

Susceptibility of Apple Fruit to *Botryosphaeria dothidea* and Isolate Variation

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ABSTRACT

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The susceptibility of apple fruit, cultivar Golden Delicious, to *Botryosphaeria dothidea* was investigated by inoculating fruit in the orchard three times during the growing seasons of 2 successive yr. Immature and mature fruit were equally susceptible to *B. dothidea*; both required a 1- to 1.5-mo incubation period before symptom development. The time of infection of apple fruit in the orchard by *B. dothidea* was investigated by sampling apples at three locations throughout the season and monitoring disease development in the laboratory. This test demonstrated that fruit can become infected within 7 wk of petal fall, even though macroscopic symptoms do not occur until later. Variation in isolate aggressiveness was investigated with five isolates of *B. dothidea* collected from infected fruit and limb cankers of trees in four locations in North Carolina. Detached apple fruit were inoculated with the five isolates, and fruit sections from the inoculated regions were removed and examined for disease development over 1 mo. All isolates were pathogenic but varied in aggressiveness.

Infection by *Botryosphaeria dothidea* (Moug.: Fr.) Ces. & De Not. results in fruit rot (white rot or bot rot) and limb cankers on apples (*Malus* × *domestica* Borkh.) in warm growing regions of the world. The disease accounts for up to 50% fruit loss (11) and extensive limb loss in the southeastern United States. Recently, *B. dothidea* has been associated with limb cankers in the mid-Atlantic apple-growing region of the United States (J. Rytter, *personal communication*).

Although fruit rot symptoms often are not noticed until 6–8 wk before harvest (6), results from previous studies differ as to the time of initial infection. Eid (3) presented evidence that initial infections occur soon after the fruit are formed. He determined that fruit infection occurs before late May and that fruit are susceptible throughout the season. Other investigators found that infections are uncommon until the soluble-solids levels reach 10.5% (6). Several mechanisms of resistance of young fruit to rot have been suggested. Sitterly and Shay (9) proposed that low soluble-sugar con-

centrations provide resistance in immature fruit, and Hwang (5) suggested that concentrations of phenols, reducing sugars, fruit acids, and amino acids, or unspecified physiological or morphological alterations resulting from the interaction of these components, may be involved in the resistance of young fruit. Soluble solids are monitored by growers to determine fruit maturity, and a preventative spray program, based on application of benomyl or thiophanate-methyl, is initiated when the soluble-solids level reaches 10% (1,6).

The morphological and physiological characteristics of individual isolates of *B. dothidea* vary greatly on potato-dextrose agar: mycelium color ranges from white to yellow, gray, or olive (4,5); aerial mycelia may be sparse or abundant; and rates of mycelial growth and sporulation are different. These *in vitro* differences do not necessarily indicate variation in aggressiveness. If variation is present, however, it could influence the results of research, since controlled studies typically employ a limited number of isolates.

Differences in pathogenicity and virulence among isolates of *B. dothidea* have been reported on apple, but most of the work has been done on the development of stem and trunk cankers rather than on fruit rot (2,3). Latorre (7) studied differences in pathogenicity and virulence (expressed as the ability to rot fruit) among five isolates of *B. dothidea*. All were pathogenic but varied in viru-

lence. Sutton (12) also found that isolates varied in their ability to rot fruit (termed pathogenicity); however, isolates recovered from cankers were generally as pathogenic as those from apple fruit. No significant differences were found among collection locations; differences in disease severity among locations were attributed to environmental conditions rather than isolate variation. In both studies, lesion size was the only measure of variation among isolates.

The purpose of our study was to determine the time of fruit infection by *B. dothidea* and to compare the pathogenicity and aggressiveness of the isolates of *B. dothidea* used in our study.

MATERIALS AND METHODS

Orchard inoculations. Apple fruit were inoculated in the orchard to determine when the fruit became susceptible. The experiment was initiated each year at the conclusion of the June drop. In 1990, the experiment was conducted at the Mountain Horticultural Crops Research Station (MHCRS), Fletcher, North Carolina. On 18 June, 16 July, and 14 August, 25 fruit on each of six non-sprayed Golden Delicious trees were inoculated. Fruit on three of the trees were not wounded before inoculation; fruit on the other three trees were wounded. The wounds, 1–2 mm deep, were made with a tool made of five no. 1 insect pins pushed through a cork stopper.

Inoculum was prepared from 2–3-wk-old cultures of isolates 1501, 1502, CB1, CB2, SH4, SH6, and RFC of *B. dothidea* that had been grown on potato-dextrose agar under continuous fluorescent light and at room temperature (~22 C). Deionized water was poured into each petri dish, and the surface of the mycelial mat was scraped to remove the conidia. The resulting suspensions were mixed and blended for 15 sec, filtered through a double layer of cheesecloth, and the inoculum standardized to 1×10^5 conidia per milliliter. The area to be inoculated on each fruit was marked with a wax pencil. Fruit were inoculated by placing in each marked spot a 2.5- × 2.5-cm piece of four-ply laboratory towel that had

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been dipped in the conidial suspension. A 6- × 6-cm piece of aluminum foil was used to hold the towel in place and to prevent drying. Each fruit was wrapped with a second piece of aluminum foil. After 48 hr, the aluminum foil and the inoculum were removed, and the apples were left on the tree until harvest (17 September) or until symptoms developed in the inoculated area. Fruit symptomless at harvest were held at room temperature to allow latent infections to develop symptoms. After 2 wk at room temperature, fruit without symptoms were considered free of infection. Binomial data based on the presence or absence of disease were recorded.

The experiment was repeated in 1991 at the Central Crops Research Station (CCRS), Clayton, North Carolina, because of insufficient fruit set at MHCRS. Five Golden Delicious trees were selected, and 10 fruit on each tree were inoculated without wounding on 21 June, 15 July, and 21 August. The same inoculation procedures were followed as in 1990, but this time the fruit were harvested after 2 wk to avoid fruit loss by bitter rot caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. in Penz. A 2- × 2-cm portion of the inoculated area of each fruit was cut into nine pieces (each approximately 0.6 × 0.6 cm with 4 mm of underlying tissue), removed, and placed in a 52-mm-diameter petri dish lined with a moist paper towel. A piece of aluminum foil between the towel and the apple pieces limited the growth of mycelia between apple sections. The dishes were sealed with Parafilm and left at room temperature (~22 C) under continuous light until sporulation was visible on the fruit surface (usually 1 mo). Data were recorded as the number of pieces out of nine exhibiting sporulation. Soluble solids were recorded each week during both seasons by averaging refractometer readings from 10 fruit collected in the orchard.

During the inoculation period both years, the temperature under the aluminum foil wrap was measured every 15 min with a thermistor attached to a micrologger. On one fruit in each of the four quadrants of the tree, a thermistor was placed beneath a piece of wet towel, which was covered with the aluminum foil. A fifth thermistor monitored ambient temperature in the center of the tree.

In 1990, the experimental design was completely randomized. In 1991, it was a randomized complete block, with each of the five trees being a block. The two experiments were analyzed separately because of the different designs. Data were transformed with arcsine \sqrt{Y} before the analysis. The effect of inoculation date on disease was tested using tree(date) as the error term in both analyses (8). Means were calculated from nontransformed data.

Season-long survey of natural infection. In 1990, 20 asymptomatic apples of the cultivar Golden Delicious were harvested each week between 3 July and 31 August from a row (about 30 trees) of nonsprayed trees at the Sandhills Research Station (SRS), Jackson Springs, North Carolina. In the laboratory, the fruit were washed, their surfaces sterilized with a 0.53% NaOCl solution, rinsed with tap water, and allowed to air dry. Two sides of each apple were sectioned as described above, placed in petri dishes, sealed, and placed on a laboratory bench. Data were recorded as the number of pieces with pycnidia of *B. dothidea* out of 18 total pieces for each apple. The soluble solids were determined each week by averaging refractometer readings for 10 apples.

In 1991, Golden Delicious apples were sampled from nonsprayed trees at three locations in North Carolina: CCRS (10 June to 19 August), SRS (5 June to 28 August), and MHCRS (11 June to 13 September). At CCRS and MHCRS, artificially inoculated branches were placed in trees in three five-tree groups to ensure sufficient inoculum. Branch prunings (0.5–1 cm in diameter) were steam sterilized in a soil cart and inoculated on 5 March by spraying them with a suspension of *B. dothidea* conidia (6.2×10^4 conidia per milliliter). Following inoculation, the cover was kept on the soil cart and the branches were kept moist by sprinkling water on them daily for the first week after inoculation. Pycnidia developed within 2 wk, after which branches were cut to 0.75-m lengths and tied together in bundles of six. In April, four bundles were tied in the top branches of each tree in each block. Fruit set at SRS was not sufficient to hang inoculated branches in individual trees, and fruit were collected from a row of 30 trees. Ten fruit from each of the three blocks at CCRS and MHCRS, and 20 fruit from the row at SRS were sampled each week. Only one side of each fruit was sampled, because analysis of the data from 1990 showed that the number of infected pieces was the same for each side ($P = 0.05$).

Meteorological data were obtained from automatic weather stations at each research station. Petal fall occurred on 19 April (SRS 1990), 23 April (CCRS 1991), 25 April (MHCRS 1991), and 22 April (SRS 1991). Harvest occurred on 31 August (SRS 1990), 26 August (CCRS 1991), 19 September (MHCRS 1991), and 27 August (SRS 1991).

In 1990, and at SRS in 1991, the experiment was analyzed as a completely randomized design. In the other two locations in 1991, it was performed as a randomized complete block. Each experiment was analyzed individually because of the differences in environment and fruit age. Data were normalized with the arcsine \sqrt{Y} transformation of the

number of pieces infected out of nine. Simple correlations were run between disease incidence, time (sample date), and soluble solids (8). Means of non-transformed data were plotted against time.

Isolates used for pathogenicity and aggressiveness test. The five isolates used in this study were collected in North Carolina. Two were collected from CCRS (CB1 and CB3), one from the Hope Orchard, Cleveland Co. (1500), one from SRS (SH6), and one from Research Unit 2, Raleigh (1502). Isolates collected from CCRS were from branch cankers; all other isolates were from rotten fruit.

Nonsprayed Golden Delicious apples from CCRS or MHCRS were used in pathogenicity and aggressiveness studies. Inoculation procedures were the same as above except that fruit were preconditioned at 28 C for 12 hr before inoculation and were not wrapped with a second piece of aluminum foil as in the orchard study. Four wounded and four nonwounded fruit were inoculated with 10^5 conidia per milliliter for each isolate, then were placed in moist chambers and incubated at 28 C for 48 hr. (In a preliminary study, 28 C was optimum for each isolate studied.) After incubation, each fruit was disinfested with 95% ethanol to eliminate surface inoculum and prevent any additional infection. Soluble solids were recorded before each run by averaging refractometer readings of 10 fruit.

Following incubation, the five wounded areas were removed with a no. 2 cork borer, and nonwounded fruit were sectioned and placed in a petri dish lined with a moist paper towel, as before. The experiment consisted of four runs (replications over time); only nonwounded fruit were included in the last two runs.

Fruit pieces were examined for the presence of tissue necrosis and pycnidia at 10, 14, 17, 23, 27, and 30 days after inoculation. The ability of an isolate to colonize fruit pieces was interpreted as evidence of pathogenicity; the time until sporulation was taken as a measure of aggressiveness. Disease occurrence was confirmed by sporulation of *B. dothidea* on the surface of the apple pieces.

Data were recorded as the number of pieces infected per plate and were transformed using the arcsine \sqrt{Y} transformation. The slope of the disease-progress curve for each isolate for days 1 through 17 and 1 through 30 were calculated. The analysis of variance for the first two runs included isolate, wounding, and wounding × isolate terms; for the last two runs, only isolate was included in the model statement.

RESULTS

Field inoculations. The means for the two experiments were not directly com-

parable, because in 1990 the data were recorded only as the presence or absence of disease, and in 1991 they were recorded as the number of rotted fruit pieces out of nine. In 1990, means among trees within treatments (replications) were significantly different ($P = 0.05$), which was one reason the experimental design was modified in 1991 to have fruit on the same five trees inoculated each time. Inoculation date had no significant ($P = 0.05$) effect on the amount of disease that developed. The mean percent of infected fruit or fruit pieces ranged from 9.0 to 9.2% in 1990, and from 32.0 to 35.8% in 1991 (Table 1). An incubation period of 1.5 mo was observed after the inoculation in June 1990; the incubation period was approximately 1 mo following the second and third inoculations. In 1991, symptoms developed 1.5 mo after each inoculation. During the course of each experiment, the temperature beneath the aluminum foil averaged 23.2 C each year; ambient air in the center of the tree averaged 23.1 C. Temperatures under the aluminum foil ranged from 15.2 to 33.8 C, which is favorable for the growth of *B. dothidea* (3,4).

Season-long survey of natural infection. In 1990, no infected fruit were found on 3 July when this study was initiated. However, symptoms were observed for all sampling dates after 3 July (Fig. 1). The amount of disease was not correlated with either sample date ($P = 0.12$) or soluble solids ($P = 0.27$). Soluble solids, however, increased over time.

In 1991, symptoms developed on fruit collected on all dates and from all locations except SRS on 5 June (Figs. 2-4). The amount of disease was not significantly correlated with the sample date at CCRS ($P = 0.18$) and SRS ($P = 0.12$), but was correlated with it at MHCRS ($r = 0.83$, $P = 0.02$). The amount of disease was not correlated with soluble solids at any location ($P = 0.16$ for CCRS, $P = 0.26$ for SRS, and $P = 0.11$ for MHCRS). The amount of infection detected was smallest during the first two sampling dates at all locations. The percent of infected fruit pieces was never at its highest level on the last two dates, but rather one or two peaks occurred midseason.

Rainfall at SRS was below average from June to August 1990 (with only 1.9 cm falling in June), and in May 1991. The other locations experienced normal or above-normal rainfall and temperatures throughout the 1991 season.

Isolate study. All isolates were pathogenic, causing white rot symptoms whether or not the apples had been wounded. The increase in the number of apple pieces showing disease generally was greatest during the first 17 days after inoculation (Fig. 5). In all cases the slopes of the disease-progress curves were steeper from day 0 to day 17 than from day 0 to day 30. For days 1-17, the slope

Table 1. Percent diseased fruit or diseased-fruit pieces resulting from inoculation with conidia of *Botryosphaeria dothidea* at various times throughout the growing season

| Location ^a | Inoculation date | Soluble solids (%) | Infected fruit or fruit pieces (%) |
|-----------------------|------------------|--------------------|------------------------------------|
| MHCRS | 18 Jun 1990 | 7.7 | 9.2 ^b |
| | 16 Jul 1990 | 8.9 | 9.0 ^b |
| | 14 Aug 1990 | 10.4 | 9.2 ^b |
| CCRS | 21 Jun 1991 | 9.7 | 32.0 ^c |
| | 15 Jul 1991 | 10.2 | 30.0 ^c |
| | 21 Aug 1991 | 12.7 | 35.8 ^c |

^aMHCRS = Mountain Horticultural Crops Research Station, Fletcher, North Carolina; CCRS = Central Crops Research Station, Clayton, North Carolina.

^bMean percent infected fruit based on 75 fruit (25 from each of three trees). There was no difference ($P = 0.05$) among inoculation dates.

^cMean percent infected fruit pieces based on 50 fruit (10 from each of five trees). There was no difference ($P = 0.05$) among inoculation dates.

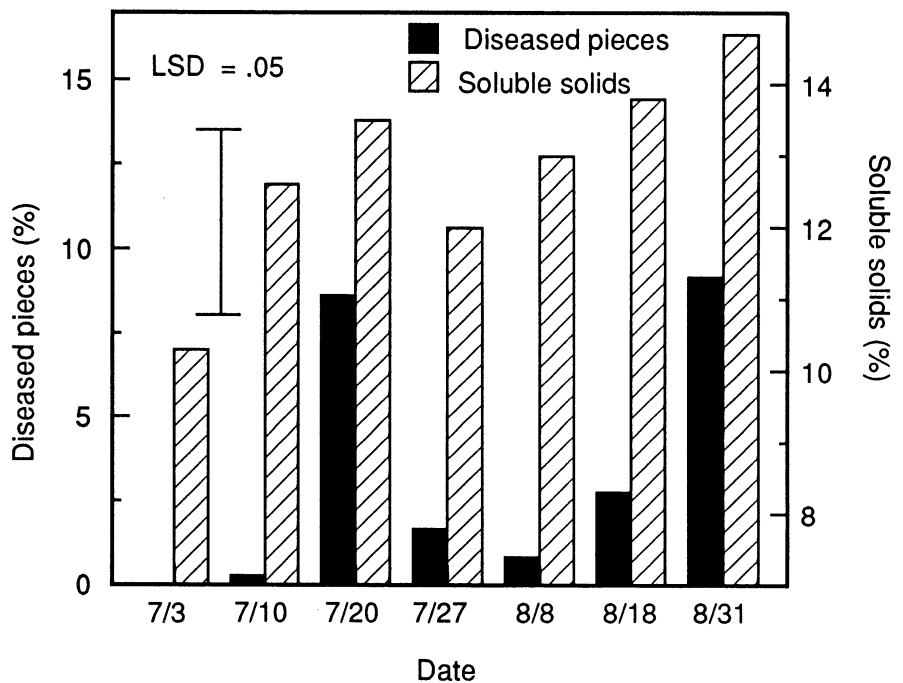


Fig. 1. Percent diseased fruit pieces and soluble solids at the Sandhills Research Station from 3 July to 31 August 1990. Percent diseased fruit pieces based on nine sections from each of 20 fruit on each sample date. $LSD_{0.05}$ bar represents least significant difference for percent diseased pieces.

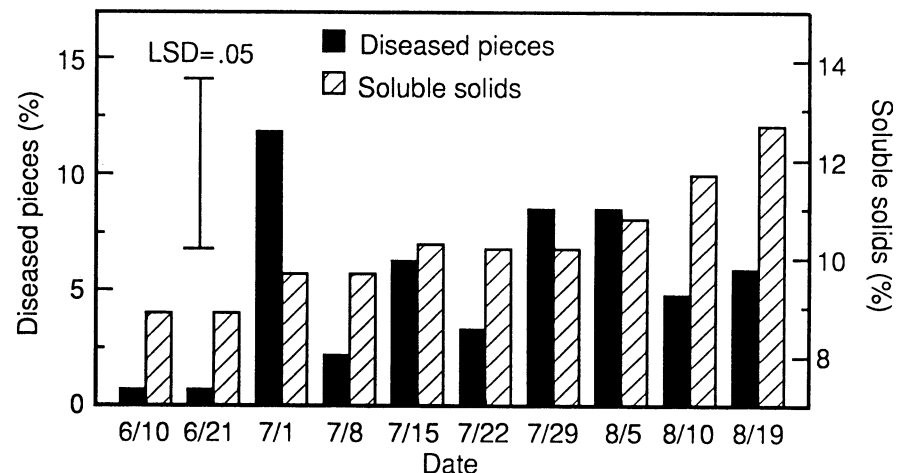


Fig. 2. Percent diseased fruit and soluble solids at the Central Crops Research Station from 10 June to 19 August 1991. Percent diseased fruit pieces based on nine sections from each of 30 fruit on each sample date. $LSD_{0.05}$ bar represents least significant difference for percent diseased pieces.

of the disease-progress curve was greatest for CB1 and least for SH6; the slopes of isolates CB3, 1501, and 1502 were intermediate. For the 30-day period, the only slopes that were significantly different from one another were CB1 and SH6. The *F* test for overall mean percent diseased pieces for each isolate at day 30 was not significant because of the large error term (test \times isolate); however, a mean separation test indicated that CB1 was significantly different from 1502 and SH6 ($P = 0.05$).

DISCUSSION

The results of our study provide evidence for early-season infections of apple fruit by *B. dothidea* which remain latent until the fruit begin to ripen. We were able to successfully infect fruit in the orchard in mid-June each year and isolate *B. dothidea* from naturally infected

symptomless fruit in mid-June at each sample location in 1991.

Several researchers have speculated that, although fruit rot symptoms are primarily noticed late in the season, infection may occur any time after petal fall (3). Sutton has shown that inoculum for infection is present throughout the season (10,12), suggesting that infection can occur whenever environmental conditions are favorable. In 1991, symptoms developed on fruit collected on the earliest sampling date at all locations except SRS, indicating that infection can occur as early as 7 wk after petal fall.

Natural infection did not occur at SRS by the first sampling date of either year. This is probably because of low inoculum level (inoculated branches were not placed in trees) and below-average rainfall (June through August 1990 and May 1991). The decrease in disease occurrence

observed on the last sampling dates may be the result of harvesting only symptomless fruit; many fruit infected early in the growing season would have developed symptoms by August and would not have been selected on the later sampling dates.

In our study, fruit were susceptible from early June until harvest, and infection was not correlated with an increase in soluble solids. This is in contrast to the results of Kohn and Hendrix (6), who reported little infection before the sugar content in fruit reached 10.5%. Furthermore, we were able to successfully inoculate nonwounded fruit both in the field and in the laboratory (*unpublished*) throughout the growing season. It is possible that the use of fungicide-treated fruit in some of Kohn and Hendrix's (6) studies prevented infection from occurring. Their inoculation techniques also were different from ours. The use of inoculum-soaked laboratory towels is a very efficient inoculation procedure that has been used successfully in our laboratory with *B. obtusa* (Schwein.) Shoemaker, *C. gloeosporioides*, and *B. dothidea*. It assures a uniform deposition of inoculum and sufficient moisture during the inoculation procedure. In addition, incubation of fruit pieces in moist chambers for 30 days allows time for latent infection to develop. Kohn and Hendrix (6) evaluated inoculated fruit after 7 days of incubation; however, they did not isolate from any symptomless fruit to determine if latent infections were present. The two studies also used different apple cultivars. They used Delicious; we used Golden Delicious. Cultivars may differ in susceptibility or in duration of the incubation period; however, there are no data in the literature on cultivar susceptibility. Eid (3) found Rome Beauty susceptible throughout the season.

B. dothidea appears to have a long incubation period in immature and mature fruit; symptom expression in the orchard appears to be correlated with the development of soluble solids in the fruit. Although we did not design our experiment to determine the length of the incubation period, we seldom observed symptoms in the orchard until mid-August. These data generally support the findings of Kohn and Hendrix (6) that "active rot lesions" seldom occur until the soluble solids reach 10.5%. However, if the level of soluble solids is to be used as a guide for initiating a spray program, our data indicate that a lower level (approximately 8.5%) should be used. The fluctuation of soluble solids from week to week and their range when natural infections were detected (8.9–9.7%) indicate that the soluble-solids level may not be a reliable indicator. A prediction model, perhaps based on temperature and wetness duration, may be more useful in timing sprays.

Our results, although based on only

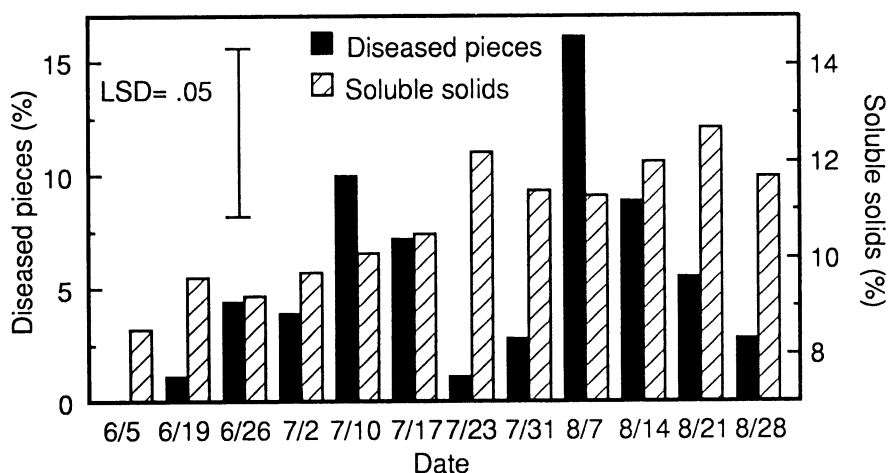


Fig. 3. Percent diseased fruit and soluble solids at the Sandhills Research Station 5 June to 28 August 1991. Percent diseased fruit pieces based on nine sections from each of 20 fruit on each sample date. $LSD_{0.05}$ bar represents least significant difference for percent of diseased pieces.

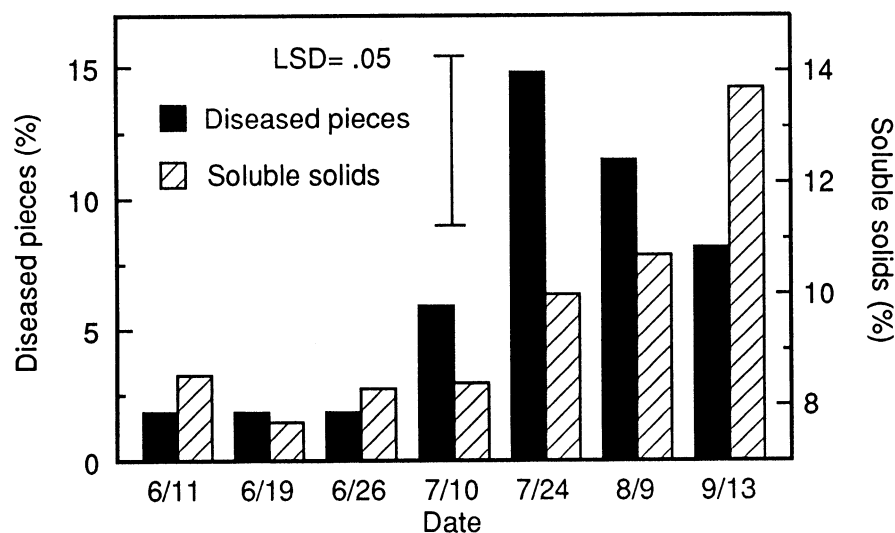


Fig. 4. Percent diseased fruit and soluble solids at the Mountain Horticultural Crops Research Station from 11 June to 13 September 1991. Percent diseased fruit pieces based on nine sections from each of 30 fruit on each sample date. $LSD_{0.05}$ bar represents least significant difference for mean percent of diseased pieces.

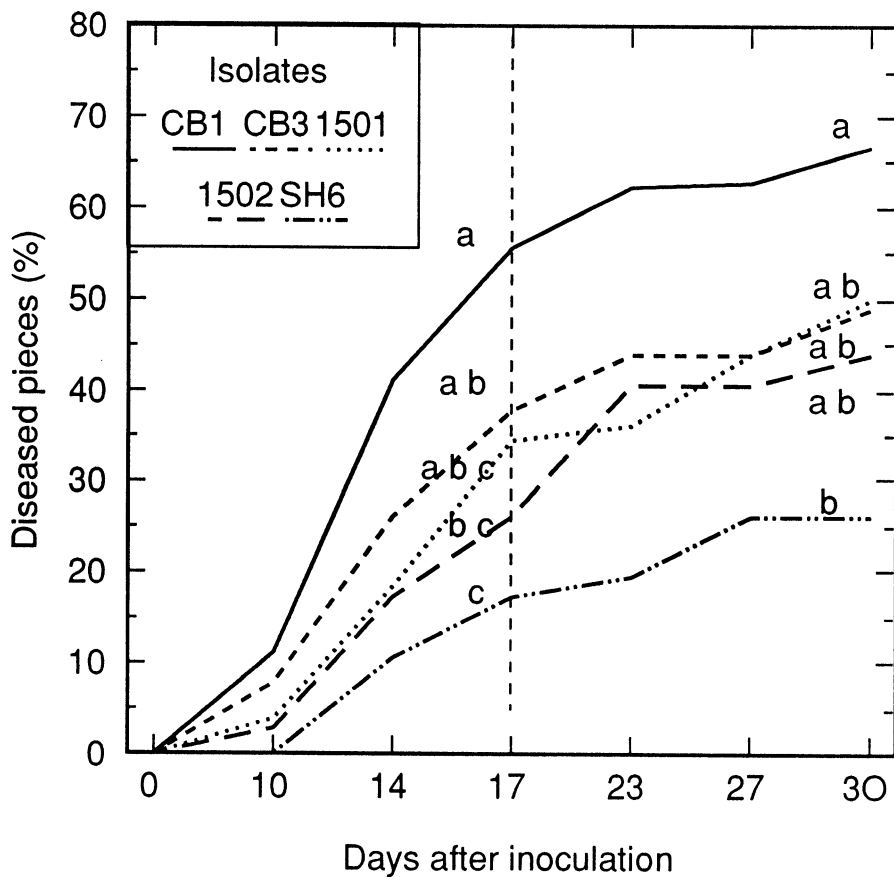


Fig. 5. Percent diseased fruit pieces for five isolates of *Botryosphaeria dothidea* following inoculation of nonwounded Golden Delicious fruit. Slopes were calculated for day 0-17 and 0-30. Lines not labeled with the same letter have slopes that are significantly different (Duncan's multiple range test, $P = 0.05$).

five isolates, corroborated those of previous studies (7,12) showing that there is a significant difference in aggressiveness among isolates of *B. dothidea*. These studies, however, examined only the final amount of disease, rather than the rate of symptom development (7,12). The two factors should be used together to

describe the aggressiveness of an isolate, because measures of the rate of disease progress and of overall disease did not yield the same results. Inclusion of a standard isolate or isolates would be helpful in comparing results from experiments conducted with different isolates. The slopes of the disease-progress curves

for different isolates may not level off or converge as quickly in the field as they did in our study, because the disease increase would be limited more by environmental conditions and the amount of fruit on the tree, not by apple pieces. Convergence was observed mainly in the first run, when four of the five isolates resulted in 90% or more infection.

LITERATURE CITED

1. Brown, E. A., and Britton, K. O. 1986. *Botryosphaeria* diseases of apple and peach in the southeastern United States. *Plant Dis.* 70:480-484.
2. Brown, E. A., and Hendrix, F. F. 1981. Pathogenicity and histopathology of *Botryosphaeria dothidea* on apple stems. *Phytopathology* 71:375-379.
3. Eid, R. F. 1959. Etiology of and control of *Botryosphaeria ribis* on apple. M.S. thesis. University of Delaware, Newark.
4. Fulkerson, J. F. 1956. *Botryosphaeria ribis* and its relation to a rot of apples. Ph.D. thesis. North Carolina State University, Raleigh.
5. Hwang, B. K. 1983. Contents of sugars, fruit acids, amino acids and phenolic compounds of apple fruits in relation to their susceptibility to *Botryosphaeria ribis*. *Phytopathol. Z.* 108:1-11.
6. Kohn, F. C., Jr., and Hendrix, F. F. 1983. Influence of sugar content and pH on development of white rot on apples. *Plant Dis.* 67:410-412.
7. Latorre, B. A., and Toledo, M. V. 1984. Occurrence and relative susceptibility of apple cultivars to *Botryosphaeria* canker in Chile. *Plant Dis.* 68:36-39.
8. SAS Institute, Inc. 1985. SAS User's Guide: Statistics. SAS Institute, Inc., Cary, NC.
9. Sitterly, W. R., and Shay, J. R. 1960. Physiological factors affecting the onset of susceptibility of apple fruit to rotting by fungus pathogens. *Phytopathology* 50:91-93.
10. Sutton, T. B. 1981. Production and dispersal of ascospores and conidia by *Physalospora obtusa* and *Botryosphaeria dothidea* in apple orchards. *Phytopathology* 71:584-589.
11. Sutton, T. B. 1990. White rot. Pages 16-18 in: Compendium of Apple and Pear Diseases. A. L. Jones and H. S. Aldwinckle, eds. American Phytopathological Society, St. Paul, MN.
12. Sutton, T. B., and Boyne, J. V. 1983. Inoculum availability and pathogenic variation in *Botryosphaeria dothidea* in apple production areas of North Carolina. *Plant Dis.* 67:503-506.