Inoculum Availability and Pathogenic Variation in *Botryosphaeria dothidea* in Apple Production Areas of North Carolina

T. B. SUTTON, Department of Plant Pathology, and J. V. BOYNE, Department of Entomology, North Carolina State University, Raleigh 27650

ABSTRACT

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Ascospores and conidia of *Botryosphaeria dothidea* were trapped in rainwater at four orchard monitoring sites in the mountains and Piedmont of North Carolina in 1976 and 1977. The relative proportion of *B. dothidea* ascospores and conidia trapped in rainwater varied by location and growing season. All isolates collected from wood or rotting fruit were pathogenic on apple fruit. Isolates of *B. dothidea* collected within various production areas in the Piedmont and mountains of North Carolina varied in pathogenicity, but the amount of variation generally was consistent from location to location. White rot disease of apple fruit may be more severe in Piedmont growing areas than mountain growing areas because of the warmer mean temperatures during the growing season or prior to harvest.

White rot, caused by *Botryosphaeria dothidea* (Moug. ex Fr.) Ces. et de Not. (imperfect stage *Dothiorella* sp.), is one of the most important diseases of apple fruit in the southeastern United States. Ascospores and conidia of *B. dothidea*, produced in perithecia and pycnidia in dead wood, are released during rain throughout the growing season and are dispersed in rainwater or by wind (7). Fruit infection can occur any time during the growing season, but fruit rot symptoms are most common just prior to harvest. In North Carolina, white rot is more severe in orchards in the lower elevation Piedmont growing areas than in the mountain growing areas.

Isolates of *B. dothidea* vary in morphology and pathogenicity. Fulkerson (2,3) found that isolates grown on potato-dextrose agar (PDA) could be grouped into six classes based on mycelial pigmentation and five classes based on mycelial morphology. Isolates also varied in their ability to produce pycnidia in culture. Forty-seven of the 48 isolates that Fulkerson collected from various hosts rotted apple fruit, but only 35 isolates caused a rapid rot. Most isolates induced more rot at 23.8 or 18.3 C than at 29.4 or 12.8 C (2,3). However, Kohn and Hendrix (5) found that rot developed most rapidly at 26–32 C.

This study was undertaken to characterize variation in inoculum availability, spore dispersal, and pathogenicity of *B. dothidea* in North Carolina.

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MATERIALS AND METHODS

Inoculum production and release. Spore production and release were studied during 1976 and 1977 at orchards in Lincoln County (Piedmont, 371-m elevation), Alexander County (Piedmont, 420-m elevation), Henderson County (mountains, 877-m elevation), and Mitchell County (mountains, 792-m elevation). Naturally infected 1-yr-old apple branches (prunings) were collected at each site from piles of prunings around the orchard's perimeter and placed in cages measuring 30.5 × 30.5 cm, constructed with 5.08-cm mesh wire and supported on legs about 30 cm above the ground. To ensure that representative samples of colonized prunings were obtained, prunings were removed from three to five areas of large brush piles or from at least three small brush piles in each orchard. There was only one large brush pile at the Mitchell County site. Prunings were selected with darkened papulate bark, which is characteristic of apple bark colonized by *B. dothidea* or *Physalospora obtusa* (Schw.) Cke.

One cage and trap was located at each site. Cages were loosely filled so that rainwater could drip freely from the branches. A portion of the rainwater dripping from the prunings was collected beneath each cage with a 10.5-cm-diameter funnel inserted in a 400-ml plastic bottle. Ten milliliters of a 5% copper sulfate solution was placed in each bottle to prevent spore germination. Bottles were changed weekly when it rained. The number of spores in each bottle was determined by filtering a 1- or 5-ml sample through a 25-mm-diameter (1.2 μm pore size) gridded filter and counting three grids at random. The numbers of *P. obtusa* ascospores and conidia trapped in the rainwater were used as references to compare the numbers of *B. dothidea* ascospores and conidia trapped. *P. obtusa*, cause of apple black rot, is a common inhabitant of dead apple wood and its spores are dispersed similarly to those of *B. dothidea* (7).

In 1976, traps were monitored from 3 March to 31 August at the Lincoln County orchard, 18 March to 31 August at the Alexander County orchard, 17 March to 1 September at the Henderson County orchard, and 22 March to 31 August at the Mitchell County orchard. In 1977, traps were monitored from 14 March to 9 September at the Lincoln county orchard, 4 March to 1 September at the Alexander County orchard, 28 March to 1 September at the Henderson County orchard, and 3 April to 27 June at the Mitchell County orchard. Temperature and relative humidity were measured at each site with a recording hygrothermograph located 1.5 m above the ground in a standard instrument shelter, and rainfall was measured with a fence-post rain gauge.

Isolates. In 1976, dead wood infected with *B. dothidea* was collected from a research orchard near Clayton, NC (eastern Piedmont); the Lincoln County monitoring site; the Alexander County site; and at the Mountain Horticultural Crops Research Station (MHCRS),

Fletcher, NC (mountains) and incubated in moist chambers in the laboratory for 2–3 days at room temperature. Cirrhi extruding from pycnidia were streaked across water agar plates and single spores were selected and transferred to PDA plates. One isolate was retained from each twig piece. The number of isolates obtained from the MHCRS, Clayton, Alexander County, and Lincoln County orchards was 31, 22, 20, and 8, respectively. An additional 25 isolates were obtained from rotting fruit collected from various orchards across the state.

In 1981, isolations were made from rotting fruit collected from a random group of orchards in Montgomery County (eastern Piedmont), Lincoln and Cleveland Counties (Piedmont), Henderson and Mitchell Counties (mountains), and from the orchard at Clayton. Twenty-five isolates were obtained from the Lincoln/Cleveland County area, 25 from Clayton, 22 from Montgomery County, and 17 from the Henderson/Mitchell County area.

All isolates collected produced the *Dothiorella* stage when grown on PDA or oatmeal agar under continuous light.

Pathogenicity studies. Pathogenicity studies were conducted on Golden Delicious apples in 1977 and 1981. Fruit were picked on 19 August 1976 and stored for about 5 mo at 2 C; in 1981, fruit were harvested on 8 November and stored at 5 C for 2 wk. Fruit were selected for uniform size and ripeness. Before inoculation, apples were washed with a mild detergent, rinsed in tap water, and surface-sterilized for 30 sec in a 1% NaOCl solution. Inoculations were made by cutting a shallow well (5 mm deep) in the side of the fruit midway between the blossom and stem end with a sterile 4-mm cork borer. *B. dothidea* isolates were grown on PDA, and mycelial plugs were cut from the margins of actively growing cultures with a 4-mm cork borer and inserted mycelial surface downward into the well. The 106 isolates collected in 1977 were used in the 1977 test; the 89 isolates collected in 1981 were used in the 1981 test. The wound was covered with a piece of masking tape in 1977. In 1981, the wound was covered with a small piece of sterile cotton that was moistened with sterile distilled water and held in place with masking tape. The inoculated fruit were placed on trays so that they did not touch one another, and the trays were enclosed in polyethylene bags. Fruit were incubated for 1 wk at 20, 25, and 30 C in 1977 and for 5 days at 25 and 30 C in 1981. Three fruit were used for each isolate-temperature combination. Uninoculated apples and apples inoculated with PDA

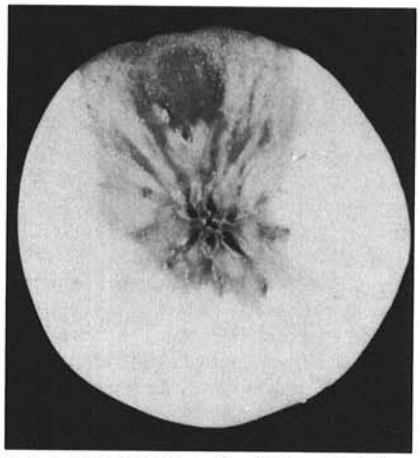

Fig. 1. Cross section of a Golden Delicious apple showing progression of *Botryosphaeria dothidea* from the point of inoculation to the core region.

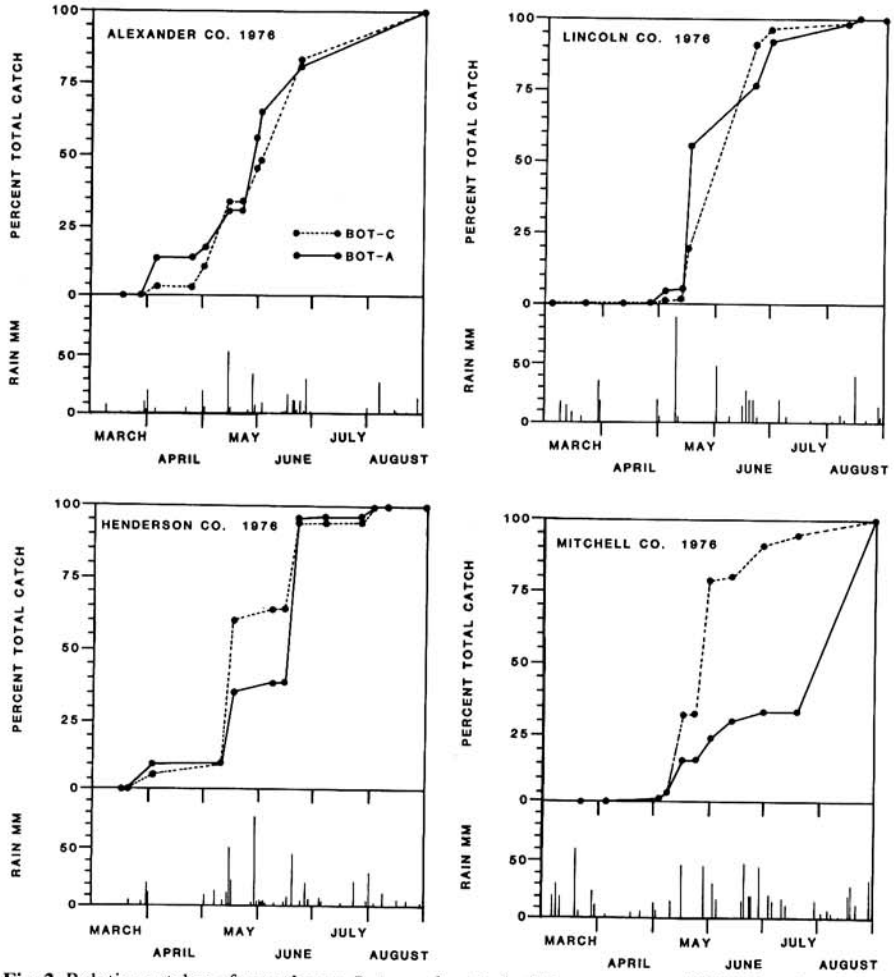

Fig. 2. Relative catches of waterborne *Botryosphaeria dothidea* ascospores (BOT-A) and conidia (BOT-C) and rainfall amounts from orchard sites in Alexander, Lincoln, Henderson, and Mitchell counties during 1976.

Table 1. Numbers and proportions of *Botryosphaeria dothidea* and *Physalospora obtusa* ascospores and conidia trapped during 1976 and 1977[a]

Location	Year	*B. dothidea*		*P. obtusa*	
		Ascospores	Conidia	Ascospores	Conidia
Mitchell	1976	5.9×10^4 (0.4)[b]	2.0×10^5 (1.3)	8.5×10^4 (0.6)	1.5×10^7 (97.7)
	1977	7.2×10^3 (0.2)	5.5×10^4 (1.8)	5.4×10^4 (1.8)	2.9×10^6 (96.2)
Henderson	1976	8.0×10^5 (21.8)	2.7×10^6 (73.7)	4.0×10^4 (1.1)	1.2×10^5 (3.4)
	1977	1.6×10^5 (20.2)	4.1×10^5 (53.7)	2.1×10^3 (0.3)	2.0×10^5 (25.8)
Alexander	1976	1.7×10^5 ((2.5)	1.1×10^6 (16.4)	1.1×10^6 (17.2)	4.2×10^6 (63.9)
	1977	3.0×10^5 ((5.9)	3.3×10^6 (63.6)	2.6×10^5 (0.6)	1.5×10^6 (29.9)
Lincoln	1976	1.6×10^6 ((3.6)	2.2×10^7 (48.5)	1.1×10^6 (2.5)	2.0×10^7 (45.4)
	1977	2.0×10^5 ((4.2)	7.7×10^5 (15.5)	1.9×10^5 (3.9)	3.7×10^6 (76.4)

[a]Totals from one trap at each location.
[b]Number of spores trapped followed by proportion (%) in parentheses.

plugs served as controls in both tests.

The amount of rot was determined by cutting the fruit radially through the inoculation well and estimating the percentage of the cross-sectional area affected. This method provided a satisfactory estimate of pathogenicity because of the nature of disease development in fruit. After infection, as a lesion begins to enlarge about the point of infection, a cylinder of necrotic tissue usually extends to the core region. As the disease progresses, the fungus invades the core and advances into the flesh of the fruit until the entire fruit is rotten (Fig. 1). In 1981, the Horsfall-Barratt system (4) was used to estimate the amount of rot. The ratings were transformed to percent infection for analysis.

RESULTS

Inoculum production and release. *B. dothidea* ascospores and conidia were trapped in rainwater at all sites in 1976 and 1977. The total number of spores trapped in rainwater during the season ranged from 7.72×10^5 at the Henderson County site in 1977 to 4.47×10^7 at the Lincoln County site in 1976 (Table 1). Proportionally fewer *B. dothidea* spores than *P. obtusa* spores were trapped at the Mitchell County site during both years. No consistent trend was observed at the other sites; the relative proportion of *B. dothidea* spores varied from year to year. To compare seasonal dispersal patterns, trap catches are presented as a percentage of the seasonal total catch (Figs. 2 and 3).

There were no distinct patterns or trends in the time of spore release associated with the various apple-growing regions in North Carolina in 1976 and 1977. At all locations, ascospores and conidia of both fungi were detected in water traps beneath the prunings following the first rain after the traps were established and were generally trapped throughout the season. Most spores were trapped from late March to early July at all locations. The pattern of spore catch of each spore type was closely related to the rainfall at a particular site. In 1976, April was very dry at all sites and significant spore catch was delayed until rain in May at the Lincoln, Henderson, and Mitchell County sites (Fig. 2). Another dry period occurred during July and early August at the Lincoln, Mitchell, and Alexander County sites, and few additional spores were trapped during the remainder of the sampling period. At Henderson County, abundant spores were trapped from early May until August, but only a few were trapped during light rains in August.

In 1977, spores were abundant during heavy rains in late March at the Lincoln and Mitchell County sites (Fig. 3). The Mitchell County data were not collected after 27 June because traps blew away during heavy thunderstorms in mid-July and mid-August. At the Lincoln County site, little rain occurred after early July and few spores were trapped during July and August. Very few spores were trapped after mid-July at the Henderson County site, although rainfall was abundant during August.

Pathogenicity tests. The rotten portion of fruit incubated at 20 C was generally darker in color and firmer than the rotten portion of fruit incubated at 25 or 30 C. At 30 C, inoculations with most isolates resulted in a light tan, watery rot. In the 1977 test, more rot ($P = 0.05$) occurred at 25 than 20 C in fruit inoculated with isolates from all locations, and more rot occurred at 30 than 25 C in fruit inoculated with isolates from all locations except Clayton (Table 2). At 30 C, most fruit had rotted after 1 wk. All isolates were pathogenic but varied in the amount of rot they induced (Table 2). There were significant differences between the amounts of rot caused by isolates collected within each location. Within each incubation temperature, there were differences in the mean amount of rot induced by isolates collected from each area; however, these differences were not

Table 2. Cross-sectional area (%) of Golden Delicious fruit rotted at 20, 25, or 30 C by isolates of *Botryosphaeria dothidea* collected in 1976

Source Location	Number of isolates	Cross-sectional area (%) rotted at 20 C Mean	20 C SD[x]	25 C Mean	25 C SD	30 C Mean	30 C SD
Isolates from wood							
Clayton	22	25.60 a[y]	6.99	86.28 a	16.05	88.63 b	17.25
Lincoln County	8	23.12 ab	3.39	58.75 c	25.40	96.45 a	2.26
Alexander County	20	27.16 a	7.42	63.66 c	20.08	94.33 a	5.83
MHCRS[z]	31	14.14 c	5.03	72.68 b	17.78	81.12 c	23.22
Isolates from fruit	25	21.72 b	9.81	77.34 b	18.03	90.03 ab	14.84

[x] Standard deviation about the mean.
[y] Means within columns with the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.
[z] Mountain Horticultural Crops Research Station.

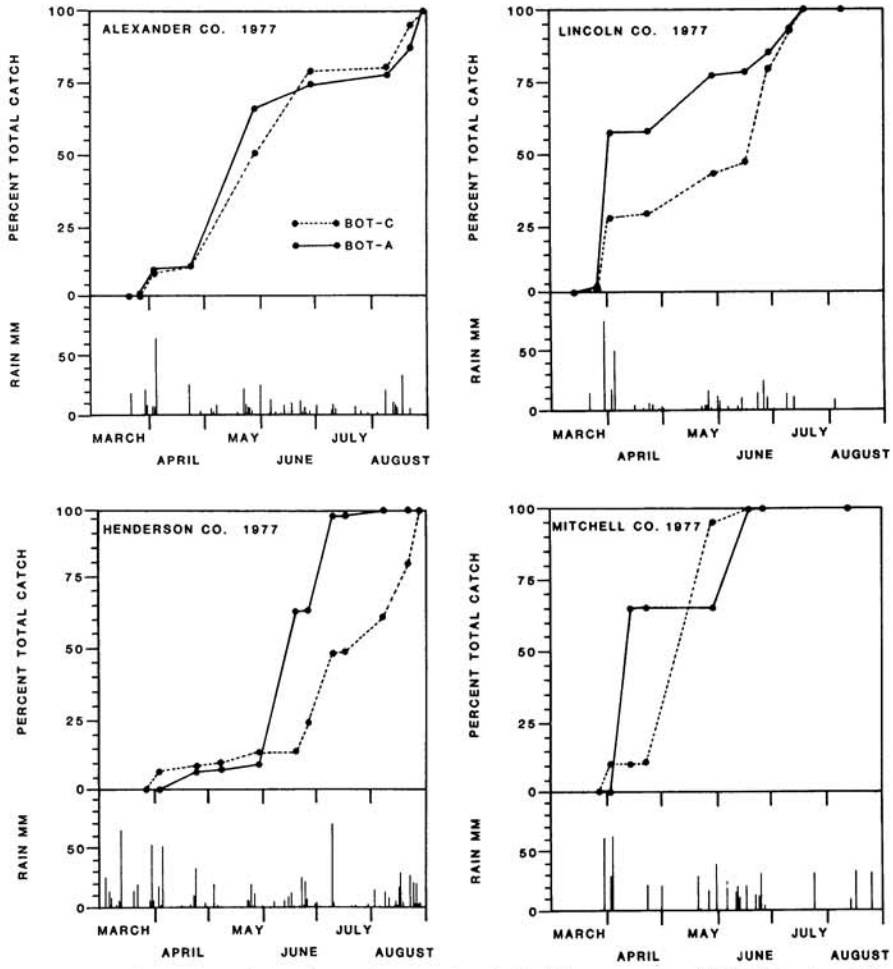

Fig. 3. Relative catches of waterborne *Botryosphaeria dothidea* ascospores (BOT-A) and conidia (BOT-C) and rainfall amounts from orchard sites in Alexander, Lincoln, Henderson, and Mitchell counties during 1977.

Table 3. Cross-sectional area (%) of Golden Delicious fruit rotted at 25 and 30 C by isolates of *Botryosphaeria dothidea* collected in 1981

Location	Number of isolates	Cross-sectional area (%) rotted at 25 C Mean	25 C SD[y]	30 C Mean	30 C SD
Lincoln/Cleveland	25	23.09 a[z]	21.69	74.69 a	30.70
Henderson/Mitchell	17	14.16 b	17.48	61.73 b	35.99
Clayton	25	14.96 b	11.97	69.02 ab	31.83
Montgomery	22	11.03 b	10.92	67.92 ab	34.51

[y]Standard deviation about the mean.
[z]Means within columns with the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

consistent between temperatures (Table 2). Isolates collected from dead apple wood were as pathogenic as those collected from fruit.

In the 1981 test, more rot ($P = 0.05$) occurred at 30 than 25 C at all locations. Isolates from the Lincoln/Cleveland County area caused the greatest amount of rot at both temperatures (Table 3). All isolates were pathogenic but varied in the amount of rot induced. There were significant differences ($P = 0.05$) in the amount of rot induced by isolates collected within each area except the Lincoln/Cleveland area.

DISCUSSION

Although spores of *B. dothidea* were trapped at only four orchard sites over 2 yr, the data suggest that the relative amount of inoculum present in orchards differs from year to year. Proportionally fewer *B. dothidea* spores were trapped at the Mitchell County area. No consistent differences in the relative number of *B. dothidea* spores were found between the Henderson County site and the two Piedmont locations although white rot is generally less prevalent in orchards in the Henderson County area.

B. dothidea ascospores and conidia were released during rain and were dispersed in rainwater from mid-March through August at all sites during both seasons. These data are consistent with previous results that showed that rainfall amount and duration were the most important factors in determining the time and number of spores released (7).

The pathogenicity tests conducted in this study confirm previous results that *B. dothidea* isolates vary in pathogenicity. Based on the fruit rot tests, the amount of variation was generally similar from location to location. Isolates obtained from infected dead wood were as pathogenic as those obtained from apple fruit.

The fruit rot test should provide a relative measure of pathogenicity of isolates because *B. dothidea* is primarily a wound pathogen. Sensitivity of the fruit rot test, however, is influenced by fruit ripeness. Although fruit were selected for uniform ripeness, undoubtedly some of the variation in pathogenicity of isolates can be attributed to differences in fruit ripeness.

The variation in pathogenicity of isolates to *B. dothidea* may not affect disease development in the orchard to any appreciable extent. All isolates were able to cause a fruit rot that would render the fruit unsuitable for market. Furthermore, any secondary inoculum produced on rotten fruit probably contributes little to the epidemic when compared with the copious quantities of inoculum produced in pycnidia and perithecia in dead wood.

It does not appear that the variation in the incidence of white rot between mountain and Piedmont growing areas of North Carolina is the result of inoculum availability or differences in the pathogenicity of *B. dothidea* isolates. Variation in environmental conditions between the two regions may be an important factor in determining white rot severity. Although the exact conditions favoring fruit infection have not been determined, Kohn and Hendrix (5) were unable to obtain fruit infection below 20 C. The mean temperatures for May through August in the Lincoln, Alexander, Mitchell, and Henderson County areas (based on NOAA Climatological Stations at Lincolnton, Wilkesboro, Celo, and Hendersonville) are about 22.6, 21.5, 17.9, and 22.9 C, respectively. Although the mean temperature in Mitchell County is only 3.5–5.0 C cooler than the other sites, this may be enough to influence the degree of infection.

The time of fruit ripening may also have an effect on disease incidence in a particular location. Kuć et al (6) have shown that apples become more susceptible to infection by *B. dothidea* as fruit mature. In the Piedmont counties, fruit ripen in mid-August to early September, whereas in the mountain counties, fruit ripen from early to late September. During the period of fruit ripening in the Piedmont counties, temperatures would be warm and favorable for infection. In the mountain counties, however, temperatures would have begun to cool and would be less favorable for infection. For example, in Lincoln County, the mean temperature for August is 24.1 C, compared with 18.7 C for September at the Henderson County area.

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