Primary Infection of Apple Buds by *Botryosphaeria obtusa*

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ABSTRACT

Smith, M. B., and Hendrix, F. F., Jr. 1984. Primary infection of apple buds by *Botryosphaeria obtusa*. Plant Disease 68:707-709.

Infestation of apple buds with *Botryosphaeria obtusa* occurs during the winter in Georgia. Primary infection may occur as early as the silver-tip stage of bud phenology. This study indicates that application of one fungicide spray at silver-tip can replace the standard five sprays starting at the prepink stage of bud phenology.

Botryosphaeria obtusa (Schw.) Shoemaker (3), the cause of black rot and frogeye leafspot disease of apple (*Malus sylvestris* Mill. var. *domestica* (Borkh.)), caused fruit losses of 25–50% in the southern United States as early as 1912 (2,7). In 1951, this disease caused more losses to apples in Georgia than all other pests combined (5). Today, black rot is usually controlled by both proper sanitation and application of effective fungicides at 6- to 10-day intervals from prepink through petal fall.

The present spray schedule is based on the premise that infection by the black rot fungus does not occur until after the prepink stage of bud development. Taylor (6) proposed that the leaves and sepals were the first susceptible tissues exposed to infection, with blossom-end rot usually the result of early sepal infection. Since then, Beisel et al (1) have shown that, in Georgia, buds are frequently infested with the black rot fungus before bud swell.

Evidence is presented to show that black rot infection may occur as early as the silver-tip stage of bud development and that early-season infections may be controlled with a single timely application of an effective, long-lasting fungicide rather than the currently recommended series of sprays applied from early prepink through petal fall.

MATERIALS AND METHODS

Inoculum preparation. *Spore suspension. B. obtusa* was isolated from rotting apples, grown on acidified potato-dextrose agar (APDA) made from fresh potatoes, and purified via hyphal-tip transfers. The cultures were sealed with Parafilm and incubated at 26 C under soft-white fluorescent lights (45 μE m^{-2} s^{-1}) for 3 wk.

Fifteen to 20 plates were needed to make 10 L of a 10^6 conidia per milliliter spore suspension. Entire cultures were added to a 0.01% Tween suspension and macerated for 5 min in a Waring Blendor. The spore concentration was verified using a hemacytometer.

To test the pathogenicity of the inoculum, a portion of the spore suspension was used to inoculate apples. Sterilized dissecting needles were dipped into the inoculum and used to puncture an area of the apple. The apples were incubated at 26 C for 1 wk, resulting in 100% fruit rot. *B. obtusa* was reisolated from all of the rotted apples.

Twigs. Apple twigs measuring about 1 × 15 cm were placed on top of a wire screen in a glass jar. Water was added to the bottom of the jar, just below the level of the screen, and the jars were covered and autoclaved. The lid contained a section of plastic tubing filled with cotton. The twigs were inoculated with 10 ml of the spore suspension, resealed, and incubated at 26 C under soft-white fluorescent lights (45 μE m^{-2} s^{-1}) for 6 wk.

Sample processing. The stage of bud development and date of sampling were recorded for each sample. Samples were divided into thirds, with one-third untreated, one-third surface-sterilized, and one-third cut in half before surface-sterilization in a 0.525% sodium hypochlorite/10% EtOH solution for 2 min before plating on APDA. Before the tight-cluster bud development stage, buds were cut in half longitudinally. Once the tight-cluster stage was reached, each of the individual flowers was cut in half. The plates were incubated at 26 C under soft-white fluorescent lights (45 μE m^{-2} s^{-1}) for 2 wk. The percentages of buds from which *B. obtusa* was recovered were recorded.

When buds are surface-sterilized before plating, fungus beneath the bud scales appears to remain viable, thus the presence of live fungus is not necessarily indicative of bud infection. If buds were first cut in half longitudinally, the vaporizing sterilization solution could penetrate under the cut surfaces of the bud scales to kill the spores lodged between the scales. Any fungus remaining viable after this procedure would more likely be indicative of bud infection. Histological data are being collected to test this hypothesis and preliminary findings support this view.

Although the incidence of contamination by other organisms such as *Epicoccum, Pestalotia, Phomopsis, Phoma, Alternaria, Rhizopus, Penicillium,* and *Aspergillus* was high, especially in the untreated samples, it was still fairly easy to ascertain the presence of *B. obtusa* using the following criteria: 1) mycelial color and texture, 2) type and placement of fruiting bodies, and 3) spore characteristics such as size, color, and surface texture.

Bud inoculations. In order to pinpoint the first susceptible bud development stage, two Red Delicious trees at the University of Georgia horticulture farm in Athens were inoculated by spraying 10 L of a 10^6 conidia per milliliter spore suspension on the trees on 5 November 1981 and 1 January 1982 and then monitored. On 4 February 1982, 25 15-cm pieces of dead wood covered with pycnidia of *B. obtusa* were placed throughout the top of each tree. Beginning at bud swell and before silver-tip, trees were subjected to overhead irrigation whenever 2 days elapsed without rainfall.

Dormant buds were collected on 6 January and 4 February. Seventy-five bud samples were collected daily from the onset of the silver-tip stage on 4 March through one-half in green on 17 March.

Buds were collected on 8 March from uninoculated trees of the same Red Delicious cultivar located within 20 m of the inoculated trees. These trees were not subjected to overhead irrigation. Samples were processed as described previously.

Comparison of leaf buds, flower buds, and individual flowers at the tight-cluster stage. It is difficult to differentiate between leaf and flower buds before green-tip, thus a combination of leaf and flower buds was used in our experiments. To determine if infestation levels of *B. obtusa* varied with bud type, a comparison was made among leaf buds, flower buds, and individual flowers at the tight-cluster stage of bud development for the

This research was supported by SEA Grant 70-59-21311-2-105-2 and state Hatch funds allocated to the Georgia Experiment Station. Portion of a Ph.D. dissertation submitted by the first author.

Accepted for publication 21 February 1984.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

incidence of *B. obtusa*. Seventy-five samples of each type were collected from each of four trees in orchard 1 in central Georgia. The samples were processed as described previously.

Natural infection. In order to determine when infection of apple buds by *B. obtusa* occurred, natural infection was monitored in two central Georgia orchards where no fungicides were applied before petal fall. Seventy-five buds were collected from each of four Red Delicious trees at both orchards at monthly intervals from December 1981 through April 1982, with one exception.

Buds were accessible from only one of the four trees in orchard 1 during the March sampling. These trees were in a neglected orchard and consequently were very large, with fruiting wood restricted to the tips of twigs. A severe pruning before our March sampling rendered all but the inaccessible top twigs barren of fruiting wood. Apples were harvested from these trees and processed as described previously.

Spray tests. Spray tests were conducted at the Mountain Experiment Station at Blairsville, GA, in 1980 on Red Delicious, Golden Delicious, Detroit Red, and Rome Beauty trees. Each cultivar was subjected to the following three treatments: 1) a single application of captafol at 5 lb a.i./A at bud swell followed by the full recommended pesticide spray schedule (spray recommendations) after petal fall, 2) season-long spray recommendations with fungicide applications beginning at the early prepink stage of bud phenology, and 3) insecticides only (control). All fungicides were applied with an airblast sprayer. There were four replicates per treatment, each replicate containing two trees. Twenty-five apples were collected from each replicate on 1 June, on 10 August, and at harvest. Apples were surface-sterilized, the four sides removed with a sterile knife, the core with the attached calyx end sliced into sections, plated, and processed as described previously. Detroit Reds were harvested on 17 August, Golden and Red Delicious on 7 September, and Rome Beauty on 25 September.

An additional spray test was conducted at the same location during 1980 on a block of Red Delicious trees. The block was subdivided into the following four treatments: 1) two applications of captan 50WP at 2 lb a.i./A on 27 February and 20 March, 2) a single application of captan 50WP at 2 lb a.i./A on either 16 March or 3) 27 March, and 4) insecticides only (control). The normal spray recommendations were resumed after petal fall. There were four replicates per treatment, each replicate containing four trees. Twenty-five apples were collected from each replicate at harvest on 7 September and processed as described previously.

The Apple Spray Guide for North Georgia recommends application of captan 50WP at 4 lb/A at the following stages of bud development for control of primary black rot infections: early prepink, 1 wk after prepink, and sprays at 7- to 10-day intervals during bloom.

RESULTS

Bud inoculations. No *B. obtusa* was recovered from any of the cut and surface-sterilized buds from inoculated trees until the beginning of the silver-tip stage of bud development, when 4% of the buds yielded this fungus (Table 1). During green-tip, *B. obtusa* was recovered from 16–24% of the cut and surface-sterilized buds. At one-half in green, 10–24% of the cut and surface-sterilized buds yielded *B. obtusa*.

Buds collected from uninoculated trees of the same Red Delicious cultivar located within 20 m of the inoculated trees yielded significantly less *B. obtusa*. Whereas 8% of the untreated buds were infested with *B. obtusa*, none of the surface-sterilized or cut and surface-sterilized buds yielded this fungus.

Comparison of leaf buds, flower buds, and individual flowers at the tight-cluster stage. There were no statistically significant differences in the mean incidence of *B. obtusa* in untreated leaf buds, flower buds, and individual flowers. These levels were 92, 96, and 92%, respectively. Each flower bud develops into a cluster of five individual flowers. Because there were no statistically significant differences between the individual flowers and entire flower buds, all individual flowers within an infested cluster probably became infested. Only entire buds were used in our other experiments.

There were no significant differences in the mean incidence of *B. obtusa* between surface-sterilized or cut and surface-sterilized leaf and flower buds after silver-tip. *B. obtusa* was recovered from 84 and 90% of the surface-sterilized leaf and flower buds and from 88 and 90% of the cut and surface-sterilized leaf and flower buds. These data show that bud type apparently had no effect on the incidence of *B. obtusa*.

Natural infection. Fifty-two percent of the untreated dormant buds from orchard 1 were infested with *B. obtusa* as early as December (Table 2). Infestation levels rose throughout the next 3 mo, and 95% of the buds were infested by mid-March. The inoculum potential, using the amount of dead wood as a rough estimator, was not as high in the second orchard, where infestation levels increased from 37% in December to 80% by mid-March.

Significantly less *B. obtusa* was recovered from the surface-sterilized buds. Infestation levels increased from 46% in December to 88% by mid-March in orchard 1 and from 16% in December to 28% by mid-March in orchard 2. *B. obtusa* was not recovered from any of the cut and surface-sterilized samples until the tight-cluster stage, when 89 and 16% of the buds from orchards 1 and 2, respectively, were infested.

Spray tests. Fluctuations in the incidence of *B. obtusa* within treatments were probably due to the small sample size (25 apples per replicate with four replicates per treatment). Because the trends were the same in all four cultivars, data were combined so that overall trends could be seen more readily (Fig. 1). Overall, application of one captafol spray at bud swell provided as much protection against black rot as the five early-season applications of captan used in the spray-recommendation treatment. Both of these treatments provided significantly more protection than the control. Trees in the captafol and spray-recommendation plots were relatively free of frogeye leafspot and rotted fruit, whereas trees in

Table 1. Incidence of *Botryosphaeria obtusa* associated with apple buds at various stages of bud development in inoculated trees at the University of Georgia horticulture farm in Athens

Stage of development (%)[a]	Untreated	Surface-sterilized	Cut and surface-sterilized
Dormant			
6 Jan.	20	12	0
4 Feb.	48	28	0
Silver-tip			
4 Mar.	24	12	4
5 Mar.	20	12	12
6 Mar.	16	12	8
7 Mar.	48	20	12
8 Mar.	52	28	20
Green-tip			
9 Mar.	56	28	24
10 Mar.	64	32	20
11 Mar.	36	24	16
12 Mar.	28	26	20
One-half in green			
13 Mar.	28	20	20
14 Mar.	40	24	24
15 Mar.	40	16	16
17 Mar.	16	12	10

[a] Percentage of 25 buds per treatment per sample date.

Table 2. Incidence of *Botryosphaeria obtusa* associated with apple buds at various stages of bud development in two central Georgia orchards

Orchard	Sample treatment	Stage of development (%)[y] Dormant 9 Dec.	Dormant 26 Jan.	Silver-tip 24 Feb.	Tight-cluster 18 Mar.	Small apples 12 Apr.
1	Untreated	52 a	76 a	92 a	95 a	n/a
	Surface-sterilized	46 a	52 b	65 b	88 a	41
	Cut and surface-sterilized	0 b	0 c	0 c	89 a	n/a
2	Untreated	37 a	58 a	78 a	80[z]	n/a
	Surface-sterilized	16 b	12 b	18 b	28[z]	12
	Cut and surface-sterilized	0 c	0 c	0 c	16[z]	n/a

[y] Data are means of four replicates with 25 buds or apples per replicate. Mean separation is by Duncan's multiple range test ($P = 0.05$). Data for orchards 1 and 2 were analyzed separately. n/a = Not applicable. Small apples were only subjected to surface-sterilization.

[z] These values represent the mean of 25 buds.

the control plot showed extensive amounts of both frogeye leafspot and rotted apples.

The mean incidence of *B. obtusa* was 5% for Red Delicious trees sprayed with captan on both 27 February and 20 March, 1% for trees sprayed on 16 March, and 13.2% for trees sprayed on 27 March. Fifteen percent of the apples in the control plot were infected. (Fisher's least significant difference for these data at 0.05 = 5.8.) The mean incidence of *B. obtusa* was thus significantly lower in apples sprayed with captan before 20 March. The first heavy rain after silver-tip on these trees did not occur until the evening of 20 March, when more than 3 in. of rain was recorded. Before this point, less than 0.1 in. of rain was received on any single date while the buds were in the silver-tip stage.

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 are released in abundance during February and March (1). Our data indicate that primary infection may occur as early as silver-tip when sufficient moisture is present.

In North Carolina, however, ascospores have been implicated as the major infective unit, with major releases during rainy periods from mid-March through May and of conidia from mid-March through August (4). These spore releases occur too late in the season to cause primary blossom infections, and as a result, infection by *B. obtusa* is usually not a problem in North Carolina until later in the season.

Black rot is the primary fungal disease affecting apples early in the season in Georgia. Up to five captan sprays are currently applied to apples from prepink through petal fall to control this disease, with additional sprays applied toward the end of the season to control secondary infections. Because captan does not control the other early-season fungal diseases, additional fungicides, such as dodine (Cyprex) and ferbam, are applied for scab, rust or mildew control only when these diseases become a problem. A single application of a persistent fungicide such as captafol at bud swell appears to reduce the inoculum level as much as the five applications of captan from early prepink through petal fall. If rain is received between silver-tip and early prepink, the five currently recommended applications are begun after initial primary infections have occurred. Considering the cost of buying and applying fungicides, a reduction in early-season sprays applied to apples from five to one should result in significant economic savings for Georgia apple growers.

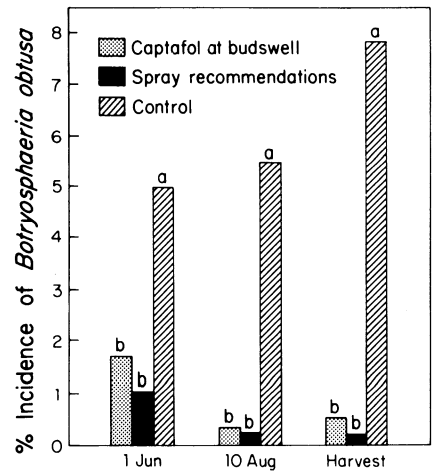

Fig. 1. Incidence of *Botryosphaeria obtusa* in apples on 1 June, on 10 August, and at harvest at Blairsville, GA. Data are means of 16 replicates with 25 apples per replicate. Mean separation is by Duncan's multiple range test ($P = 0.05$).

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