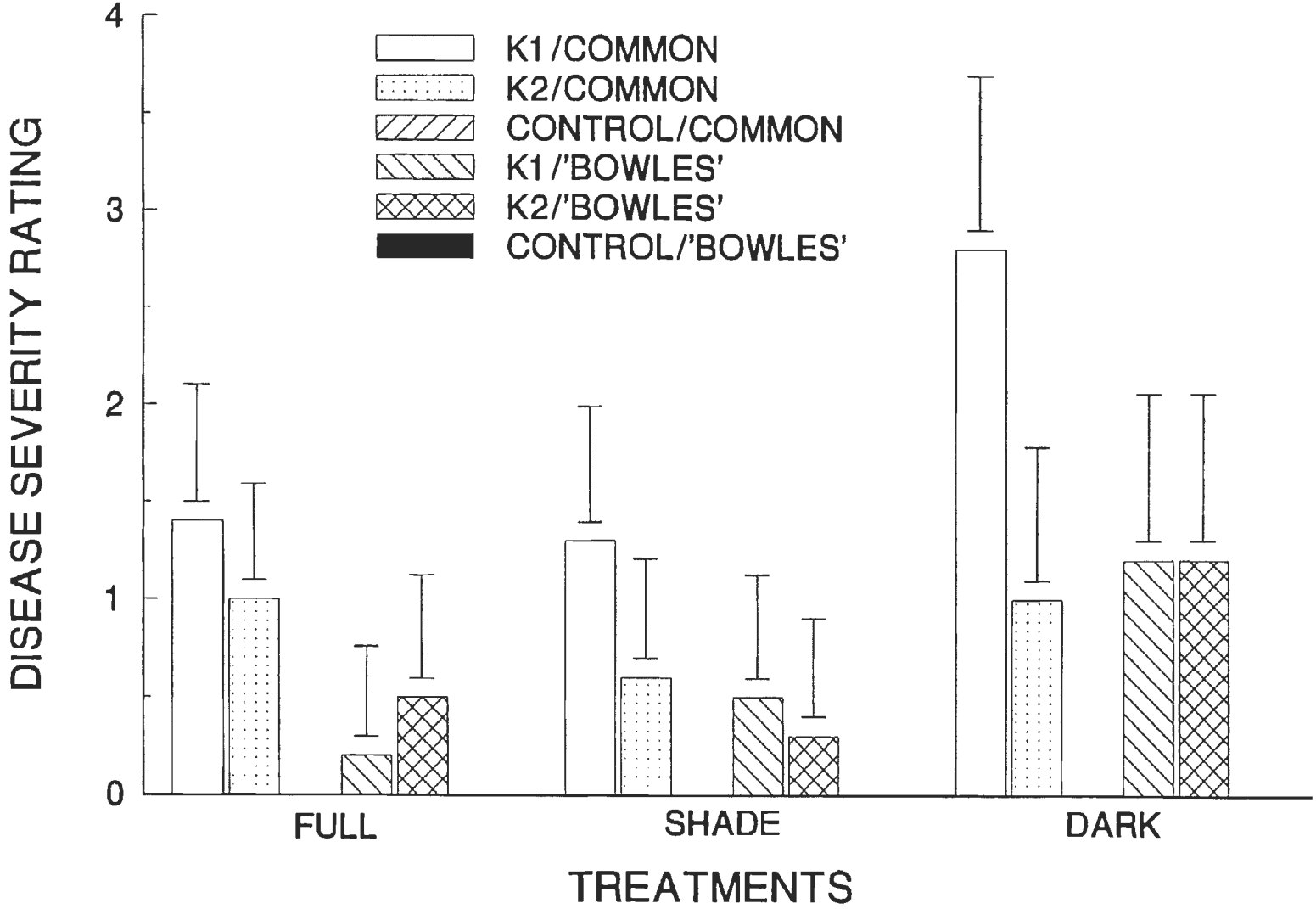

DISEASE SEVERITY RATING
4
3
2
1
0
K1 /COMMON
K2/COMMON
CONTROL/COMMON
K1 /'BOWLES'
K2/'BOWLES'
CONTROL/'BOWLES'
FULL
SHADE
DARK
TREATMENTS

Figure 2.2. Disease severity rating of two isolates of *C. gloeosporioides* (K1 and K2) on common periwinkle and 'Bowles' periwinkle leaves in full light, shaded light and dark. Rating scale: 0 = no lesions, 1 = leaf spots, 2 = <50% necrosis on leaf tissue, 3 = >50% necrosis on leaf tissue, and 4 = total necrosis of leaf tissue. Legend key: K1 = *C. gloeosporioides* isolate from 'Bowles' periwinkle, K2 = *C. gloeosporioides* isolate from 'Emerald Gaiety' evergreen wintercreeper, Control = no inoculum, Full = petri dishes placed under uninhibited lighting, Shade = petri dishes placed under 50% shade cloth, and Dark = petri dishes wrapped in aluminum foil. Vertical bars indicate 95% confidence intervals.

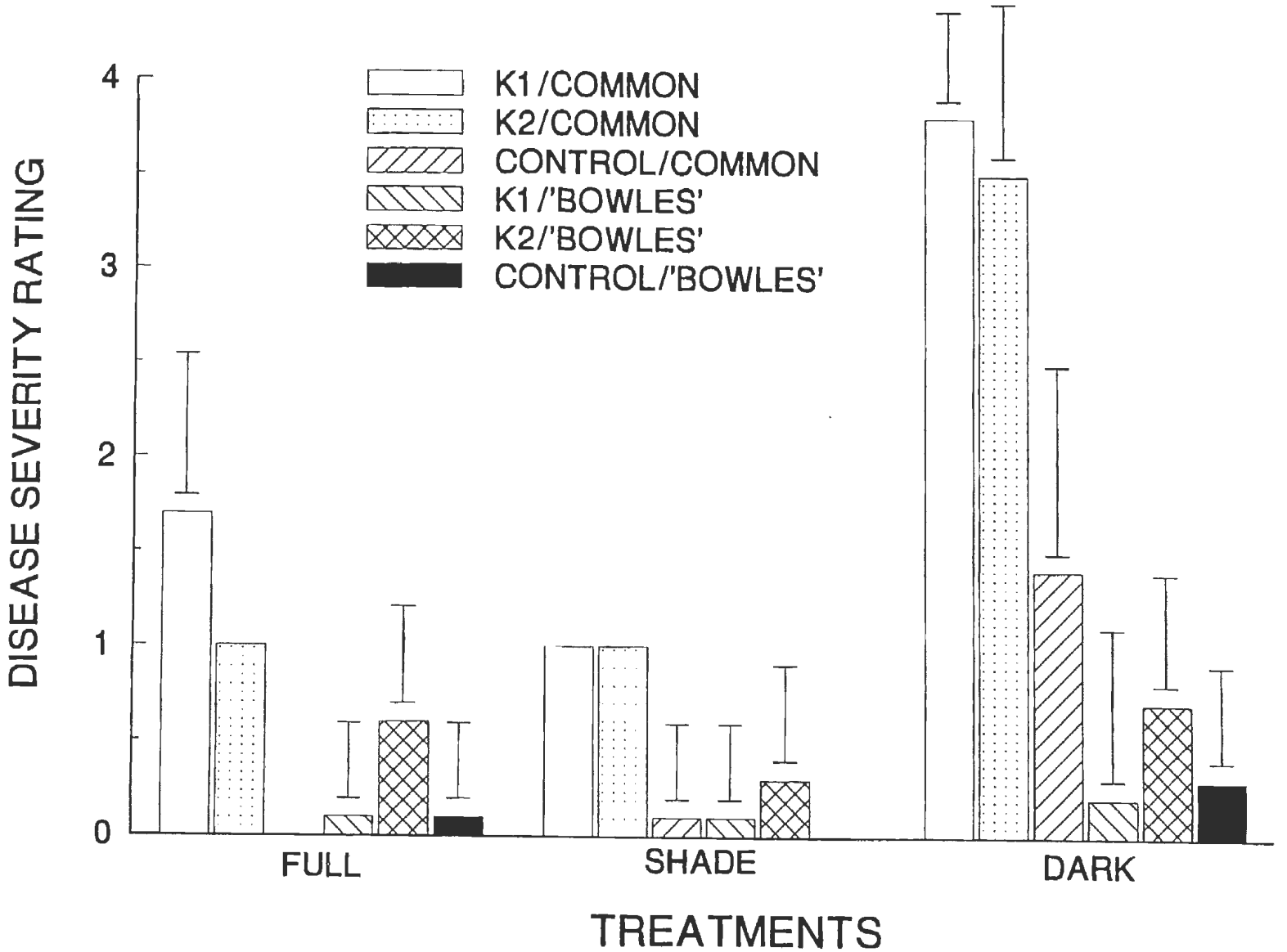

DISEASE SEVERITY RATING
4
3
2
1
0
K1/COMMON
K2/COMMON
CONTROL/COMMON
K1/'BOWLES'
K2/'BOWLES'
CONTROL/'BOWLES'
FULL
SHADE
DARK
TREATMENTS

Figure 2.3. Disease severity of two isolates of *C. gloeosporioides* (K1 and K2) on common periwinkle and 'Bowles' periwinkle leaves under four light treatments. Rating scale: 0 = no lesions, 1 = leaf spots, 2 = <50% necrosis on leaf tissue, 3 = >50% necrosis on leaf tissue, and 4 = total necrosis of leaf tissue. Legend key: K1 = *C. gloeosporioides* isolate from 'Bowles' periwinkle, K2 = *C. gloeosporioides* isolate from 'Emerald Gaiety' evergreen wintercreeper, Control = no inoculum, Full = leaves in petri dishes placed under uninhibited lighting, Shade = leaves in petri dishes placed under 50% shade cloth, Dark = leaves in petri dishes wrapped in aluminum foil, and Dark Initiated = leaves in petri dishes received 18 h of darkness prior to 14/10h day/night cycle. *Dark initiated data represents results from Detached Leaf Study II and III. Vertical bars indicate 95% confidence intervals.

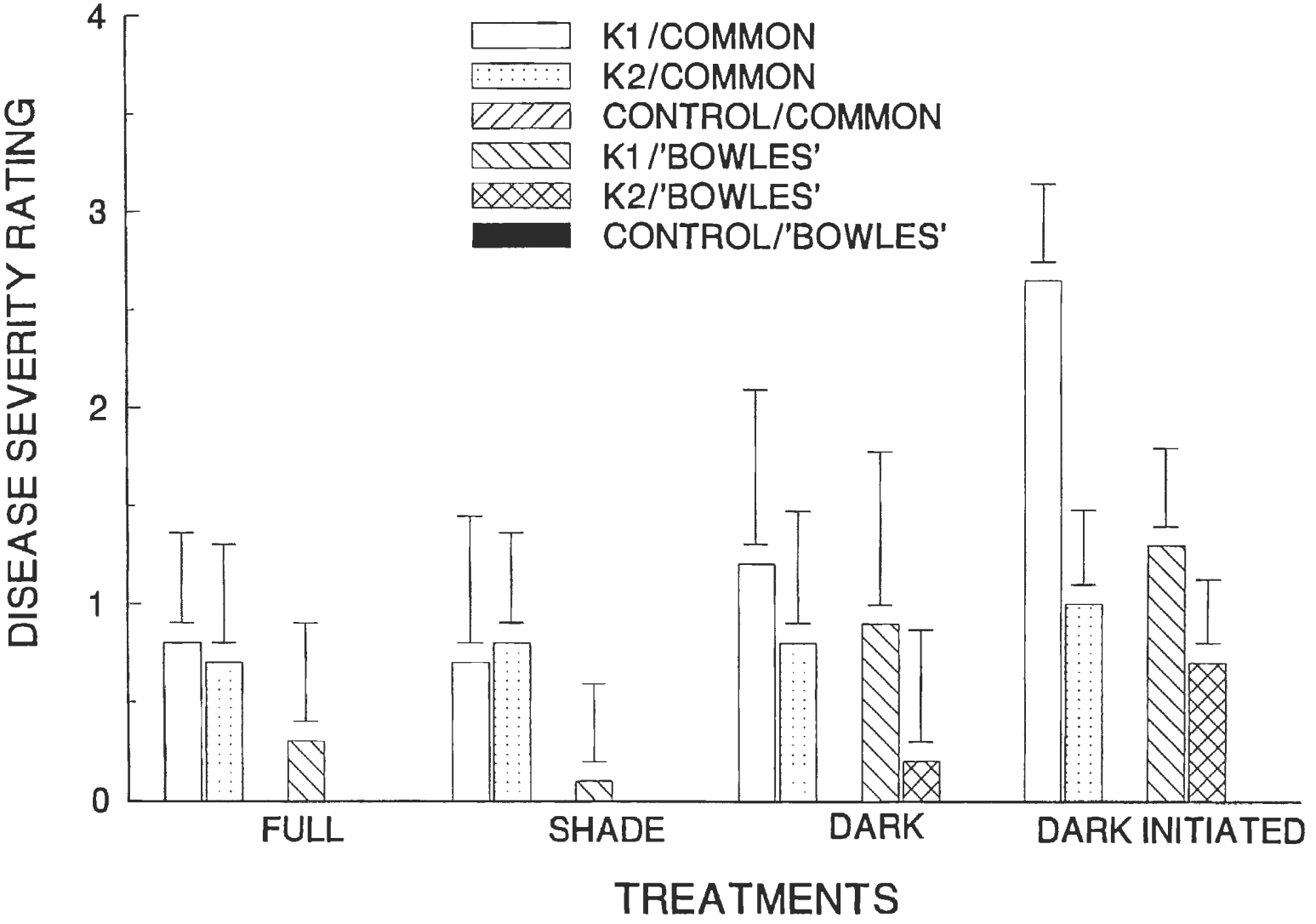

K1 /COMMON
K2/COMMON
CONTROL/COMMON
K1 /'BOWLES'
K2/'BOWLES'
CONTROL/'BOWLES'
DISEASE SEVERITY RATING
0
1
2
3
4
FULL
SHADE
DARK
DARK INITIATED
TREATMENTS

Figure 2.4. Disease incidence of *C. gloeosporioides* (K1) on 'Bowles' periwinkle stems placed in various environments for 45 h after inoculation, then in a growth chamber at 22°C or 28°C. Legend key: Mist = shoots misted after inoculation within a mist chamber in a greenhouse, Dark = shoots placed in dark cabinet for 48 h after inoculation, Shade = shoots placed under 50% shade cloth within respective growth chambers after inoculation, Full = shoots placed in respective growth chambers after inoculation, and Control = shoots placed in respective growth chambers without inoculum. Vertical bars indicate 95% confidence intervals.

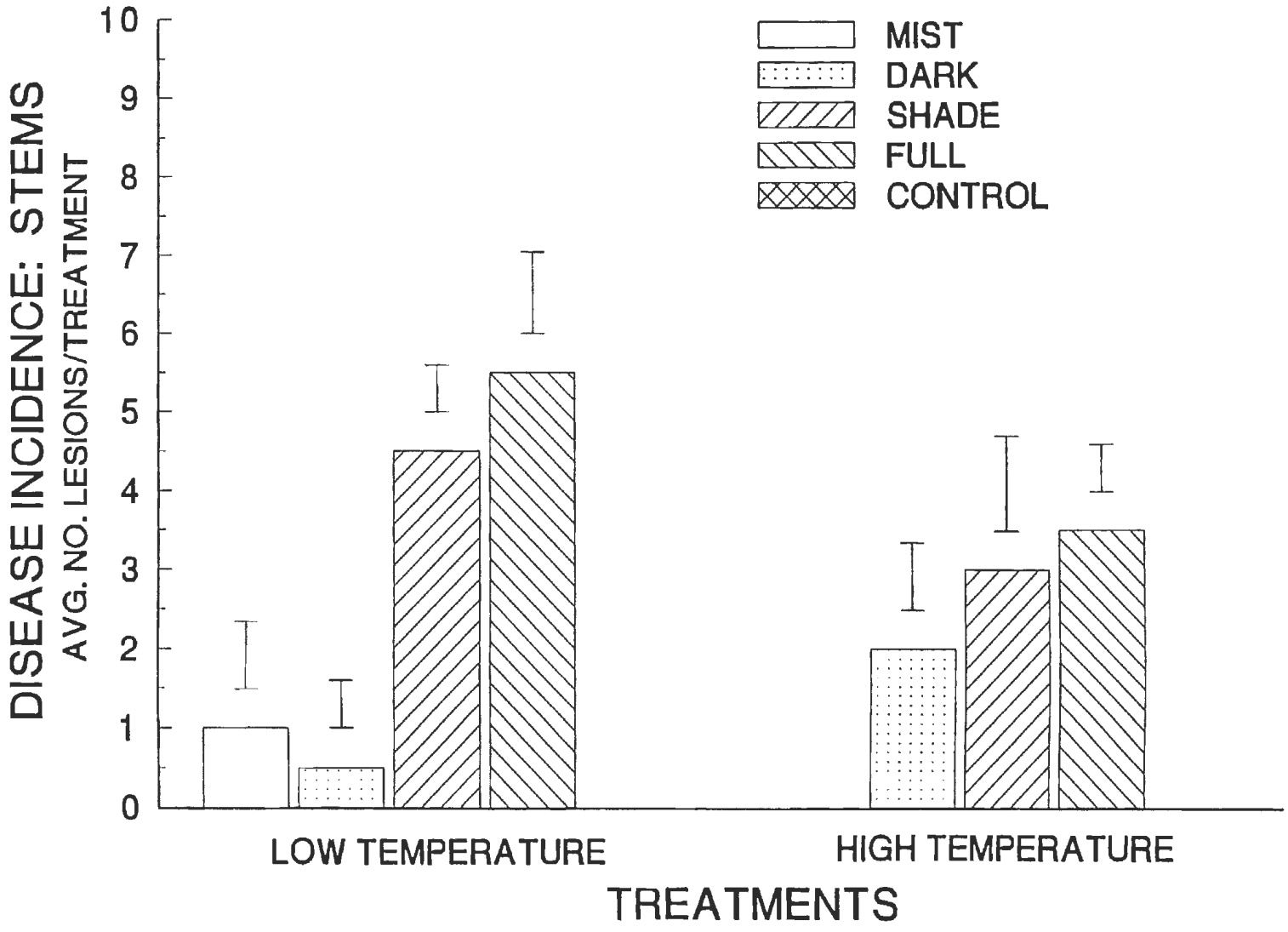

DISEASE INCIDENCE: STEMS
AVG. NO. LESIONS/TREATMENT
10
9
8
7
6
5
4
3
2
1
0
MIST
DARK
SHADE
FULL
CONTROL
LOW TEMPERATURE
HIGH TEMPERATURE
TREATMENTS

Figure 2.5. Disease incidence of *C. gloeosporioides* (K1) on 'Bowles' periwinkle leaves placed in various environments for 45 h after inoculation, then in a growth chamber at 22°C or 28°C. Legend key: Mist = shoots misted after inoculation within a mist chamber in a greenhouse, Dark = shoots placed in dark cabinet for 48 h after inoculation, Shade = shoots placed under 50% shade cloth within respective growth chambers after inoculation, Full = shoots placed in respective growth chambers after inoculation, and Control = shoots placed in respective growth chambers without inoculum. Vertical bars indicate 95% confidence intervals.

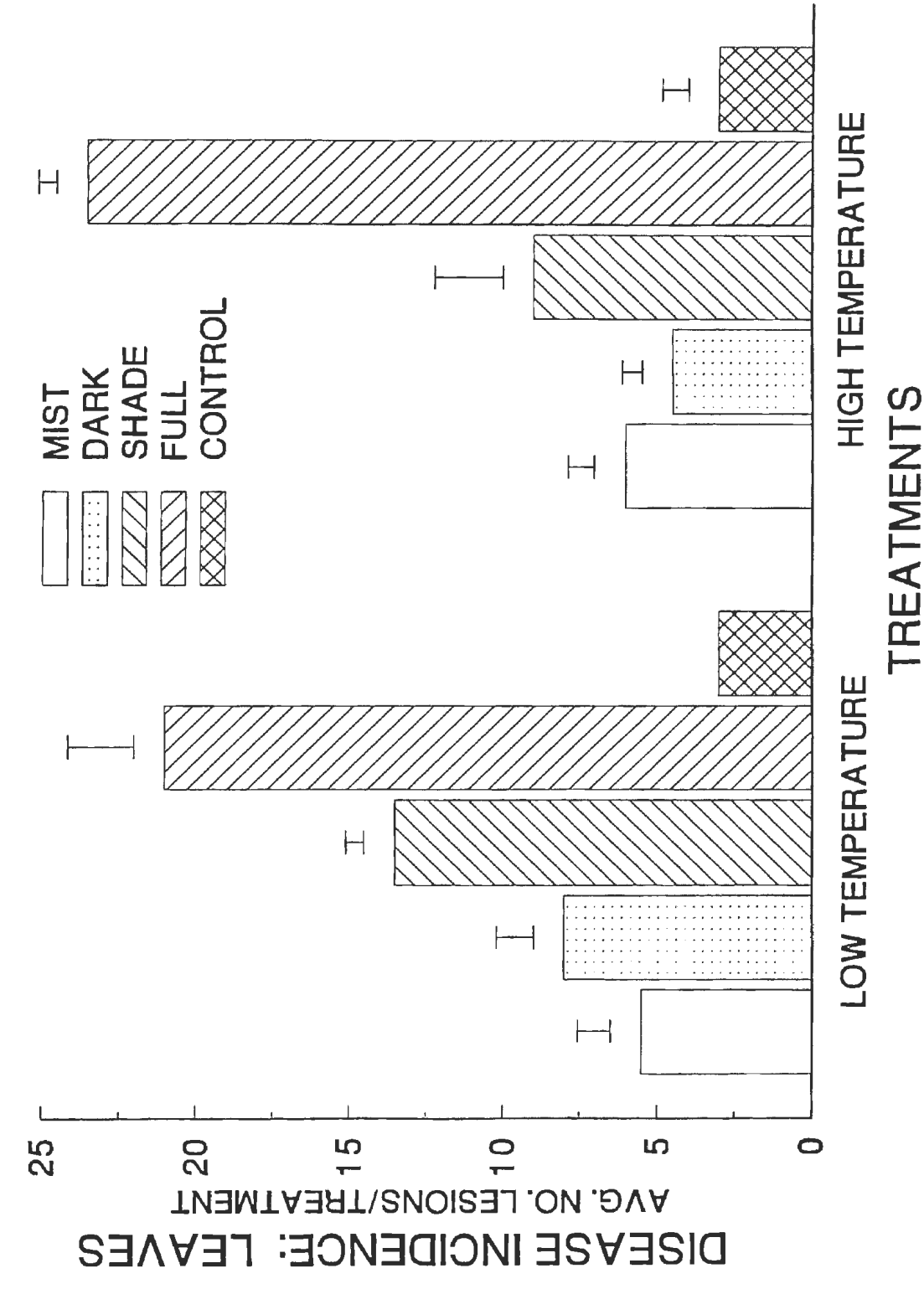

DISEASE INCIDENCE: LEAVES
AVG. NO. LESIONS/TREATMENT
25
20
15
10
5
0
MIST
DARK
SHADE
FULL
CONTROL
LOW TEMPERATURE
HIGH TEMPERATURE
TREATMENTS

Figure 2.6. Disease incidence on 'Bowles' periwinkle shoots of two isolates of *C. gloeosporioides* (K1 isolated from 'Bowles' periwinkle and K2 isolated from 'Emerald Gaiety' evergreen wintercreeper) under full light and shaded light (50% shade cloth). Control shoots (C) received no inoculum. Vertical bars indicate 95% confidence intervals.

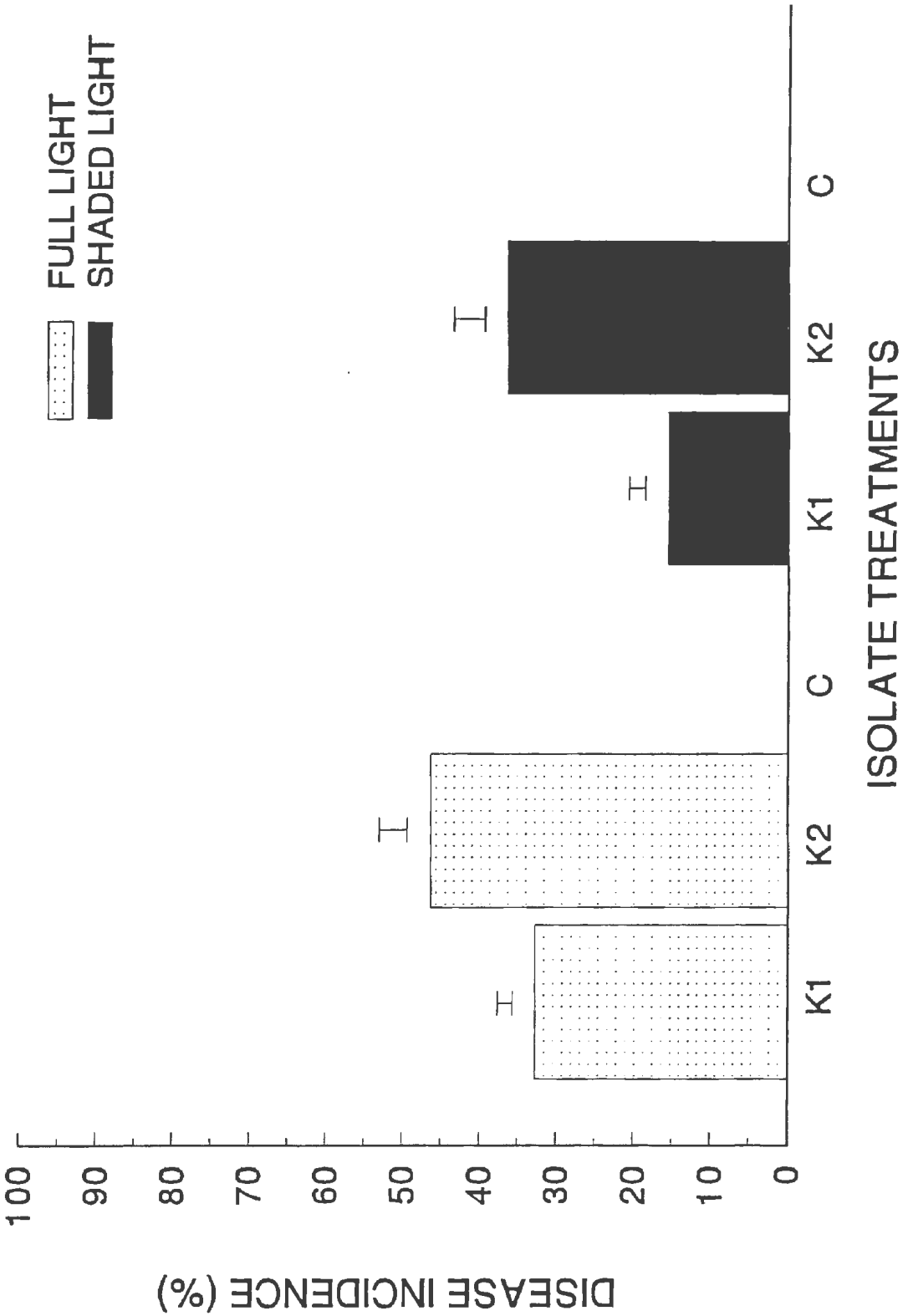

FULL LIGHT
SHADED LIGHT
DISEASE INCIDENCE (%)
100
90
80
70
60
50
40
30
20
10
0
K1
K2
C
K1
K2
C
ISOLATE TREATMENTS

Figure 2.7. Disease incidence of two isolates of *C. gloeosporioides* (K1 isolated from 'Bowles' periwinkle and K2 isolated from 'Emerald Gaiety' evergreen wintercreeper) under full light and shaded light (50% shade cloth). Control shoots received no inoculum. Vertical bars indicate 95% confidence intervals.

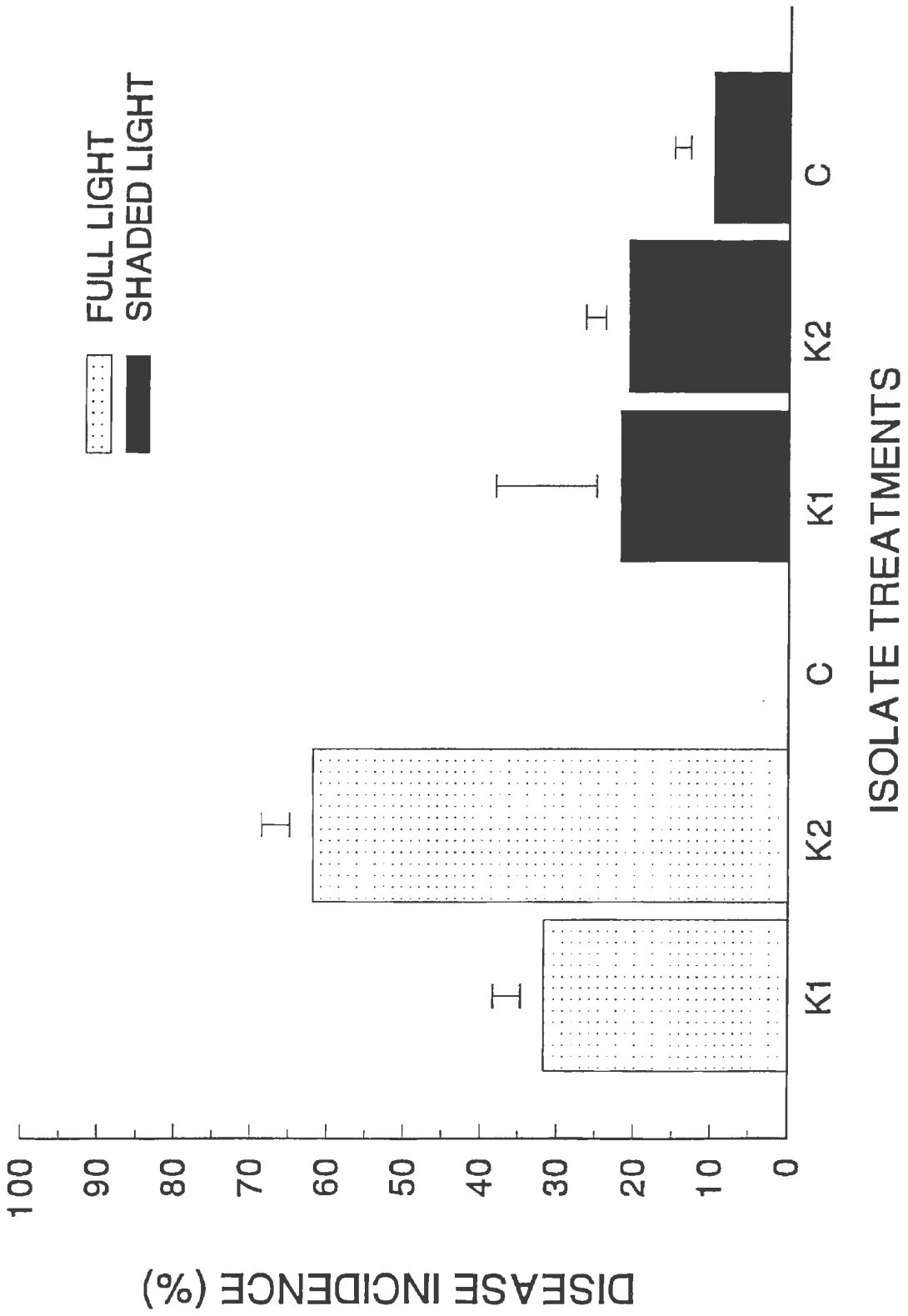
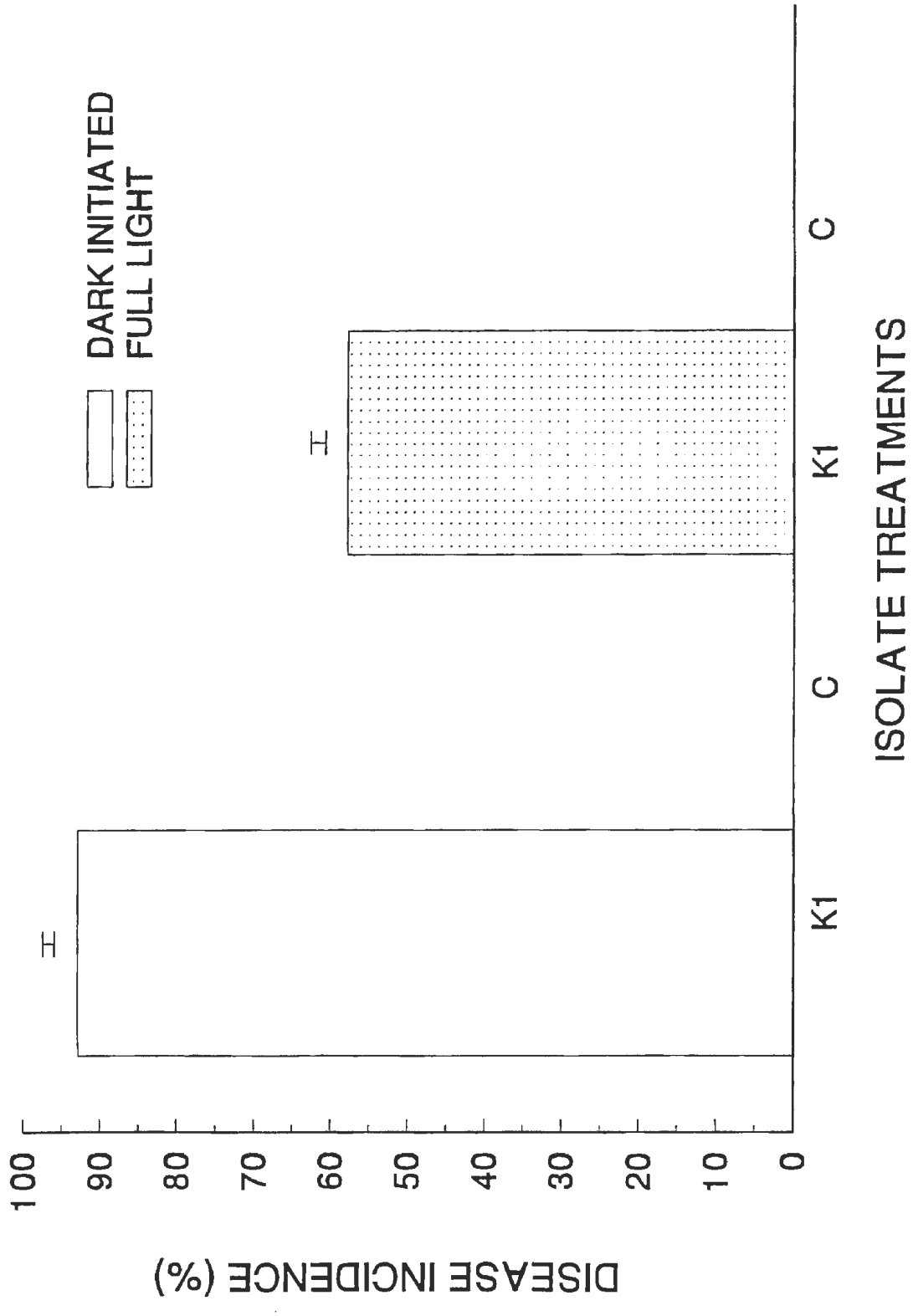

FULL LIGHT
SHADED LIGHT
DISEASE INCIDENCE (%)
100
90
80
70
60
50
40
30
20
10
0
K1
K2
C
K1
K2
C
ISOLATE TREATMENTS

Figure 2.8. Disease incidence of one isolate of *C. gloeosporioides* (K1) isolated from 'Bowles' periwinkle on 'Bowles' periwinkle exposed to 17 h of darkness after inoculation (dark initiated) and full light. Control (C) shoots received no inoculum. Vertical bars indicate 95% confidence intervals.


DARK INITIATED
FULL LIGHT
DISEASE INCIDENCE (%)
100
90
80
70
60
50
40
30
20
10
0
K1
C
K1
C
ISOLATE TREATMENTS

CHAPTER III

EFFECTIVENESS OF SEVEN FUNGICIDES IN CONTROLLING FOLIAR DISEASES OF COMMON PERIWINKLE (*VINCA MINOR* L.)

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ABSTRACT

Production of common periwinkle (*Vinca minor* L.) and 'Bowles' periwinkle (*V. minor* L. 'Bowles') has declined due to foliar diseases caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. in Penz. and *Phoma exigua* Desmaz. var. *inoxydabilis* Boerema & Vegh in Vegh et al. Leaf and stem spots, anthracnose and stem dieback were associated with *C. gloeosporioides* which was the most frequently occurring pathogen. *P. exigua* var. *inoxydabilis* was found with leaf spots. This study was conducted to determine whether several labeled and experimental fungicides could control foliar diseases in these cultivars. A study was conducted outdoors during two consecutive summers. Plants were sprayed weekly with thiophanate methyl/mancozeb (1.79 g liter^{-1}), propiconazole (0.95 ml liter^{-1}), thiophanate methyl (1.58 ml liter^{-1}), triforine (3.95 ml liter^{-1}), cyproconazole (0.79 ml liter^{-1}), triforine--CC 17461 (3.95 ml liter^{-1}), or CGA 173506 (0.47 g liter^{-1}). Thiophanate methyl/mancozeb was most effective at reducing foliar dieback during both seasons. Dry weights of plants treated with thiophanate methyl/mancozeb were significantly greater at the end of the second summer, than those of plants treated with the other fungicides or the untreated control plants. Five concentrations of eight fungicides (thiophanate methyl/mancozeb, propiconazole, thiophanate methyl, triforine, cyproconazole, chlorothalonil, cupric hydroxide, and mancozeb) were used to test inhibition of mycelial growth *P. exigua* var. *inoxydabilis* and two isolates of *C. gloeosporioides* on fungicide amended agar. Propiconazole inhibited growth of *P. exigua* var. *inoxydabilis* (100%) and both isolates of *C. gloeosporioides* (>96%). Cyproconazole completely inhibited mycelial growth of *P. exigua* var. *inoxydabilis*. Thiophanate methyl/mancozeb partially inhibited growth (50%) of *C. gloeosporioides*.

Keywords: 'Bowles' periwinkle, *Colletotrichum gloeosporioides*, chlorothalonil, cupric hydroxide, cyproconazole, mancozeb, *Phoma exigua* var. *inoxydabilis*, propiconazole, thiophanate methyl, thiophanate methyl/mancozeb, and triforine.

Abbreviations: PDA = potato-dextrose agar, PPF = photosynthetic photon flux.

INTRODUCTION

Common periwinkle (*Vinca minor* L.) is a relatively low maintenance shade plant once established within a landscape. For this reason, sales to homeowners and landscapers have increased. However, the numbers propagated and produced by growers has declined, and common periwinkle has even been eliminated from some inventories. The reason for this decline is the difficulty of implementing effective control methods for foliar diseases during the production. Nursery growers have estimated plant losses ranging from 10% to 60% of total production for each season (Mark Andrews, Greenleaf Nursery, Park Hill, OK and Gary Percefull, Arrowhead Growers, Tulsa, OK, personal communication, 1992).

Foliar disease symptoms include leaf and stem spots, anthracnose, and stem dieback. The two fungal pathogens frequently observed on diseased common periwinkle from nursery stock producers were *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. in Penz. and *Phoma exigua* Desmaz. var. *inoxydabilis* Boerema & Vegh in Vegh et al. (Chapter II.). In addition, growers have indicated that the common periwinkle has more disease problems than cultivars such as 'Bowles' periwinkle (*Vinca minor* L. 'Bowles').

Effective chemical control of foliar diseases is difficult. Previously, benomyl was used in spray programs for common periwinkle disease outbreaks; however, the recent removal of ornamental plant uses from its label has necessitated finding a suitable

substitute. Fungicides must be tested to determine those which are effective in controlling common periwinkle foliar disease symptoms and which do not adversely effect plant quality. The objective of this study was to determine effective chemical controls using labeled and experimental fungicides.

MATERIALS AND METHODS

Field Studies of Fungicidal Control. Rooted cuttings of common periwinkle were established in 3.8 liter pots containing a medium consisting of pine bark : peat : sand (1:1:1 by volume) amended with 4.7 kg m^{-3} 17N-3.6P-10K slow release fertilizer (Osmocote, Grace-Sierra, Milpitas, CA), 3.0 kg m^{-3} gypsum, 3.0 kg m^{-3} dolomite and 0.9 kg m^{-3} micronutrients (Micromax, Grace-Sierra). Plants were grown outdoors under full sun (maximum photosynthetic photon flux (PPF) of 1275 µmol m^{-2} s^{-1}) on a black ground cover fabric. The plants received approximately 1.3 cm water daily by overhead-sprinkler irrigation. After a 6-week establishment period, plants were sprayed weekly to runoff with a CO_2-pressurized backpack sprayer with an approximate output of 0.2 liters m^{-2} until runoff with thiophanate methyl/mancozeb (dimethyl [(1,2-phenylene)-bis (iminocarbonothioyl)] bis [carbamate]) and a combination of zinc ion and manganese ethylenebis dithiocarbamate) at 1.8 g $liter^{-1}$, propiconazole (1-[2-(2',4'-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl-methyl]-1*H*-1,2,4-triazole) (Ciba Geigy, Greensboro, NC) at 0.93 ml $liter^{-1}$, thiophanate methyl (dimethyl [(1,2-phenylene)-bis (iminocarbonothioyl)] bis [carbamate]) (Grace-Sierra) at 1.6 ml $liter^{-1}$, triforine (N,N'-[1,4-piperazinediylbis (2,2,2-trichloroethylidene)] bis [formamide]) (Ortho, Chevron Chemical Co., San Ramon, CA) at 4.0 ml $liter^{-1}$, cyproconazole (2-(4-chlorophenyl)-1-(1*H*-1,2,4-triazol-l-yl)-butan-2-ol) (Sandoz Crop Protection, Des Plaines, IL) at 0.8 g $liter^{-1}$, triforine--CC 17461 (N,N'-[1,4-

piperazinediylbis (2,2,2-trichloroethylidene)] bis [formamide] with micro emulsion) (Ortho, Chevron Chemical Co.) at 4 ml liter^{-1}, CGA 173506 (4-(2-2-difluoro-1,3-benzodioxol-4-yl) pyrrole-3-carbonitrile) (Ciba Geigy) at 0.4 g liter^{-1}, and no fungicide was applied to the control plants. Fungicide applications began 5 May 1992 and the experiment was terminated on 12 October 1992. Plants were rated every other week throughout the growing season by three independent raters on a scale of one to five (1 = no foliar symptoms, 2 = 1 to 33% necrotic plant tissue, 3 = 34 to 66% necrotic plant tissue, 4 = 67 to 99% necrotic plant tissue, and 5 = 100% necrotic tissue). Upon termination of the study, roots and shoots were harvested and oven-dried at 44°C for seven consecutive days and weighed.

This trial was repeated the following summer with a few modifications. Rooted cuttings of 'Bowles' periwinkle were established and treated with fungicides as described above except that 1) common periwinkle plants were added for cultivar disease severity comparison, 2) plants were placed in a shadehouse with a maximum PPF of 945 µmol m^{-2} s^{-1}, 3) an additional fungicide treatment consisting of alternating weekly sprayings of thiophanate methyl/mancozeb with propiconazole was added, 4) fungicide applications began 13 May 1993 and the experiment was terminated on 21 September 1993, and 5) only shoots were harvested for dry weight results.

All plants in both trials were healthy with no foliar disease symptoms at the time of establishment. Plants were not artificially inoculated with fungal spores, but received natural inoculation through the growing environment.

A completely randomized design was used for each experiment. There were 20 'Bowles' periwinkle plants per fungicide treatment in the first summer trial and 15 plants

each of common periwinkle and 'Bowles' periwinkle plants per fungicide treatment in the second trial. Analysis of variance procedures and least significant difference (LSD) tests were used to determine differences between fungicide treatments in both studies and cultivar differences in the second trial.

Inhibition of Fungal Mycelial Growth by Fungicides In Vitro. One isolate of *C. gloeosporioides* from common periwinkle (K1) and from 'Emerald Gaiety' evergreen wintercreeper (*Euonymus fortunei* (Turcz.) Hand.-Mazz. 'Emerald Gaiety') (K2), and one isolate of *P. exigua* var. *inoxydabilis* from common periwinkle (K4) were isolated from infected areas of plants. Leaf and stem pieces with spots and anthracnose symptoms were plated onto 9 cm plastic petri dishes containing potato-dextrose agar (PDA - 39 g liter^{-1} of sterilized distilled deionized water and autoclaved at 120°C for 20 minutes). The pathogens were identified by culture growth and spores. Techniques described by Boerema (1976) were used to identify *P. exigua* var. *inoxydabilis* from small leaf spots. Leaf and stem pieces with spots and anthracnose were characteristic of *C. gloeosporioides* (as also described in Chapter II).

Each isolate was grown on PDA amended with the following fungicides and rates (g ai liter^{-1}: thiophanate methyl/mancozeb, 0, 0.8, 1.6, 2.4, and 4.8; propiconazole, 0, 0.2, 1.0, 1.2, and 2.4; thiophanate methyl, 0, 0.2, 0.5, 0.9, and 1.8; triforine, 0, 0.1, 0.2, 0.3, and 0.5; cyproconazole, 0, 0.04, 0.1, 0.2, and 0.3; chlorothalonil, 0, 0.9, 17.8, 35.6, and 71.1; cupric hydroxide, 0, 0.3, 0.5, 6.2, and 12.5; and, mancozeb, 0, 0.7, 15.0, 30.0, and 60.0. These rates correspond to 0, half the lowest recommended rate, the lowest recommended rate, highest recommended rate, two times the highest recommended rate, and four times the highest recommended rate for each fungicide. Cultures were grown

for six days in 9 cm plastic petri dishes. Petri dishes were wrapped in foil to eliminate exposure to light and placed in a dark incubator at 27±1°C for 6 days. Mycelial growth was measured every two days. A completely randomized design with three plates for each isolate and fungicide was used, and the study was repeated in time. Analysis of variance procedures and regression analysis were used to determine treatment differences.

RESULTS

Field Studies of Fungicidal Control. During the first year, disease symptoms began to appear on plants in all treatments beginning 4 June 1992 (Figure 3.1) and symptoms increased throughout the growing season. From 4 June through 29 July 1992 thiophanate methyl/mancozeb provided the most suppression of foliar symptoms among the fungicides tested. At harvest, shoot dry weights of the thiophanate methyl/mancozeb-treated common periwinkle plants were greater than shoot dry weights of plants receiving any other fungicide treatment or untreated controls (Table 3.1). Root dry weights in common periwinkle were not different regardless of fungicide treatment.

During the second summer trial, plants receiving propiconazole and thiophanate methyl/mancozeb in alternate weeks did not perform as well as those receiving thiophanate methyl/mancozeb only, but they were rated higher than plants from all other fungicide treatments and control plants beginning 28 June 1993 for both cultivars (Table 3.2). Plants treated with cyproconazole had significantly higher ratings than those of any other treatment beginning 10 June for both cultivars. At harvest, plants receiving the thiophanate methyl/mancozeb treatment had the highest dry weights (Table 3.1). Plants treated with cyproconazole had the lowest weights compared to plants from all other treatments.

Inhibition of Fungal Mycelial Growth by Fungicides In Vitro. Propiconazole at all rates (except for K2 at 1.1 ml liter^{-1}) completely inhibited mycelial growth of all isolates tested (Table 3.3, 3.4, and 3.5). The cyproconazole had the second highest percent inhibition at all rates for all fungal isolates. The thiophanate methyl/mancozeb combination gradually increased percent inhibition as rates increased for each isolate. The compounds thiophanate methyl, mancozeb, cupric hydroxide, and chlorothalonil had the lowest percentage of inhibition against all isolates.

DISCUSSION

With the loss of benomyl, it is encouraging that several fungicides have shown satisfactory activity against the two major pathogens, *C. gloeosporioides* and *P. exigua* var. *inoxydabilis*. The *in vitro* study findings of the three fungicides, propiconazole, cyproconazole, and with the thiophanate methyl/mancozeb fungicide combination, showed good inhibition, with propiconazole having the best results. Performance of fungicides in the field did not always correspond with the *in vitro* studies. Thiophanate methyl/mancozeb and thiophanate methyl/mancozeb alternated with propiconazole were the best treatments overall in the field.

The fungal pathogen *C. gloeosporioides* was observed in both field studies more frequently than the fungus *P. exigua* var. *inoxydabilis*. During the first trial, plants rapidly declined after 17 June. The first half of the summer was more mild and wet than the second half. These conditions could have influenced fungal sporulation, growth, and disease severity on the plants and coincided with published findings of Chakraborty et al. (1990). He reported that after inoculation of *C. gloeosporioides* on *Stylosanthes scabra*, optimal infection occurred when plants received 16 h of leaf wetness, temperatures were

20 to 30°C, and there was high relative humidity after inoculation for the initial 12 h. In addition, growers have stated that the disease symptoms first occur in spring. Then, as new growth becomes vigorous, the plants flourish and appear healthy. As growth slows during summer, symptoms appear again. New flush growth overcomes the diseased areas; therefore, infected leaves that remain on the soil surface may provide inoculum for future infection when conditions are favorable for the spores to be activated. Thus, this may be one reason for decreased effectiveness of fungicidal applications.

During the second summer, the shaded environment favored plant growth and influenced fungicide effectiveness. Disease susceptibility of common periwinkle and 'Bowles' periwinkle were compared in the second trial. Disease severity of the 'Bowles' periwinkle was lower regardless of fungicide treatment. This indicated that common periwinkle had a greater degree of susceptibility to disease infection. This finding supported common periwinkle growers. Growers have noted that cultivated lines are more resistant than the native species (Gary Percefull, Arrowhead Growers, Tulsa, OK, and Preston Warren, Warren and Son Nursery, Oklahoma City, OK, personal communication). This may be due to the more stringent propagation conditions during the propagation of cultivated lines or to the selection of more resistant lines along with the selection of horticultural qualities, such as particular flower color, flower size, foliage shape, foliage color, and growth habit. Propagation material for native lines are taken directly from the wild and may already be infected with pathogens. Since this material is genetically highly diverse, susceptibility would be maintained. Much work on *Glomerella cingulata* (Stoneman) Spauld. and H. Schrenk.) (the perfect stage of *C. gloeosporioides*) infecting numerous cultivars of camellia (*Camellia* L. sp.) differentially

has been documented (Baxter, 1991; Baxter, et al., 1988; Dickens and Cook, 1989). More resistant common periwinkle cultivars would reduce the amount of disease present in plant material.

The fungicides tested were either systemic or protective compounds. Systemic fungicides included propiconazole, cyproconazole, and thiophanate methyl. All other fungicides except the thiophanate methyl/mancozeb combination of a systemic plus a protectant were protectant fungicides. Plants treated with thiophanate methyl/mancozeb had lower disease severity ratings in both field studies. This combination of systemic and protectant action was apparently more effective at suppressing disease symptoms throughout each growing season than the other fungicides. Although, the systemic fungicides propiconazole and cyproconazole inhibited mycelial growth most *in vitro*, these systemics alone did not prove the most effective in the field studies. An interesting finding was that the thiophanate methyl/mancozeb combination was more effective in inhibiting *in vitro* mycelial growth than either fungicide alone at the same concentrations.

Cyproconazole has similar properties to propiconazole, both are sterol-inhibitors. The severity ratings of cyproconazole treated plants in the second summer was higher than that of plants treated with all other fungicides. These higher ratings were due to phytotoxicity symptoms rather than foliar disease symptoms. Phytotoxicity was not as apparent during the first summer possibly due to the different growing environments of the two studies. The amended agar results showed complete mycelium growth inhibition with *P. exigua* var. *inoxydabilis* and increasing inhibition with *C. gloeosporioides* as concentrations increased. The difference between the high percentage of mycelial inhibition compared to the disease severity ratings in the field studies may have resulted

from continuous exposure of pathogens to the fungicide medium. Breakdown of fungicides in the field may have occurred from exposure to irrigation, rainfall, and sunlight.

The fungicides chlorothalonil, mancozeb, and thiophanate methyl are commonly used by Oklahoma growers. Our studies indicate that these fungicides are not the most effective of those available.

The fungicide mancozeb has been used for control of diseases caused by *Colletotrichum* Corda in Sturm. sp. on alfalfa (Broscious and Kirby, 1988) and as a protectant for northern leaf blight (*Exserohilum turcicum* (Pass.) K. J. Leonard & E. G. Suggs) (Bowen and Pedersen, 1988). Dickens (1990) conducted a fungicide trial for the control of white rust (*Puccinia horiana* Henn.) with propiconazole, mancozeb, and triforine. After sporulation of the white rust, three sprayings of propiconazole were required to kill telia and triforine did not kill the telia. When propiconazole was used as a protectant and only sprayed once as were mancozeb and triforine applied, infection was only reduced with all fungicides tested.

Chlorothalonil has been used to control numerous diseases on ornamentals, turf, and vegetables. The conidia and sclerotia of tomato anthracnose (*Colletotrichum coccodes* (Wallr.) S. J. Hughes) were shown *in vitro* to be sensitive to chlorothalonil (Dillard, 1988). Barnard (1984) tested chlorothalonil and copper hydroxide for the most effective control against stem canker (*Cylindrocladium scoparium* Morg.) on Eucalyptus seedlings (Barnard, 1984). No phytotoxicity was apparent with copper hydroxide, which showed promise, but chlorothalonil had the most effective control (Barnard, 1984).

Neither compounds showed satisfactory inhibition of mycelial growth in our *in vitro* studies, and were not compared in the outdoor screening trials.

While some of the fungicides tested suppressed disease symptoms, no fungicide controlled the foliar diseases in actively growing plants. The chemical compounds recommended for use on ornamentals (common periwinkle) as a preventative treatment throughout the season include the thiophanate methyl/mancozeb combination and propiconazole, if used alternately with thiophanate methyl/mancozeb. There was some plant resistance with the common periwinkle cultivar 'Bowles'.

With additional research on control by fungicides and cultural practices, results from these studies have the potential to increase common periwinkle production in Oklahoma. This ground cover is a source of profitable income, if production costs that include disease control can be reduced. In the future, Oklahoma producers could potentially supply the state's demand for saleable plants and eliminate the need to import these. Eventually, successful marketing could enable Oklahoma to be a production source for surrounding states.

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Table 3.1. Dry weight (g) of shoots and roots of common periwinkle and 'Bowles' periwinkle receiving weekly fungicide applications throughout the 1992 and 1993 growing seasons.

	1992		1993
Fungicide Weight	Shoot Dry Weight	Root Dry Weight	Shoot Dry
Control	0.6	1.2	18.3
Thiophanate methyl/mancozeb	1.9	1.3	24.3
Propiconazole	0.5	1.1	14.7
Thiophanate methyl	0.3	0.8	17.2
Triforine	0.8	1.6	15.3
Cyproconazole	0.8	1.9	8.0
Triforine (CC 17461)	0.8	1.0	18.3
CGA 173506	0.7	1.1	18.1
Thiophanate methyl/mancozeb + Propiconazole	--	--	21.9
Significance ($LSD_{0.05}$)			
Fungicide (F)	0.93	NS	2.48
Cultivar (C)	--	--	NS
F x C	--	--	NS

Table 3.2. Visual ratings of common periwinkle and 'Bowles' periwinkle receiving weekly fungicide applications throughout the 1993 growing season. Rating scale: 1 = no foliar symptoms, 2 = 1 to 33% necrotic plant tissue, 3 = 34 to 66% necrotic plant tissue, 4 = 67 to 99% necrotic plant tissue, and 5 = 100% necrotic tissue.

Cultivar	Fungicide	May 13	May 24	June 10	June 28	July 15	July 28	Aug 12	Aug 23	Sept 9	Sept 20
						Visual Rating					
Common Periwinkle	Cont	1.0	1.1	1.1	1.8	2.6	2.7	3.2	3.2	3.0	3.1
	TMM	1.1	1.1	1.0	1.2	1.6	2.0	2.2	2.6	2.3	2.5
	PROP	1.0	1.0	1.2	1.7	2.5	2.9	2.9	3.1	3.0	3.1
	TM	1.0	1.1	1.2	1.9	2.6	3.0	2.9	3.1	2.9	3.0
	TRIF	1.0	1.1	1.1	1.8	2.3	2.8	2.9	3.0	3.1	3.0
	CYP	1.0	1.1	1.8	2.9	3.4	3.3	3.5	3.7	3.8	3.9
	TRIFCC	1.0	1.0	1.1	2.0	2.6	2.7	2.8	3.1	3.0	3.0
	CGA	1.0	1.1	1.2	1.7	3.4	2.7	2.9	3.3	3.2	3.1
	PROPTMM	1.0	1.0	1.1	1.4	2.1	2.3	2.6	2.7	2.7	2.8
'Bowles' Periwinkle	Cont	1.0	1.2	1.1	1.7	2.1	2.8	2.9	3.2	3.3	3.4
	TMM	1.0	1.2	1.2	1.4	1.8	1.9	2.3	2.5	2.5	2.5
	PROP	1.0	1.2	1.2	1.8	2.4	2.7	2.9	3.2	3.1	3.2
	TM	1.0	1.1	1.1	1.8	2.3	2.6	2.7	3.2	3.2	3.2
	TRIF	1.1	1.2	1.3	1.9	2.4	2.6	2.9	3.1	3.1	3.1
	CYP	1.0	1.2	1.7	3.0	3.2	3.3	3.4	3.7	3.7	3.7
	TRIFCC	1.0	1.4	1.3	1.8	2.2	2.6	2.7	3.0	3.0	3.0
	CGA	1.0	1.2	1.2	1.8	2.1	2.6	2.6	3.1	3.2	3.3
	PROPTMM	1.0	1.2	1.2	1.5	2.0	2.2	2.6	2.9	3.0	3.2
Significance ($LSD_{0.05}$)											
Cultivar (CV)		0.02	0.05	NS	NS	0.08	0.69	0.79	NS	0.08	0.08
Fungicide (F)		NS	NS	0.12	0.16	0.16	0.15	0.17	0.17	0.17	0.17
CV x F		0.01	0.08	NS	NS	0.21	NS	NS	NS	NS	0.15

Table 3.3. Growth inhibition of *Colletotrichum gloeosporioides* isolated from 'Bowles' periwinkle (K1) grown on fungicide amended agar.

Fungicide	Active Ingredient (g ai liter^{-1})	Percent Inhibition
Thiophanate methyl/ mancozeb	0.0	0.0
	0.8	50.5
	1.6	66.3
	2.4	76.9
	4.8	82.6
	linear	*
	quadratic	*
Propiconazole	0.0	0.0
	0.2	100.0
	1.0	100.0
	1.2	100.0
	2.4	100.0
	linear	**
	quadratic	**
Thiophanate methyl	0.0	0.0
	0.2	1.2
	0.5	5.6
	0.9	4.6
	1.8	8.1
	linear	NS
	quadratic	NS
Triforine	0.0	0.0
	0.1	57.5
	0.2	67.6
	0.3	77.9
	0.5	87.6
	linear	**
	quadratic	**

Cyproconazole	0.0	0.0
	0.04	66.3
	0.1	77.2
	0.2	86.7
	0.3	96.4
	linear	**
	quadratic	**
Chlorothalonil	0.0	0.0
	0.9	45.7
	1.8	55.4
	3.6	60.5
	7.1	65.4
	linear	**
	quadratic	**
Cupric hydroxide	0.0	0.0
	0.3	8.3
	0.5	12.9
	6.2	92.6
	12.5	92.1
	linear	**
	quadratic	**
Mancozeb	0.0	0.0
	0.7	6.2
	1.5	7.2
	3.0	11.1
	6.0	14.7
	linear	NS
	quadratic	NS

NS,*,**Nonsignificant or significant at $P = 0.05$ or 0.01, respectively in respect to linear or quadratic responses.

Table 3.4. Growth inhibition of *Colletotrichum gloeosporioides* isolated from 'Emerald Gaiety' evergreen wintercreeper (K2) grown on fungicide amended agar.

Fungicide	Active Ingredient (g ai liter^{-1})	Percent Inhibition
Thiophanate methyl/ mancozeb	0.0	0.0
	0.8	78.0
	1.6	74.4
	2.4	83.8
	4.8	81.7
	linear	*
	quadratic	*
Propiconazole	0.0	0.0
	0.2	96.8
	1.0	100.0
	1.2	100.0
	2.4	100.0
	linear	**
	quadratic	**
Thiophanate methyl	0.0	0.5
	0.3	35.7
	0.5	39.3
	0.9	55.4
	1.8	53.7
	linear	NS
	quadratic	NS
Triforine	0.0	0.0
	0.1	61.4
	0.2	73.5
	0.3	81.2
	0.5	86.1
	linear	**
	quadratic	**

Cyproconazole	0.0	0.0
	0.04	70.8
	0.1	81.2
	0.2	85.1
	0.3	93.7
	linear	**
	quadratic	**
Chlorothalonil	0.0	0.0
	0.9	48.7
	1.8	55.6
	3.6	59.0
	7.1	68.8
	linear	**
	quadratic	**
Cupric hydroxide	0.0	0.0
	0.3	9.4
	0.5	14.3
	6.2	98.0
	12.5	94.6
	linear	**
	quadratic	**
Mancozeb	0.0	0.0
	0.7	4.4
	1.5	6.9
	3.0	6.6
	6.0	12.5
	linear	NS
	quadratic	NS

NS,*,**Nonsignificant or significant at $P = 0.05$ or 0.01, respectively in respect to linear or quadratic responses.

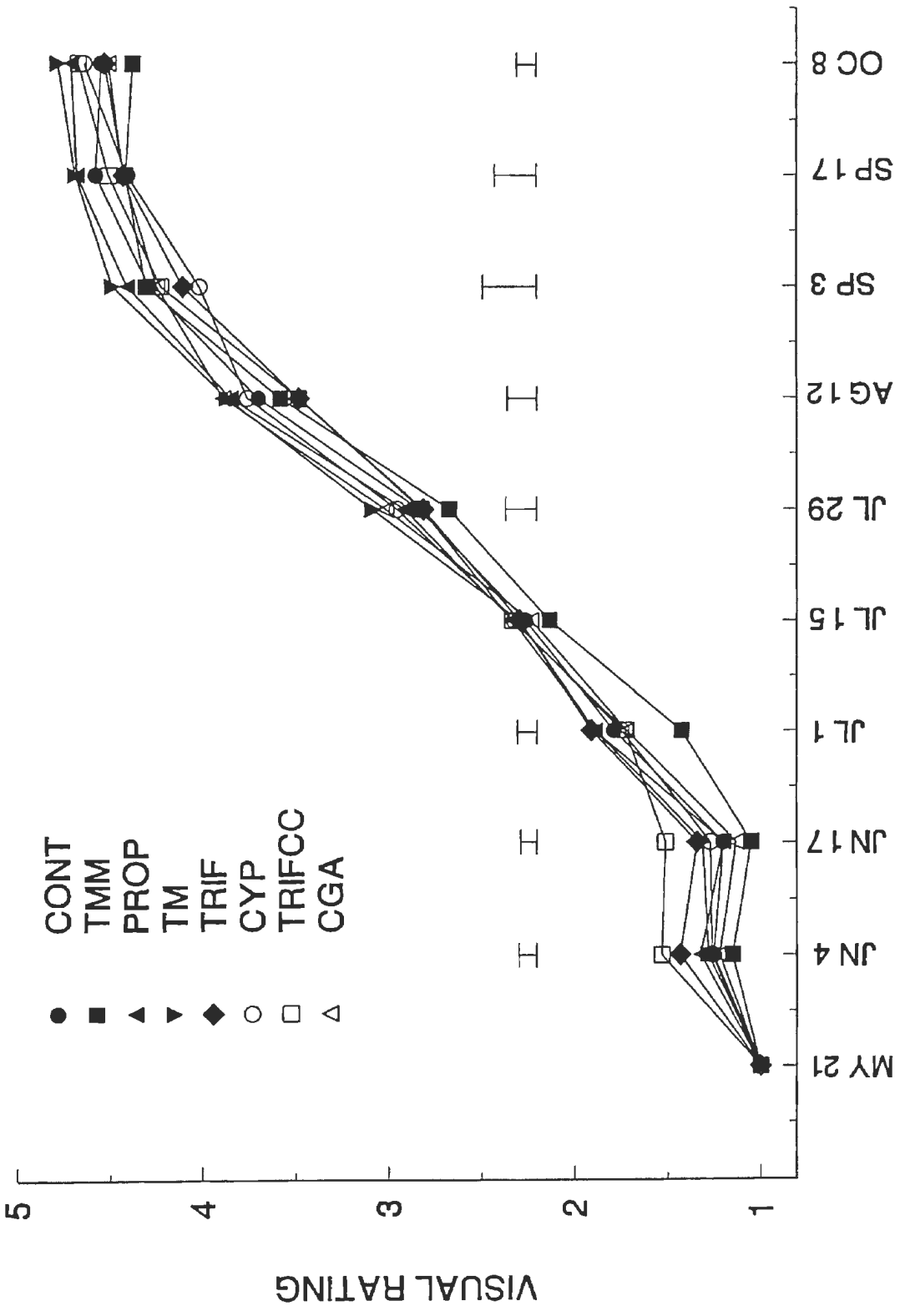
Table 3.5. Growth inhibition of *Phoma exigua* var. *inoxydabilis* (K4) grown on fungicide amended agar.

Fungicide	Active Ingredient (g ai liter^{-1})	Percent Inhibition
Thiophanate methyl/ mancozeb	0.0	0.0
	0.8	89.6
	1.6	92.9
	2.4	94.4
	4.8	94.4
	linear	*
	quadratic	*
Propiconazole	0.0	0.0
	0.2	100.0
	1.0	100.0
	1.2	100.0
	2.4	100.0
	linear	**
	quadratic	**
Thiophanate methyl	0.0	0.0
	0.2	1.9
	0.5	1.2
	0.9	3.3
	1.8	3.7
	linear	NS
	quadratic	NS
Triforine	0.0	0.0
	0.1	38.1
	0.2	58.2
	0.3	69.5
	0.5	82.5
	linear	**
	quadratic	**

Cyproconazole	0.0	0.0
	0.04	100.0
	0.1	100.0
	0.2	100.0
	0.3	100.0
	linear	**
	quadratic	**
Chlorothalonil	0.0	0.0
	0.9	47.9
	1.8	51.5
	3.6	59.4
	7.1	67.6
	linear	**
	quadratic	**
Cupric hydroxide	0.0	0.0
	0.3	0.0
	0.5	0.0
	6.2	79.8
	12.4	83.9
	linear	**
	quadratic	**
Mancozeb	0.0	0.0
	0.7	20.2
	1.5	26.9
	3.0	31.1
	6.0	42.1
	linear	NS
	quadratic	NS

NS,*,**Nonsignificant or significant at $P = 0.05$ or 0.01, respectively in respect to linear or quadratic responses.

Figure 3.1. Visual ratings of 'Bowles' periwinkle receiving weekly fungicide applications throughout the 1992 growing season. Rating scale: 1 = no foliar symptoms, 2 = 1 to 33% necrotic plant tissue, 3 = 34 to 66% necrotic plant tissue, 4 = 67 to 99% necrotic plant tissue, and 5 = 100% necrotic tissue. Vertical bars indicate $LSD_{0.05}$.


VISUAL RATING
5
4
3
2
1
MY 21
JN 4
JN 17
JL 1
JL 15
JL 29
AG 12
SP 3
SP 17
OC 8
CONT
TMM
PROP
TM
TRIF
CYP
TRIFCC
CGA

CHAPTER IV

EFFECT OF SPRINKLER AND DRIP IRRIGATION ON THE SPREAD OF *COLLETOTRICHUM GLOEOSPORIOIDES* DURING PRODUCTION OF COMMON PERIWINKLE AND 'BOWLES' PERIWINKLE

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ABSTRACT

Common periwinkle (*Vinca minor* L.) production has declined due to foliar diseases. Disease symptoms include leaf spot, anthracnose, and stem dieback. Cultural practices are suspected to influence disease incidence and severity. This study compared overhead sprinkler and drip irrigation on common periwinkle and 'Bowles' periwinkle in the spread of anthracnose caused by *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. in Penz. The incidence of foliar lesions did not differ among plants of irrigation systems until week 5 when plants receiving drip irrigation had more lesions than those receiving sprinkler irrigation. 'Bowles' periwinkle had fewer lesions than common periwinkle, suggesting that 'Bowles' periwinkle is not as susceptible to *C. gloeosporioides* as common periwinkle.

Keywords: anthracnose, leaf spot, stem dieback.

Abbreviations: PPF = photosynthetic photon flux.

INTRODUCTION

Common periwinkle (*Vinca minor* L.) is an ornamental ground cover (Huner, et al, 1988) popular to homeowners and landscape personnel for several reasons; it tolerates poor soils, dry sites, shade, and has been recommended for use in urban areas under stress (Corley, 1986). Once properly established in the landscape, common periwinkle is a relatively low maintenance plant; however, plant survival and vigor are decreased if diseased plants are introduced into a planting with improper cultural practices. Cultural practices which contribute to worsened disease symptoms include exposure to too much sunlight, excessive moisture, and poor air movement around wet foliage. The number of common periwinkle plants produced has decreased and even been eliminated from nursery inventories due to foliar disease (Mark Andrews, Greenleaf Nursery, Park Hill, OK and Gary Percefull, Arrowhead Growers, Tulsa, OK, personal communication). Disease symptoms include stem and leaf spots, anthracnose, and stem dieback.

In previous studies (Chapter II), two fungal pathogens reported on nursery samples were *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. in Penz. and *Phoma exigua* Desmaz. var. *inoxydabilis* Boerema & Vegh in Vegh et al. *Colletotrichum* Corda in Sturm. spp. have been found in Florida and North Carolina and *P. exigua* var. *inoxydabilis* in California on common periwinkle (Farr et al., 1989). Hawthorne and Otto (1986) reported that many of these fungi infect plants through wounds that are either natural or artificial.

The pathogenicity of *C. gloeosporioides* (the imperfect stage of *Glomerella cingulata* (Stoneman) Spauld. and H. Schrenk.) has been determined on other ornamental plants. Numerous reports concerning camellia cultivars (*Camellia* L.) associated with

dieback caused by this fungus have been published (Baxter, 1991; Baxter et al., 1988). The causal agent of anthracnose on evergreen wintercreeper (*Euonymus fortunei* (Turcz.) Hand.-Mazz. 'Emerald n' Gold' and 'Gaiety') has been identified as *C. gloeosporioides* (Mahoney and Tattar, 1980).

Irrigation techniques may contribute to the spread of spores of these pathogens. Overhead sprinklers can splash water containing spores from infected plant parts to non-infected areas (Larry Madden, Ohio State University, personal communication). Increased air circulation has been suggested as a means of control by propagators (Preston Warren, Warren and Son Nursery, Oklahoma City, OK, personal communication). Overcrowding encourages increased infection, due to less air circulation to dry the moist plant parts.

The objective of this research was to compare the distribution of *C. gloeosporioides* irrigated by sprinkler and drip irrigation. The common periwinkle and 'Bowles' periwinkle (*Vinca minor* L. 'Bowles') cultivars also were compared for resistance against *C. gloeosporioides*.

MATERIALS AND METHODS

Rooted cuttings of common periwinkle (5 cm by 5 cm by 6.25 cm deep pots) and 'Bowles' periwinkle (10 cm liners) were transplanted 18 December 1992 to 3.8 liter pots containing a medium consisting of perlite : vermiculite : peat (2:1:1 by volume) amended with 5.6 kg m^{-3} 25N-4P-8K slow release fertilizer (Osmocote, Grace-Sierra, Milpitas, Calif.), 0.6 kg m^{-3} micronutrients (Micromax, Grace-Sierra), and 2.3 kg m^{-3} dolomite. Common periwinkle plants were sprayed to runoff with thiophanate methyl (dimethyl [(1,2-phenylene)-bis (iminocarbonothioyl)] bis [carbamate]), thiophanate methyl/mancozeb (dimethyl [(1,2-phenylene)-bis (iminocarbonothioyl)] bis [carbamate]) and a combination

of zinc ion and manganese ethylenebis dithiocarbamate), and with propiconazole (1-[2-(',4'-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl-methyl]-1*H*-1,2,4-triazole) periodically before 3 April 1993 to assure plants were free of foliar pathogens for the beginning of the study. Insect control during establishment and throughout the study was accomplished by spraying the plants to runoff weekly with diazinon (0-0-diethyl-O-[2-isopropyl-6-methyl-4-pyrimidinyl] phosphorothiate), insecticidal soap, with (Orthene) acephate (0,S-Dimethyl acetylphosphoramidothioate), and with malathion (0,0-dimethyl dithiophosphate of diethyl mercaptosuccinate). All plants were fertilized at 3-week intervals with 20N-4.3P-16.6K water soluble fertilizer (Peter's Peat Lite Formula, Grace-Sierra) at 22.5 mg N liter^{-1} and micronutrients (S.T.E.M., Grace-Sierra) at 0.06 g liter^{-1} until the study was initiated. Plants were established in a polyethylene greenhouse under long days provided by a 2 h night interruption with incandescent lighting and a minimum/maximum temperature of 10/30°C. During establishment plants were hand watered as necessary.

Twenty-four plants of each cultivar were inoculated with a spore solution of *C. gloeosporioides* 27 April 1993 using the techniques described in Chapter II. C. gloeosporioides were grown in 9 cm plastic petri dishes on a medium consisting of potato-dextrose agar (39 g of potato-dextrose agar with 1 liter of distilled deionized water and autoclaved at 120°C for 20 minutes) for 14 days for spore production. A hemacytometer (Baxter Healthcare Corporation, McGaw Park, IL) was used to estimate the concentration of spores in the spore solution at 1.56×10^4 ml^{-1}. Spore solution was sprayed on plant parts until runoff with a hand mist sprayer. After inoculation, plants were placed into two growth chambers by cultivar. Both growth chambers remained dark the first 18 h, then were programmed for a day/night cycle consisting of 14 h of light and

10 h of darkness. Both chambers were maintained at 28°C, greater than 90% relative humidity, and mist was applied to all plants for 3 minutes every 13 minutes while the lights were on to maintain leaf wetness. Photosynthetic photon flux (PPF) at plant height was 310 µmol m^{-2} s^{-1} for common periwinkle plants and 315 µmol m^{-2} s^{-1} for 'Bowles' periwinkle. Plants remained in the growth chamber for 6 days until stem and leaf spots and anthracnose appeared. On 3 May 1993, inoculated plants were moved to the greenhouse and placed on benches so that each inoculated plant or non-inoculated control plant was surrounded by eight non-infected plants. The PPF in the greenhouse was 490 µmol m^{-2} s^{-1} at plant height. Once in the greenhouse, the plants received 2.5 cm water nightly with overhead sprinklers (Nifty Nozzles, American Plant Products, Oklahoma City, OK) or drip tubes (Dramm, Manitowoc, WI). The evening watering insured a minimum of 10 h of leaf wetness to encourage *C. gloeosporioides* infection.

Plants were visually rated weekly by three individuals using a scale ranging from 0 to 5: 0 = no disease; 1 = trace to 3% of foliage showing symptoms; 2 = 4% to 10% of foliage with symptoms; 3 = 11% to 25% of foliage with symptoms; 4 = 26% to 50% of foliage with symptoms; and 5 = more than 50% of foliage with symptoms. The experiment was terminated during week 5 on 10 June 1993. The shoots of two replicates of each treatment from each bench were harvested and dried at 44°C for seven days and weighed. The experimental design was a split plot with irrigation as the main plot treatment and cultivar and inoculation as subplot treatments. There were three replicates with four subsamples per replicate. Analysis of variance procedures and least significant differences (LSD) tests were used to determine differences between cultivars, irrigation techniques, and inoculation treatments in the visual ratings and dry weight data.

RESULTS

Beginning at week 3, the plants associated with inoculated common periwinkle plants had higher visual ratings than those associated with non-inoculated common periwinkle plants (Table 4.1). Visual ratings of 'Bowles' periwinkle did not differ significantly at any time during the study. Irrigation methods did not affect disease incidence on either cultivar until week 5 when plants receiving drip irrigation had higher ratings than those receiving sprinkler irrigation.

When the study was terminated, the plants surrounding inoculated common periwinkle plants had higher dry weights than those surrounding non-inoculated common periwinkle plants (Table 4.2), but the non-inoculated 'Bowles' periwinkle had higher dry weights than the inoculated 'Bowles' periwinkle. Plant dry weights were not affected by irrigation methods.

DISCUSSION

Cultural practices implemented in greenhouse environments often provide optimum conditions for fungal spores to be produced, disseminate, germinate, and infect plants. Production of common periwinkle plants often occurs within a minimum amount of space under shade with overhead irrigation. The increased moisture and shade requirements provide higher humidity and lower light intensity for the clear spores to proliferate. The leaf surfaces remain moist for a long period of time for spores to infect plant material after dissemination. Sprinkler irrigation would provide an inoculum dispersal method.

High humidity and temperature, and a specific amount of darkness following initial inoculation affect *C. gloeosporioides* disease incidence and severity (Chapter II). Trevorrow et al. (1988) and Chakraborty et al. (1990) have noted specific time periods,

temperatures, and humidity levels for infection of *C. gloeosporioides* to occur. This period of leaf wetness is the foundation for many forecasting models for numerous similar plant pathogens (Louis and Cooke, 1985; Royle and Butler, 1986; Jones, 1986).

Abraham et al. (1988) isolated, identified, and confirmed the pathogenicity of *C. gloeosporioides* in *Artocarpus incisa*. Prolonged disease symptoms resulted in twig die-back. In addition to the infection on directly inoculated twigs, infection that initially began on the leaf lamina, spread to the petiole, then throughout the plant. Infection on the common periwinkle and 'Bowles' periwinkle plants in this research began with a single spot either on the leaf surface or on stems. As the infection grew, necrosis increased on infected plant parts. This study was terminated due to shoot growth which covered infected areas, causing inaccurate visual ratings.

Cultivar resistance is another potential method of disease control. Growers have noted that cultivated lines are more resistant than native varieties. This may be due to the more stringent propagation conditions for cultivated lines, or to the selection of more resistant lines as other horticultural qualities, such as particular flower color, flower size, foliage shape, foliage color, and growth habit are selected. Propagation material for native lines are taken directly from the wild and may already be infected with pathogens.

Successful control of *Glomerella cingulata* (Stoneman) Spauld. and H. Schrenk. (the perfect stage of *C. gloeosporioides*) on camellias has been accomplished by using a combination of techniques including 1) dipping cuttings in fungicides prior to planting, 2) removal of infected plant parts and immediately burning them, and 3) avoiding irrigation techniques that cause splashing of spores (Can et al., 1978). The use of

resistant cultivars also aids in reducing this disease in camellias. Resistant common periwinkle cultivars should reduce disease severity and incidence.

It has been noted that environmental conditions, cultural practices, cultivar resistance, plant nutrition, and chemical control influenced disease incidence and severity. A better understanding of these practices would improve the production techniques and growth quality of common periwinkle. The results of these findings may even encourage greater production in Oklahoma. Although these diseases have been more prevalent in nurseries, infected plants transplanted into landscapes have the potential of also being affected; therefore, increased knowledge of common periwinkle may encourage sales and popularity.

While providing some information, the results of this experiment are inconclusive. Therefore, further research is necessary to confirm that spores of *C. gloeosporioides* can be spread by the splashing of irrigation water. 'Bowles' periwinkle appears more resistant than common periwinkle, but further research is needed to confirm this observation.

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Table 4.1. Weekly visual ratings of inoculated and non-inoculated common periwinkle and 'Bowles' periwinkle receiving drip or sprinkler irrigation. Rating scale: 0 = no foliar symptoms, 1 = trace to 3%, 2 = 4% to 10%, 3 = 11% to 25%, 4 = 26% to 50%, and 5 = greater than 51% of the plant having foliar symptoms.

	Visual Rating				
Treatment	1	2	3	4	5
			Irrigation		
Drip	0.2	0.8	0.8	1.2	2.1
Sprinkler	0.3	0.8	1.0	0.9	2.0
			Cultivar x Inoculation Interaction		
Common Inoculated	0.3	0.7	0.8	0.9	1.8
Common Non-inoculated	0.2	0.4	0.6	0.7	1.3
'Bowles' Inoculated	0.4	1.0	1.1	1.3	2.4
'Bowles' Non-inoculated	0.3	1.0	1.1	1.4	2.7
Significance ($LSD_{0.05}$)					
Irrigation (I)	NS	NS	NS	NS	0.1
Cultivar (C)	NS	NS	NS	NS	NS
I x C	NS	NS	NS	NS	NS
Inoculation (IN)	NS	NS	NS	NS	NS
I x IN	NS	NS	NS	NS	NS
IN x C	NS	NS	0.5	1.1	1.7
I x C x IN	NS	NS	NS	NS	NS

Table 4.2. Dry weights (g) of inoculated and non-inoculated common periwinkle and 'Bowles' periwinkle receiving drip or sprinkler irrigation.

Cultivar	Treatment	Dry Weight (g)
Common	Inoculated	10.9
	Non-inoculated	10.5
'Bowles'	Inoculated	10.4
	Non-inoculated	11.5
Significance ($LSD_{0.05}$)		
Irrigation (I)		NS
Cultivar (C)		NS
I x C		NS
Inoculation (IN)		NS
I x IN		NS
C x IN		1.63
I x C x IN		NS

CHAPTER V

SUMMARY

Common periwinkle (*Vinca minor* L.) is a ground cover that is most often placed in shaded areas of landscapes. The popularity of this ornamental plant has increased in recent years since it is a relatively low maintenance plant once established. Despite its popularity, the number of plants in production has declined, and it has been eliminated from some grower inventories, primarily due to foliar diseases which occur during production. The severity of the disease problem has increased due to a lack of effective control methods. The diseases also appear in common periwinkle plants in landscapes.

Foliar disease symptoms include leaf and stem spots, anthracnose, and stem dieback. The isolation of fungal organisms from infected plant parts revealed two major causal agents, *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. in Penz. and *Phoma exigua* Desmaz. var. *inoxydabilis* Boerema & Vegh in Vegh et al. The fungal pathogen *C. gloeosporioides* was more prevalent on diseased samples, hence this pathogen was the primary focus of this research. Optimum conditions for infection in the reported studies conducted under controlled conditions included 1) a temperature of 28°C, relative humidity greater than 90%, an initial dark period of 16 to 18 h followed by normal light (120 to 151 μmol m^{-2} s^{-1}) with a day/night cycle consisting of 14 h and 10 h of darkness.

This research also tested the ability of two cultivars, common periwinkle and 'Bowles' periwinkle, to survive and grow in the presence of foliar pathogens. Growers stated that disease severity on common periwinkle was greater than with cultivated lines.

This may be attributed to the diverse genetic background and harvesting techniques of the common periwinkle, since common periwinkle used for propagation is typically collected from the wild. In contrast, cultivated lines such as 'Bowles' are normally propagated from stem cuttings with careful attention to proper propagation techniques.

With increasing pesticide restrictions and labeling requirements, the number of effective fungicides has rapidly decreased. Several fungicides were evaluated in this research for disease control in the field and *in vitro* studies. The fungicides most effective at inhibiting mycelial growth *in vitro* were propiconazole, cyproconazole, and a thiophanate methyl/mancozeb combination. Four fungicides commonly used in spray programs in the industry were chlorothalonil, cupric hydroxide, mancozeb, and thiophanate methyl. These four fungicides did not satisfactorily inhibit mycelial growth in *in vitro* studies. Field studies determined that thiophanate methyl/mancozeb alone or in rotation with propiconazole, effectively controlled the disease complex with no phytotoxicity. Propiconazole alone did not control disease symptoms in the field. Cyproconazole was phytotoxic during the second summer.

Cultural practices may influence disease spread and severity. Producers most commonly use overhead irrigation methods such as hand watering with a hose, mist, or sprinkler irrigation. These methods can spread spores through the splashing of water. An experiment was conducted to determine pathogen dispersal; however, results were inconclusive and further research is necessary to confirm that spores can be spread by the splashing of water.

Disease incidence and severity may be reduced significantly by utilizing our knowledge of infection requirements and altering production practices of common

periwinkle so that environmental conditions are less favorable for pathogen survival and spread. The use of resistant cultivars and appropriate chemicals, when available, will encourage more widespread production and use of common periwinkle as a landscape plant.

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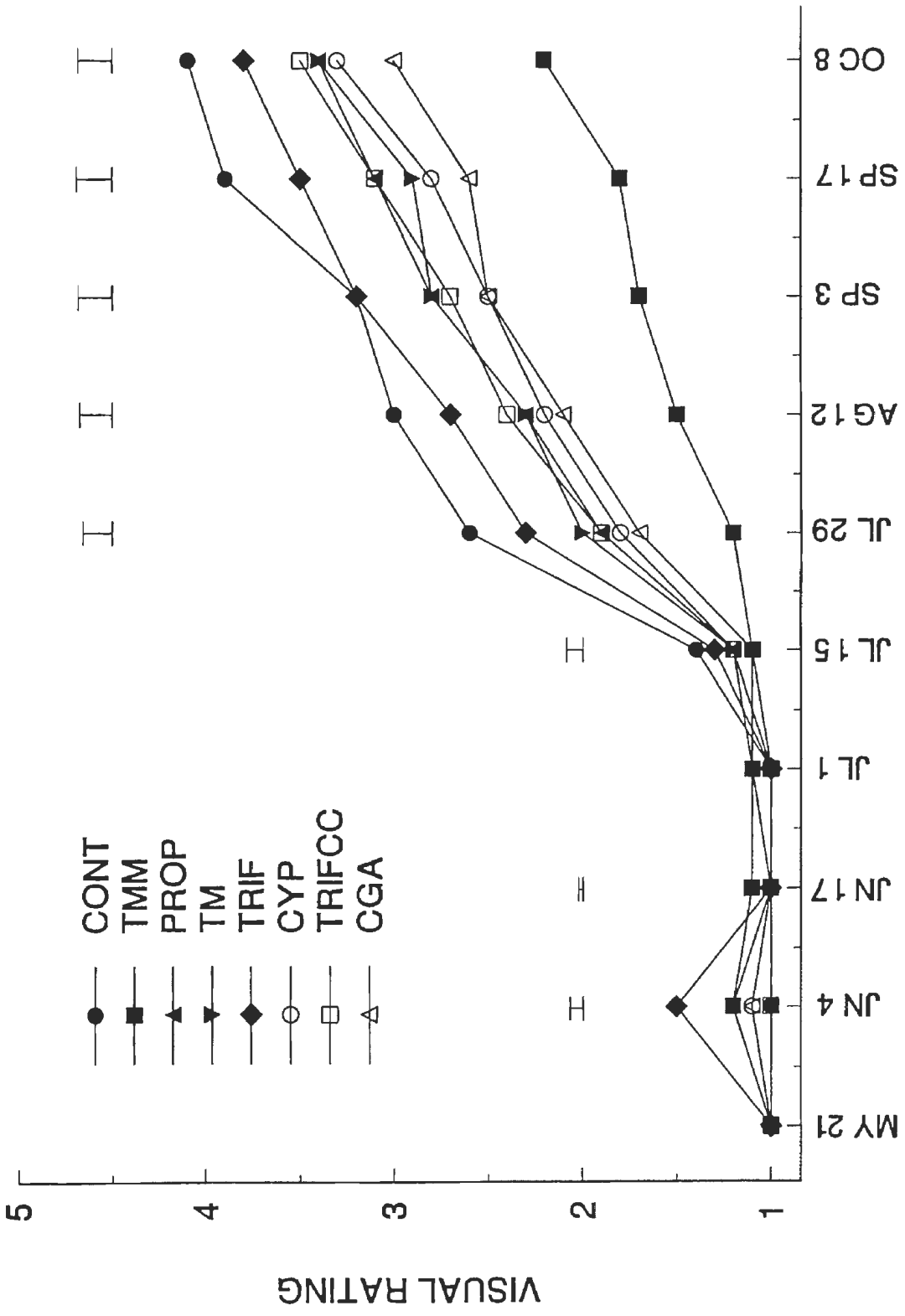
APPENDIX

EFFECTIVENESS OF SEVEN FUNGICIDES IN CONTROLLING FOLIAR DISEASES OF 'EMERALD GAIETY' EVERGREEN WINTERCREEPER (*EUONYMUS FORTUNEI* (TURCZ.) HAND.-MAZZ. 'EMERALD GAIETY')

Table A.1. Dry weights (g) of shoots and roots of 'Emerald Gaiety' evergreen wintercreeper receiving weekly fungicide applications throughout the 1992 growing season.

Fungicide	Shoot Dry Weight	Root Dry Weight
Control	11.2	8.7
Thiophanate methyl/mancozeb	30.3	15.8
Propiconazole	18.7	11.0
Thiophanate methyl	17.2	9.9
Triforine	14.3	8.9
Cyproconazole	20.7	10.7
Triforine - CC 17461	17.0	9.5
CGA 173506	19.7	10.6

Figure A.1 Visual ratings of 'Emerald Gaiety' evergreen wintercreeper receiving weekly fungicide applications throughout the 1992 growing season. Rating scale: 1 = no foliar symptoms, 2 = 1 to 33% necrotic plant tissue, 3 = 34 to 66% necrotic plant tissue, 4 = 67 to 99% necrotic plant tissue, and 5 = 100% necrotic tissue. Vertical bars indicate $LSD_{0.05}$.


VISUAL RATING
5
4
3
2
1
MY 21
JN 4
JN 17
JL 1
JL 15
JL 29
AG 12
SP 3
SP 17
OC 8
CONT
TMM
PROP
TM
TRIF
CYP
TRIFCC
CGA

VITA 2

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