Influence of Environment and Inoculum Density on Penetration and Colonization of Sycamore Leaves by *Apiognomonia veneta*

VERNON AMMON, Associate Professor, Department of Plant Pathology and Weed Science; BENSON GRAVES, Senior Entomologist, Division of Plant Industry; and DOT GRIFFIN, Postdoctoral Assistant Professor, Mississippi State University, Mississippi State 39762

ABSTRACT

Ammon, V., Graves, B., and Griffin, D. 1990. Influence of environment and inoculum density on penetration and colonization of sycamore leaves by *Apiognomonia veneta*. Plant Dis. 74:989-991.

Leaves of sycamore (*Platanus occidentalis*) seedlings were inoculated with conidial suspensions of *Apiognomonia veneta* to identify optimum temperature, postinoculation period of free moisture, and inoculum concentration required for penetration and colonization. Penetration was consistently high in artificially inoculated seedlings incubated at 100% relative humidity for 16 or more hours at 22–23 C. Inoculum containing 10^5 conidia per milliliter was optimum for seedling infection.

American sycamore (*Plantanus occidentalis* L.) will help to satisfy a projected 114% increase in demand for hardwood products by the year 2030 (4) if selected constraints are overcome. A major limiting factor is the lack of information regarding resistance to anthracnose, the most widespread and potentially most damaging disease of sycamore. Anthracnose is caused by the ascomycete *Apiognomonia veneta* (Sacc. & Speg.) Höhn. (= *Discula platani* (Peck) Sacc. [3]) and occurs in North and South America, Europe, Asia, and Australia. It is endemic throughout the range of sycamore, and periodic disease outbreaks have been reported in Illinois (1) and Mississippi (7). Growth loss and reduced wood quality occur in heavily infected trees (2). The amount of damage, characterized in order of appearance as canker formation (including bud and twig blight), shoot blight, and leaf blight, is determined primarily by the incidence of foliar infection. *A. veneta* directly penetrates leaf surfaces and colonizes subcuticular tissues where it frequently becomes latent (9). The fungus grows from colonized leaves into twigs. Growth of *A. veneta* continues in fall and winter unless limited by cold temperature. Colonization of twigs during the host's dormant period causes a necrosis of cambial and bark tissues, buds, and twigs, and induces canker development. Shoot blight is initiated when *A. veneta* grows from twig cankers into healthy shoots emerging from buds (5). Because an anthracnose epidemic is most likely to occur in intensively managed plantations, a better understanding of environmental factors that influence initial stages of disease development is needed.

Characterization of environmental conditions that favor infection by *A. veneta* could identify silvicultural practices to reduce disease losses and assist in the identification and development of genetically improved, disease-resistant sycamore selections. The objectives of this study were to determine the effects of inoculum density, temperature, and leaf wetness duration on penetration and colonization of sycamore foliage by *A. veneta*.

Published with the approval of the director of the Mississippi Agricultural and Forest Experiment Station.

Accepted for publication 4 June 1990 (submitted for electronic processing).

MATERIALS AND METHODS

Plant culture. Sycamore seed from three mid-South sources was selected. Seeds were sown on the surface of a growth medium of 2:1 (v:v) peat:sand, lightly covered with the same mixture, and misted with water at 5-min intervals for 7 days under greenhouse conditions. Two weeks after emergence, individual seedlings were transplanted to peat pellets and grown an additional 42 days. Plants were watered daily. Sycamore seedling production was similar for all experiments.

Inoculum production. *A. veneta* was obtained from a natural infection of sycamore at Stoneville, Mississippi. Stock cultures were maintained on modified sycamore agar (40 g of finely ground sycamore leaves, 5 g of $CaCo_3$, 300 ml of V-8 juice, 15 g of agar, and 640 ml of distilled water [9]). Inoculum was produced by transferring mycelial plugs from stock cultures to fresh modified sycamore agar in plastic petri dishes. Cultures were incubated 14 days on a 12-hr diurnal light period at 24 C. Distilled water was added to each petri dish and conidia were dislodged with a sterile transfer loop. Conidial suspensions were combined in a beaker and separated from mycelial fragments by filtering through Whatman No. 4 filter paper under vacuum. Conidial concentration was adjusted to the desired density with a hemacytometer.

Influence of temperature on spore germination. A suspension of *A. veneta* spores prepared as previously described was adjusted to 5×10^4 spores per milliliter. Six individual drops of spore suspension were placed on clean slides with a 1-ml pipette. The slides were then placed in moist chambers and incubated at 8, 16, 24, 32, or 36 C. Spore germination (germ tube length = 1.5 × spore length) was determined by counting 100 spores in each of six replications after 4, 16, and 24 hr of incubation. The experiment was conducted as a completely randomized design, repeated, and the data were pooled. Analysis of variance was conducted and means were separated by Duncan's new multiple range test.

Controlled environment studies. Two experiments were conducted in controlled environment chambers (Percival Model E-540, Percival Refrigeration and Manufacturing Co., Inc., Des Moines, IA) containing cool-white fluorescent and incandescent lamps. Day length was based on a light period of 12 hr per day. Air temperatures were maintained at ± 1 C.

Unless stated otherwise, both experiments were conducted with the following general experimental conditions. Experimental units consisting of 24 8-wk-old seedlings in plastic boxes containing moistened paper towels were acclimated to the temperature(s) being tested for 4 hr before inoculation. In each experiment, seedlings were inoculated with an aerosol-propelled atomizer to apply 48 ml of spore suspension per experimental unit. Boxes were closed and placed in growth chambers for 48 hr. During this period, lids were removed at appropriate times. Following incubation in the growth chamber, experimental units were moved to a greenhouse where they remained for two additional weeks. Colonization of sycamore leaves was established by isolating *A. veneta* from inoculated plants. Ten 0.5-cm leaf disks were removed from each plant. Epiphytic

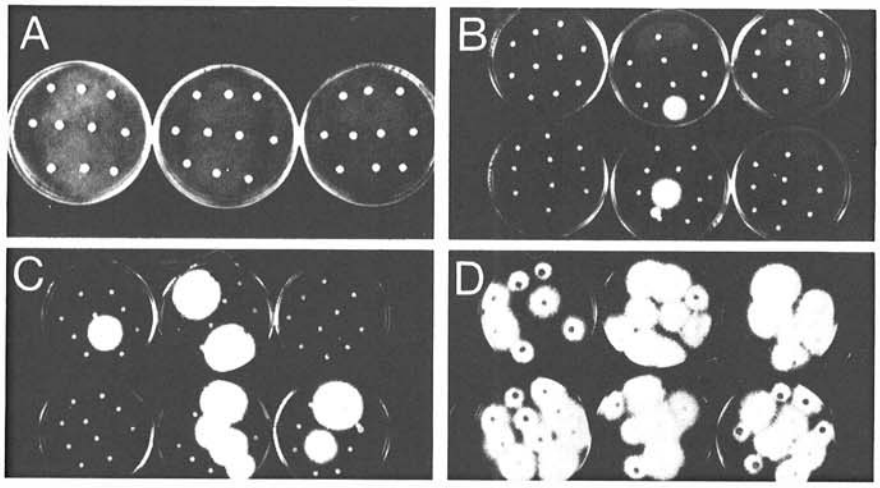

Fig. 1. Leaf disk assay used to determine endophytic growth of *Apiognomonia veneta* from sycamore leaf tissue after 10 days. **(A)** Leaf disks removed from uninoculated seedlings. **(B)** Growth of *A. veneta* from leaf disks removed from inoculated sycamore seedlings that had been surface-disinfested and incubated at 22 C and continuous wetness for 0.25 hr, **(C)** 4 hr, and **(D)** 16 hr.

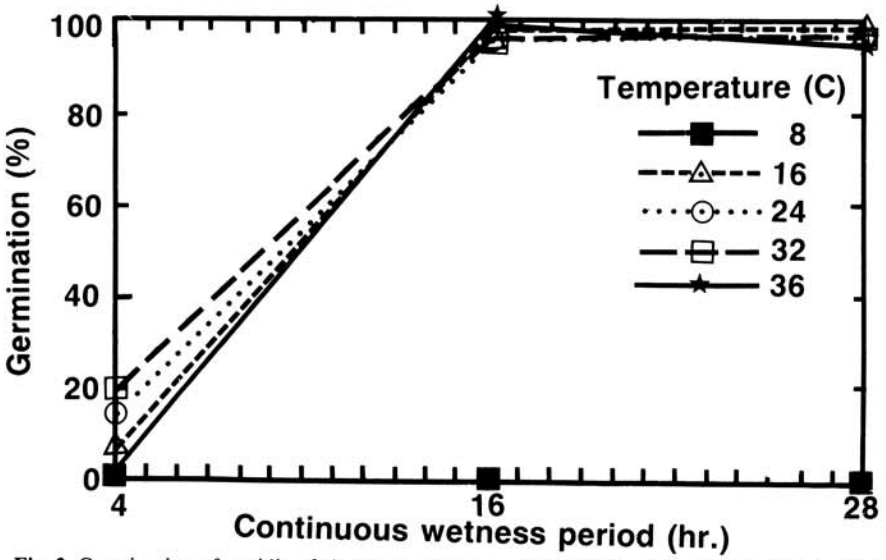

Fig. 2. Germination of conidia of *Apiognomonia veneta* incubated at five different temperatures and three different periods of continuous wetness. No germination occurred at 8 C regardless of inoculation period.

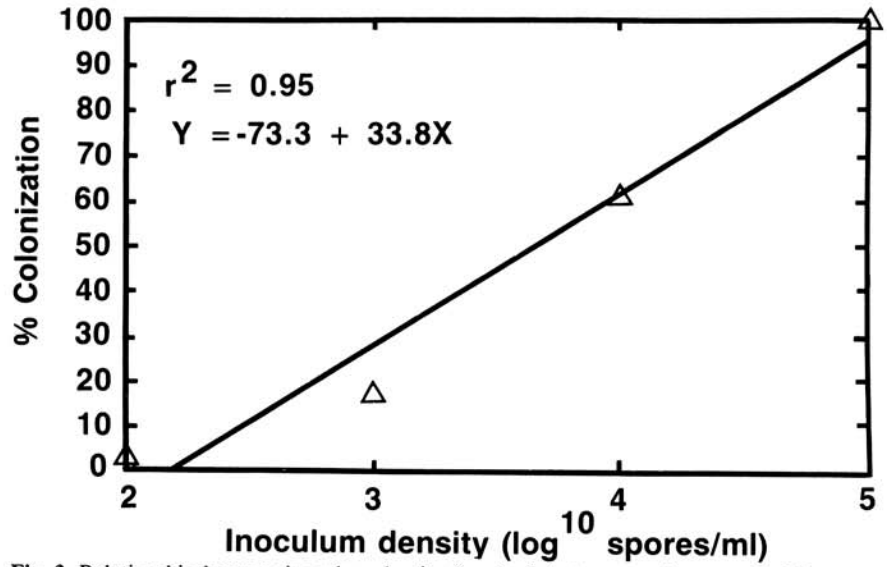

Fig. 3. Relationship between inoculum density ($\log_{10}$) of *Apiognomonia veneta* and frequency of colonization on 8-wk-old sycamore seedlings after 28 hr of continuous leaf wetness at 22 C.

growth of *A. veneta* was differentiated from endophytic growth by disinfesting leaf pieces for 20 sec in 70% ethyl alcohol and for 1 min in 1% NaOCl followed by three successive rinses in sterile, distilled water. Leaf disks were placed in petri dishes containing modified sycamore agar and incubated for 10 days at 22 C. A plant was considered colonized if *A. veneta* grew from at least one leaf disk (Fig. 1).

Influence of inoculum density on colonization. Groups of 24 plants were randomly selected and inoculated with a spore suspension adjusted to 10^2, 10^3, 10^4, and 10^5 spores per milliliter. Plants were incubated in growth chambers as previously described at 22 C and continuous leaf wetness was maintained for 28 hr. The presence of *A. veneta* was determined as described earlier. Treatments were replicated in a randomized complete block design. The experiment was repeated and the data were combined for analysis. Linear regressions were computed with transformed (percent infection:arcsine square root; inoculum density:$\log_{10}$) or nontransformed data.

Influence of temperature and continuous leaf wetness on colonization. Groups of 24 plants were inoculated with 10^5 spores per millileter and placed in growth chambers adjusted to 11, 16, 22, 27, 33, or 38 C. Covers were removed from randomly selected groups of plants after 0.25, 4, 16, and 28 hr of continuous wetness. Plants were incubated and evaluated as previously described. The experiment was conducted as a completely randomized design, repeated, and the data were combined for analysis. Data were analyzed using analysis of variance and means were separated by Fisher's protected LSD where appropriate.

RESULTS

Influence of temperature on spore germination. *A. veneta* conidia failed to germinate at 8 C (Fig. 2). Germination was poor, ranging from 2% to 19% at 4 hr of incubation for all temperature treatments. Germination approached 100%, however, at temperatures from 16 C to 36 C when conidia were incubated at continuous wetness for at least 16 hr (Fig. 2).

Influence of inoculum density on colonization. Colonization increased linearly when 10^2–10^5 conidia per milliliter were applied to 8-wk-old sycamore seedlings and maintained under conditions of continuous leaf wetness for 28 hr at 22 C (Fig. 3). The linear regression with nontransformed colonization and transformed ($\log_{10}$) inoculum density data was significant ($P = 0.05$; $r^2 = 0.95$ [Fig. 3]).

Influence of temperature and continuous leaf wetness on colonization. Endophytic growth of *A. veneta* in inoculated sycamore seedlings was difficult to detect at 11 C regardless of

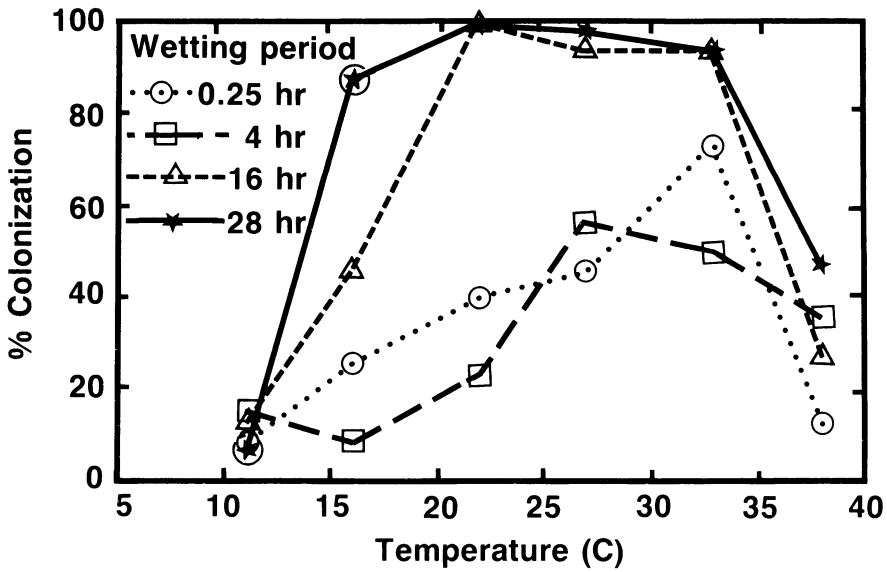

Fig. 4. Anthracnose incidence as a function of temperature and wetness duration. Sycamore seedlings were inoculated with a suspension containing 10^5 conidia per millileter of *Apiognomonia veneta*, incubated in moist chambers at the temperatures indicated, and removed after the desired wetting period had elapsed. All seedlings were subsequently incubated in a common greenhouse maintained at 22 C.

duration of leaf wetness (Fig. 4). Colonization of tissues by *A. veneta* increased significantly when inoculated seedlings were maintained in postinoculation environments of increasing temperature and lengthened periods of continuous wetness (Figs. 1B,C,D, and 4). The fungus could be recovered from over 90% of the leaf disks from those inoculated seedlings that had been incubated at temperatures ranging from 16 C to 33 C in growth chambers where continuous leaf wetness was maintained for periods from 6 hr to 28 hr in duration.

DISCUSSION

Conidia represent the most important form of primary inoculum produced by *A. veneta* (9,11). The significance to disease incidence and severity is conditioned by a number of environmental constraints (6,8), as well as the amount of inoculum produced. Data presented here demonstrate the linear relationship between inoculum density and anthracnose incidence. An inoculum density of 10^5 on seedling leaves whose surfaces are wet and likely to remain so for several hours and where the ambient temperature is approximately 22 C will result in disease incidence approaching 100% in susceptible genotypes. These data also provide evidence that disease incidence can be moderated even under favorable environmental conditions if the resource manager or homeowner employs management tactics designed to reduce inoculum levels.

Previous investigations (9) demonstrated that conidia germinate on wet leaves within 6 hr and that conidial germination approaches 100% if the duration of leaf wetness is maintained for at least 16 hr at temperatures ranging from 16 C to 36 C. These conditions are satisfied frequently throughout the natural range of sycamore.

Direct penetration of the cuticle by infection pegs emanating from appressoria can occur as soon as 6 hr after inoculation if some rather broad leaf surface temperature and moisture requirements are satisfied. Subsequent host reactions, however, are not fully understood. Earlier studies (9) suggest that there may be subtle differences between environmental requirements for penetration of leaf surfaces and maintenance of endophytic growth of *A. veneta* and the requirements for expression of the characteristic leaf blight phase of this disease. Scanning and transmission electron micrographs of inoculated sycamore leaves incubated at room temperature provide strong corroborative evidence of the ability of *A. veneta* to directly penetrate the cuticular layer and grow inter- and intracellularly without accompanying visual symptoms (9). Similar instances of latent infection with anthracnose fungi were reported by Simmonds (10), who demonstrated that *Gloeosporium* and *Colletotrichum* can persist just below the cuticle (subcuticular hyphae) or in cell walls of outer cell layers in some species of tropical fruits without causing visible symptoms until the fruit ripens.

Observations of latent infection lend credibility to the possibility that many apparently healthy sycamore leaves are colonized by *A. veneta* long before blighting occurs. Leaf-disk assays of apparently healthy sycamore leaves could provide information about the prevalence of latent infection in natural stands of sycamore. A more thorough understanding of the significance of latent infections characteristic of this organism is suggested, especially before large-scale screenings for host resistance are conducted.

The incidence of recovery of *A. veneta* measured in these investigations, where inoculum density was held constant at 10^5 spores per millileter, goes beyond substantiating that sycamore anthracnose occurs frequently under cool (16 C), moist conditions (free water on leaf surfaces for 28 hr [5,12]). It shows that severe epidemics could occur at temperatures approaching 30 C if free water could be maintained on leaf surfaces for 16 to 28 hr. This information could be useful in reducing disease incidence in sycamore plantations growing in the South. More attention to tree spacing and thinning operations to improve air circulation may prove to be a relatively inexpensive, environmentally sound disease management tactic.

LITERATURE CITED

1. Boewe, G. H., Campana, R. J., and Schneider, I. R. 1954. Sycamore anthracnose severe in Illinois. Plant Dis. Rep. 38:597-598.
2. Briscoe, C. B. 1969. Establishment and early care of sycamore plantations. USDA For. Serv. Res. Pap. SO-50. 18 pp.
3. Farr, D. F., Bills, G. F., Chamuris, G. P., and Rossman, A. Y. 1989. Fungi on Plants and Plant Products in the United States. American Phytopathological Society Press, St. Paul, MN. 1252 pp.
4. Forest Service, 1984. Your nation's timber: Problems and opportunities. U. S. Dep. Agric. For. Serv. Misc. Publ. 1440. 23 pp.
5. Neely, D., and Himelick, E. B. 1963. Temperature and sycamore anthracnose severity. Plant Dis. Rep. 47:171-175.
6. Neely, D., and Himelick, E. B. 1967. Characteristics and nomenclature of the oak anthracnose fungus. Phytopathology 57:1230-1236.
7. McCracken, F. I., and Filer, T. H. 1971. Sycamore anthracnose epidemic in Mississippi-Yazoo delta. Plant Dis. Rep. 55:93-94.
8. Milne, K. L., and Hudson, H. J. 1987. Artificial infection of leaves of the London plane by ascospores and conidia of *Apiognomonia veneta*. Trans. Br. Mycol. Soc. 88:399-401.
9. Seifers, D., and Ammon, V. 1980. Mode of penetration of sycamore leaves by *Gloeosporium platani*. Phytopathology 70:1050-1055.
10. Simmonds, J. H. 1941. Latent infection in tropical fruits discussed in relation to the part played by species of *Gloeosporium* and *Colletotrichum*. Proc. R. Soc. Queensl. 52:92-102.
11. Sinclair, W. A., Lyon, H. H., and Johnson, W. T. 1987. Diseases of Trees and Shrubs. Comstock Publishing Associates, Cornell University Press, Ithaca, NY. 574 pp.
12. Yarwood, C. E. 1951. Defoliation by a rain-favored, a dew-favored, and a shade-favored disease. Phytopathology 41:194-195.