Natural Inoculation of Apple Buds by *Botryosphaeria obtusa*

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ABSTRACT

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Black rot, caused by *Botryosphaeria obtusa*, is a major disease of apples in Georgia. Inoculum was found on and in buds as early as October, and reached levels as high as 77% at the silver tip stage. Bud infestation was evenly distributed through the tree, and was less in trees that had been sprayed with fungicides in previous years than in trees which had not been sprayed. There were no differences in bud infestation levels among Red Delicious, Golden Delicious, Rome Beauty, and Detroit Red cultivars.

The black rot and frogeye leafspot disease of apple (*Malus domestica* Borkh.) caused by *Botryosphaeria obtusa* (Schw.) Shoemaker was first described in 1879 by Peck (3) in New York. While fruit losses of 25–50% occurred in the southern United States as early as 1912 (8), the economic importance of this disease was not fully realized in Georgia until 1951 when a survey by Taylor (5) revealed that in that year black rot had caused more loss of apples than all other pests combined.

Prior to 1955, *B. obtusa* was considered primarily a wound parasite. Halsted (1) and Sturgis (4) reported results from both field and laboratory inoculation tests showing that if the skin was not broken prior to inoculation, fruit would remain sound. This

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explained why lesions frequently surrounded insect and hail injuries.

Taylor (6) found that wounding was not a prerequisite for fruit infection, with blossom-end rot usually originating from early sepal infection. Taylor also proved that although rotting does not appear until the fruit begins to ripen, infections caused by *B. obtusa* could occur from bud break in the spring until harvest. Early fruit infection results in premature ripening and fruit often drop before the rot is evident on the surface.

Up to five sprays with captan are currently applied to apples from prepink to petalfall to control black rot in apples. Considering recent escalations in the cost of control with fungicides, proper timing of sprays becomes increasingly important. The following studies were designed to determine when inoculum arrives at the infection court, and which spore types serve as inoculum.

MATERIALS AND METHODS

Surveys were conducted in the apple production areas of central and north Georgia. Delicious-type cultivars Top Red and Starkrimson were sampled unless otherwise noted. Fifty buds per tree were randomly selected from each of four replicate trees. Buds were plated on natural (made from fresh potatoes) acidified potato-dextrose agar (APDA) and incubated at 26 C under cool-white fluorescent lights (45 $\mu E \cdot m^{-2} \cdot S^{-1}$) for 2 wk. Half of the buds were surface-sterilized by submersion in a solution containing 0.525% sodium hypochlorite and 10% ethanol for 3 min. Preliminary bimonthly bud surveys were conducted from October 1978 through April 1979, in five orchards in northern Georgia and two in central Georgia.

More extensive bud surveys were conducted from December 1981 through March 1982 in northern Georgia and from December through February in central Georgia. These were the periods with the greatest incidence of bud infestation during the preliminary study. Four replicate trees were selected from ten orchards in northern Georgia and four in central Georgia. Monthly bud surveys were also conducted from January 1982 through March 1982 in an abandoned orchard in Henderson County, North Carolina.

To assess the effect of control practices in previous years on infestation levels within the same orchard, a preliminary bud survey was conducted at both Gray (in central Georgia) and at the Mountain Experiment Station (Blairsville, in northern Georgia) from July 1979 through February 1980. Two blocks of 10 trees were selected at each orchard. The spray guide plot had received all of the fungicidal sprays recommended by the Georgia Extension Service from 1976 through 1980, whereas the check plot had received no fungicidal sprays. A total of 50 buds were randomly collected from the innermost eight trees in each plot at bimonthly intervals. A similar study was conducted at the Mountain Experiment Station at Blairsville during the same time interval. Twenty-five buds were tested on each sample date.

This study was expanded in 1981–1982 to determine both the effect of control practices and cultivar differences on infestation by *B. obtusa* levels in dormant buds within the same orchard. Thirty-two trees at the Mountain Experiment Station were selected and treated according to a randomized complete block design. The spray guide treatment had received all of the recommended fungicidal sprays for the previous 5 yr, whereas the check treatment had received only insecticides during that period. The cultivars used were Vance Delicious or Starkrimson Delicious, Golden Delicious, Detroit Red, and Rome Beauty. Buds were collected monthly from December 1981 to March 1982. Half of the buds were surface-sterilized prior to plating. The incidence of *B. obtusa* in unsterilized and surface-sterilized dormant buds over time was determined.

The remaining experiments were conducted to determine the inoculum spore type and the method of spore dispersal. A Kramer-Collins 7-day spore trap was maintained for 24 hr/day, all year at the Mountain Experiment Station, Blairsville, from 1978–1982. The trap was suspended at mid-canopy height, between two trees. Windblown spores were trapped on the surface of a spore tape coated with Vaseline. The tape was cut into 2-hr segments, and plated on APDA weekly. The hours during which spores of *B. obtusa* were collected were recorded.

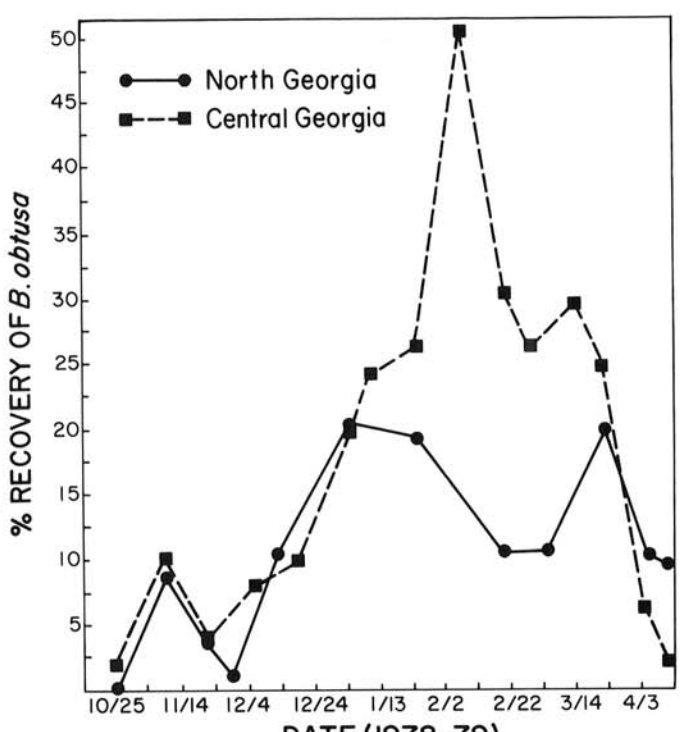

Fig. 1. Recovery of *Botryosphaeria obtusa* from unsterilized buds of Red Delicious apples from five orchards in northern Georgia and two orchards in central Georgia. Fishers least significant difference for central Georgia = 25, and for northern Georgia = 15.

RESULTS

B. obtusa was recovered from both surface-sterilized and unsterilized buds from all of the orchards sampled in the October 1978 to April 1979 preliminary survey. The incidence over time of *B. obtusa* on and within unsterilized dormant apple buds in selected orchards in northern and central Georgia is shown in Fig. 1. A separate composite curve is shown for each of the two areas. Each data point represents the mean of five orchards for northern Georgia or two for central Georgia.

The incidence of *B. obtusa* in unsterilized dormant buds was <2.5% for both northern and central Georgia in October. Infestation levels in both areas was <10% during November, but increased steadily throughout December. Thereafter, infestation levels for the two areas differ significantly. Bud infestation levels in north Georgia gradually declined throughout January and February. The incidence of bud infestation increased again in March when the buds reached the silver tip stage, with ~20% of the buds infested by the time they had reached the 12.7-mm (half-inch) green stage at the end of the month. This was followed by a sharp decline, through the tight cluster stage. Infestation was greater in central Georgia, reaching a peak in late February, then declining through early April.

In northern Georgia, the incidence of *B. obtusa* in the sterilized dormant buds was <5% from October through February. This was followed by a sharp rise in bud infestation by the 12.7-mm (half-inch) green stage in March. In April, bud infestation levels began a seasonal decline. In central Georgia, on the other hand, bud infestation levels rose throughout January and early February. The infestation level dropped rapidly during the rest of February, and continued to decline in March and April, with only 2% of the apples infected by 11 April.

Average bud infestation levels rose gradually throughout the study which began in December 1981 and ended in February 1982 in central Georgia and in March in northern Georgia. Buds in both areas had reached the silver tip stage by the end of the survey. Table 1 shows the mean incidence of both surface-sterilized and unsterilized buds infested with *B. obtusa* for the 10 northern Georgia and four central Georgia orchards. There was significant variation in the bud infestation levels from orchard to orchard each of the 3 mo. No *B. obtusa* was isolated from any of the buds from Henderson County, North Carolina, during January or March, but the fungus was isolated from 7 and 2% of unsterilized and surface-sterilized buds, respectively, during February.

In 1979, in central Georgia, no *B. obtusa* was found in unsterilized dormant buds from trees in the spray guide plot from August through September. Following current practices, no fungicides were applied from October to March. The incidence of *B. obtusa* increased throughout October, dropped during November, then rose again from December through February. Although no *B. obtusa* was found in unsterilized buds from trees in the check plot until the middle of September, the incidence of *B. obtusa* increased throughout September and October. It dropped during November, rose again from December through January, then decreased through mid-February.

The incidence of *B. obtusa* in 1979–1980 surface-sterilized dormant buds from both plots over the same time period was measured. *B. obtusa* was not found in surface-sterilized buds in the spray guide plot until October. The infestation level dropped at the beginning of November, began rising by the middle of that month, and continued to increase throughout December and January. No *B. obtusa* was found in surface-sterilized buds from the check plots until the middle of September. Infestation levels fluctuated below 8% through December, and then increased steadily until the end of January. No *B. obtusa* was found in unsterilized buds from trees in the spray guide plot at Blairsville from August through October. From November through March the incidence of *B. obtusa* fluctuated below 12%. Although no *B. obtusa* was found in unsterilized buds from trees in the check plot until September, the percent incidence of *B. obtusa* increased quickly throughout September and October. It dropped during November, but rose steadily through February and reached a new high by the beginning of March.

Infestation levels for the four cultivars were compared in 1981–1982 for both the spray guide and check treatments, unsterilized and surface-sterilized buds, within each date. A summary of these data is presented in Fig. 2 and Table 2. Statistically significant differences among cultivars were found only in unsterilized and surface-sterilized buds from the check block in March. Unsterilized buds of cultivar Rome Beauty had significantly higher infestation levels of *B. obtusa* than those of either cultivar Golden Delicious or Detroit Red. In the comparison between surface-sterilized buds for the same treatment and time period, the buds of Red Delicious had significantly higher infestation levels than those of Golden Delicious, but there was no significant difference between the infestation levels in the buds of Golden Delicious, Detroit Red, or Rome Beauty.

Spores were recovered from the trap during a total of 46, 94, 12, and 30 hr for the years 1978–1981. The majority of these spore releases did not coincide with periods of heavy bud infestation. In 1978, they occurred from 19 April to 30 August; in 1979 from 27 February to 6 December; in 1980 from 13 June to 20 August and in

TABLE 1. Incidence of *Botryosphaeria obtusa* in surface-sterilized and unsterilized buds of Red Delicious apples in 10 orchards in northern Georgia, four orchards in central Georgia, and one orchard in Henderson County, North Carolina

Location and month	Incidence of *B. obtusa* (%)[z] Sterile	Unsterile
Northern Georgia		
December	2 a	9 a
January	5 ab	16 b
February	7 b	26 c
March	14 c	26 c
Central Georgia		
December	22 a	42 a
January	20 a	50 a
February	29 a	64 b
Henderson County, NC		
January	0	0
February	2	7
March	0	0

[z]Data are means of four replicates with 25 buds per replicate. Mean separation is by Duncan's multiple range test ($P=0.05$). Data for northern and central Georgia were analyzed separately.

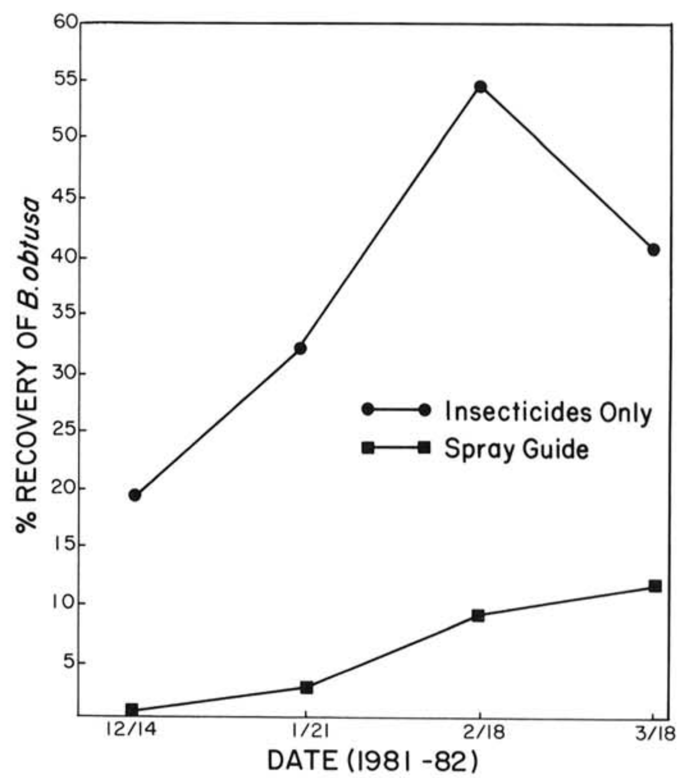

Fig. 2. Percent recovery of *Botryosphaeria obtusa* from unsterilized buds of Red Delicious, Golden Delicious, Detroit Red, and Rome Beauty apple trees in plots that received either the full recommended pesticide spray schedule (spray guide) or insecticides only in the preceding 5 yr. Differences between values at each data point are statistically significant, $P=0.05$.

TABLE 2. Percent incidence of *Botryosphaeria obtusa* in buds of four apple cultivars during the winter of 1981–1982 at the Mountain Experiment Station at Blairsville in northern Georgia

Cultivar	Unsterilized[a] Dec	Jan	Feb	Mar	Surface-sterilized Dec	Jan	Feb	Mar
Detroit Red	21 ax	31 ax	50 ax	28 bx	7 ax	1 ax	30 ay	22 aby
Golden Delicious	5 ax	25 axy	38 ay	30 bxy	6 ax	3 ax	37 ay	15 bxy
Red Delicious	32 ax	50 axy	72 ay	40 abxy	13 ay	10 ay	42 ax	39 ax
Rome Beauty	19 ax	22 ax	57 ay	63 ay	5 ax	15 axy	41 ayz	31 abz

[a]Within columns, means followed by a common letter (a and b) are not significantly different, $P=0.05$; within sterilization treatments and rows, means followed by a common letter (x, y, and z) are not significantly different, $P=0.05$, according to Duncan's multiple range test.

1981 from 16 February to 4 September. Thus, it is unlikely that windblown spores would serve as a major inoculum source in this area. Rainsplashed and insect dispersed conidia are probably the primary inoculum sources. To assess this hypothesis, four check blocks of Red Delicious trees were selected at Gray, GA, on 30 October 1978. Two replicate trees were sampled in each block. Ten twigs, bearing at least five buds each, were taken from each of nine loci on every tree. Samples were taken from the upper, mid-, and lower canopy levels in all four quadrants. There were no significant differences in the percentage of *B. obtusa* recovered from buds collected at the nine sampling locations.

DISCUSSION

Conidia, which appear to be the most important infective unit involved in apple black rot in Georgia, are produced on dead bark throughout the year. These spores occur in abundance in February and March and are released during rainy periods when temperatures are above 15 C (2). When pycnidia embedded in dead bark become watersoaked, and temperatures are above 15 C, conidia are forced through the ostiole onto the surface of the bark then are carried, possibly by rainsplash and insects, to the dormant buds. The incidence of *B. obtusa* in unsterilized apple buds over time indicates the periods of inoculum release and/or transport, whereas the incidence of *B. obtusa* in sterilized buds over time portrays the actual buildup of inoculum within the bud scales and possibly subsequent bud infection.

The second peak in the production of conidia in June is associated with twigs killed by fireblight in early spring (6). Spores of *B. obtusa* mature in pycnidia in these twigs in June. The even distribution of *B. obtusa* within a tree may be indicative of insect dispersal. It should be noted, however, that throughout this and subsequent experiments, rotted apples were also found in cone-shaped zones beneath dead wood in trees that had not been sprayed with fungicides. This distribution pattern is indicative of dispersal by rainsplash, and probably represents secondary infection during the summer.

Spore trap data collected in North Carolina implicate ascospores as the major infective unit, with major releases of ascospores during rainy periods from mid-March through May and of conidia from mid-March through August (7). In contrast to Georgia, infection by *B. obtusa* is usually not a problem until late in the season in North Carolina. This is supported by the low bud infestation levels recorded from the North Carolina orchard during our study. Major spore releases occur too late in the season to cause primary blossom infections.

Since conidia are spread only over short distances in Georgia, as indicated by the different levels of infestation in the fungicide and unsprayed plots which were side by side, the primary inoculum source for bud infestation must be located either within the canopy or near each tree. Thus, the level of bud infestation in any particular tree is dependent on the amount of dead wood in or near each tree and the application of protective fungicides. The presence of localized infection centers coupled with the limited dispersal of this fungus accounts for the high degree of variability in bud infestation levels from orchard to orchard in the same general area and even from tree to tree.

Sanitation and protective fungicides appear to be the key to black rot control. Twenty-five square millimeters of dead wood colonized by this fungus can yield 2×10^6 spores (6). Thus, unless a conscientious effort is made to remove the dead wood, the inoculum pressure in a tree can increase rapidly. Protective sprays cannot compensate for poor sanitation practices. Likewise, sanitation alone will not suffice as it is usually impossible to remove all of the dead wood.

Applications of protective fungicides are typically begun at the early pre-pink stage of bud development, which normally occurs at the beginning of April in North Georgia. This timing is based on the supposition that the tips of leaves and sepals are the first susceptible tissues exposed to infection (6). Our data suggest that prior to pre-pink stage, inoculum is getting inside the bud scales. This may mean that fungicides should be applied earlier.

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