Role of Conidia of *Botryosphaeria dothidea* in the Natural Spread of Peach Tree Gummosis

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I wish to thank Lewis Zellner for technical assistance.
Accepted for publication 25 October 1978.

ABSTRACT

WEAVER, D. J. 1979. Role of conidia of *Botryosphaeria dothidea* in the natural spread of peach tree gummosis. Phytopathology 69:330-334.

Symptoms of peach (*Prunus persica*) tree gummosis were visible 9–13 mo after healthy limbs were exposed for 30-day periods to conidia of *Botryosphaeria dothidea* produced on naturally infected peach wood. Experimental inoculations in July caused significantly more infections than those in March, April, or May. Branches inoculated in June with a suspension of conidia had symptoms after only 3 mo. Swollen lenticels and sunken lesions developed on 1 or 2 yr old branches; only sunken lesions formed on 3 yr old branches. Conidia were first detected in rainwater traps on 22 March, and the greatest numbers were collected in late July and early August 1977. Numbers of conidia declined in December and none were detected in rainwater after 30 December. The rainwater collection from limbs diseased for 1–2 yr yielded from 2 to 138 times as many conidia as limbs diseased for 4–5 yr. The optimum temperatures for germination and germ-tube growth of conidia obtained from diseased wood or agar cultures were 25–35 C and 30 C, respectively. Warm wet weather favored the release and germination of conidia that infected peach bark through lenticles and induced gummosis symptoms.

Additional key words: lenticels, *Prunus persica*.

Bark gummosis, a serious disease of peach (*Prunus persica* (L.) Batsch.) trees in Georgia, is caused by *Botryosphaeria dothidea* (Moug. ex Fr.) Ces. & de Not. (11). Symptoms include sunken necrotic lesions (1–2 cm in diameter) in bark around lenticels and gum exudation from diseased lenticels. On young branches infected lenticels become swollen but gumming usually does not occur.

The disease has spread steadily since it was first observed in 1971 in Peach County, GA, and some commercial orchards in Alabama are now affected by gummosis. The infection process or spread of the disease in peach orchards is not well understood. I postulated that the fungus invades through the lenticels because every infection has a lenticel at its center (11). Mature conidia were present in numerous pycnidia in the bark of diseased trees (11), on peach wood pruned from diseased trees and left on the ground, and on dead branches left on diseased trees (12).

Experiments were initiated to study the release of conidia from the bark of diseased peach trees and to determine the role of conidia in the spread of gummosis.

MATERIALS AND METHODS

Inoculation with conidia from naturally diseased wood. Peach wood (2.5–3.5 cm diameter) naturally infected by *B. dothidea* was collected from commercial orchards with severe symptoms of gummosis late in February 1976. The wood had been pruned from the trees 4–6 wk earlier. Pieces of wood bearing numerous pycnidia were cut into 10-cm lengths, allowed to dry at room temperature, and stored in polyethylene bags. This procedure allowed the infected pieces to be stored for several weeks in good condition.

A few days before use as a source of inoculum, pieces of the wood were rehydrated by incubation for 3 days at 100% humidity and 20 C. Conidia from rehydrated wood were tested for viability. Thin sections of pycnidia from each of six inoculum pieces were placed on a drop of water in the center of petri dishes containing 1.5% water agar; a glass rod was used to spread the conidia over the agar surface. Plates were incubated at 25 C for 4 hr, 200 conidia per plate were counted, and the percent germination was calculated. Each time prunings were rehydrated, 95–100% of the conidia germinated.

Three year old trees of Loring peach were used in inoculation studies. The trees were grown at the Southeastern Fruit & Tree Nut Laboratory orchard Byron, GA, in an area where gummosis was not present. Three scaffold limbs on each of eight trees were inoculated at monthly intervals from March through July 1976. Two pieces of infected wood were tied to the adaxial surface of the limb approximately 1 m distal from the trunk, left for 30 days, and then removed. Tests to determine the viability of the conidia were repeated. On each inoculation date, three limbs on each of two trees were tagged with ribbon and left as uninoculated controls. Limbs were examined for disease symptoms at 2–3 wk intervals during the next 18 mo. Attempts were made to isolate *B. dothidea* from bark infections as previously described (11).

Inoculation with conidia produced in vitro. Conidia were produced from two isolates of *B. dothidea* from naturally diseased peach bark. The fungus was grown on oatmeal agar (10) at 26 C under continous fluorescent light (2,14) at 21,520 lux (2,000 ft-c). Large numbers of pycnidia formed within 8 days and by the 12th day numerous cirrhi had extruded from the pycnidia. A conidial suspension was made by flooding the plates with sterile water and gently scraping the agar surface with a bent glass rod. The suspension was filtered through four layers of sterile cheesecloth to remove mycelial fragments, and the concentration of conidia was determined with a hemacytometer. Inoculum contained an average of 4,500 conidia per milliliter.

The conidial suspension was used within 1 hr to inoculate branches of healthy, 4 yr old Loring peach trees in June 1977. A 15-cm section of 1, 2, and 3 yr old branches on each of six trees was selected for inoculation and marked by tying a piece of flagging ribbon at each end. The suspension was sprayed with a hand-operated atomizer until the bark was completely wet. The inoculated area was wrapped immediately with six layers of cheesecloth saturated with sterile water and covered with polyethylene film to maintain high humidity. Two branches per tree, similarly treated after being sprayed with sterile water, served as controls. Conidial viability was 98.7% immediately after all branches were inoculated. The polyethylene and cheesecloth were removed from the branches 6 days after inoculation. Branches were observed at 2–3 wk intervals for development of disease symptoms, and *B. dothidea* was isolated from bark infections as previously described (11).

Effect of temperature on conidial germination. Conidia were obtained from pycnidia on diseased peach wood and from oatmeal agar cultures as described earlier. Eighteen petri dishes containing

1.5% water agar were seeded with conidia from each source and three plates of each were incubated for 4 hr at 10, 15, 20, 25, 30, and 35 C. A minimum of 200 conidia per plate were observed and the percentage that germinated at each temperature was calculated. The length of germ tubes on 15 conidia per plate also was measured, and the averages were calculated. This experiment was performed twice.

Waterborne conidia from diseased limbs. Bertrand and English's technique (1) was used to collect rainwater runoff from diseased peach tree limbs from 10 January 1977 to 10 January 1978. I selected four trees that had been diseased for 1 to 2 yr and four trees diseased for 4 to 5 yr based on field observations. A trap was set to collect the rainwater runoff from one diseased scaffold limb on each tree. Ten milliliters of 5.0% copper sulfate solution was added to each bottle before use to prevent spore germination (T.B. Sutton, *personal communication*). Water in the traps was collected within 24 hr after rainfall. Instruments to record daily maximum and minimum air temperatures and rainfall were approximately 0.3 km from the collection stations.

I modified the technique of Sutton et al (9) to determine the number of conidia in the collection bottles. Water in each bottle was thoroughly mixed, a 10-ml sample was removed and put in a test tube, and the volume of the remaining water was measured in a graduate cylinder. Conidia in each tube of water were stained by mixing with 0.2 ml of cotton blue in lactophenol. The stained sample was filtered through a 47-mm diameter (0.80-μm pore size) gridded filter and the conidia in 10 randomly selected grid squares were counted. Grid counts were adjusted to give the total number of conidia in each bottle.

Data were subjected to an analysis of variance, and Duncan's multiple range test was used, when appropriate.

RESULTS

Inoculation with conidia from naturally diseased wood. Symptoms of gummosis were observed on 15 April 1977, more than 1 yr after branches were first inoculated and 9 mo after the last inoculations (Table 1). Typical dark brown, sunken lesions in the bark beneath lenticels were evident and gum later exuded from many of the infections. When numbers of infections were counted in April, significantly fewer infections were observed on limbs inoculated in July than in April.

Numbers of infections on each branch were counted again late in August 1977, and the average number per inoculation date had increased 1.8–9.9 times compared with counts in April. Branches inoculated in July had significantly more infections than branches inoculated in March, April, or May, but not in June. No infections occurred on control branches. *B. dothidea* was isolated from 34 of 38 bark infections.

All infections occurred within or below the inoculated area on the branch. Rainfall, which occurred from 5 to 11 times during each monthly inoculation period, presumably was responsible for dispersal of conidia. Based on infection counts made in August, however, the coefficient of correlation between rainfall and numbers of infections for each month was low ($r = 0.354$) and not significant. The mean daily air temperature during each inoculation period was significantly correlated with numbers of infections ($r = 0.784$; $P = 0.05$).

Inoculation with conidia produced in vitro. Symptoms of gummosis were first observed 12 wk after inoculations. Some lenticels on inoculated branches appeared swollen and protruded farther above the bark surface than lenticels on noninoculated branches (Fig. 1A,B). Two weeks later the swelling in lenticels was more obvious and sunken necrotic areas in the bark beneath some lenticels also had appeared (Fig. 1C). Infections were observed only in the portions of the branches that had been inoculated and wrapped with cheesecloth. None of the controls was diseased.

Swollen lenticels and sunken lesions were counted; the mean numbers of infections for branches of each age are presented in Table 2. Fourteen weeks after inoculation more than twice as many infections were observed on 1 or 2 yr old branches than on 3 yr old branches. Infections increased after 20 wk, and the number was similar on branches of all ages. Swollen lenticels and sunken lesions were observed on 1 and 2 yr old branches, but only sunken lesions occurred on the 3 yr old branches. The pathogen was isolated from 42 of 45 bark infections sampled.

Effect of temperature on conidial germination. Temperature effects on germination of conidia from pycnidia on peach wood and from oatmeal agar cultures were similar (Table 3). Conidia germination after 4 hr at 10, 15, 20, 25, 30, and 35 C was 0, 20, 90, 98, 99, and 99%, respectively.

Germ tube development also was similar for both conidial sources. Development after 4 hr incubation was greatest at 30 C (153.1 and 138.2 μm) with reductions in germ tube length of about 33% at 25 and 35 C, 66% at 20 C, and 89% at 15 C.

Waterborne conidia collected from diseased limbs. Daily maximum and minimum air temperatures and the log of the average numbers of conidia collected in rainwater runoff from diseased limbs after each rainfall from 10 January 1977 to 10 January 1978 are presented in Fig. 2. Conidia were first detected on 22 March after several days of minimum temperatures ranging as high as 7–13 C and maximum temperatures reaching 26–28 C for the first time in 1977. Thereafter, conidia were present in water from each rainfall until late December.

Average numbers of conidia collected from limbs diseased for 1–2 yr and for 4–5 yr fluctuated similarly for each rainfall during the test period ($0.16–1{,}898.90 \times 10^3$ for the 1–2 yr group; $0.02–513.93 \times 10^3$ for the 4–5 yr group). Spore concentrations in rainwater ranged from 1 to 2,563 conidia per milliliter for the 1–2 yr group and from 1 to 1,836 per milliliter for the 4–5 yr group. Yields of conidia from the 1–2 yr group were from 2 to 138 times as great as yields from the 4–5 yr group. Maximum numbers of conidia were collected from both groups of limbs in late July and early August, after several days when high maximum (33–37.5 C) and minimum (12–22 C) air temperatures were recorded. Numbers of conidia gradually declined in December and none were detected after 19 December for the 4–5 yr group. Conidia were present in

TABLE 1. Development of gummosis on peach tree limbs experimentally inoculated with conidia from peach wood bearing pycnidia of *Botryosphaeria dothidea*

Limbs exposed to inoculum	Conditions during inoculation period		1977 bark infections (mean no. per limb) on:[z]	
	Days with rainfall (no.)	Mean daily temperature (C)	15 April	24 August
1976				
March 2–April 2	11	14.5	2.6 ab	7.6 b
April 2–May 2	5	18.7	6.2 a	11.0 b
May 4–June 4	7	22.5	5.3 ab	9.5 b
June 3–July 3	11	26.8	2.9 ab	13.8 ab
July 9–August 9	6	30.7	2.3 b	22.8 a
Controls, not exposed inoculum			0.0	0.0

[z] Number of infections was determined by counting sunken lesions around lenticels or lenticels with gum exuding. Values followed by different letters are significantly different, ($P = 0.05$) using Duncan's multiple range test.

rainwater from the 1–2 yr group on 24 and 30 December but not in the last rainwater sample collected on 6 January 1978.

No significant correlation was found between the total number of conidia collected and the volume of rainfall (r = 0.352), the concentration of conidia and the volume of rainfall (r = 0.286), or the total number of conidia collected and the mean air temperature (r = 0.390).

DISCUSSION

Inoculation experiments on other host plants have indicated that *B. dothidea* is usually a wound parasite (3,6,7,13,14). However, the fungus invades unwounded stems of blueberry (5), elm (4), and almond (2). I reported previously (11) that each necrotic bark infection occurred beneath a lenticel and hypothesized that conidia

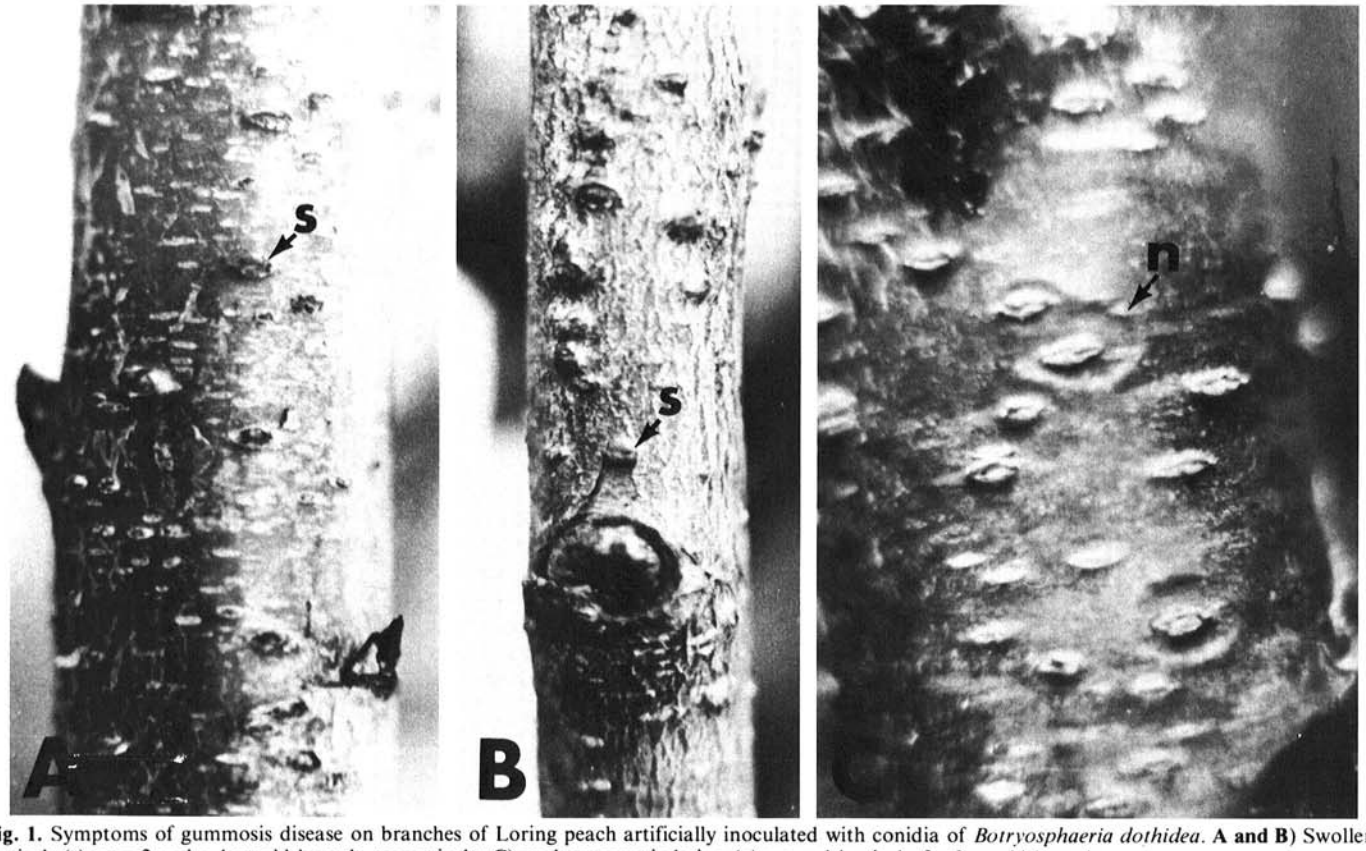

Fig. 1. Symptoms of gummosis disease on branches of Loring peach artificially inoculated with conidia of *Botryosphaeria dothidea*. **A and B)** Swollen lenticels (**s**) on a 2 and a 1 yr old branch, respectively; **C)** sunken necrotic lesion (**n**) around lenticel of a 3 yr old branch.

TABLE 2. Development of gummosis symptoms in lenticels of peach branches after artificial inoculation with conidia of *Botryosphaeria dothidea*

Branch age (yr)	No. of branches inoculated	Mean no. of infections per branch (weeks after inoculation)		Disease symptoms[z]	
		14	20	Swollen lenticel	Sunken lesion
1	15	10.6	17.8	+	+
2	16	8.2	13.9	+	+
3	6	4.0	18.6	−	+
Controls	12	0.0	0.0	−	−

[z] Symptoms present (+) or absent (−) 20 wk after inoculation.

TABLE 3. Effect of temperature on germination and germ tube development on water agar of conidia of *Botryosphaeria dothidea* from pycnidia produced in vitro and in vivo

Source of conidia	Spore germination (%), after 4 hr[y] at temperature (C):						Germ tube length (μm), after 4 hr[z] at temperature (C):					
	10	15	20	25	30	35	10	15	20	25	30	35
Peach wood	0	21.6	90.3	98.0	99.5	98.2	0	17.0	48.7	98.0	153.1	96.3
Oatmeal agar	0	20.2	89.2	98.4	99.8	99.7	0	15.7	51.0	100.7	138.2	98.0

[y] A minimum of 200 conidia were observed on each of three plates per treatment; data are averages of two experiments.
[z] Fifteen germ tubes were measured on each of three plates per treatment; data are averages of two experiments.

initiated infections through the lenticels. This study supports that hypothesis. Healthy peach branches experimentally inoculated with a conidal suspension developed gummosis symptoms within 3.5 mo and each infection was centered at a lenticel. Symptoms produced on 1, 2, or 3 yr old branches were identical to those on naturally diseased trees.

Conidia from pycnidia on naturally diseased peach wood also induced gummosis symptoms, but a considerably longer time (9–13 mo) was required. Fungi such as *B. dothidea* (*Dothiorella* sp. imperfect stage) that form conidia in a pycnidium usually release spores in response to wetting. Rainfall during the inoculation periods was responsible for the release and dispersal of the conidia, which was confirmed by conidia in rainwater runoff from diseased limbs. However, I did not attempt to determine if other factors affected spore release.

Cultures of *B. dothidea* isolated from peach gummosis infections are favored by high temperatures. The optimum temperature for mycelial growth was 28 C, but good growth occurred at 36 C and slight growth at 38 C (11). Isolates of *B. dothidea* from other hosts also grew well at 28 C, but little or no growth was reported above 35 C (2,7,8,14). Maximum germination and germ tube development of conidia for peach isolates occurred at 25–35 C. However, I did not establish a maximum temperature for germination of conidia. In other studies conidia germinated well after 4–6 hr at room temperature (2) or at 25–30 C (5).

The stimulatory effect of high temperature on germination and germ tube growth of conidia and on mycelial growth may account for the rapid symptom development on peach bark inoculated with conidia in June. It also may account for the greater number of infections on branches inoculated with diseased wood in July compared with that in the preceding month when temperatures were less favorable for the fungus. English et al (2) also reported that in California *B. dothidea* caused less severe infections in almond branches inoculated in winter than in those inoculated in spring, summer, and fall.

Conidia were released from naturally diseased peach bark during spring, summer, and fall, indicating the wide temeprature range in which conidia of *B. dothidea* are released in the field. Although air temperature was not correlated with numbers of conidia collected in rainwater, peak conidial production occurred in late July and early August—one of the warmest times of the year. More inoculum was produced on newly infected trees than on trees that were diseased for several years.

Windblown rain during the spring and summer probably spread *B. dothidea* in peach orchards. It is not known if infection occurs during fall when conidia are still being released from diseased bark. Because spore release and infection by *B. dothidea* may occur during a long period, a program to control the disease with fungicides may last for several months each year.

The involvement of ascospores in spreading the fungus has not

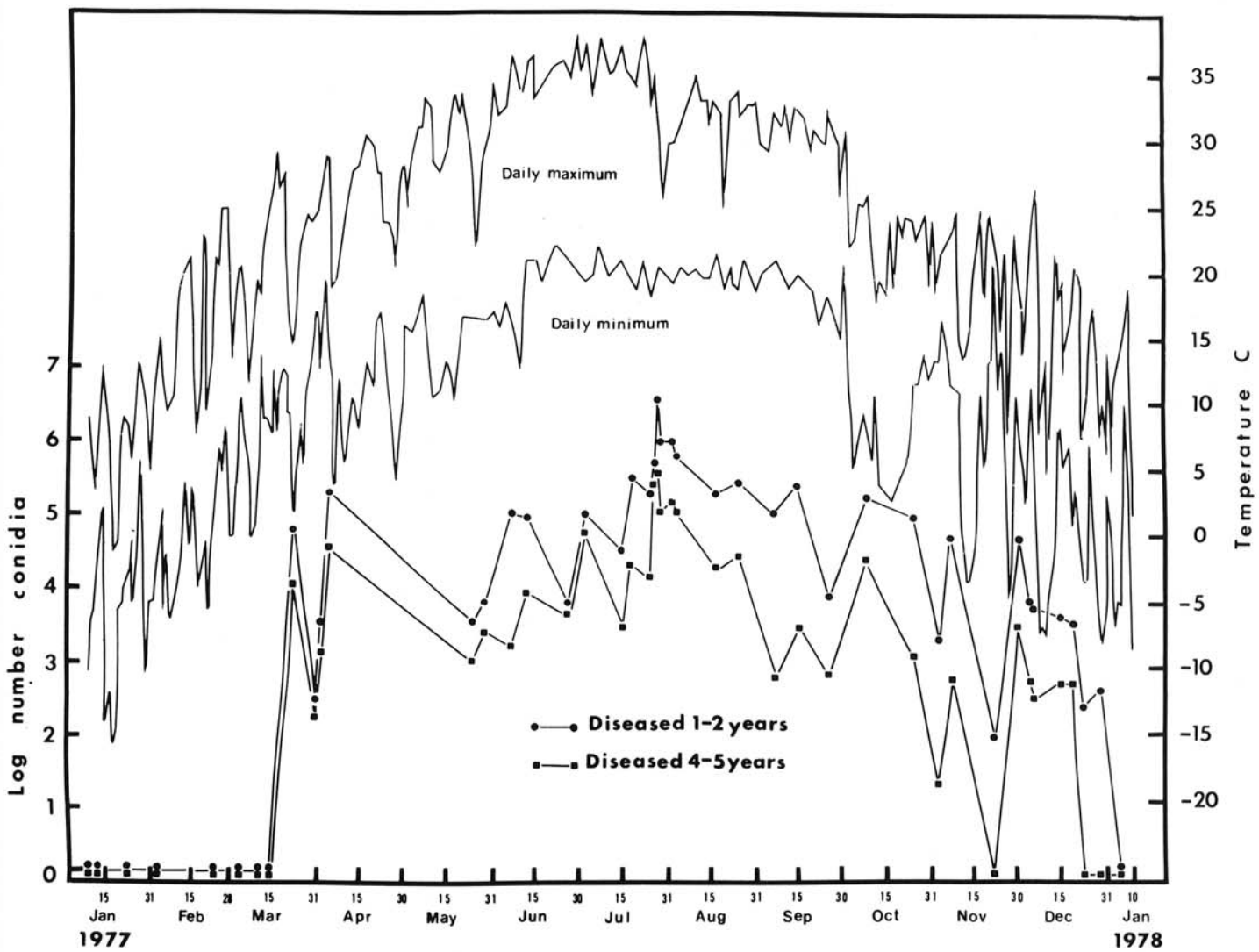

Fig. 2. Daily maximum and minimum air temperatures at Byron, GA, and the log of numbers of conidia of *Botryosphaeria dothidea* in rainwater runoff from peach limbs affected by gummosis for 1–2 yr and for 4–5 yr. Each point represents a day when rainfall occurred and gives the log of average numbers of conidia collected from four limbs in each group.

been determined. Although perithecia containing mature ascospores are occasionally found in diseased peach bark (11), ascospores were not detected in rainwater collected from diseased limbs in this study. However, the failure to detect ascospores may have been because the counting technique was inadequate or because ascospores were too few or may be windblown and airborne. If the wind carries ascospores, they could be involved in long-distance spread of peach tree gummosis.

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