Spore Release and Infection Periods of *Botryosphaeria dothidea* on Blueberry in North Carolina

T. C. CRESWELL, Graduate Research Assistant, and R. D. MILHOLLAND, Professor, Department of Plant Pathology, North Carolina State University, Raleigh 27695-7616

ABSTRACT

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Infections of blueberry plants wounded and exposed to natural inoculum of *Botryosphaeria dothidea* for one 2-wk period of the growing season were most numerous during May and June. Disease occurrence on these plants was positively correlated to log of spore count, minimum temperature, and mean temperature. The mean interval between inoculation and symptom appearance varied between 1 and 14 wk, but a majority of plants became symptomatic after 4–6 wk. Incubation times were shorter for inoculations in May than in March, April, or June. Infections occurred during most of the year, and the average incubation time was about 4–6 wk. Pruning wounds provided the greatest number of infection sites for *B. dothidea*. Conidia were present in rainwater during most of the year but were barely detectable for several weeks from December to February. The log of total numbers of conidia collected in rainwater was positively correlated to rainfall amount, temperature, and relative humidity during the current week. Spore counts were often more strongly correlated to temperature and relative humidity values from the previous week than to values from the current week.

Blueberry stem blight caused by *Botryosphaeria dothidea* (Moug.:Fr.) Ces. & de Not. (syn. *B. ribis* Gross. & Dug.) is a serious disease problem for the North Carolina blueberry industry. Both highbush (*Vaccinium corymbosum* L.) and rabbiteye (*V. ashei* Reade) blueberry are attacked by the pathogen. Symptoms consist of reddening, chlorosis, and drying of leaves and dieback of individual branches resulting in a pecan-brown vascular discoloration. Loss of fruit production and eventual death of entire plants occur if infected branches are not removed before the fungus reaches the base of the plant. Milholland (2) found that infections are possible on both wounded and nonwounded stems but necrosis and dieback occur only after wound inoculation. The production and dispersal of inoculum of *B. dothidea* have been studied in apple (4,5) and peach (6) but not in blueberry. The purpose of this research was to determine periods of spore release for *B. dothidea* on blueberry and to determine the times of stem infection.

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MATERIALS AND METHODS

Time of infection. Tests to determine the period when most infections occur on blueberry by *B. dothidea* were conducted in Craven County, North Carolina, during 1985 and 1986. Vigorously growing 2-yr-old Bluechip or Croatan plants in 15.2- or 20.3-cm-diameter clay pots were maintained in ground beds at North Carolina State University until needed. Then, at 2-wk intervals they were placed in the soil beneath mature blueberry plants heavily infected with *B. dothidea*. Ten pruning cuts were made on each test plant at the start of each 2-wk exposure period to enhance the possibility of infection. Plants were left in the field for a 2-wk period of exposure to natural inoculum except in three instances in 1985 (the periods ending 10 May, 17 May, and 16 August) in which plants were replaced after a 1-wk exposure. Plants were then removed from the field and returned to the ground beds. Isolations from four wound sites on each plant were made 3–4 mo later to determine infection. Six plants each of Bluechip and Croatan in 1985 and 15 Bluechip plants in 1986 were used for each exposure period. The experiment was conducted from April through September 1985 and from March to August 1986. Controls consisted of 39 plants of each cultivar in 1985 and 72 Bluechip plants in 1986 that were kept in ground beds and not exposed to natural inoculum.

Incubation periods. The incubation period (interval between inoculation and symptom expression) for artificially inoculated and naturally infected blueberry plants was determined in tests in Duplin and Craven counties, North Carolina, in 1986. The primary factors to be investigated were the effect of temperature or disease development and the length of the incubation period under natural conditions. Two fields of the Bluechip were selected in Duplin County: site A with 155 2-yr-old plants and site B with 212 3-yr-old plants. Only plants that appeared healthy and were free from stem blight symptoms at the beginning of the season were included in the tests. In each field, eight plants were inoculated on each of five dates at approximately 4-wk intervals beginning 7 March. Plants were inoculated each month to determine the incubation period for *B. dothidea* in blueberry stems infected during March, April, May, June, and July. The remaining noninoculated (healthy) plants (115 at site A and 172 at site B) were examined weekly for symptoms of stem blight. A similar experiment was done in Craven County in a field of 200 1-yr-old Bluechip plants and a field of 86 2-yr-old cultivar cv. Harrison plants. Five plants of each cultivar were inoculated monthly beginning 6 March. At both sites, each plant to be inoculated was wounded three times by removing a tangential section of stem tissue approximately 1 × 2 cm to expose the vascular tissue. Each wound site was covered with cheesecloth soaked in a suspension containing conidia and some mycelium from three isolates of *B. dothidea*. The suspension was obtained by flooding four 2-wk-old oatmeal agar cultures of each isolate with 10 ml of deionized water per culture and scraping the surface of the plate with a razor blade before grinding the mixture in a mortar and pestle and straining it through several layers of cheesecloth. Inoculation sites were sealed with Parafilm overlaid with aluminum foil for a 2-wk period, after which time the covers were removed. The inoculated and noninoculated plants (the balance of the healthy appearing plants at each site) were observed weekly for appearance of stem blight symptoms, and isolations were made from symptomatic plants to confirm the presence of the fungus.

Sites of infection. Sites of natural infection were determined in blueberry fields in Duplin County in 1985 and 1986 by removing the bark of symptomatic plants to reveal the extent of tissue discoloration or necrosis. The point of entry of the pathogen was assumed to be at a wound or break in the epidermis or bark of the plant surrounded by the most severely discolored or necrotic tissue, since *B. dothidea* requires a wound for typical symptom development to occur in blueberry (1). The site of entry was recorded as being at a pruning wound, a growth crack or other injury, or a bud (on the stem of the original rooted cutting) existing belowground as a result of planting or as being of undetermined location. The percent natural infection of pruning wounds by *B. dothidea* was also determined by isolating from 100 symptomless pruning stubs taken from apparently healthy Bluechip plants in Craven and Duplin counties in March 1986; pruning is usually done in January and February.

Spore release. Spore traps were used to study the release of inoculum of *B. dothidea* from infected blueberry stems. Spore release data were obtained in Duplin and Craven counties in 1985 and 1986 by placing rainwater spore traps beneath the branches of Croatan bushes heavily infected with *B. dothidea*. The traps consisted of 500-ml plastic bottles mounted under a 10.5-cm-diameter plastic funnel lined with Saran cloth. Spore germination was prevented by placing 10 ml of 5% copper sulfate solution in each bottle at the beginning of the trapping period. Six traps were maintained at each of the two sites, and bottles were changed weekly from 26 April 1985 to 20 September 1985 and from 2 February 1986 to 20 August 1986 in Craven County and from 5 April 1985 to 20 August 1986 in Duplin County. Adequate spore counts were assured in Duplin County by placing 10–15 diseased stem segments (5–10 cm long) in the funnels of six additional traps at the beginning of the trapping season. Spore concentrations in rainwater were determined as described by Sutton (4). Total amount of water accumulating in each trap was measured in 1985. It was then determined that an average of 9.7 ml of water accumulated for each millimeter of rainfall, and this factor was used in calculating total spore catch for each trap in 1986.

Daily temperature and humidity were recorded with a hygrothermograph in a standard U.S. Weather Bureau instrument shelter at each location, and total accumulated rainfall in millimeters was measured weekly.

Analysis of data. Meteorological factors were examined for their relationship to spore production and release by simple correlation analysis. Factors used in the analysis were conidial concentration in trapped water (SPORES), natural log of total number of conidia (LOGSPORES), mean temperature (MEANTEMP), maximum temperature (MAXTEMP), minimum temperature (MINTEMP), mean relative humidity (MEANRH), maximum relative humidity (MAXRH), minimum relative humidity (MINRH), and millimeters of rainfall (RAIN). For each meteorological variable listed above, variables were created to represent the values for that variable for the previous week (e.g., RAIN1) and for 2, 3, and 4 wk past (e.g., RAIN2, RAIN3, and RAIN4). These variables were also included in the correlation analysis of inoculum release and disease incidence data. Disease incidence data recorded as percentages were transformed where appropriate by the transformation square root of $(n + 0.5)$, where n = percentage of plants showing symptoms of stem blight. Time of infection data was analyzed by simple correlation analysis comparison of disease incidence data to meteorological variables and spore count for the first week of each 2-wk exposure period and for the 2-wk exposure period means for these values.

RESULTS

Time of infection. Plants wounded and exposed to natural inoculum for 2-wk periods developed stem blight symptoms after each exposure period. The greatest number of plants from which *B. dothidea* was isolated had been exposed to inoculum during April and June in 1985 and during June and July in 1986 (Fig. 1). Percent infection ranged from 0 to 67 ($\bar{x}$ = 31) for Croatan and from 8 to 83 ($\bar{x}$ = 50) for Bluechip in 1985. In 1985, infection for both cultivars was least during the period ending 10 May (Fig. 2A). Infection in 1986 ($\bar{x}$ = 67%) ranged from 40% in the period ending 3 May to 87% in each of the periods ending 14 and 28 June (Fig. 2B). Control plants had no stem blight symptoms.

In 1985, neither spore count variables (spores per milliliter of rainwater and total spore count) nor meteorological variables were positively correlated with the mean values of percent infection occurring in the two cultivars. Disease occurrence in 1986 was, however, positively correlated to LOGSPORES, MINTEMP, and MEANTEMP values for the first week of each exposure period ($P < 0.05$) (Table 1). Percent infection data were not positively correlated to the 2-wk exposure period mean for spore count and meteorological variables.

Incubation periods. The time required for symptoms to be expressed after inoculation of Harrison plants in Craven County was approximately 3 wk when plants were inoculated on 2 April and 28 May 1986. No symptoms developed on plants inoculated on 6 March, 30 April, or 24 June. The percentage of plants showing symptoms of stem blight after inoculation on 2 April and 28 May was 40 and 100, respectively. Stem blight symptoms appeared on 100% of Bluechip plants inoculated on 28 May, with an incubation period of 2 wk.

Relatively few of the noninoculated plants observed for disease development became infected with *B. dothidea* in Craven County in 1986. The percentages of noninoculated Bluechip plants becoming symptomatic on 14 May, 28 May, 11 June, 2 July, and 20 August were 8.0, 2.5, 1.0, 2.0, and 0.5, respectively. *B. dothidea* was isolated from 64% of the plants showing symptoms. The percentage of the 61 noninoculated Harrison plants showing symptoms on 16 June and 2 July was 4.8 for each date, with *B. dothidea* being isolated from all symptomatic plants. Symptom appearance in Bluechip plants was not correlated with total spore count, or with spores per milliliter of rainwater, or with any of the meteorological parameters examined. Correlation of symptom appearance of Harrison plants with meteorological data and spore counts was not attempted.

Table 1. Correlation of spore count and meteorological variables to disease incidence in plants exposed to natural inoculum in Craven County in 1986

Variable[a]	Correlation coefficient[b]
SPORES	0.39 ns
LOGSPORES	0.59*
RAIN	0.47 ns
MAXTEMP	0.50 ns
MINTEMP	0.58*
MEANTEMP	0.62*
MAXRH	0.50 ns
MINRH	0.46 ns
MEANRH	0.51 ns

[a] All variables were analyzed as 1-wk averages. SPORES = Concentration of conidia of *Botryosphaeria dothidea* per milliliter of rainwater in spore traps, LOGSPORES = natural log of total number of conidia per milliliter, RAIN = millimeters of rainfall, MAXTEMP = maximum temperature, MINTEMP = minimum temperature, MEANTEMP = mean temperature, MAXRH = maximum relative humidity, MINRH = minimum relative humidity, MEANRH = mean relative humidity. Time of infection data were analyzed by simple correlation analysis comparison of disease incidence data to meteorological variables and spore count for the first week of each 2-wk exposure period means for these values.

[b] * = Significant at $P = 0.05$, ns = not significant.

The greatest number of plants infected in Duplin County resulted from inoculations on 7 April (Table 2). The mean incubation time for the four inoculation dates on which infection occurred in Duplin County was 42 days, with the shortest period occurring after inoculation on 5 May and the longest occurring after inoculation on 7 March. No symptoms developed on plants inoculated in July at sites A and B. Stem blight symptoms were more numerous in the noninoculated Bluechip plants at sites A and B in Duplin County than in Craven County. Stem blight symptom appearance in noninoculated plants was maximum in Duplin County during May ($\bar{x}$ = 9 and 4% at sites A and B, respectively) and July ($\bar{x}$ = 2 and 4% at sites A and B, respectively). Incidence of symptoms on individual observation dates ranged from 0.5 to 18.3%. The pathogen was isolated from 82 and 65% of the plants showing symptoms at sites A and B, respectively. Attempts to correlate the mean disease incidence values for sites A and B to meteorological parameters for the week in which the symptoms appeared and to variables for 1, 2, 3, 4, and 5 wk previous to symptom appearance produced no significantly positive correlations.

Sites of infection. A total of 84 and 103 diseased plants were examined during 1985 and 1986, respectively. Pruning wounds made between the 1984 and 1985 growing seasons accounted for 61 and 68% of apparent infection sites in 1985 and 1986, respectively. The other categories of apparent infection sites (growth cracks or injuries, belowground buds, and undetermined) accounted for 15, 11, and 13% of infection sites, respectively, during 1985 and for 27, 0, and 5% of infection sites, respectively, in 1986.

Isolation of *B. dothidea* from pruning stubs from apparently healthy plants revealed 20 and 13% infection in samples from Duplin County and Craven County, respectively.

Spore release. At the Craven County site (Figs. 2A and B), spore concentration ranged from 0 to 181 conidia per milliliter of rainwater and total catch per week

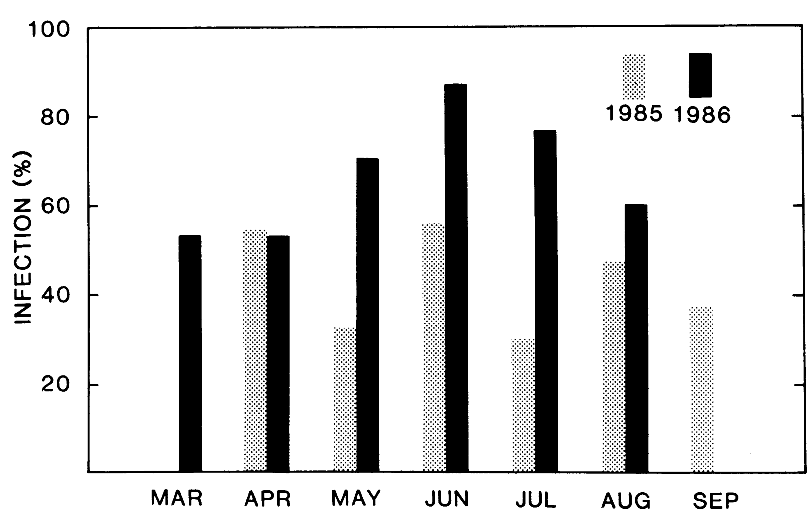

Fig. 1. Infection of blueberry exposed to natural inoculum of *Botryosphaeria dothidea* for 2-wk periods during 1985 and 1986.

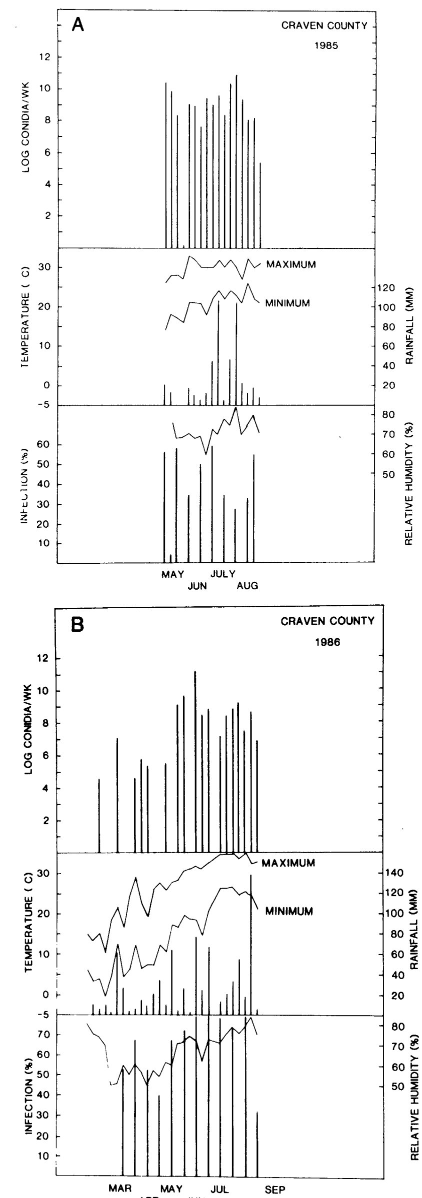

Fig. 2. Mean weekly maximum air temperatures, mean relative humidity, total rainfall, percent disease incidence of blueberry exposed to natural inoculum for 2-wk periods, and $\log_e$ of total number of conidia of *Botryosphaeria dothidea* collected in rainwater traps in Craven County, NC, in **(A)** 1985 and **(B)** 1986. Data on the number of conidia represent the means of six spore traps.

ranged from 0 to more than 60,000 conidia. Spore counts remained at relatively constant levels throughout most of the 1985 trapping period (Fig. 2A) and from late May to August 1986 (Fig. 2B). Counts were extremely low from 5 February to 14 May in 1986. Analysis of spore count data for 1985 and 1986 for the Craven County site (Table 3) revealed no significant correlations of spore concentration to the variables tested. Log_e of total spore catch was positively correlated ($P = 0.5$) to RAIN, MAXTEMP, MINTEMP, MEANTEMP, MAXRH, MINRH, and MEANRH in 1986.

Highest spore concentrations occurred during June and July in 1985 and during May and June of 1986 (Fig. 3) in Duplin County. Total spore counts were greatest in July in 1985 and in May and July in 1986. Periods of spore release in baited and nonbaited traps in Duplin County were similar during the first 5 wk in 1986, but the numbers of spores in nonbaited traps each week were extremely low (0.7, 0.0, 0.1, 2.0, and 0.6 conidia/ml) when compared with baited traps (10.7, 0.0, 12.8, 6.8, and 1.2). Therefore, only counts from baited traps were measured for the remaining 15 wk. Both concentration and total count fell to very low levels in the December–February period and in April 1986.

Significantly positive correlation of all temperature variables to spore concentration and log_e of total spore count was noted for Duplin County (Table 4) in both years. Log_e of total spore count in 1986 was also correlated to RAIN and three RH variables. In almost every instance, the previous week's values for the temperature variables were more strongly correlated to spore count variables than the values for the current week. Conidial concentration was positively correlated to MEANRH1 but not MEANRH in 1985 and 1986.

DISCUSSION

In 1986, percent infection of plants exposed to natural inoculum was greatest for the two exposure periods occurring during the 4 wk of greatest spore catch (Fig. 2B), hence the positive correlations to LOGSPORES. Poor correlation of infection with spore trap catch in 1985 and weak correlation in 1986 may have resulted because conditions that favor spore release may not necessarily be favorable for infection. Large numbers of spores may be released during heavy rains, but light rain may be more conducive to spore deposition and infection. Incubation times varied substantially at both the Craven County and Duplin County sites. If the mean incubation time of 42 days at the Duplin County site is used as an estimate, then a majority of plants that became symptomatic during the season were probably infected in March and April. By contrast, Weaver (6) found that the greatest number of infections of peach branches by conidia of *B. dothidea* from diseased wood occurred in July. He attributed this to favorable temperatures for germination of conidia and mycelial growth during July.

Our results suggest that many infections at both sites likely occurred in March and April of 1986, during which time maximum temperatures were within the range for optimum germination and germ tube growth for conidia of *B.*

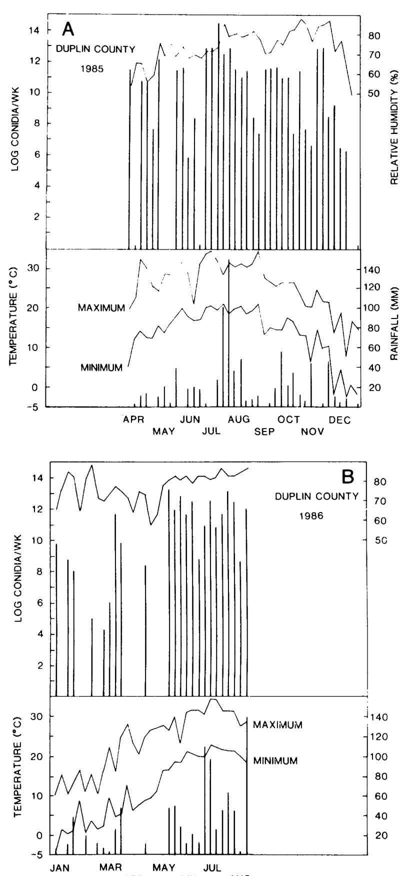

Fig. 3. Mean weekly maximum and minimum air temperatures, mean relative humidity, total rainfall, and log_e of total number of conidia of *Botryosphaeria dothidea* collected in rainwater traps in Duplin County, NC, in **(A)** 1985 and **(B)** 1986. Data on the number of conidia represent the means of six spore traps.

Table 2. Percent infection of Bluechip plants inoculated with *Botryosphaeria dothidea* or not inoculated in Duplin County in 1986

	Inoculated			Noninoculated	
Planting site	Date[a]	Infection[b] (%)	Incubation period[c] (days)	Observation period[d]	Infection (%)
A	7 March	14	45	March	0.0
B	7 March	38	99	March	0.0
A	7 April	75	30	April	0.2
B	7 April	88	74	April	0.1
A	5 May	25	7	May	8.7
B	5 May	63	23	May	4.2
A	2 June	38	26	June	0.6
B	2 June	50	35	June	0.2
A	1 July	0	···	July	1.6
B	1 July	0	···	July	4.3

[a] Eight plants were inoculated on each date with a composite conidial suspension of three isolates of *B. dothidea.*
[b] Percentage of eight plants showing stem blight symptoms by 1 October.
[c] Number of days required for first symptoms to appear after inoculation with *B. dothidea.*
[d] Month during which symptoms appeared; each month, 115 plants at site A and 172 plants at site B were examined.

Table 3. Correlation of meteorological variables to concentration of conidia of *Botryosphaeria dothidea* and natural log of total conidia trapped in rainwater in Craven County, North Carolina, in 1985 and 1986

	Pearson correlation coefficients[b]			
	Conidia/ml rainwater		Log_e (total conidia)	
Variable[a]	1985 coeff.	1986 coeff.	1985 coeff.	1986 coeff.
RAIN	0.02 ns	0.01 ns	0.46 ns	0.39*
MAXTEMP	−0.39 ns	0.29 ns	0.22 ns	0.69***
MINTEMP	−0.30 ns	0.33 ns	0.27 ns	0.77***
MEANTEMP	0.01 ns	0.32 ns	0.37 ns	0.77***
MAXRH	0.10 ns	0.27 ns	0.08 ns	0.40*
MINRH	0.36 ns	0.14 ns	0.33 ns	0.41*
MEANRH	0.35 ns	0.22 ns	0.29 ns	0.38*

[a] All variables were analyzed as 1-wk averages. RAIN = millimeters of rainfall, MAXTEMP = maximum temperature, MINTEMP = minimum temperature, MEANTEMP = mean temperature, MAXRH = maximum relative humidity, MINRH = minimum relative humidity, MEANRH = mean relative humidity.
[b] * = Significant at $P = 0.05$, *** = significant at $P = 0.001$, ns = not significant.

Table 4. Correlation of meteorological variables to concentration of conidia of *Botryosphaeria dothidea* and natural log of total conidia trapped in rainwater in Duplin County, North Carolina, in 1985 and 1986

	Pearson correlation coefficients[b]			
	Conidia/ml rainwater		Log_e (total conidia)	
Variable[a]	1985 coeff.	1986 coeff.	1985 coeff.	1986 coeff.
RAIN	0.18 ns	0.16 ns	0.22 ns	0.61**
MAXTEMP	0.31 ns	0.35*	0.46**	0.48**
MAXTEMP1	0.52***	0.44**	0.33*	0.50**
MINTEMP	0.44**	0.47*	0.37*	0.57***
MINTEMP1	0.45**	0.54*	0.42**	0.67***
MEANTEMP	0.40**	0.41*	0.53***	0.51**
MEANTEMP1	0.44**	0.51**	0.29 ns	0.59**
MAXRH	0.18 ns	0.22 ns	−0.04 ns	0.17 ns
MINRH	0.22 ns	0.09 ns	−0.04 ns	0.24 ns
MINRH1	−0.14 ns	0.21 ns	−0.13 ns	0.46**
MEANRH	0.21 ns	0.30 ns	−0.02 ns	0.63**
MEANRH1	0.34 ns	0.38 *	0.12 ns	0.59***

[a] All variables were analyzed as 1-wk averages. RAIN = millimeters of rainfall, MAXTEMP = maximum temperature, MAXTEMP1 = maximum temperature of previous week, MINTEMP = minimum temperature, MINTEMP1 = minimum temperature of previous week, MEANTEMP = mean temperature, MEANTEMP1 = mean temperature of previous week, MAXRH = maximum relative humidity, MINRH = minimum relative humidity, MINRH1 = minimum relative humidity for previous week, MEANRH = mean relative humidity, MEANRH1 = minimum relative humidity of previous week.

[b] * = Significant at $P = 0.05$, ** = significant at $P = 0.01$, *** = significant at $P = 0.001$, ns = not significant.

dothidea (6). However, the presence of infected but symptomless pruning stubs in March at both locations implies that some infections may have occurred earlier in the year or at the time of pruning in late fall or winter. Pruning wounds appeared to be the primary site of entry for the pathogen for most naturally occurring infections in both 1985 and 1986. In North Carolina, highbush blueberry cultivars are generally pruned heavily from late November to February during the first few years of field growth to shape the plant and remove excess flower buds. A delay or elimination of nonessential pruning operations for susceptible cultivars and a delay in pruning until times of low inoculum, i.e., during the coldest and driest months of winter, might reduce the number of infections occurring each year. Also, previous studies (1) showed a general trend toward fewer infections occurring with increasing wound age, which probably reflects a wound healing response. Schreiber (3) found that pruning wounds on *Rhododendron* decreased in susceptibility to *B. dothidea* over a period of 8 wk.

Conidia of *B. dothidea* were present in rainwater traps throughout the growing season at both locations. Weaver (6) found that rainfall was responsible for the release and dispersal of conidia of *B. dothidea* from diseased peach limbs. Sutton (4) demonstrated that average and maximum temperature variables relating to rainfall amount and duration were positively correlated to the numbers of conidia of *B. dothidea* caught in rainwater traps in apple orchard sites. In our study, the amount of rainfall was also generally correlated with total spore catch but not with concentration of spores in the trap water. The lack of correlation of rainfall to spore concentration probably results from dilution with continuing rainfall. Although duration of rainfall was not recorded, this factor could be as important as absolute amount. The positive correlation of temperature variables to spore catch at the Duplin County site agrees with the findings of Sutton (4). Values of meteorological variables for the previous week were more strongly correlated to spore release in Duplin County than were values for the current week. Additional in-depth studies will be required to determine the exact role of temperature and relative humidity on spore maturation, release, and infection of blueberry by *B. dothidea*.

In general, the use of fungicides for stem blight control has proved to be ineffective when applied from bloom to the start of leaf fall (Milholland, *unpublished*). The ineffectiveness of fungicides probably reflects the fact that inoculum of *B. dothidea* is present most of the year. Stem blight infections occur through pruning wounds and damage from mechanical harvesting during a large part of the season, and a single infection can kill the plant.

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