Influence of Water Stress on Susceptibility of Nonwounded Peach Bark to *Botryosphaeria dothidea*

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ABSTRACT

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Botryosphaeria dothidea invades bark of peach trees through wounds and lenticels. The effect of water stress on disease development at lenticels was studied in 1-yr-old peach trees that were inoculated by applying suspensions of conidia on nonwounded stems. From May to September 1985, inoculated trees were irrigated daily or had water withheld for 2, 4, or 6 days in each 8-day cycle. At the end of the test, trees with the 6-day withholding had higher mean values for number of gum exudation sites and number of lesions and a higher percentage of bark necrosis than trees in the other treatment groups ($P = 0.05$). In 1986, the effect of severe water stress at or near the time of inoculation was determined by stopping daily irrigation of trees 2 days before inoculation and starting irrigation again after leaf water potential had dropped to below −3.0 MPa. This was done with four different sets of trees from April to October. In another test with a similar objective, trees in various stages of wilt in May 1986 were inoculated and then subjected to 96 ± 2% relative humidity for 7 days to maintain water potential levels. In neither of the latter two types of tests was water stress or water potential at the time of inoculation shown to be related to disease development. To determine the effect of severe water stress after inoculation, the trees inoculated in April or June 1986 had daily irrigation interrupted after varying periods. Water was withheld until leaf water potential was below −3.0 MPa. Based on observations in November, stress imposed 2–6 mo after inoculation resulted in a dramatic increase in number of lesions and in percentage of bark necrosis. Stress imposed during the period of August to October consistently caused an increase in disease severity. This has significance because commercial peach orchards (even those equipped with irrigation systems) generally are not irrigated after fruit harvest, which occurs between May and August.

Botryosphaeria dothidea (Moug. ex Fr.) Ces. & de Not. (*B. ribis* Gross. & Dugg.) invades peach (*Prunus persica* (L.) Batsch) trees through wounds and lenticels (2,4,8,11,17,18) in production areas of the southeastern United States (11,12). Invasion through lenticels leads to localized infections manifested as sunken necrotic lesions and gum exudation on the trunk and scaffold limbs. On young branches, the fungus frequently causes swelling in areas surrounding lenticels due to hyperplasia of cells beneath the outer bark. Because of the characteristic swelling of bark, the disease is known in Japan as "peach blister canker" (2,8). In the United States and China (4), it is referred to as a "gummosis" disease of peach trees.

The reported time period between inoculation or nonwounded peach trees with *B. dothidea* and symptom expression varies from 2 wk to many months (4,11,18). This variability may be due in part to environment and tree vigor. Considering that environmental stress is known to predispose a number of tree hosts to invasion by *B. dothidea* through wounds (6,7,9,10,14,16), possibly stress influences the development of infections by *B. dothidea* at lenticels on peach trees. On apple trees, *B. dothidea* has been reported to reside in lenticels and to invade the cortical tissue beneath lenticels when moisture stress develops (5). In this report, greenhouse experiments were conducted to study the effect of water stress on the reaction of nonwounded peach trees to *B. dothidea*.

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MATERIALS AND METHODS

Host and greenhouse conditions. One-year-old peach trees obtained from a nursery were placed in 23-cm-diameter pots with peat:vermiculite:soil (1:1:1) in February. The cultivar Summergold on Loring rootstock was used in 1985 and Coronet on Nemaguard rootstock was used in 1986. In all tests, trees were fertilized with Osmocote 13-13-13 and irrigated with a computer-controlled system (ChronTrol CD model, Lendberg Enterprises, Inc., San Diego, CA). Tubes supplied water to individual pots, filling them to capacity once every day at 10:00 a.m., except when water was withheld to study the effects of water deficits on disease development. During experiments conducted between April and November, greenhouse temperature and relative humidity varied widely (3–37 C, 15–100% RH), depending on outside conditions.

Inoculation procedure. An isolate of *B. dothidea* from diseased peach trees in Dooley County, Georgia, previously designated PI-5 (11), was used in all inoculations. The fungus was transferred from Difco potato-dextrose maintenance medium to Difco oatmeal agar for production of conidia. Cultures were subjected to a diurnal light period of 15 hr for 19–23 days at 25 C. Unless otherwise indicated, inoculations were made by applying, with a paintbrush, 10^5 conidia per milliliter of aqueous suspension of *B. dothidea* to a section of the peach stem extending from the bud union to the first branch (15–30 cm long). Noninoculated check trees received water only. The stems of both inoculated and noninoculated check trees were wrapped with three layers of moist cheesecloth, then one layer of Parafilm. Wrappings were removed after 6–7 days.

Effect of different irrigation schedules. In 1985, the frequency at which inoculated peach trees received water was varied. Some trees were irrigated daily without interruption and others had water withheld the last 2, 4, or 6 days in each 8-day cycle of an automatic system. On 10 May, which was at the end of the second 8-day cycle, inoculations were made with *B. dothidea* (8×10^5 conidia per milliliter of suspension). Although some trees had not received water for 6 days before inoculation, no signs of water stress were visible at this time. For each schedule, 19 inoculated and three noninoculated trees were used. All trees in the experiment were in one randomized complete block. The irrigation schedules were continued for 19 wk, or 16 complete cycles.

Effect of severe water stress at time of inoculation. Daily irrigation of trees in 1986 was stopped 2 days before inoculation and not resumed until after wilting was severe and leaf water potential was below −3.0 MPa (7–17 days of withholding). Water potential was measured with a pressure bomb (15) using three of the youngest, fully expanded leaves on each plant. The test was performed four times, with inoculations made on 25 April, 17 June, 20 August, and 19 October. For each date, water was supplied daily without interruption to four inoculated trees but withheld from four inoculated and two noninoculated trees. Both the inoculated and noninoculated stems were covered with moist cheesecloth and Parafilm for the same period that water was withheld.

In another test in 1986, peach trees with varying water potentials were inoculated and placed in environmental chambers where plant water potentials were stabilized, using the method of Schoeneweiss (13,14). On each of three successive days in early May, water withholding was started for 11 trees. Eight days after water withholding was begun, 33 trees were in various stages of wilt. Inoculations were made at this time (14 May) on 30 trees from which water was withheld and on eight that were irrigated daily. Three trees that had water withheld and three trees irrigated daily were used as noninoculated checks. Trees were inoculated by applying a spore suspension of *B. dothidea* with an airbrush to a 20-cm section of the stem extending upward from the bud union. To determine the effect of high relative humidity (as opposed to free moisture) on infectivity of nonwounded bark, 10 cm of the stem was wrapped with moist cheesecloth and Parafilm and 10 cm was left unwrapped. Half of the trees were wrapped along the upper 10 cm of stem and half were wrapped below. The plants were randomized and placed in complete darkness in three different 1.3-m^3 environmental chambers maintained at 96 $\pm$ 2% relative humidity and 25 $\pm$ 1 C. After 48 hr, water potentials of three leaves per plant were measured with a pressure bomb. After a total of 7 days, water potentials were again measured, cheesecloth and Parafilm were removed, and trees were transferred back to the greenhouse, where daily irrigation was resumed.

Effect of severe water stress after inoculation. Peach trees were inoculated on either 13 April or 17 June 1986 and daily irrigation was interrupted after varying periods. Whenever water was withheld, the trees were closely monitored until severe wilt was observed and the average water potential of three leaves per plant was below −3.0 MPa. Daily irrigation was then resumed. Ten April-inoculated trees were irrigated daily without interruption, and 24 had water withheld beginning 13 May, 17 June, 5 September, or 17 October; six trees were used per date. Four June-inoculated trees were irrigated daily without interruption, and eight (four per date) had water withheld beginning 20 August or 17 October. On each of the dates that inoculated trees had water withheld, two noninoculated check trees brushed with water only and wrapped in April or June also had water withheld.

Tree response and data collection. Water withholding periods that led to severe wilt and leaf water potentials of below −3.0 MPa varied from 6 to 18 days. Generally, 50 to nearly 100% of the leaves were lost during or soon after the withholding period. Senescent leaves were avoided when water potential was being measured. Defoliation was particularly severe when trees were stressed in late summer and early fall. When trees were under different irrigation schedules in 1985, signs of water stress were visible only during 2–7 June, when outside temperatures exceeded 37 C. At that time, five of the 19 plants from which water was withheld for 6 days in the 8-day cycle wilted and eventually lost 25–50% of their leaves.

Blisters, necrotic lesions, and gum exudation sites associated with lenticels were counted and their numbers calculated per 10 cm^2 of inoculated bark area. Gum deposits were also removed from trees and weighed to determine gum weight per 10 cm^2 of bark area. In addition, lesion diameters were measured and total percentage of inoculated bark area showing necrosis was calculated.

For the 1985 test involving different irrigation schedules, data were examined by analysis of variance. In one 1986 test, regression analysis was used to determine whether plant water potentials maintained under high humidity at the time of inoculation related to disease development. In all other cases, comparisons were made using Student's t test.

RESULTS

Only trees inoculated with *B. dothidea* showed disease symptoms. The symptoms involved localized infections at lenticels and were the same as those previously described and shown to be caused by *B. dothidea* (2,4,8,11,17,18). None of the noninoculated trees, whether stressed or not, were diseased.

Different irrigation schedules. Gum exudate was first noticed on trees 5 wk after inoculation (18 June) (Fig. 1). The gum originated from bark infections initiated at lenticels. Fourteen weeks after inoculation (27 August), 72% of the trees with the greatest water withholding (6 days in each 8-day cycle) had exuded gum. None of the inoculated trees that were irrigated daily showed gum until the final observation on 18 September, 19 wk after inoculation. Trees with the 6-day water withholding had more gumming sites per 10 cm^2 of bark after

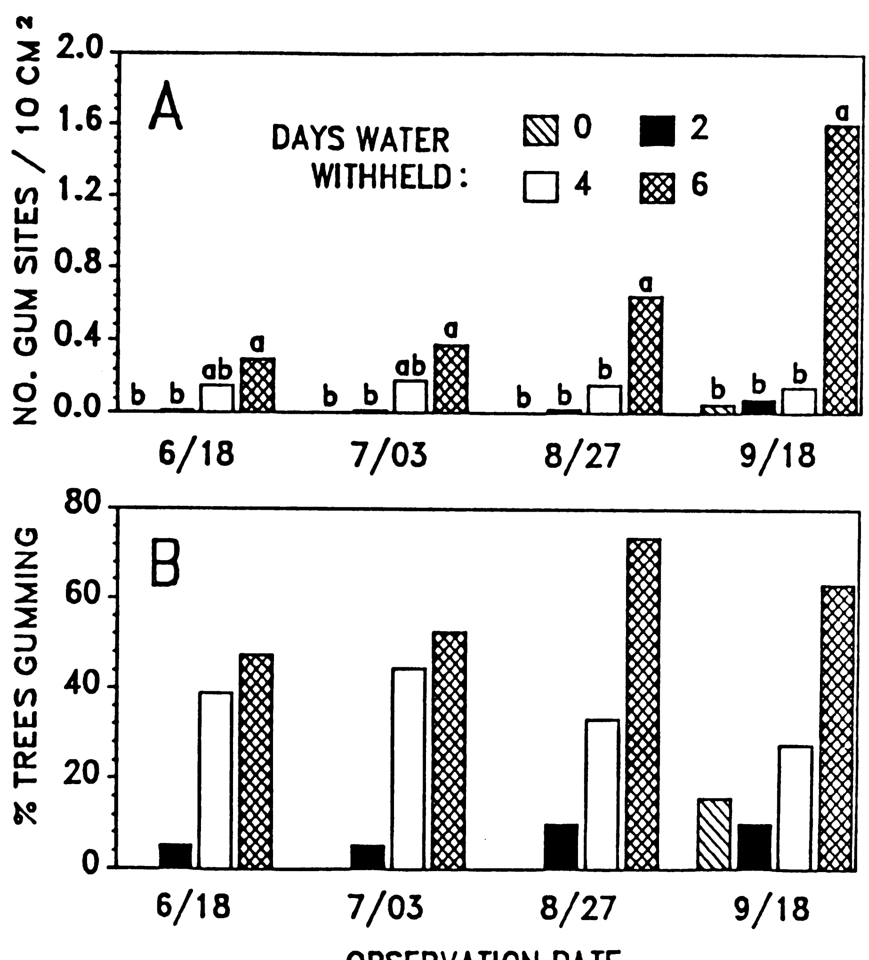

Fig. 1. Gumming reaction of nonwounded peach trees to *Botryosphaeria dothidea* after inoculation on 10 May 1985 and intermittent withholding of daily irrigation (water was withheld for varying periods in each 8-day cycle). **(A)** Mean number of gum exudation sites per 10 cm^2 of inoculated bark surface. For each date, bars with the same letter are not different ($P = 0.05$) according to Duncan's multiple range test. **(B)** Percentage of 19 trees with gum exudate.

14 and 19 wk than did the other trees. After 19 wk, they also exuded a greater amount of gum per 10 cm^2 of bark (Fig. 2A). No differences in gum exudation were detected among the other treatment groups.

Although gum originated where sunken lesions had developed surrounding lenticels, not all lesions exuded gum. Surface area of bark lesions ranged from 3 to 39 mm^2. Nineteen weeks after inoculation, trees with the greatest water withholding had a higher percentage of bark necrosis ($P = 0.05$) (Fig. 2B) and a higher number of lesions per 10 cm^2 of bark ($P = 0.05$) (Fig. 2D) than other trees. Again, no differences were observed among the other treatment groups.

Swollen areas at lenticels were first noticed 13 wk after inoculation. At 19 wk, between 90 and 100% of the trees in each treatment group had blisters. No differences were detected in the number of blisters per 10 cm^2 of bark (Fig. 2C).

Severe drought stress at time of inoculation. Despite repeated tests, in no case was water stress or water potential at the time of inoculation shown to be related to disease development.

A comparison of trees irrigated daily with those that had water withheld until water potential was below −3.0 MPa showed no differences in symptom parameters measured on 5 November ($P = 0.05$). Trees inoculated in August or October did not show symptoms, with the exception of a single nonstressed tree inoculated in August that showed blisters. Blisters developed on over 80% of the trees inoculated in April or June. Stressed and nonstressed trees inoculated in April had means of 18.7 and 23.8 blisters per 10 cm^2 of bark, respectively. Less than 50% of the trees inoculated in these months developed necrotic lesions. Stressed and nonstressed trees inoculated in April had means of 0.20 and 0.23 lesions per 10 cm^2 of bark, respectively; those inoculated in June had means of 1.15 (standard error of ±0.85) and 0.06, respectively. Gumming was minimal for trees inoculated in April or June, with only five of 16 trees with gum exudate.

Results were similar when trees in various stages of wilt in May were inoculated and then held for 7 days at high relative humidity to stabilize and maintain water potential levels. Average leaf water potential after 7 days of incubation was 9% greater than after 48 hr. Measurements for the two dates were averaged together and recorded as representing the relative water potential of each tree. Minimum and maximum water potentials for trees were −4.0 and −0.17 MPa, respectively. Average water potential for all inoculated trees was −1.95 MPa, with a standard deviation of ±1.18. Disease symptoms did not begin appearing until September. The trees were closely examined, and the symptom parameters described above were measured on 7 November. Analysis of regression of the symptom parameters on water potential showed that none of

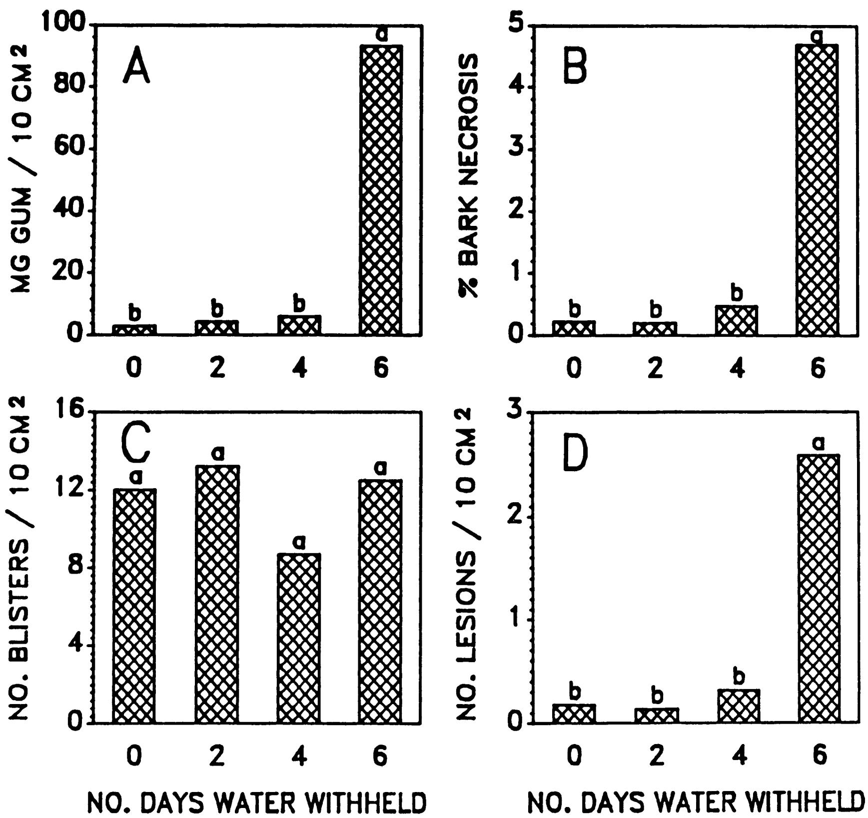

Fig. 2. Reaction of nonwounded peach trees to *Botryosphaeria dothidea* as observed on 19 September 1985 after inoculation on 10 May and intermittent withholding of water for varying periods in each 8-day cycle. **(A)** Weight of gum exudate in milligrams per 10 cm^2 of inoculated bark surface. **(B)** Percentage of total inoculated bark area that was necrotic. **(C)** Number of blisters and **(D)** number of necrotic lesions per 10 cm^2 of bark area. Bars with the same letter are not different ($P = 0.05$) according to Duncan's multiple range test.

Table 1. Reaction of nonwounded peach trees to severe water stress subsequent to inoculation with *Botryosphaeria dothidea*[a] in April or June 1986

			Symptom parameters measured 19 November[d]			
Date trees inoculated	Date water withholding initiated[b]	No. of days water withheld[c]	No. of blisters/ 10 cm^2 of bark (± SE)	No. of gum sites/ 10 cm^2 of bark (± SE)	No. of lesions/ 10 cm^2 of bark (± SE)	Percent bark necrosis (± SE)
16 April	Nonstressed	0	4.3 (±1.6)	0.0	0.4 (±0.1)	0.5 (±0.2)
16 April	13 May	8–10	1.7 (±0.6)	0.0	0.0	0.0
16 April	17 June	6–7	4.6 (±1.5)	0.2 (±0.1)	4.3 (±0.8)*	4.5 (±0.8)*
16 April	5 September	7–11	0.0*	1.8 (±0.5)*	6.2 (±1.3)*	9.0 (±2.3)*
16 April	17 October	7–18	0.6 (±0.6)	0.7 (±0.4)	3.9 (±0.9)*	7.4 (±1.4)*
17 June	Nonstressed	0	4.9 (±2.7)	0.0	0.1 (±0.1)	0.2 (±0.2)
17 June	20 August	7–8	0.0	6.7 (±1.6)*	23.6 (±3.6)*	32.6 (±8.0)*
17 June	17 October	10–14	1.6 (±0.6)	0.0	8.2 (±1.8)*	16.4 (±4.3)*

[a]Stem was brushed with 10^5 conidia per milliliter of suspension and wrapped with moist cheesecloth and Parafilm.
[b]Ten April-inoculated trees and four June-inoculated trees were irrigated daily without interruption (nonstressed). Starting on each date, water was withheld from six April-inoculated and four June-inoculated trees.
[c]Water was withheld until leaf water potential was below −3.0 MPa.
[d]Asterisks indicate that mean is different ($P = 0.05$) from mean for nonstressed trees inoculated on same date, based on Student's *t* test.

the slopes of the parameters were different ($P = 0.05$) from zero.

During the 7-day incubation period under high humidity conditions, no moisture was visible on the surface of stem sections not covered by moist cheesecloth and Parafilm. These sections had a mean of 8.1 blisters per 10 cm^2 of bark, which was significantly lower than the mean of 36.1 blisters per 10 cm^2 of bark for wrapped sections ($P = 0.05$). For other disease measures, no differences were shown between wrapped and open inoculation methods. For both wrapped and open sections, the mean number of lesions per 10 cm^2 of bark was 1.0. The mean number of gum exudation sites per 10 cm^2 of bark for wrapped and open stem sections was 0.25 and 0.09, respectively.

Severe water stress after inoculation. Water stress imposed 2–6 mo after the April or June inoculations resulted in an increase in disease severity based on number of lesions per 10 cm^2 of bark and percentage of bark necrosis determined in November (Table 1). Trees inoculated in April and stressed in May did not develop necrotic lesions.

Gum exudate was observed on only one of 10 nonstressed trees inoculated in April (represented by one site) and on none of the nonstressed trees inoculated in June. However, 44% of all inoculated trees stressed after May exuded gum. In terms of the number of gumming sites per 10 cm^2 of bark, only those trees stressed in August or September had statistically higher mean values than did the nonstressed trees ($P = 0.05$) (Table 1). Only three of the 10 trees stressed in October exuded gum during the 5-wk period before the trees were examined in November.

Blisters were observed on all nonstressed trees when the trees were examined in November, but only 44% of all trees stressed after May had blisters. A statistical difference in numbers of blisters per 10 cm^2 of bark was shown only for trees stressed in September ($P = 0.05$) (Table 1).

DISCUSSION

The imposition of water stress on nonwounded peach trees 2–6 mo after the trees were inoculated dramatically increased disease severity based on the extent of lesion development and bark necrosis. In repeated tests, water stress at the time of inoculation did not affect disease development. The latter results are in contrast to wound-inoculation studies with other tree hosts (6,14).

Stress at the time of inoculation had no effect even when stressed trees were inoculated and held for 7 days in a high-humidity chamber to maintain low water potential as described by Schoeneweiss (13,14). When the effect of high humidity on infectivity was studied as a secondary objective, it was found that blisters and lesions developed on some bark sections not wrapped with moist cheesecloth and Parafilm. Although the number of infections represented by blisters were fewer for open than for wrapped stem sections, the test indicated that *B. dothidea* can invade nonwounded peach bark under high relative humidity as well as wet conditions.

On nonwounded peach bark, it appears from the test results that water availability to the plant does not influence the initiation of infection by *B. dothidea*. On the other hand, exactly when the bark became infected is not known. The fungus may have invaded the cortex soon after inoculation or it may have resided epiphytically in the outer dead bark before invading the cortex via lenticels. In a previous study (11), *B. dothidea* was recovered at a 90% frequency rate from the outer cork layers of bark in and around lenticels of healthy peach trees. The trees had been inoculated 17 mo earlier with a nonpeach isolate of *B. dothidea* that does not cause infections at peach lenticels.

The development of blisters may be a defense reaction by the tree. Swelling is due to hyperplasia of peridermal and cortical cells near the lenticel (1; *unpublished*). This may have the effect of compartmentalizing the pathogen. The intermittent withholding of water over a 19-wk period in 1985 appeared not to affect blister development. On the basis of tests in 1986, however, water stress may result in fewer blisters. Blisters and lesions can form as a direct result of infections at lenticels, or lesions can develop at sites where blisters were previously formed. The latter is evident from observations of newly formed lesions located inside larger areas that earlier had become swollen. This may occur more frequently when trees are under water stress.

When trees were inoculated and simultaneously stressed during the months of August or October, infection rarely occurred on either the stressed or nonstressed check trees. This is consistent with field studies in which newly planted peach trees were inoculated every 21 days from February to December (*unpublished*); trees inoculated in August or later developed few symptoms even after 1 yr.

It is significant that water stress imposed during the period from August to October (following inoculations in April or June) consistently resulted in an increase in bark necrosis caused by *B. dothidea*. Even commercial peach orchards equipped with irrigation systems in the southeastern United States generally are not irrigated after the fruit are harvested between May and August. This situation may be comparable to the effect of postharvest moisture stress on Cytospora canker of French prune in California (3). Field studies will be necessary to determine whether irrigation as an orchard management practice is effective in reducing damage to peach trees caused by *B. dothidea*.

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