Influence of Sugar Content and pH on Development of White Rot on Apples

FRANK C. KOHN, JR., Former Graduate Research Assistant, Department of Plant Pathology and Plant Genetics, and F. F. HENDRIX, Professor, Plant Genetics, University of Georgia, Athens 30602

ABSTRACT

Kohn, F. C., Jr., and Hendrix, F. F. 1983. Influence of sugar content and pH on development of white rot on apples. Plant Disease 67:410-412.

Apples were collected at weekly intervals from an orchard in Georgia beginning 3 wk after petal fall and continuing until harvest. At each sampling date, puncture-wounded and unwounded apples were spray-inoculated with *Botryosphaeria dothidea* conidia at a concentration of about 1×10^5/ml by using a chromatographic spray bottle at 10 psi. Inoculated fruit were incubated in moist chambers at 30 C and the percentage that developed rot was recorded after 7 days. The sugar content in a subsample of fruit was measured by determining percent soluble solids with a refractometer. The pH of the apple filtrates was also measured. No lesions developed until sugar content reached about 10.5%, which occurred 8 wk before harvest in 1979. Lesion formation continued to increase with increasing sugar content until harvest, when sugar content reached 13.8% and rot incidence was 100%. Regression analysis indicated a significant association (r^2 = 72.3%) between sugar content and rot incidence. Greatest mycelial dry weight production in vitro occurred with a 20% sucrose level in the medium.

The ascomycetous fungus *Botryosphaeria dothidea* (Moug. ex Fr.) Ces. & de Not. (=*B. ribis* Gross. & Dug.) causes a serious fruit rot and canker disease on apple trees in the midwestern and southeastern United States. Losses to fruit rot can reach 100% in the absence of control measures (8). The organism was originally described as the cause of currant cane blight (7) and was subsequently found to cause fruit rot (5) and trunk canker (12) of apple. The fungus is quite ubiquitous, occurring on a wide variety of woody plants (14,16).

B. dothidea produces a soft light-colored rot on apple fruit referred to as white rot or bot rot. In many cases, this rot is visually indistinguishable from black rot caused by *B. obtusa* (Schw.) Shoemaker, making isolation of the pathogen necessary for correct identification of the disease (8).

Several investigators (2,4,6,8–10, 15,17,18) have noted that apple fruit in the field are not rotted until midsummer, although Drake (2) believes that many primary infections are initiated soon after the fruit are formed. Sitterly and Shay (15) attributed the breakdown in resistance in immature fruit to a physiological factor or factors induced as the fruit approaches maturity. They treated fruitbearing limbs with a respiration inhibitor, maleic hydrazide, and found that this hastened the onset of fruit rot symptoms. In another experiment, apple fruit were infused with sucrose and fructose beginning on 1 June, resulting in the invasion of apple tissue about 20 days earlier than in untreated fruits. They postulated that there is a "threshold" value of sugar accumulation in maturing fruit that must be crossed before *B. dothidea* is able to use apple tissue as a substrate for growth and production of apple-rotting enzymes.

Wallace et al (17,18) proposed a more complex mechanism to explain the resistance of immature apples. They found that the amount of water-insoluble material in apple pulp tissue (eg, insoluble proteins and pectins) declined at about the time fruit became susceptible to *B. dothidea* and suggested that this decrease coupled with the exchange of polyvalent cations for potassium in the protopectin constituents of apple cell walls was responsible for the loss of resistance in maturing fruit.

It is possible that fungitoxic compounds in immature fruit are responsible for early season resistance of apples to *B. dothidea*. Kuć et al (9), however, tested resistant apples for fungitoxic compounds and found that *B. dothidea* grew on resistant apple slices after the cells were ruptured either by autoclaving, freezing, or grinding.

Drake and Moore (3), in a study on the growth of *B. dothidea* in semisynthetic liquid medium, found that good growth of the fungus was supported over a pH range of 4–7.5. Sucrose, several other sugars, and pectin supported superior growth, but utilization of starch as a carbon source was significantly lower.

Control of *B. dothidea* would be improved if a method were available to determine when fruit becomes susceptible. The primary objectives of this study were to determine if there is a correlation between susceptibility and fruit characteristics commonly measured by growers and to determine when *B. dothidea* occurs in apples in the field. The effects of differing sucrose levels in a synthetic medium on the growth rate of *B. dothidea* were also determined.

MATERIALS AND METHODS

Inoculum. *B. dothidea* isolates were obtained from infected fruit collected from orchards in Gray (central Georgia) and Blairsville (northern Georgia) beginning in 1979. It was necessary to maintain fresh cultures by continuous isolation because the organism lost its ability to sporulate in culture and rot the fruit after repeated transfers. The isolates were grown and maintained on acid natural potato-dextrose agar (APDA) in petri dishes at 29 C under continuous fluorescent light. Because it was desirable to use mass cultures as inoculum to more closely simulate field conditions, cultures were not single-spored.

Sampling and inoculation. To determine when fruit becomes susceptible, Red Delicious apples were collected at weekly intervals in 1979 beginning 3 wk after petal fall from trees in Gray that had been sprayed according to the recommended apple spray guide for central Georgia (11) and from a control plot sprayed only with insecticides. At each sampling from Gray in 1979, 20 apples from the spray-guide treatment were washed in a mild detergent solution, rinsed with distilled water, and allowed to dry. Before inoculation, 10 of the apples were wounded with a puncturing tool consisting of five dissecting needles taped into a bundle.

To determine when fruit infection was occurring in the field, fruit of several cultivars from two locations were sampled in 1976 and 1979. Sampling in 1976 consisted of cultivars Detroit Red, Golden Delicious, and Red Delicious from northern Georgia and Red Delicious from central Georgia. In 1979, sampling was from the cultivar Red Delicious in northern and central Georgia. Samples were taken of each cultivar at weekly intervals beginning 3 wk after petal fall from trees that had been sprayed according to the recommended apple spray guide for central or northern

Present address of first author: Mobay Chemical Company, Tifton, GA 31794.

This work was supported by state and Hatch Act funds allocated to the Georgia Experiment Station and USDA, ARS, Grant funds.

Accepted for publication 13 September 1982.

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.

Georgia (11) and from a control plot sprayed only with insecticides. The treatments contained four replicates and were arranged in a complete randomized block design. At each sampling date, 100 apples from each treatment (25 per replicate) were surface-sterilized, with 0.25% sodium hypochlorite and 10% ethanol, sectioned longitudinally, and entire apples were plated on APDA. Plates were incubated under continuous fluorescent light at room temperature (20 C) for 2 wk, after which the percentage of plates containing *B. dothidea* was recorded.

At each sampling from central Georgia in 1979, 20 apples from each replicate of the spray-guide treatment were washed in a mild detergent solution, rinsed with distilled water, and allowed to dry. Before inoculation, 10 of the apples were wounded as described earlier; the remaining 10 apples were inoculated without wounding. The apples were inoculated with a spray suspension of *B. dothidea* conidia at a concentration of about 1×10^5/ml. Inoculated fruit were incubated at 30 C in damp chambers on metal grids placed over wet absorbent cotton. After 7 days, the number of fruit that developed rot was recorded. Isolations were made from the rotted tissue to ensure that the causal organism was *B. dothidea*.

From each sample in 1979 from central Georgia, a sample of five healthy apples from each replicate of the spray-guide treatment was taken at random and juice from these apples was extracted with a garlic press. The pH of the extract was determined. Sugar content was measured by determining percent soluble solids with a calibrated ATAGO hand refractometer (McCormick's Fruit Tree Co., Yakima, WA).

To study the effects of varying sucrose levels on the growth of *B. dothidea* in culture, 125-ml flasks containing 50 ml of Czapek-Dox solution were seeded with water agar plugs containing mycelium of *B. dothidea*. The inoculum was prepared by transferring mycelium of a single-conidial isolate of the fungus from APDA stock cultures to petri dishes containing water agar. After 2 days, plugs containing vigorously growing mycelium were cut with a sterile 4-mm cork borer from the advancing margins of the colony and transferred into flasks containing the Czapek-Dox solution. The treatments were 0, 0.1, 1, 10, 20, 40, and 60% sucrose at pH 7.3, and each treatment was replicated five times. Flasks were incubated at room temperature (20 C) on a rotary shaker. After 5 days, mycelial mats were harvested by filtering the media containing hyphal mats of *B. dothidea* through Fischer qualitative filter paper (12.5 cm). After the culture filtrate had been drawn off, each mycelial mat was rinsed with 10 ml of sterile distilled water to remove traces of medium. Before filtering, the filter paper was dried for 24 hr in a convective oven at 75 C and then weighed. After filtering, the filter paper and mycelial mats were oven-dried for 24 hr at 75 C. After drying, the filter paper and mycelial mats were weighed and the dry weight of mycelial growth for each treatment was determined.

RESULTS

Isolations from apple fruit in 1976 and 1979 did not yield appreciable levels of *B. dothidea* until the fruit were within 6–8 wk of harvest. In 1976 in central Georgia, unsprayed Red Delicious trees had no fruit infection 13 wk after petal fall, but 25% of the apples were infected 16 wk after petal fall. In northern Georgia, there were no infected Detroit Red apples 11 wk after petal fall, but 13% were infected 17 wk after petal fall. Golden Delicious had no infected fruit 11 wk after petal fall, but 73% were infected 20 wk after petal fall.

In 1979 in northern Georgia, 1% of the unsprayed Red Delicious fruit were infected 8 wk after petal fall and the sugar content was 8.4%. After 16 wk, the sugar content was 9.5% and only 2% of the fruit were infected. After 20 wk, the sugar content was as high as 10.5% and 12% of the fruit were infected. In central Georgia, infection of Red Delicious apples did not increase appreciably late in the season because of a lack of summer rain. Active rot lesions did not occur on artificially inoculated apples until the sugar content reached about 10.5%. Results in Figure 1 represent infection of wounded apples. Unwounded apples were not infected at any stage of development. Rot development began on inoculated fruit 11 wk after petal fall. After this point, the number of apples developing lesions continued to increase with increasing sugar content. At harvest, when sugar content reached 13.8%, rot incidence was 100%. Regression analysis indicated a significant association (r^2 = 72.3%) between sugar content and rot incidence (Fig. 1). The pH of juice from developing apples remained in the 3.5–3.8 range throughout the growing season and was not correlated with fruit susceptibility.

Mycelium dry weight production by the organism was greatest in liquid media when the sucrose concentration was between 10 and 60% with an optimum level of 20% (Fig. 2). Some of the weight of the mycelial mats grown at high sucrose concentrations (40 and 60%) was attributed to insufficient rinsing of the mycelial mats with a subsequent retention of sucrose residues that artificially increased the final weights. Mycelium grown at the 40 and 60% sucrose levels appeared to be distorted and irregular hyphal balls were produced.

DISCUSSION

These results show that *B. dothidea* did not begin to infect apples at appreciable levels until about 6 wk before harvest. Significant levels of *B. dothidea* isolated from apples late in the season corresponded with the period at which inoculated apples became susceptible to infection by the organism. Eid and Heuberger (4) reported that peak spore production of the pathogen also coincides with this period. *B. dothidea* is normally capable of infecting a high percentage of apples as the fruit approaches maturity (8). However, as in central Georgia in 1979, weather conditions and other factors may cause lower than usual levels of infection.

Previous studies (4,6,8–10,15,17,18) have fixed varying dates during the growing season at which fruit become susceptible. The orchard in Gray is located in the central portion of the state where much warmer temperatures prevail and the growing season begins earlier. At that location, inoculated apples became susceptible about the middle of June and harvesting occurred during the first week of August. This agrees with other observations (5,9) that apples become susceptible about 6–8 wk before harvest. Because the date at which apples become

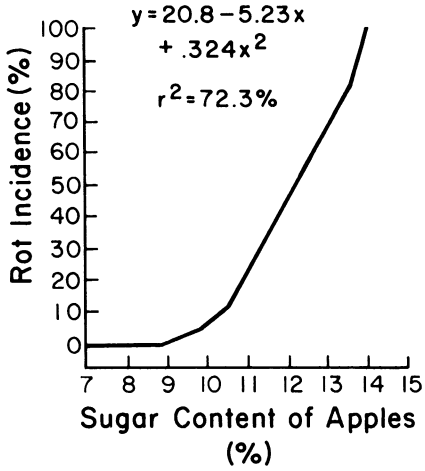

Fig. 1. Rot incidence as a function of sugar content of apples from Gray, GA, in 1979.

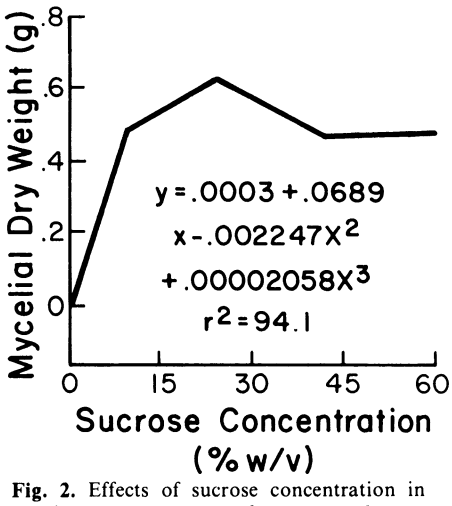

Fig. 2. Effects of sucrose concentration in medium on growth of *Botryosphaeria dothidea*.

susceptible may vary with the cultivar and the locality, our results indicate that time of year is a less important factor than sugar content of the fruit. Although sugar levels are probably not the primary factor in fruit resistance (9,17), they can indicate when developing fruit have reached the stage when they are susceptible to infection by *B. dothidea*. Numerous studies (E. A. Brown, *unpublished*; 1,16,19) have shown that *B. dothidea* is primarily a wound pathogen. E. A. Brown (*unpublished*) found that wound treatments greatly enhanced stem infections on apple, whereas Witcher and Clayton (19) stated that wounding was a prerequisite for induction of stem-blight disease of blueberry caused by *B. dothidea*. The fact that we were not able to produce infections on unwounded apples supports the results of other workers and indicates that wounding agents such as insects play an important role in the epidemiology of white rot.

Our results indicate that maximum growth of *B. dothidea* in vitro occurred at a sucrose content of 10–20% in the growth medium, which is within the range where rot development occurs. Because obvious growth occurred at sucrose levels as low as 0.1%, however, it is unlikely that sugar content alone accounts for the resistance of immature fruit as was reported by Sitterly and Shay (15). The effect of pH appears to be minimal because the range found in developing apples approximated the lower limits for growth reported by Drake and Moore (3). Kuć et al (9) discounted the possibility that substances in resistant apples that inhibit the pathogen play a major role in resistance, and they suggested that transition from resistance to susceptibility may depend on the resistance of the apple cell walls to hydrolysis by fungal enzymes. Wallace et al (17,18) proposed that the extensive cross-linking by calcium and other bivalent cations between pectins in the cell wall material may provide a steric hindrance to pectin-hydrolyzing enzymes produced by the pathogen, thereby preventing effective cleavage of pectin chains. They found that breakdown of resistance was associated with the exchange of polyvalent cations for potassium in the cross-links of pectin cell wall constituents and that this exchange facilitated the availability of cleavage sites for the fungal enzymes, resulting in apples becoming susceptible to invasion by *B. dothidea*.

Whatever the mechanism of resistance of immature apple fruit to *B. dothidea* is, these results show that there is a definite point in the development of apple fruit at which susceptibility occurs. This information could be used in an integrated control program against apple pests. Refractometers are used widely in the apple industry to measure sugar content as an aid in harvest timing. By testing apples during the growing season, growers should be able to time their spray programs so that fungicides can be applied when they are most needed and will be most effective. These results show that sprays to control *B. dothidea* should begin when the sugar content in apples reaches 10% and continue until harvest.

LITERATURE CITED

1. Crist, C. R., and Schoeneweiss, D. F. 1975. The influence of controlled stresses on susceptibility of European white birch stems to attack by *Botryosphaeria dothidea*. Phytopathology 65:369-373.
2. Drake, C. R. 1971. Source and longevity of apple fruit inoculum, *Botryosphaeria ribis* and *Physalospora obtusa*, under orchard conditions. Plant Dis. Rep. 55:122-126.
3. Drake, C. R., and Moore, L. D. 1966. Effect of pH and carbon on growth of the apple rot fungus, *Botryosphaeria ribis*. (Abstr.) Phytopathology 56:876.
4. Eid, R. F., and Heuberger, J. W. 1959. Etiology of *Botryosphaeria ribis* on wood and fruit of the Rome apple. Phytopathology 49:523.
5. Fenner, E. A. 1925. A rot of apples caused by *Botryosphaeria ribis*. Phytopathology 15:230-234.
6. Fulkerson, J. F. 1960. *Botryosphaeria ribis* and its relation to a rot of apples. Phytopathology 50:394-398.
7. Grossenbacher, J. G., and Dugger, B. M. 1911. A contribution to the life history, parasitism, and biology of *Botryosphaeria ribis*. N.Y. State Agric. Exp. Stn., Geneva, Tech. Bull. 18:113-190.
8. Hendrix, F. F., Powell, W. M., and McGlohon, N. E. 1978. Apple diseases in Georgia. Fruit South 2(4):112-116.
9. Kuć, J., Williams, E. B., Maconkin, M. A., Ginzel, J., Ross, A. F., and Freedman, L. J. 1967. Factors in the resistance of apple to *Botryosphaeria ribis*. Phytopathology 57:38-42.
10. Lewis, G. D., and Shay, J. R. 1963. Control of summer diseases of apple. Plant Dis. Rep. 37:84-87.
11. McGlohon, N. E. 1978. Apple spray guide for middle Georgia. Coop. Ext. Serv., Univ. Ga. Bull. 740.
12. Putterill, V. A. 1919. A new apple tree canker. South Afr. J. Sci. 16:258-272.
13. Shay, J. R., and Sitterly, W. R. 1954. Botryosphaeria canker of apple. Phytopathology 44:505.
14. Shear, C. L., Stevens, N. E., and Wilcox, M. S. 1925. *Botryosphaeria* and *Physalospora* in the eastern United States. Mycologia 17:98-107.
15. 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.
16. Smith, C. O. 1934. Inoculations showing the wide host range of *Botryosphaeria ribis*. J. Agric. Res. 49:467-476.
17. Wallace, J., Kuć, J., and Draudt, H. N. 1962. Biochemical changes in the water-insoluble material of maturing apple fruit and their possible relationship to disease resistance. Phytopathology 52:1023-1027.
18. Wallace, J., Kuć, J., and Williams, E. B. 1962. Production of extra-cellular enzymes by four pathogens of apple fruit. Phytopathology 52:1004-1009.
19. Witcher, W., and Clayton, C. N. 1963. Blueberry stem blight caused by *Botryosphaeria dothidea* (*B. ribis*). Phytopathology 53:705-712.