Seasonal Infection of Nonwounded Peach Bark by *Botryosphaeria dothidea*

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ABSTRACT

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Conidial suspensions of *Botryosphearia dothidea* were applied at various concentrations to the nonwounded stems of 1-yr-old peach trees maintained under wet conditions for periods that varied. Bark necrosis increased with spore concentration and the duration of bark wetness. Similar inoculations made at 3-wk intervals during 1987 and 1989 revealed that infection occurred from March through August with a peak period from about late April through July or early August. A significant correlation was found between disease severity and temperature for a 3- to 6-wk period after inoculation. In two commercial orchards, stems of newly planted peach trees were periodically exposed to natural inoculum of *B. dothidea*. At 1-mo intervals from April 1988 through December 1989, water-excluding covers were removed from preselected trees for a 1-mo period. Infections occurred more frequently in the second season of growth (particularly during June and July) than in the season after planting. Disease severity was positively correlated with the availability of waterborne spores of *B. dothidea*. Nonwounded bark of 1- to 2-yr-old peach trees is susceptible to invasion by *B. dothidea* during most of, if not the entire, growing season. The amount of infection during this period depends on inoculum availability and environmental conditions.

Botryosphaeria dothidea (Moug.:Fr.) Ces. & De Not. invades nonwounded bark of peach (*Prunus persica* (L.) Batsch) and causes localized infections associated with lenticels (1,2,4,9–11,14,15). On young stems and branches, blisters may form due to hyperplasia of peridermal and cortical cells surrounding diseased tissue near the lenticel opening (1; P. L. Pusey, *unpublished*). On older branches and trunks, sunken necrotic lesions, which are often accompanied by copious gum exudation, appear at multiple sites. Expansion and coalescence of lesions can eventually girdle and kill major branches or the entire tree. This disease occurs in peach production areas of the southeastern United States (12,13) where it is referred to as peach tree fungal gummosis. It is a problem also in Japan (2), China (4), and Australia (J. Slack, *personal communication*). The causal agent is a physiologic race of *B. dothidea* that is specific to peach (2,6,11,12).

Sunken lesions and gum exudation are generally first noticed on young peach trees during the third season after planting. The time of infection leading to the development of these symptoms in the southeastern United States is unclear. Waterborne conidia of *B. dothidea* are potentially available most of the year (9), but conditions required for invasion of nonwounded bark by this fungus have not been elucidated. According to Weaver (15), who exposed peach branches to infected woody tissues at monthly intervals from March through July, inoculations in July resulted in more infections than in other months.

Reliable data relating to the conditions and time of infection by *B. dothidea* are needed to develop a practical management strategy that includes sanitary measures and proper timing of protective agents. Therefore, the following study was conducted.

MATERIALS AND METHODS

Inoculum level and period of wetness. To study the effects of fungal spore concentration and duration of bark wetness on disease severity, inoculations were performed with 1-yr-old budded peach trees (cv. Summergold). Trees were planted in 23-cm-diameter pots filled with peat:vermiculite:soil (1:1:1, v/v) and maintained in a greenhouse beginning in January 1987. Inoculation was made on 12 May 1987 with a virulent isolate of *B. dothidea* that was collected in 1981 from a peach tree in Dooley County, GA (10,12,13). The fungus was stored on silica gel at −20 C (8). Conidia were produced in culture on Difco oatmeal agar that was subjected to diurnal light of 15 h for 19–23 days at 25 C. Aqueous suspensions of 10^4, 10^5, or 10^6 conidia per milliliter were applied with a soft brush to a 20-cm section of each tree stem above the bud union. To maintain surface wetness on trees held in the greenhouse, the stems were wrapped with three layers of moist cheesecloth and then one layer of Parafilm. Wrappings were removed after 24, 48, 72, or 144 h. Each spore concentration and period of wetness was replicated four times. Stems of control trees were brushed with water and wrapped for 144 h.

Trees were checked periodically for disease development. In October of the following season, necrotic lesions and gum exudation sites were counted and numbers per unit area of bark were calculated. Lesion diameters were measured and total percentage of inoculated bark area showing necrosis was determined. In cases of extensive necrosis, outer bark was removed and the percentage of necrosis was estimated visually.

Periodic artificial inoculation. To establish when nonwounded bark of peach trees can be infected by *B. dothidea*, given that spore inoculum is present, field-grown trees were inoculated at various times during the year. In January 1987 and 1989, 1-yr-old budded peach trees (cv. Majestic) were planted at a spacing of 1.2 m in rows 6 m apart. Inoculum was prepared as in the preceding experiment. Suspensions of 10^5 conidia per milliliter were brushed onto a 20-cm section of each stem and covered with moist cheesecloth and Parafilm to maintain wetness for 7 days. At 3-wk intervals from 28 January to 23 December 1987 and from 24 February to 20 December 1989, six trees of the current-year planting were inoculated. Trees were evaluated as described above. Total percentage of bark necrosis was determined 1 or 2 yr after the year of inoculation, in October.

Natural inoculation. To determine when nonwounded bark of newly planted peach trees becomes naturally infected by *B. dothidea*, the stems of trees were exposed to natural inoculum at various times during the first 2 yr after planting. Two commercial orchards (cv. Summergold) planted in January or February 1988 were selected for the study. Plantings referred to as Lane and Evans orchards were near Fort Valley, GA, and were surrounded by older trees with severe symptoms of peach tree gummosis. An 18-cm-long cover made of two halves of 7.6-cm-diameter polyvinyl chloride plastic pipe cut longitudinally and then reconnected with duct tape was attached to the stem of each tree. (The pipe was changed to 12.5 cm in diameter the second year to accommodate growth.) A strip of polyurethane foam rubber, 2.5 cm thick and slightly wider than the gap between the tree and the pipe, was placed around the tree at the top and bottom of the cover to hold the cover in place and to allow the tree to expand. The cover was further secured with a wooden stake placed in the ground and taped to the cover. Acrylic caulking with silicone was used to seal the top to keep waterborne fungal spores from the covered area. At 1-mo intervals from April 1988 through December 1989, the covers on six trees in each orchard were removed for 1 mo. In January 1990, all covers were removed.

Release of waterborne conidia of *B. dothidea* was monitored in 1988 and 1989 by placing a trap below each of two mature trees in adjacent orchards where inoculum was detectable. These trees exhibited severe gummosis symptoms and were located 12–18 m to the north or east of the young trees under study. Spores were trapped as described previously (3,9) with some modification. A 15-cm-diameter funnel was attached with wire to a scaffold limb, and acrylic-silicone caulking was used to channel water into the funnel. The funnel was connected by a polyurethane tube to a 3.8-L bottle that was changed whenever it rained. Ten milliliters of a 5.0% copper sulfate solution was added to each clean bottle to prevent spore germination. Conidia were counted after filtering a 10-ml sample through a 25-mm-diameter gridded filter (1.2 μm pore size). The membrane was mounted in acid-fuchsin-lactophenol containing 1% polyvinyl alcohol and then examined at 320× magnification. For each orchard site, the total numbers of spores collected in two traps were combined.

Trees in commercial plantings were evaluated the year after removal of all plastic covers. A disease severity index was calculated by dividing the number of gum exudation sites on the covered bark by the number of gumming sites on an equivalent noncovered area immediately above the covered area.

Environmental monitoring. Weather information from the U.S. Weather Bureau substation at Byron, GA, was collected approximately 1.0 km from artificially inoculated trees, 14.5 km from the Lane orchard, and 16.9 km from the Evans orchard. Temperature was recorded with a 7-day hygrothermograph, and rainfall was measured with a 7-day universal rain gauge. Vegetative wetness was recorded on a strip chart recorder connected to a series of five wetness sensors (5).

In all experiments, trees were in a completely randomized design. Effects of artificially varying the inoculum level and the period of wetness were evaluated using linear regression analysis. Disease data from other experiments were analyzed with an analysis of variance. Correlation analyses were used to study the relationship between disease severity and temperature in periodic artificial inoculations and between disease severity and inoculum availability in periodic natural inoculations.

RESULTS

Inoculum level and period of wetness. Seventeen months after inoculation, the percentage of inoculated bark that became necrotic increased with the inoculum level and the duration of wetness (Fig. 1). Regression of percent necrosis on each variable was highly significant ($P < 0.001$). The highest spore concentration (10^6 conidia per milliliter) combined with the longest wetness period (144 h) resulted in the death of all trees inoculated. Noninoculated control trees did not exhibit disease symptoms.

Periodic artificial inoculation. During the period from autumn in 1987 or 1989 (before all periodic inoculations had been made) through spring of the following year, blisters were observed on many inoculated stems. By the end of the second season, symptoms were primarily sunken necrotic lesions. Disease severity was greater ($P \leq 0.05$) in trees inoculated during March through August than in uninoculated controls (Fig. 2). The peak period of infection occurred from about late April through July or early August.

In correlation analyses of disease severity and temperature, expression of the latter as daily maximum, daily minimum, or daily average produced the same results. However, when the temperature covariable was expressed as an average for various periods beginning at inoculation, different results were observed. When daily average temperature was averaged for a 1-wk period after inoculation (during which wetness was maintained), *r* values were only 0.39 to 0.47, depending on how disease severity was measured (number of lesions, number of gumming sites, or percent bark necrosis), and no significant correlation was found ($P \leq 0.05$). In contrast, when daily average temperature was averaged for a 3- or 6-wk period after inoculation, *r* values were 0.54–0.59 (significant at $P \leq 0.05$) or 0.64–0.73 (significant at $P \leq 0.01$), respectively.

Natural inoculum. Infection due to natural inoculum was more evident for peach bark exposed in the second season than in the first season after planting (Fig. 3). Disease severity and inoculum levels were generally higher in the Lane orchard than in the Evans orchard.

In the Lane orchard, disease indexes for June and July, 1989, were significantly higher than mean indexes ($P \leq 0.05$) for all other months except August 1989. Disease indexes for trees exposed during 11 mo of the 21-mo period were greater than that of control trees which were protected from exposure to conidia. Infection appeared to develop in some months not expected on the basis of artificial inoculations (e.g., December 1988, February 1989, and November 1989). Numbers of gumming sites on bark ranged from a mean of 0.8 on control trees to 26.5 on trees exposed in June 1989.

At the Evans orchard, a disease index greater than that for the control was found only for trees exposed to conidia in August 1989. In that month, the mean number of gumming sites was 9.2, the highest of all months, compared with 1.3 for the unexposed control.

Waterborne conidia were detected in most months, with the highest numbers generally occurring during the period from about June through September (Fig. 3). Correlations were made between total monthly spore number and either disease index or number of gumming sites for the period from March through August, since this is when infection can occur on the basis of artificial inoculations. No significant correlation was shown for 1988 ($P \leq 0.05$). When the disease index for 1989 was analyzed as covariable, *r* values for the Lane and Evans orchards were 0.77 ($P = 0.07$) and 0.81 ($P = 0.05$), respectively; when using number

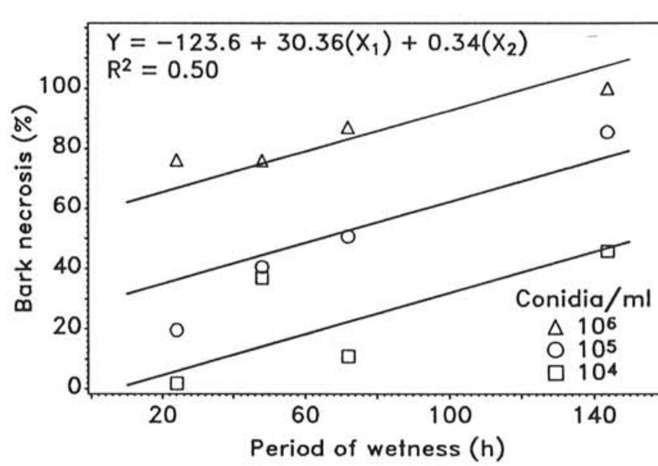

Fig. 1. Effect of inoculum level and period of wetness on percentage of inoculated peach bark that became necrotic 17 mo after nonwounded stems were brush-inoculated with conidia of *Botryosphaeria dothidea*. On the basis of regression analysis, effects of both variables were significantly different from zero ($P \leq 0.001$), but the interaction was nonsignificant ($P = 0.7$). In the linear equation, X_1 = log spore concentration and X_2 = duration of wetness. Each symbol represents the mean of four replicates.

of gumming sites per tree or number of gumming sites per unit area of bark as the covariable, r values for Lane and Evans orchards were 0.93 ($P \leq 0.001$) and 0.61–0.62 ($P \leq 0.2$), respectively. Analysis for the period from February through November, during which differences were detected in the Lane orchard (Fig. 3), gave approximately the same results.

Weather information was helpful only in general application because long distances (14–17 km) between the weather station and commercial orchard sites did not allow for local differences in temperature and rainfall. Disease indexes and spore numbers for 1989 tended to be higher when temperatures were higher, and spore numbers were relatively low when rainfall was low. During May 1989, when a high rate of infection might be expected on the basis of artificial inoculations, spore numbers were low and hours of vegetative wetness were low relative to other months.

DISCUSSION

Our results demonstrate for the first time that the severity of disease incited by *B. dothidea* on nonwounded peach bark increases with spore inoculum level and duration of surface wetness. Thus, it is predictable that disease severity will be greatest when these variables are relatively high, assuming that other factors (e.g., temperature) favor infection.

Periodic artificial inoculations helped to establish the seasonal period when nonwounded bark of young peach trees can be invaded by *B. dothidea*, given that spore inoculum is present and moisture conditions are favorable. Inoculations made from March through August resulted in disease severity values that were greater than those of the uninoculated controls; inoculations made in February or after August did not. Results are consistent with the report by Weaver (15). However, since Weaver exposed trees only from March through July, information was lacking regarding the approximate beginning and ending of the infection period.

The rate of infection due to artificial inoculations was especially high from about late April through July or early August. This appears to correspond to the period when peach trees are growing vigorously in central Georgia. The possible relationship between disease severity and temperature, as indicated by correlations, may involve an effect on the pathogen and/or the host. Germination and germ-tube growth of conidia of *B. dothidea* are favored by warm temperatures (optimum of 25–35 C and 30 C, respectively) (15), as is tree physiology and growth (7).

In orchard tests that relied on natural inoculum, evaluations at the end of the third season after planting revealed that infection was more frequent in the second season than in the first. Results are consistent with the observation that gumming lesions most often appear initially in commercial peach orchards in the third year, when considering that the time required for this symptom to develop after artificial inoculation is usually 12–18 mo (11,

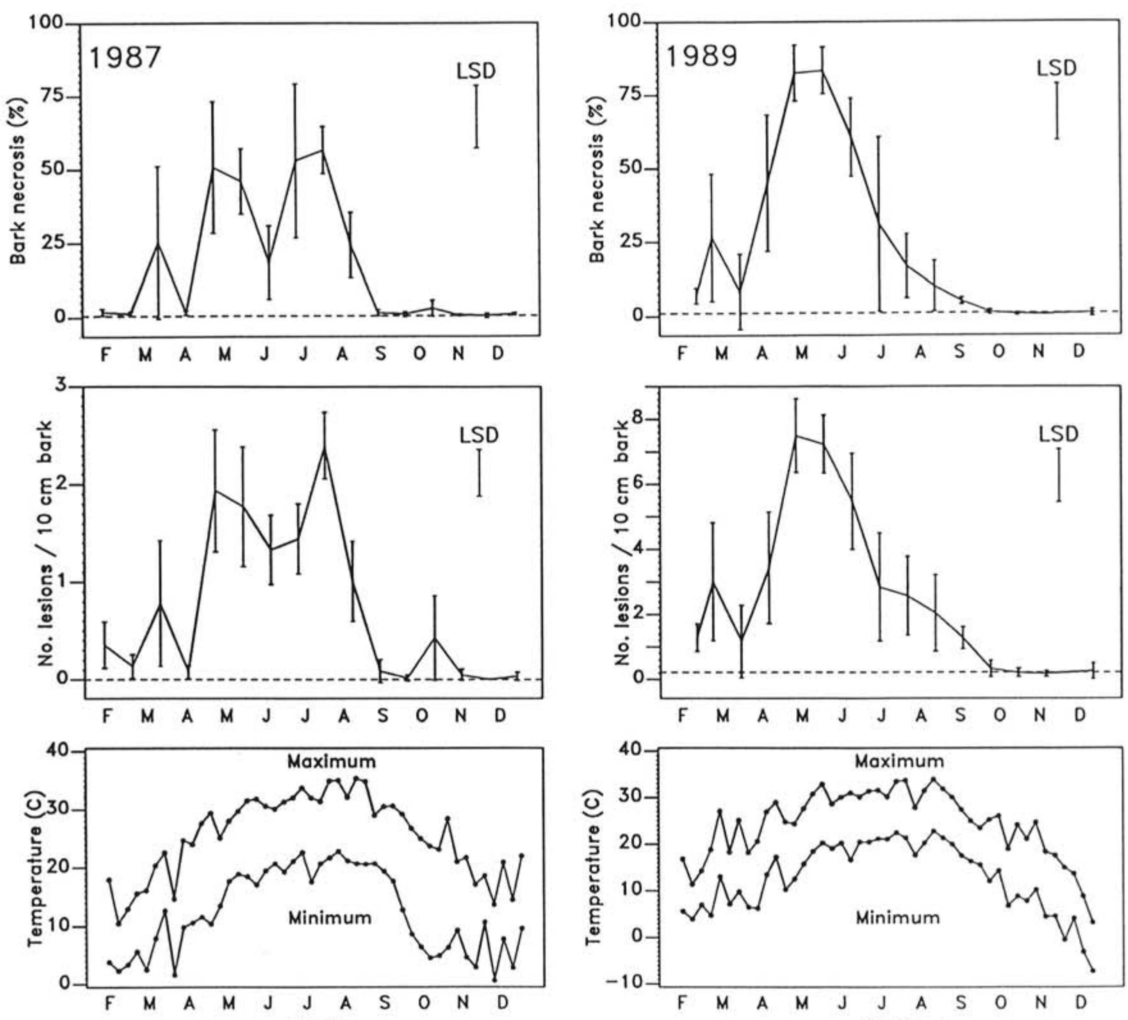

Fig. 2. Disease severity on nonwounded peach stems artificially inoculated with *Botryosphaeria dothidea* at 3-wk intervals in 1987 and 1989. A suspension of 10^5 spores per milliliter was brushed on, and wetness was maintained for 7 days. Trees were evaluated for number of lesions per 10 cm^2 of bark in October of the following year. The percentage of inoculated bark that was necrotic was determined in October 2 yr after the 1987 inoculations and 1 yr after the 1989 inoculations. Horizontal broken lines indicate values for uninoculated control trees. Vertical bars denote standard error of means or LSD ($P \leq 0.05$). Also shown are weekly averages for daily maximum and and minimum temperature during the year when inoculations were made.

12,15). The low infection rate during the first season is most likely due to a low inoculum potential. An explanation for the difference between seasons cannot be based on bark susceptibility, since first-year trees are highly susceptible in inoculation tests (10,11,12) and susceptibility of 1-yr-old branches was reported to be equal to or greater than that of 2-yr-old branches (15).

Results of spore-trapping in older adjacent orchards probably reflected the timing of spore release in the young orchards under study, but did not necessarily indicate relative levels of conidia from year to year in the test orchards. Inoculum from adjacent orchards is believed to have caused primary infections at wounds or lenticels in the test orchards, thus giving rise to secondary inoculum and an overall increase in inoculum density by the second year. Also, the volume of dead wood available for fungal colonization and sporulation was much greater in the second season than in the first. Winter pruning in the test orchards before the second season substantially increased the amount of dead wood both in trees (e.g., pruning stubs that died back to the branch collar) and on the orchard floor.

The indicated time of infection from natural inoculum sometimes was unexpected on the basis of artificial inoculations. For example, trees in the Lane orchard were shown to become infected in December 1988 when spores were not detectable. If covers were not sealed adequately with caulking when replaced on trees, waterborne spores could have entered the protected area at a later time. Alternatively, the plastic covers might have affected host susceptibility. Covered bark was subjected to continuous darkness and was insulated somewhat from outside temperatures. It became light in color and softer than uncovered bark. If changes leading to dormancy were delayed or altered and fungal invasion occurred later than usual (e.g., November 1989, Lane orchard), infection might have developed.

In short, nonwounded bark of 1- to 2-yr-old peach trees is susceptible to invasion by *B. dothidea* during most, if not the entire, growing season. Infection during this period depends on the availability of waterborne spores and on environmental conditions. Given that spore availability and moisture conditions are optimal for infection, temperature may be the primary determinant. In the southeastern United States, the potential for infection appears to be greatest in the months of May, June, and July. In new peach plantings surrounded by older diseased orchards, infections on the nonwounded trunk and lower scaffold are more frequently initiated in the second season than in the first, probably due to increased inoculum levels. Since some evidence indicates that peach bark is less susceptible to fungal invasion through lenticels as it becomes older (15; P. L. Pusey, *unpublished*), efforts to control the disease on the trunk and major branches may be necessary only in the early years of the orchard. This approach is currently being investigated in field tests with protective compounds.

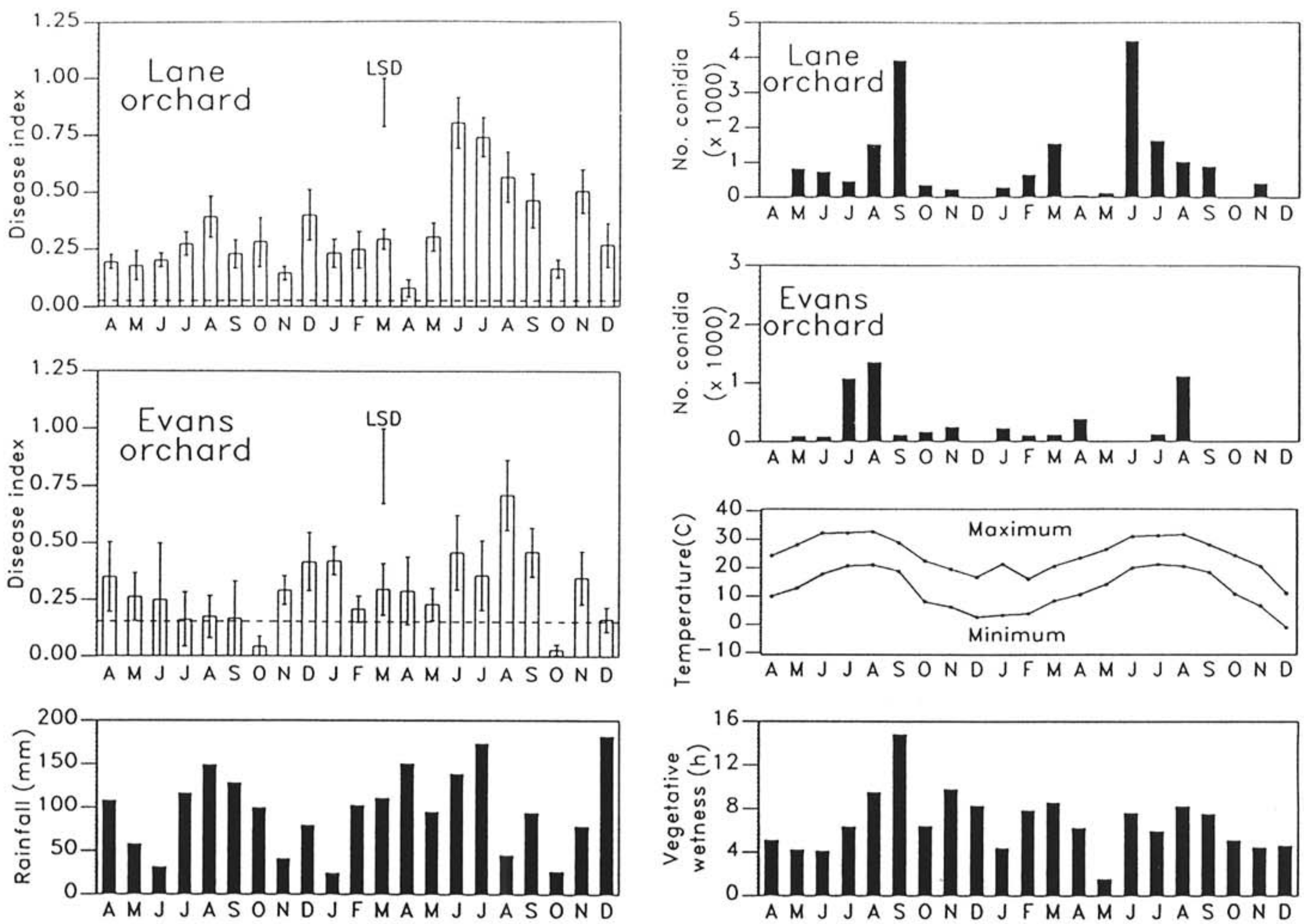

Fig. 3. Disease severity of peach trunks exposed to natural inoculum of *Botryosphaeria dothidea* at 1-mo intervals from April 1988 to December 1989. Trees were evaluated in October 1990. The disease index equals the number of gumming sites on bark that was covered (except for the 1-mo exposure) divided by the number of gumming sites on an equivalent area of noncovered bark on the same trunk. The broken horizontal line indicates the disease index for control trees that were covered the entire time. Vertical bars denote standard errors of means or LSD ($P \leq 0.05$). Also shown are monthly totals for rainfall and waterborne conidia trapped. Monthly averages are given for daily maximum and minimum temperature and daily hours of wetness.

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