Effects of Temperature, Free Moisture, and Relative Humidity on the Occurrence of Walnut Anthracnose

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ABSTRACT

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Black walnut seedlings grown in a greenhouse were artifically inoculated by brushing an aqueous suspension of *Marssonina juglandis* conidia (perfect stage: *Gnomonia leptostyla*) onto the foliage. Little infection occurred at 27 C, none at 32 C, and symptom appearance was delayed at 15 and 10 C. At the optimum temperature (approximately 21 C) more than 6 hr with free moisture on the leaflets were required for the development of significant amounts of infection. Longer wetting periods were required at lower temperatures. No infection occurred at relative humidity levels below 98% (±2%). Conidia survived for 2 wk on the leaflet surface, infecting the seedlings when moisture became available.

Additional key word: epidemiology.

Anthracnose is the most serious foliar disease of the black walnut. The causal agent, *Gnomonia leptostyla* (Fr.) Ces. & de Not. [imperfect stage: *Marssonina juglandis* (Lib.) Magn.], attacks fruit and foliage, resulting in poorly filled nuts and premature defoliation. On the leaflets, irregular necrotic areas are formed, usually ⩽5 mm in diameter, surrounded by small chlorotic halos. Small (⩽2 mm), black, necrotic flecks form on the fruit. The pathogen overwinters primarily on foliar debris, and ascospores are the primary inoculum in the spring (1, 5). The conidia serve to intensify the disease during the summer (1, 5).

Little research has been done on the etiology of walnut anthracnose. Klebahn (5) reported that perithecia were formed only in infected leaves during the winter. Berry (1) and other workers (3, 4) noted the importance of precipitation for dispersal of the ascospores and conidia. Gard (3) noted that a relative humidity (RH) at or above 70% appeared to increase the possibility of infection.

Our studies were initiated to achieve a better understanding of environmental influence on disease severity. This paper reports the effects of temperature, free moisture, and RH on the amount of infection under controlled conditions.

MATERIALS AND METHODS

Juglans nigra L. seedlings were grown singly in 2-liter metal cans containing a 3:1 (v/v) mixture of peat:sand. Lime (5.14 kg), superphosphate (2.57 kg), $MgSO_4$ (0.43 kg), and a 0.05 kg mixture of micronutrients (9% Mn, 6% Fe, 3% Cu, 3% Zn, 2% B) were added per cubic meter of root medium.

The inoculation technique used was a modification of techniques employed by Berry (1) and Klebahn (5). At the time of inoculation all seedlings ranged in age from 2 to 6 mo. Care was taken to employ similar-aged seedlings within each test. Data were recorded only from leaflets that had fully expanded at the time of inoculation. An aqueous suspension of conidia was standardized to 5.0×10^4 conidia/ml with a hemacytometer. One ml of suspension was brushed onto both surfaces of each compound leaf. Each seedling was covered with a plastic bag that was gathered and fastened around the stem beneath the lowest inoculated leaf. Noninoculated controls were brushed with tap water and bagged. Bags were removed after varying times according to the experiment.

Temperature.—The effect of temperature on infection and disease development was investigated in controlled environment chambers at 5, 10, 15, 21, 27, and 32 C, a 16-hr daily photoperiod, and an average irradiance of 13 w/m^2. Six plants were placed in a chamber 48 hr prior to inoculation. Five plants were inoculated and one served as a bagged but noninoculated control. Two wk after inoculation, the percentage of infected leaflets was recorded. All plants then were subjected to two additional weeks at 21 C to assay for latent infection.

Free moisture.—Preliminary work revealed that free moisture was necessary for infection. The interaction of temperature and duration of wetting was studied.

Under greenhouse conditions [18-30 (mean 21) C], 35 seedlings were inoculated and bagged. Bags were removed at random from five trees after 0, 3, 6, 12, 24, 48, and 72 hr. Controls were five trees not inoculated or bagged and five bagged (72 hr) but noninoculated trees. The plant leaves were dry within 5 min after uncovering.

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The percentage of leaflets infected was recorded after 14 days.

The duration of wetting required for infection at 32, 21, and 10 C was determined in controlled environment chambers set for 16-hr daily photoperiods. Twenty seedlings were inoculated and bagged, and then were incubated at each temperature. After 6, 12, 24, and 48 hr, five plants were taken from each chamber, bags were removed, and the leaves were air dried. The plants then were placed in the greenhouse (17-20 C), and the percentage of leaflets infected was recorded after 2 wk. Inoculated, nonbagged controls, as well as noninoculated controls were employed in each regime.

The ability of *G. leptostyla* conidia to survive periods without free moisture on the leaflet was investigated. Forty-eight seedlings were inoculated, allowed to remain dry for different periods of time, and then misted with an atomizer and bagged. Four inoculated and immediately-bagged trees, and four noninoculated trees, served as controls. The length of time between inoculation and rewetting was 1, 3, 6, 9, 12, 16, 20, or 24 hr and 2, 4, 8, or 14 days. Percentage of infection was recorded 2 wk after bagging. The test was conducted under greenhouse conditions (17-20 C).

Relative humidity.—A humidity cabinet (6) within a controlled-environment chamber was used for RH treatments of 100, 98, 95, 80, or 50% (±2%). Temperature was held at 27±2 C during the 16-hr daily photoperiod, 21 ±2 C during darkness.

Twelve trees were placed in the chamber 24 hr prior to inoculation for each of the five RH treatments. From 81 to 39 leaflets were inoculated on each of 10 trees, but only the 100% RH and the noninoculated controls were bagged. Visual observations were made of the free moisture status on the leaves after 15 min. The percentage of inoculated leaflets with one or more anthracnose lesions was recorded after 2 wk.

RESULTS

Temperature.—Incubation at 21 C resulted in the most infection, with 90% of the inoculated leaflets having lesions after 2 wk (Fig. 1). At 15 C, significantly fewer lesions were evident. Percentages of leaflets with symptoms 2 wk after inoculation at temperatures of 5, 10, 27, and 32 C were not significantly different from the controls [$P = .05$ by Fisher's least significant difference test (FLSD)].

Incubation of inoculated plants for an additional 2 wk at 21 C resulted in significant increases in disease severity on plants initially incubated at 5, 10, or 15 C; but no change in plants initially incubated at 21 C or higher (Fig. 1).

Free moisture.—When free moisture remained on the leaves between 12 and 72 hr, 24 to 36% of the inoculated leaflets developed one or more anthracnose lesions. This difference was not significant at $P = .01$ (FLSD = 14.33%). When leaflets were air dry within 6 hr or less after inoculation, infection levels were only 0 to 2%.

The temporal influence of free moisture on infection varied with temperature. At 32 C during the postinoculation period, only 1-3% infection occurred regardless of how long free moisture remained on the leaflet surface (Fig. 2). At 21 C, as time with free moisture increased from 6 to 24 hr, the proportion of leaves with symptoms increased from 3 to 90%. No difference was noted between trees bagged longer than 24 hr at this temperature. At a postinoculation temperature of 10 C, significant differences were noted between all time periods. As time with free moisture increased by 6, 18, and 42 hr, the proportion of leaflets that became infected increased by 12, 52, and 81%, respectively.

Although free moisture was required for infection, the conidia appeared capable of surviving at least 2 wk on the leaflet surfaces in the absence of free moisture. Dry periods of up to 6 hr following inoculation did not inhibit

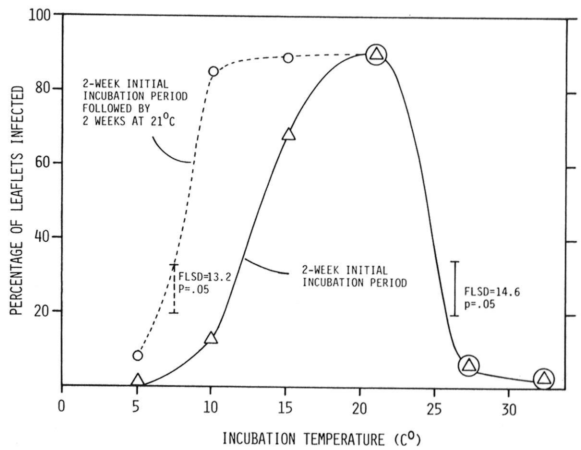

Fig. 1. Influence of the incubation temperature during 2 wk following inoculation, and an additional 2 wk of incubation at 21 C, on incidence of infection in walnut leaflets following artificial inoculation with *Gnomonia leptostyla*. Each point on the graph represents the mean percent infection for five replications.

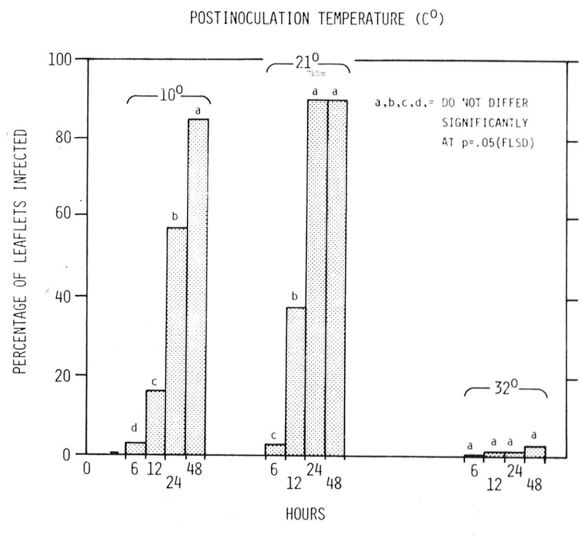

Fig. 2. Influence of the length of time free moisture remained on leaflet surfaces at three postinoculation temperatures on incidence of infection of walnut leaflets following artificial inoculation with *Gnomonia leptostyla*. Each bar on the graph represents the mean percent infection for five replications.

infection significantly (Fig. 3). After a dry period of 9 hr, infection was inhibited by 20% when compared to continuous moisture. Dry periods of over 9 hr resulted in even greater inhibition of infection. However, after periods of up to 2 wk without visible surface moisture, 8% of the inoculated leaflets became infected following rewetting. Statistical analysis of these data revealed a significant curvilinear response, represented by the equation $Y = b_1x + b_2x^2$ in which x = hours, Y = % infection, $b_1 = 1.6$, and $b_2 = -0.05$.

Relative humidity.—The anthracnose pathogen was capable of infecting susceptible tissue only within a narrow range of high RH. At 100% RH, 83% of the inoculated leaflets were infected, but only 2% were infected at 98% RH. Infection did not occur at or below 95% RH. At 15 min after inoculation, only those plants maintained at 98 or 100% RH had free moisture remaining on the leaflet surface.

DISCUSSION

Previous workers established that precipitation is required to release and disperse ascospores and conidia of the walnut anthracnose agent (1, 3, 4). Our work revealed that precipitation also supplies the free moisture required for infection. Under optimum temperature conditions, 6 hr or more of available free moisture are required for significant levels of infection. Should free moisture not be immediately available, the conidia survive dry periods of up to 6 hr without loss of infective potential, and infective potential remains detectable even after 14 days. Our tests were run in a greenhouse where attenuation of UV radiation may have enhanced conidial survival. Such data indicate that frequent periods of precipitation may not be required for an increase in disease incidence. Spores liberated by a brief shower during the day may survive until evening dew supplies the free moisture required for infection.

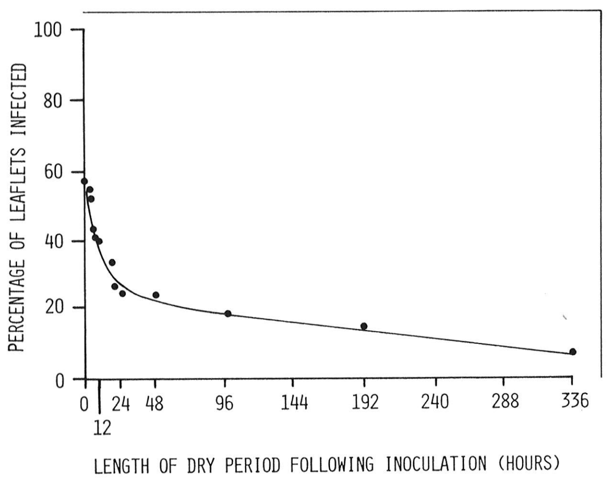

Fig. 3. Influence of length of dry period on incidence of infection in walnut leaflets artificially inoculated with *Gnomonia leptostyla*. Data were recorded after 14 days. The experiment was conducted in the greenhouse.

Based upon empirical observations made during the 1920's, it had been postulated that RH ⩾70% enhanced walnut anthracnose severity (3). Our work revealed that a much narrower range of RH, from 98-100% (±2%), is required for infection to occur. High RH promotes infection primarily through the prolongation of the period of time that free moisture remains upon the leaflet surface.

Temperature has a dramatic effect on the development of walnut anthracnose. Temperatures ranging from 15 to 21 C supported high levels of infection, and 21 C appeared optimum. These data agree with Fayret's (2) findings that 20-21 C is optimum for conidum production and growth of the pathogen. Cooler temperatures resulted in a delay of symptom expression, and higher temperatures greatly inhibited infection.

Meteorological conditions which result in high humidities, moderate temperatures, and adequate free moisture will enhance the possibility of infection by *G. leptostyla*. In areas with high incidence of anthracnose, the use of silvicultural practices which abate such conditions within the canopy is advised, although the effect of the following practices on nut yield is unknown. Adequate spacing between trees and pruning to an open center would allow more air movement within the canopy, reducing relative humidity and allowing for rapid evaporation of free moisture from the leaflet surfaces. Pruning also would favor penetration of sunlight, resulting in elevated temperatures on the leaflet surface. Planting on slopes, especially eastern or southern hillsides, would minimize dew formation, promote rapid removal of any condensed moisture, and elevate canopy temperatures. Silvicultural practices appear feasible and would minimize the need for chemical control to reduce the severity of walnut anthracnose.

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