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Identification, etiology and control of euonymus fortunei anthracnose caused by colletotrichum gloeosporioides.

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IDENTIFICATION, ETIOLOGY AND CONTROL OF *EUONYMUS FORTUNEI* ANTHRACNOSE CAUSED BY *COLLETOTRICHUM GLOEOSPORIOIDES*

A Thesis Presented

By

MATTHEW JOHN MAHONEY

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Plant Pathology

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INTRODUCTION

A leaf and stem spotting disease of Euonymus fortunei 'Emerald 'n Gold' has been detected on balled and burlapped plants in winter storage, and on container-grown plants outdoors as well as in a greenhouse at Corliss Brothers Nursery in Ipswich, Massachusetts. Corliss Bros. holds a plant patent on E. fortunei 'Emerald 'n Gold', an upright, variegated, evergreen shrub distinguished by the gold margin on its green leaves. Fully grown, this plant reaches 1.4 to 1.7 meters in height with a spread of approximately 1 meter.

Leaf disease symptoms appear as discrete, circular, dark brown lesions 0.5-3.0 mm in diameter with light tan necrotic centers, occurring on both upper and lower leaf surfaces (Fig. 1). Initial leaf lesions appear as discrete, circular, light tan spots. During the latter stages of infection a reddish discoloration may be noted in the tissue surrounding the lesion, and the necrotic lesion centers may drop out, creating a "shot-hold" appearance.

Stem lesions appear as raised, brown, circular to elliptical regions 0.5-3.0 mm in diameter with light tan centers.

I have observed symptoms similar to those of diseased E. fortunei 'Emerald 'n Gold on E. fortunei 'Emerald Gaiety' at Corliss Brothers Nursery, and on 'Emerald Gaiety' leaves received from another Massachusetts nurseryman. Recently

Fig. 1. Colletotrichum gloeosporiodes leaf lesions on Euonymus fortunei 'Emerald 'n Gold.'

I was informed by the Robert Baker Nursery of West Suffield, Connecticut that due to a severe leaf spotting disease which occurred during propagation, and in the field, they had halted all production of E. fortunei -- 'Emerald 'n Gold'.

Euonymus plants afflicted with this disease often become so heavily infected that leaf abscission and stem dieback occur, rendering the plants so unsightly as to be unsalable.

The objectives of this study were: 1) to identify the causal organism and determine its etiology as related to control; 2) to determine the susceptibility of several evergreen Euonymus fortunei cultivars to the disease; 3) to evaluate the effectivensss of several fungicides at controlling the disease in question.

C H A P T E R I

LITERATURE REVIEW

Occurrence of Colletotrichum leaf spot on Euonymus

Colletotrichum spp. on Euonymus spp. was first reported in 1911 by Heald and Wolf (17). The pathogen, Colletotrichum griseum was encountered on leaves and branches of Euonymus japonicus Thunb. at several locations in Texas. This disease was also reported in 1931 on Euonymus spp. in Alabama (3).

A thorough search of the literature has failed to reveal any further citations of Colletotrichum diseases of Euonymus spp. However, in 1971, a disease complex involving a Phytophthora sp. a Colletotrichum sp. and poor cultural practices was reported by Peterson et al in New Jersey (34). The Colletotrichum sp. was reported to be responsible for the leaf lesions.

Colletotrichum

The genus Colletotrichum is classified in the Deuteromycetes, order Melanconiales, and family Melanconiaceae (12). Colletotrichum is the imperfect stage of the Ascomycete Glomerella (12).

Colletotrichum spp. are known to be pathogenic to a wide range of host plants, many of which are economically important. The speciation within the genus Colletotrichum reflects this wide host range, with many species being

named according to the host upon which they were first encountered. Thus, there is a considerable amount of synonomy to be found among Colletotrichum spp. throughout the literature (9,39). Members of this long list of Colletotrichum spp. are known to cause leaf and stem lesions, leaf and twig blights, fruit rots and root rots.

The genus Colletotrichum is characterized by the formation of disc-shaped acervuli with dark brown setae at the edge, or among the conidiophores (1,2,4). Colletotrichum conidia are produced on simple elongate conidiophores; are hyaline, usually longer than 12 um, cylindrical, falcate or ellipsoidal, and form dark appressoria after germination (2,4). The acervuli which generally develop below the epidermis or cuticle of the host become erumpent when mature, releasing conidia in colored droplets; the color depending upon pigmentation of the conidia (1).

Colletotrichum is differentiated from the genus Gloeosporium by the presence or absence of setae in the acervulus. This distinguishing characteristic is variable, however, and it's validity has been questioned (1,12). The genera Colletotrichum and Gloeosporium are known to cause "Anthracnose" type plant diseases.

Colletotrichum gloeosporioides. C. gloeosporioides Penz., the imperfect state of Glomerella cingulata, causes disease on a wide range of plant hosts and is a common saprophyte in previously weakened plant tissue (41).

C. gloeosporioides is known to cause "withertip," a terminal twig dieback of many sub-tropical and tropical plant species including orange, Citrus, lemon grapefruit, avocado, Aucuba, cherimoya, fig, loquat, roselle, rose-mallow, royal palm, Dieffenbachia, rubber plant and mango (32,33,41) and leafspots and/or anthracnose of peach, sweet pea, yam, statice, Calendula, papaya, dogwood, Hibiscus, tasmine, Passiflora, Umbellularia, fig, Ginko, dwarf mistletoe, northern joinvetch, Populus balsamifera, and P. tremuloides (18,27,32,33,38,39,41). C. gloeosporioides is also known to be involved with canker and dieback diseases of Azalea, blackberry, bittersweet, rose, raspberry, soapberry, mountain ash, and English ivy; and bitter rot of apple and pear, and ripe rot of grapes all caused by Glomerella cingulata (41).

Anthracnoses

The modern use of the term anthracnose refers to a plant disease characterized by zonate lesions of foliage, fruit and stems, often accompanied by dieback (38). The term anthracnose is also used to describe diseases caused by the acervulus-forming fungi Colletotrichum and Gloeosporium (12,36,37,41). Other acervulus-forming members of the Melanconiaceae are also occasionally considered anthracnose fungi. These include the genera Marssonina, Pestalotia, Sphaceloma, and Monochaetia (33,38). If present, the ascigerous states of the fungi listed above are also considered as anthracnose fungi. These include the

Ascomycetes Glomerella, Gnomia, Mycosphaerella, Neofabraea, Elsinoe and Pseudopeziza (1,2,4,36,41).

Anthracnose fungi are usually characterized by subcutaneous or subepidermal acervuli, which at maturity become partially erumpent and ooze conidia in a conglutinated mass (37,41). The conidia are usually avoid to ablong, hyaline and one-celled. The spore-bearing stroma appears as a waft of thickly woven mycelium which increases in size, breaks the cuticle, and bears conidia atop short conidiophores (4,12,37). Acervuli often occur in concentric circles upon the host (37). Upon germination, conidia often become septate, and form large, brown, variable-shaped appressoria (11,12,16,20,41). Hasselbring (16) noted that the formation of appressoria is induced by a contact stimulus such as a microscope cover slip, but that in the presence of abundant nutrients the germ tube fails to form appressoria.

Anthracnose diseases affect a wide range of woody and herbaceous hosts. Disease symptoms include: zonate, necrotic leaf spots; necrotic stem cankers; soft, watery stem and petiole rots; necrotic leaf blotches; elongated, sunken stem lesions; circular, sunken, water-soaked fruit cankers; fruit and root rots; and twig dieback (7,14,18,34,36,41).

Anthracnose etiology

Primary inoculum. Anthracnose fungi overwinter in previously infected host tissue as sclerotia, mycelium, and perithecia

(14,41). They are capable of surviving on infested crop debris in soil (10,14,41), in seed coats of previously infected plants (35,41), and on previously infected foliage, twigs, stems and petioles of living plants (19,30,38). Lukezic (24) supported the theory that Colletotrichum trifolii survived in dry alfalfa debris on stored equipment and served as primary inoculum in the spring. Primary inocula in the form of conidia or ascospores are produced on previously infected plant material when environmental conditions favoring sporulation prevail (7,8,25,30,31,40, 41,42).

Anthracnose spores are spread principally by rain splash and rarely by dry winds or dews (42). Roberts found short-distance transport of Colletotrichum coffeanum Noack conidia mediated by rain splash (30). Couch and Grogan (10) reported that Marssonina panattoniana conidia were not detached when sporulating lesions were exposed to a dry wind. Their findings also showed that when a moist air stream was passed over wetted lesions, the spores were detached and moved in the wind current.

Optimum temperature for spore production of anthracnose fungi has been shown to vary slightly. Milholland (28) found optimum spore production of Gloeosporium minus at 25-30°c. Goos and Tschirsch (40) found a well defined peak in sporulation of G. musarum at 32°, and Marks et al (25) noted the optimum sporulation temperature of G. tremulae to be 24°.

Infection. Anthracnose infections are associated with periods of precipitation and high humidity (5,7,8,10,15, 22,28,30,31,41,42). Rain functions to release and disseminate spores, as well as to provide the free moisture necessary for spore germination (42). Fergus (15) found a correlation between the severity of white oak anthracnose (Gnomiaventata) and the number of days and hours of rain during late April and May. Chambers (8) noted that at least 2 consecutive days of rain accompanied by cloudiness and high humidity were necessary for infection of lima bean stems by Colletotrichum truncatum. Under laboratory conditions it has been shown that as the dew period is decreased to less than 12 hours, fewer northern joinvetch seedlings are infected with C. gloeosporioides (38).

Spore germination of anthracnose fungi is also dependent on moisture. While studying optimum conditions for germination of Gloeosporium musarum conidia, Goos and Tschirsch (40) found that conidia failed to germinate at relative humidities below 92%. Similar findings were reported by Nutman and Roberts (30), who noted that germination of C. coffeanum Noack conidia was negligible below 100% relative humidity. The optimum temperature range for spore germination and hyphal growth of most anthracnose fungi is 21-32° (13,21, 23,24,28,30,31,34,38).

Anthracnose fungi accomplish host penetration by direct cuticular penetration, through wounds, and through open

stomates (6,10,41). Appressoria often function in the direct penetration of Populus tremuloides leaves by C. gloeosporioides, Marks et al (26) found that infection pegs emerged from infection pores in the floors of the appressoria and penetrated the host cuticle primarily by mechanical pressure, and possibly assisted by chemical softening. However, direct penetration of mature leaves was found to be unsuccessful due to structural failure of the appressorium resulting from a backthrust of the infection peg bearing down on an unyielding cuticle.

Following successful penetration, anthracnose hyphae ramify both intercellularly and intracellularly through the host tissue (6,10).

Although weather conditions are the primary determinant of successful anthracnose infection, the stage of host development is also important. Fergus (15) found that Quercus alba were most susceptible to infection by Gnomia ventata when buds were opening and leaves were expanding. Similar findings were noted by Ogawa et al (31), who showed that infection of modesto ash, by Gloeosporium aridum was most severe during the initial period of bud-break and leaf development. Fisher (28) noted that infection of Citrus spp. by C. gloeosporioides is most severe during coinciding periods of high moisture, and new growth.

Anthracnose control

Control of anthracnose type plant diseases varies according to the host plant, and prevailing weather conditions. It has been established, however, that anthracnose diseases are more prevalent during moist weather conditions. Thus, chemical control measures are most beneficial at such times (5,8,28,31,41). It has also been established that anthracnose pathogens overwinter in crop debris and on previously infected foliage, twigs, stems and petioles of living plants (19,31,41). Where economically feasible, sanitation via eradicative pruning and removal of crop debris may be of value in the control of anthracnose diseases (41).

C H A P T E R II

METHODS AND MATERIALS

Pathogen Isolation and Identification

Tissue sections containing lesions were excised from E. fortunei 'Emerald 'n Gold' leaves and were surface sterilized in a 10% Chlorox solution for 5 minutes. After soaking, tissue pieces were plated on potato dextrose agar (PDA, 20 g/L distilled H_2O) and on potato carrot agar (PCA, 20 g potato extract, 20 g carrot extract, 20 g agar/L distilled H_2O). After sufficient growth and sporulation in culture the pathogen was identified to genus (4). Species identification was performed by Dr. J.A. von Arx.

Field Observation

Twenty E. fortunei 'Emerald 'n Gold' plants in 1 liter plastic containers were placed outdoors in Amherst, Massachusetts on May 22, 1978. Leaves and stems contained numerous lesions from the previous growing season. New growth was examined at 3 day intervals for evidence of new infections. A rain gauge (Edwards Manufacturing Co., Albert Lea, Minn.) and a recording hygrothermograph (Science Associates Inc., Princeton, N.J.) were utilized to monitor precipitation, temperature and relative humidity in the test plot vicinity.

Optimum Temperature for Vegetative Pathogen Growth

Five mm mycelial plugs were cut with a cork borer from

pathogen isolates grown on PCA, and plated on PCA. The cultures were incubated for 4 days in darkness at 15, 20, 25, 30, and 35°C. The average of 5 colony diameters was recorded for each temperature regime.

Optimum Spore Germination Temperature

Spores were gathered in sterile distilled water from 12-18 day old colonies grown on PCA. Spore suspensions were adjusted to approximately 1 x 10^6 - 2 x 10^6 spores/ml distilled water, utilizing a hemacytometer slide (American Optical, Buffalo, N.Y.). Spore suspensions were applied to hemacytometer slides with glass cover slips, and slides were incubated in petri dish moist chambers for 5 hours in darkness at 15, 20, 25, 30 and 35°C. After 5 hours scales were counted to determine percent spore germination. Germination was determined by the presence of a germ tube. Ten hemacytometer scales were counted for each temperature regime.

Effect of Leaf Wetness Period on Lesion Development

Rooted cuttings of E. fortunei 'Emerald 'n Gold' grown in a 1:1:1 peat, sand, perlite mix in 10 cm plastic pots were utilized. Recently emerged leaves were inoculated with 2 x 10^6 spores/ml distilled water. Spores were harvested from 12-14 day old colonies grown on PCAL (PCA with 5% lactic acid, 10 drops/ml agar added) to avoid bacterial

contamination. Spore suspensions were sprayed upon upper leaf surfaces with a hand-held atomizer after the upper leaf surfaces had been swabbed with sterile distilled water. Immediately following inoculation, plants were placed in a mist/humidity chamber consisting of a 40 x 55 x 35 cm clear polyethylene chamber with a water saturated 5 cm sand base.

The mist source consisted of an electric cool-water humidifier (Northern Co., Waynesboro, Miss.) situated within the chamber. The chamber was situated within a darkened incubator at 27°C, and mist was applied for 2.5 hours during each 24 hour period. (1 hr. at the beginning, and 1.5 hrs. after 12 hrs. in the chamber.) At 6, 12, 18, 24, and 48 hours respectively, inoculated plants were removed and transferred to a greenhouse bench. Five days after inoculation, leaves were harvested, lesions/leaf were counted with the aid of a dissecting microscope, and individual leaf areas were determined with a Li-Cor Portable Area Meter (Lambda Instruments Corp., Lincoln, Neb.). Lesions/cm^2 were calculated for each leaf. An average of 33 leaves (approx. 4 plants) was utilized in each treatment, and each treatment was replicated 3 times. Ten control leaves were sprayed with sterile distilled water and included in each treatment.

Susceptibility of E. fortunei Cultivars To Lesion Development

Utilizing the mist/humidity system of the leaf wetness

experiment, recently emerged leaves of the E. fortunei cultivars 'Emerald 'n Gold', 'Emerald Gaiety', 'Argenteo Marginatus', 'Sheridan Gold', and 'Radicans', were swabbed with sterile distilled water and sprayed with a suspension of 2×10^6 spores/ml distilled water. The plants remained in a mist/humidity chamber for 24 hours in darkness at 27°C, after which they were removed to a greenhouse bench. Five days after inoculation, lesions/cm^2 - leaf area were determined. Each treatment utilized an average of 33 leaves (approx. 4 plants), and each treatment was replicated twice. Ten control leaves were sprayed with sterile distilled water.

Protective Fungicidal Action Against Lesion Development

The following fungicides were sprayed upon the upper surfaces of recently emerged E. fortunei 'Emerald 'n Gold' leaves after swabbing with sterile distilled water: Manganese ethylene bis dithiocarbamate (Maneb W.P.) 1.7 g/L (1.36 g/L active), Methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate (Benlate 50% W.P.) 1.3 g/L (.65 g/L active), Manganese ethylene bis dithiocarbamate and zinc (Manzate 200 W.P.) 1.8 g/L (1.44 g/L active), and Tetra chloroisophthalonitrile (Daconil 2787 75% W.P.) 2.7 g/L (2.0 g/L active). Three drops of Tri-ogen spreader-sticker were added to each liter of spray material except Daconil 2787, when the manufacturer advised against the use of surfactants.

After the fungicides had dried, 2×10^6 spores/ml dis-

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

C H A P T E R III

RESULTS

Pathogen Isolation and Identification

The fungus isolates grew rapidly and produced a gray-white mycelium on PDA, but did not produce fruiting structures. On PCA, the fungus grew more slowly, producing a gray-white, septate mycelium, and concentric rings of dark acervuli in response to alternating fluorescent light and darkness. Oblong, hyaline, single-celled conidia 16 x 4.5 u were borne apically atop conidiophores within the acervuli (Fig. 2), and conidia oozed from erumpent acervuli in salmon-colored masses.

The presence of acervuli indicated a member of the Melanconiaceae and the initial absence of dark setae in the acervuli indicated that the fungus was either Gloeosporium or Sphaceloma, and not Colletotrichum. Dark setae, however, were detected at a later date in acervuli, and have been detected repeatedly since that time (Fig. 3). Thus, the fungus has been identified as a Colletotrichum sp., and more recently, Dr. J.A. von Arx has further identified the fungus as a form-species of Colletotrichum gloeosporioides Penz. (personal communication, June, 1979.).

Field Observations

Initial leaf lesion detection on outdoor plants in

Amherst, Massachusetts occurred on June 1, 1978, when several small, zonate, tan lesions were discovered on recently emerged Euonymus leaves. A Colletotrichum gloeosporioides was isolated from the infected tissue.

On June 16, 1978, and also on July 20, numerous young leaves and some new shoots exhibited disease symptoms.

New infections were observed on recently emerged foliage on July 31, at which time many of the previously infected leaves were beginning to abscise.

On August 11, 1978, after a 48 hour rainy period (8cm), extensive new infections were evident on both leaves and stems. New lesions were so numerous as to coalesce, and many leaves were abscising. After August 11, all experimental plants exhibited severe disease symptoms, such as leaf abscission and shoot dieback.

Throughout the experiment, acervuli, which appeared as small, black dots, were detected in established lesion centers, especially after periods of precipitation and high humidity.

In all cases, where new infections were detected, relative humidity was greater than 90% for at least 24 hours, less than 4 days before symptom expression, and in all cases, some precipitation occurred during the 4 day period before symptom expression (Appendix 1).

During 1979, symptom expression initially occurred on May 24, after 72 hours of rain.

During April, 1979, a Colletotrichum gloeosporioides was isolated from a 1978 leaf lesion, and disease symptoms were produced on E. fortunei 'Emerald 'n Gold' leaves inoculated with conidia harvested from the isolate.

Optimum Temperature for Vegetative Pathogen Growth and Spore Germination

Vegetative growth of Colletotrichum gloeosporioides as measured by average colony diameter at 15, 20, 25, 30 and 35°C was 0.0, 2.0, 4.5, 5.5 and 2.25 cm respectively (Fig. 4).

Initial attempts at spore germination in distilled water in hanging drop type microscope slides resulted in very low germination percentages (1.0%) even after 24 hours. The use of standard glass slides with cover slips, however, resulted in substantial increases in germination percentages (50.0%) after only 5 hours. Conidia beneath glass cover slips germinated quickly, forming appressoria, and often becoming septate (Fig. 5). Spore germination percentages, as measured with hemacytometer slides with glass cover slips, at 15, 20, 25, 30 and 35°C, were 0.95, 6.0, 59.0, 53.0 and 25.0% respectively (Fig. 6).

Effect of Leaf Wetness Period on Lesion Development

At 6, 12, 18, 24 and 48 hours of leaf wetness in a mist/humidity chamber, Colletotrichum gloeosporioides

symptom development of E. fortunei - 'Emerald 'n Gold' as measured by lesions/cm^2 - leaf area was 0.56, 0.43, 3.0, 16.0 and 18.4 lesions/cm^2, respectively (Fig. 7). These results are averages of the means of 3 trials/leaf - wetness regime.

Due to the inherent difficulty of obtaining consistent rates of infection on all intact leaves in an approximated natural system, such as a mist/humidity chamber, considerable variability in lesion development occurred in each wetness period. There was a significant increase in symptom development, however, between 18 and 24 hours leaf wetness, and between 24 and 48 hours leaf wetness. The 6, 12 and 18 hour wetness regimes were not significantly different from each other. In an attempt to more clearly indicate the effect of leaf wetness period on lesion development, data points are expressed as percentages of the sample population (leaves which fall into categories of 0, 1-10, 10-20, and 20 and above lesions/cm^2 - leaf area in Table 1.

In all trials, symptom development occurred approximately 48 hours after inoculation, and in all cases symptoms on older leaves were absent.

Susceptibility of E. fortunei Cultivars to Lesion Development

At 24 hours leaf wetness in a mist/humidity chamber, symptom development as measured by lesions/cm^2 - leaf area on E. fortunei 'Emerald 'n Gold', 'Emerald Gaiety', 'Argenteo

Marginatus', 'Sheridan Gold', and 'Radicans', was 16.0, 7.3, 9.7, 8.0 and 12.6 lesions/cm^2, respectively (Fig. 8).

Protective Fungicidal Action Against Lesion Development

At manufacturers' recommended rates, Maneb, Manzate 200, and Daconil 2787 effectively protected recently emerged E. fortunei 'Emerald 'n Gold' leaves from lesion development. Benlate reduced the lesion development, but did not completely protect the leaves. Symptom development on the Maneb, Manzate 200, Daconil 2787, and Benlate treated leaves was 0.0, 0.0, 0.0, and 1.5 lesions/cm^2 - leaf area, respectively (Table 2). All treatments differed significantly from the control treatment (13.3 lesions/cm^2 - leaf area).

Fig. 2. Colletotrichum gloeosporioides acervulus and conidia.

Fig. 3. Dark setae from a Colletotrichum gloeosporioides acervulus.

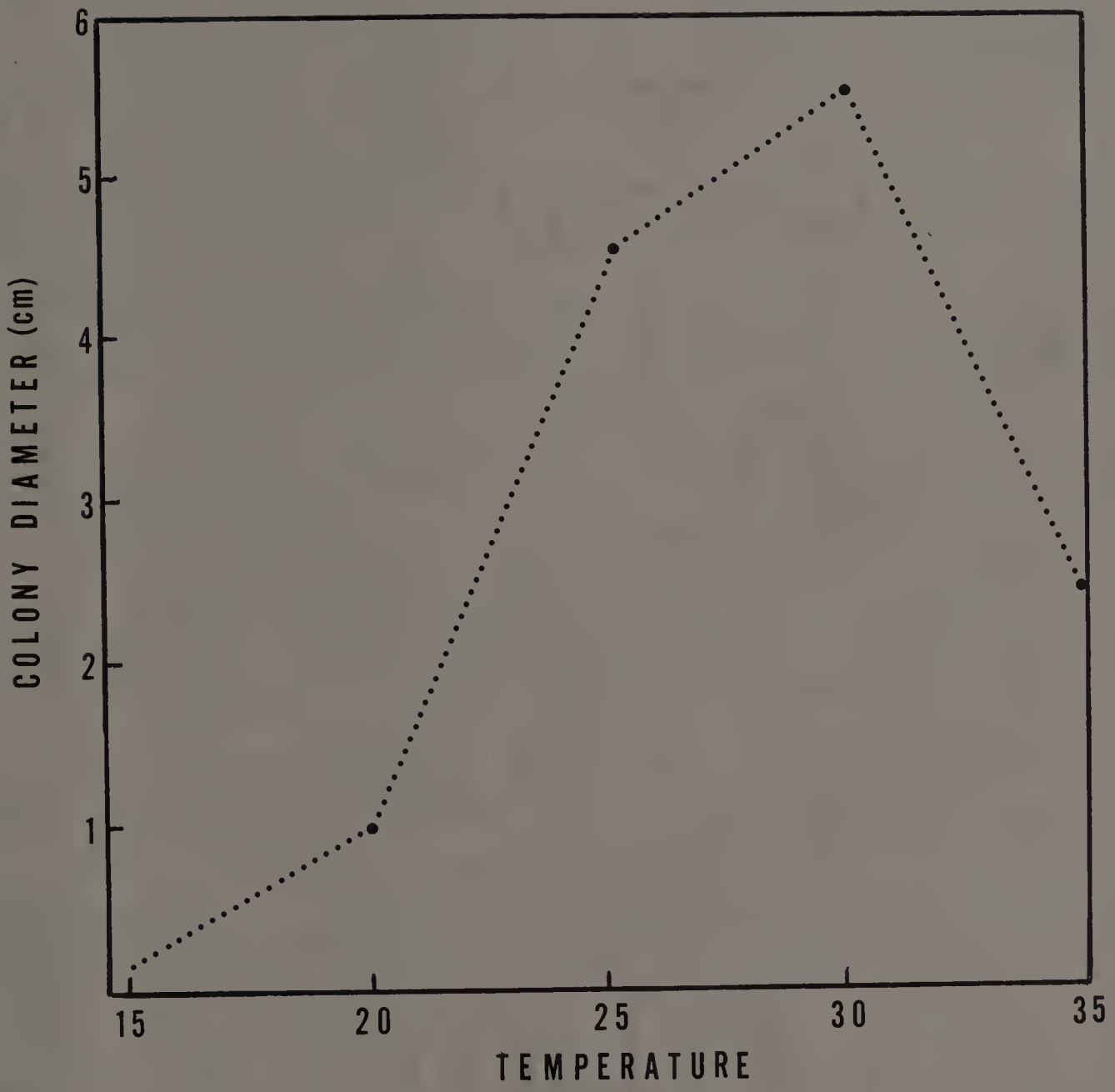
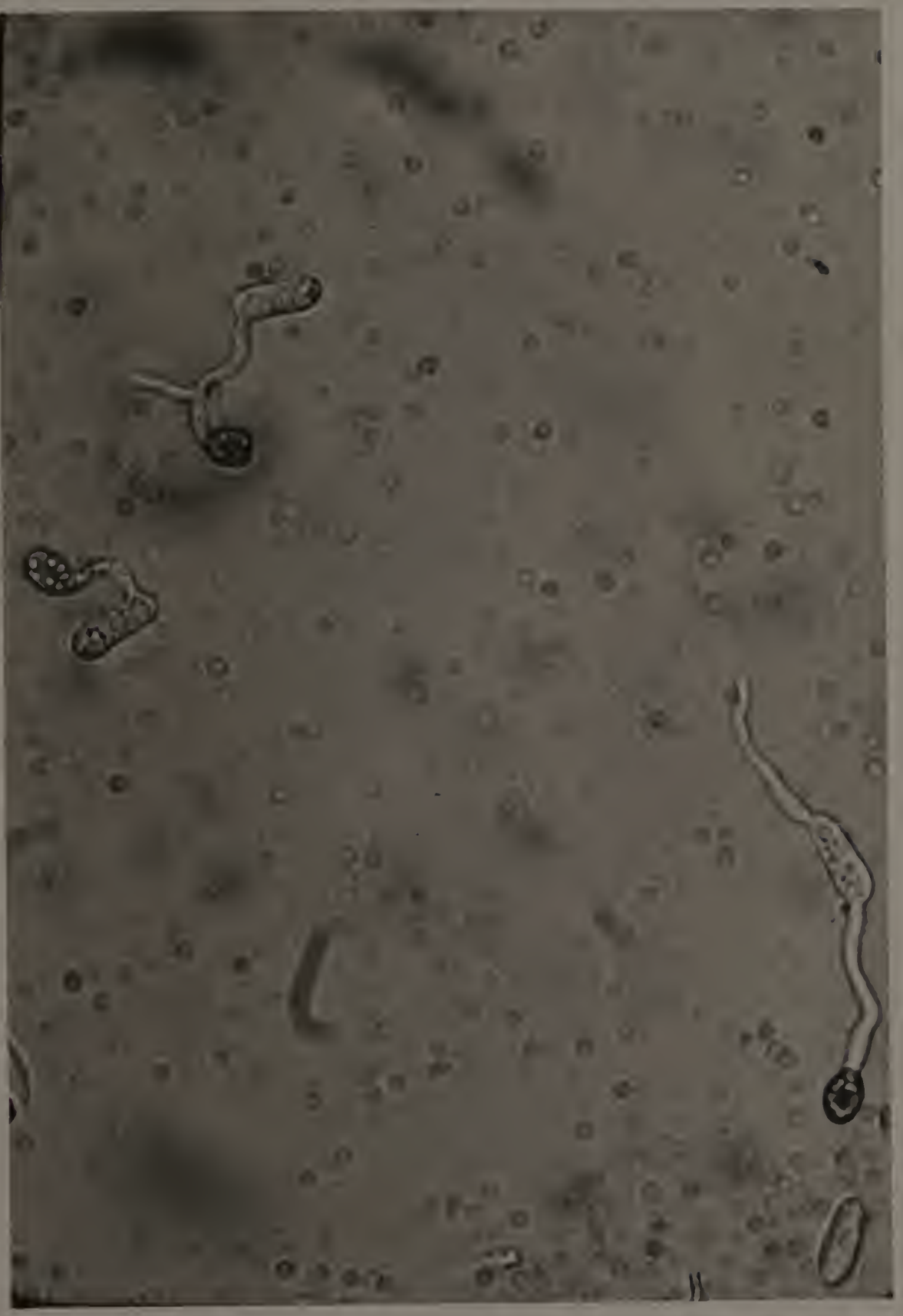


Fig. 4. Temperature effect on Colletotrichum gloeosporioides growth.

Fig. 5. Germinated Colletotrichum gloeosporioides conidia.



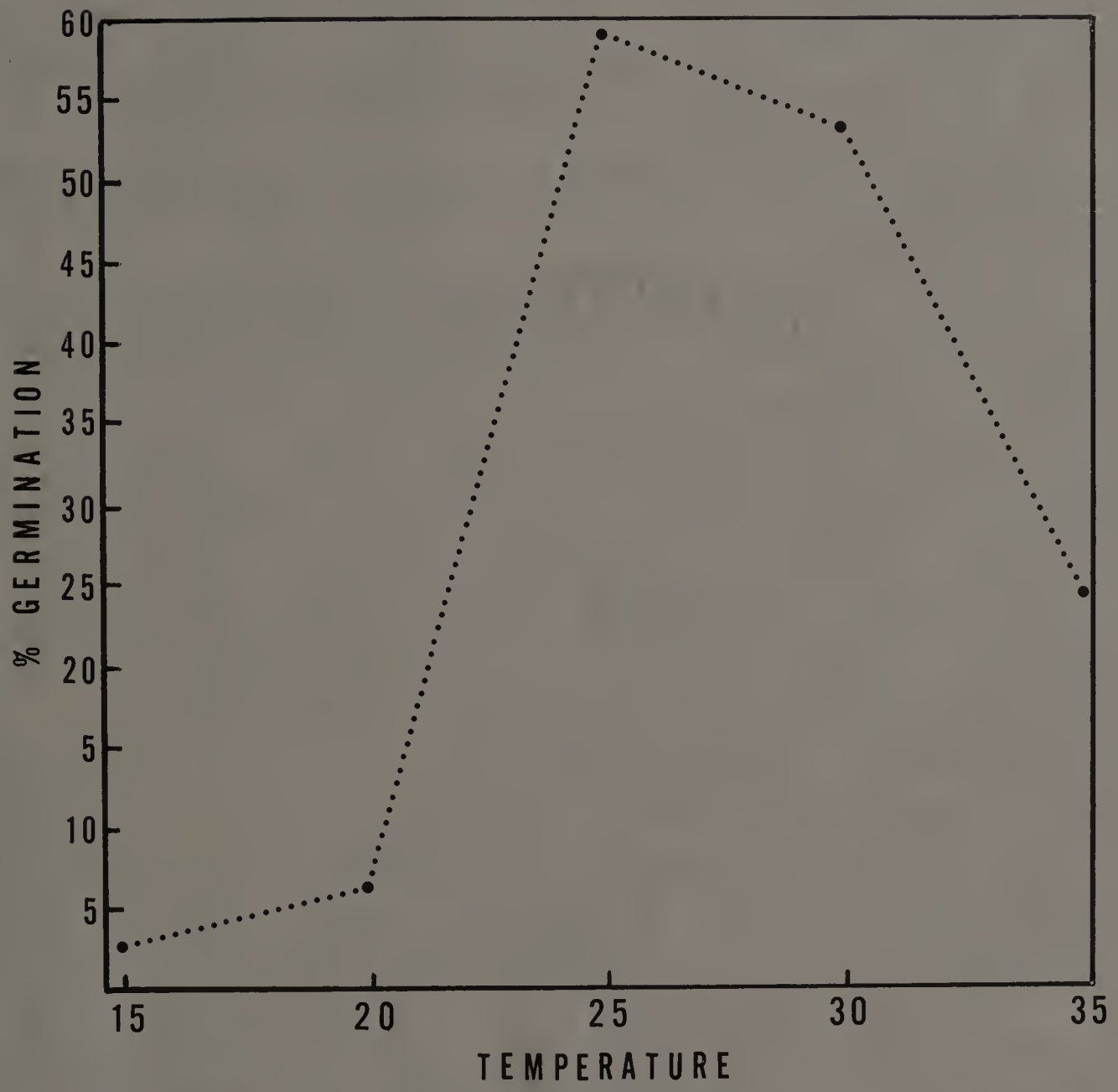


Fig. 6. Temperature effect on Colletotrichum gloeosporioides conidia germination.

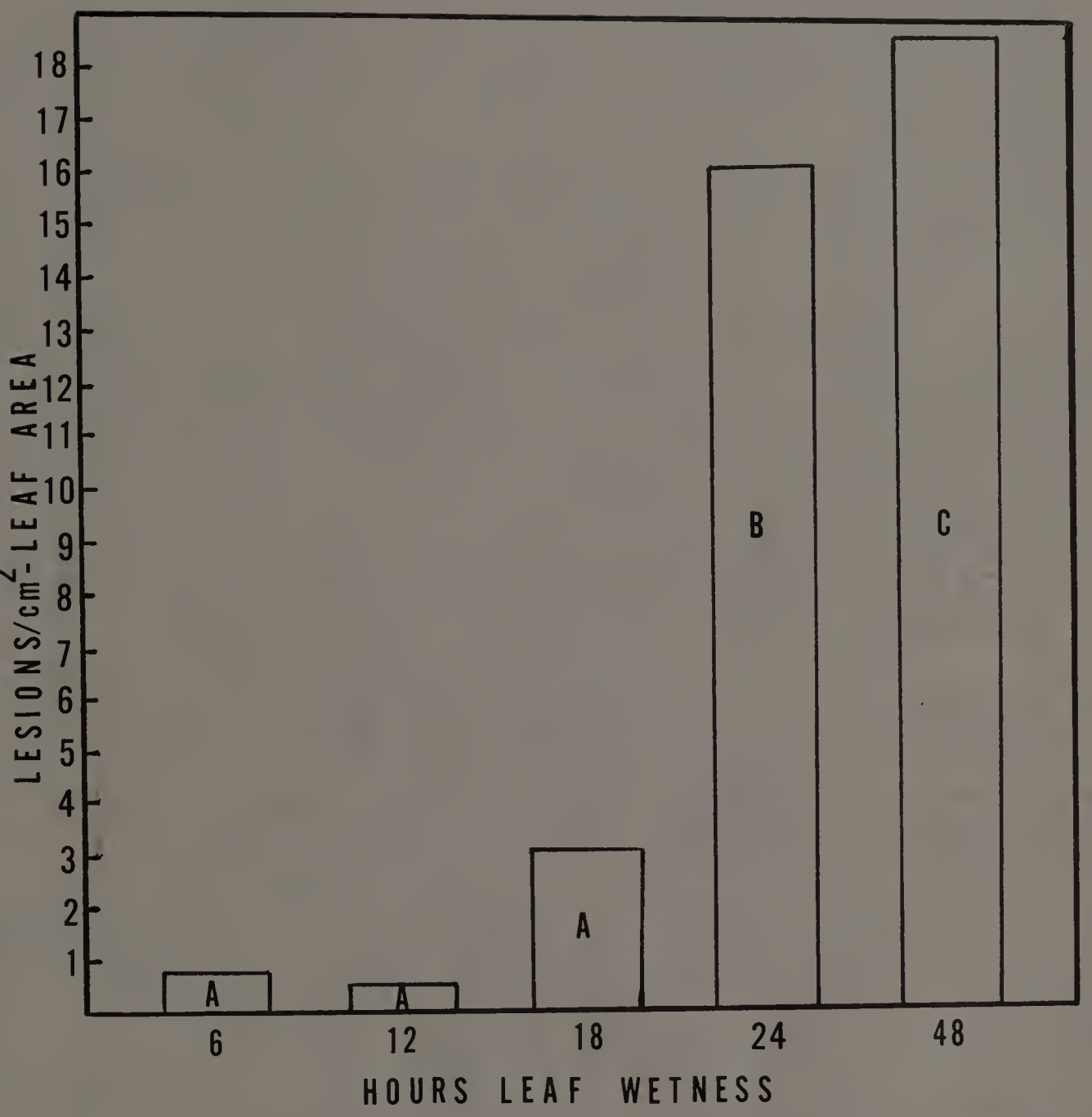


Fig. 7. Effect of leaf wetness period on Colletotrichum-gloeosporioides lesion development. Bars labeled by the same letters are not significantly different, $P \leq 0.05$, (Duncan's-multiple range test).

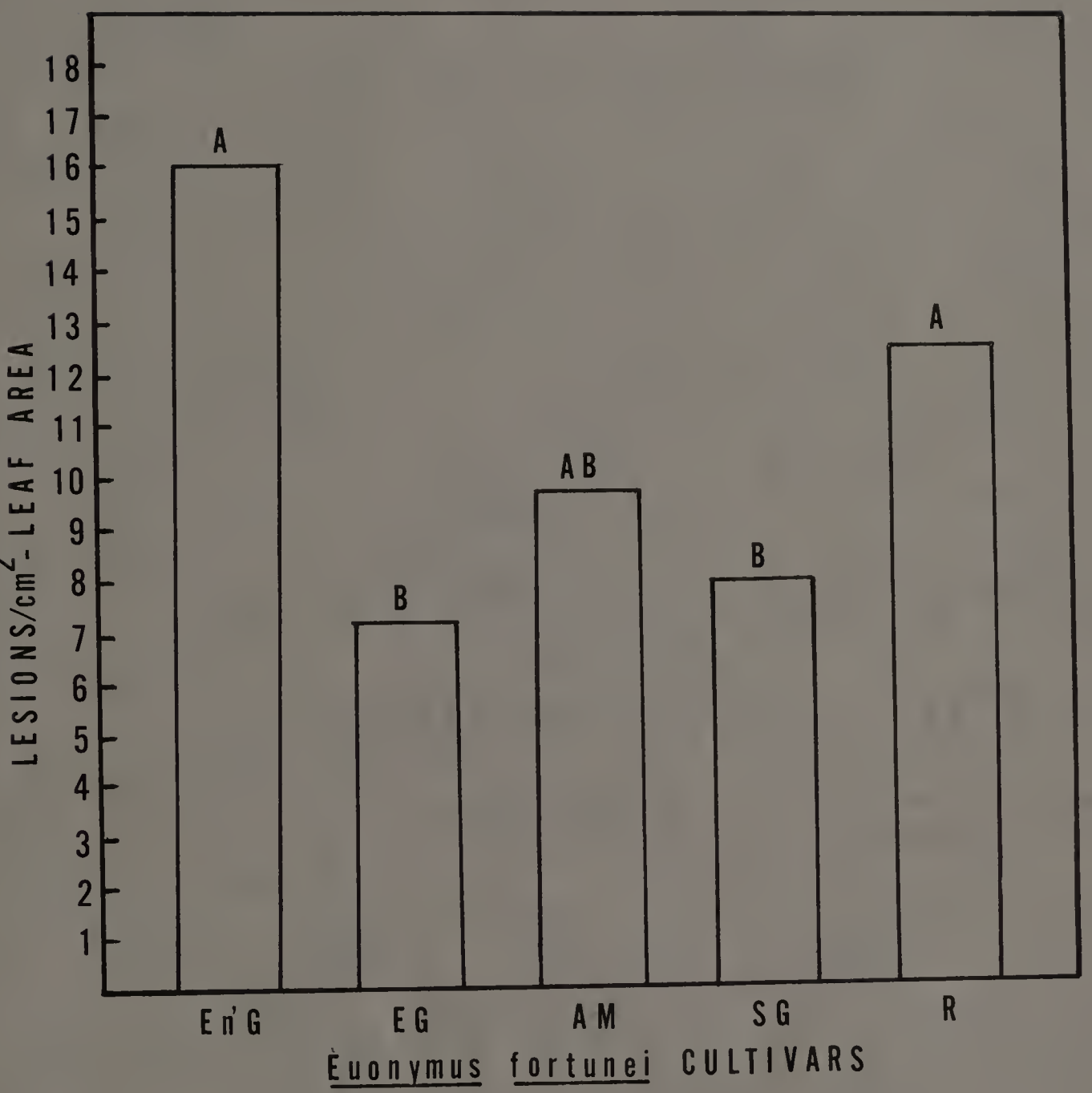


Fig. 8. Susceptibility of Euonymus fortunei cultivars to Colletotrichum gloeospoioides lesion development. En'G = Emerald n" Gold, EG= Emerald Gaiety, AM= Argenteo Marginatus, SG= Sheridan Gold, and R= Radicans. Bars labeled by the same letter are not significantly different, $P \leq 0.05$, (Duncan's multiple range test).

Table 1. Effect of leaf wetness period on Colletotrichum Gloeospoioides lesion development.[b]

Hours leaf wetness	Lesions/cm^2- leaf area			
	0	1-10	10-20	≧20
6	76.8[a]	21.7	1.5	0.0
12	70.2	29.8	0.0	0.0
18	21.4	73.4	8.0	1.5
24	2.3	57.6	11.6	28.5
48	3.3	34.8	24.6	37.3

[a] Expressed as percent of sample population (leaves).
[b] On Euonymus fortunei 'Emerald 'n Gold'

Table 2. Effect of protective fungicides on Colletotrichum gloeosporioides lesion development.

Fungicide	Lesions/cm^2- leaf area
Maneb	0.0[a]
Manzate 200	0.0[a]
Daconil 2787	0.0[a]
Benlate	1.5[b]
Control	13.3[c]

[a] Values followed by the same letter are not significantly different, P≦0.05.

C H A P T E R IV

DISCUSSION

My results indicate that the Colletotrichum gloeosporioides responsible for the Euonymus disease is a typical anthracnose-type disease organism. The fungus is capable of overwintering in previously infected host tissue, most likely as vegetative mycelium, and it appears to be dependent on periods of precipitation and high, relative humidity for spore dispersal, germination and penetration.

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

Temperature experiments indicate an optimum temperature range of 25-30^{o}C for vegetative growth and spore germination of the causal Colletotrichum gloeosporioides. Due to the formation of appressoria by germinating Colletotrichum gloeosporioides conidia, the results also indicate that a direct cuticle penetration mechanism is involved with the infection process.

Severity of Colletotrichum gloeosporioides lesion development of E. fortunei 'Emerald 'n Gold' appears to be correlated with the period of leaf wetness. A low rate of

lesion development (2 lesions/cm^2 leaf-area) was observed up to 12 hours of leaf wetness after inoculation, while a substantial increase in lesion development (14 lesions/cm^2 leaf area) occurred between 12 and 24 hours of leaf wetness. Periods of leaf wetness greater than 24 hours do not substantially enhance Colletotrichum gloeosporioides lesion development. Therefore, a minimum of 24 hours leaf wetness is required for maximum infection of E. fortunei 'Emerald 'n Gold' leaves by Colletotrichum gloeosporioides. These results, however, were obtained under laboratory conditions, utilizing a high spore load (2×10^6/ml) and optimum temperature (27°C). Thus, the minimum leaf wetness period may vary considerably under natural conditions.

The occurrence of E. fortunei anthracnose in the field has been reported to be limited to the cultivars 'Emerald 'n Gold' and 'Gaiety'. The results of my cultivar susceptibility experiments show, however, that all cultivars tested were susceptible to lesion development under laboratory conditions and that statistically 'Emerald 'n Gold' and 'Radicans' were most susceptible. It is possible that optimum infection conditions in the laboratory may overcome any amount of resistance present under natural conditions.

Maneb, Manzate 200, and Daconil 2787 completely protect E. fortunei 'Emerald 'n Gold' foliage from Colletotrichum gloeosporioides lesion development. Since the disease is most prevalent during wet weather, control practices must

be developed according to local weather conditions. Protective fungicide sprays should be applied during the moist weather periods of May and June, beginning at bud break. Sprays should also be applied whenever 24 hours, or more, of wet weather is forcasted during the remainder of the growing season.

Where overhead irrigation is utilized in Euonymus production, irrigation should be limited to midday applications to facilitate rapid drying of foliage. Propagation techniques for E. fortunei accentuate the spread of anthracnose throughout the nursery. Cuttings taken from infected mother plants result in infected propagation material. A subsequent increase in Colletotrichum gloeosporioides inoculum occurs throughout the nursery as the infected young plants are lined out or placed in container areas.

To reduce the incidence of infected cuttings, and to reduce the Colletotrichum gloeosporioides inoculum load, infected leaves and shoots should be removed from plants throughout the growing season, as well as during winter. Special attention should be paid to keeping mother plants disease free.

A combination control program consisting of sanitation measures and protective fungicide applications should considerably reduce the incidence of Euonymus anthracnose in commercial nurseries.

APPENDIX 1

Table 3. Meterologic conditions during 1978 Colletotrichum gloeosporioides infection periods.

Meterologic factors	Infection period				
	5/27-6/1	6/11-6/16	7/15-7/20	7/26-7/31	8/6-8/11
Average day temperature	24.5	18.5	24.0	23.0	23.0
Average night temperature	21.5	13.0	21.5	19.0	22.5
Precipitation (cm)	.05	.64	.71	1.52	8.25
Relative humidity ≧90%	36 hr.	24 hr.	36 hr.	24 hr.	24 hr.

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