Influence of Temperature and Moisture on Germination of Ascospores and Conidia of *Botryosphaeria dothidea*

T. B. SUTTON and L. F. ARAUZ, Department of Plant Pathology, North Carolina State University, Raleigh 27695-7616

ABSTRACT

Sutton, T. B., and Arauz, L. F. 1991. Influence of temperature and moisture on germination of ascospores and conidia of *Botryosphaeria dothidea*. Plant Dis. 75:1146-1149.

Predicted optimum temperatures for germination of conidia of isolates 1, 2, and 3 of *Botryosphaeria dothidea* in free water were 26.7, 27.8, and 29.5 C. Conidia of isolates 1 and 2 germinated at 8 C after 24 hr but none from isolate 3 germinated. Conidia of isolate 1 incubated at 95–100% relative humidity (RH) germinated after 12 hr; the percent germination declined with RH. Few conidia of isolates 2 or 3 germinated in the absence of free water. The predicted optimum temperature for ascospore germination was 24.6 C. Ascospores germinated over a wider range of RH than did conidia (92–100%); percent germination declined with RH. Ascospore germ tube length declined with RH. Air drying for approximately 20 min significantly reduced the viability of conidia. Conidia from isolates 2 and 3 were more sensitive to drying than isolate 1.

White rot, or Bot rot, caused by *Botryosphaeria dothidea* (Moug.:Fr.) Ces. & De Not. (anamorph *Fusicoccum aesculi* Corda), is an important apple (*Malus domestica* Borkh.) disease in many of the warmer apple-growing regions in the world. Losses in the southeastern United States have been extensive in some orchards. The disease is characterized by a soft watery rot, which rapidly affects the entire fruit under warm conditions. In addition to a fruit rot, *B. dothidea* also causes a serious canker disease (4,11).

Ascospores and conidia, produced in dead bark in the tree and in mummified apples, serve as primary inoculum. Both are produced throughout the growing season and are discharged and dispersed during rainfall (13,14). Conidia are generally more abundant than ascospores (13) (T. B. Sutton, *unpublished*). The exact time of fruit infection is not clear. Eid (5) presented evidence that infections occur throughout the growing season but remain latent until fruit begins to ripen. However, Kohn and Hendrix (10) found that fruit infection was rare unless soluble solids in the fruit were greater than 10.5%.

The optimum temperature for mycelial growth is approximately 30 C (9), whereas the optimum temperature for germination of conidia is 25–30 C; no germination occurs at 8.8 C after 24 hr (5). The effect of temperature on ascospore germination and the effect of relative humidity on conidia or ascospore germination have not been investigated. These data would help to determine the criteria necessary for fruit infection. Thus, the objective of this study was to determine the influence of temperature and moisture and their interaction on the germination of ascospores and conidia of *B. dothidea*.

MATERIALS AND METHODS

Conidial isolates. Three isolates of *B. dothidea* (designated as isolates 1, 2, and 3) obtained in August 1987 from rotten apples exhibiting typical white rot symptoms were maintained on potato-dextrose agar (PDA) in test tube slants at 4 C until used in the study.

Isolates were transferred to cellulose films (Flexel Sales, Inc., Covington, IN) placed on top of oatmeal agar medium (Difco Laboratories, Detroit, MI) and grown for approximately 15 days under continuous fluorescent light. Conidia were obtained by removing the cellulose films from agar plates and blending films, mycelium, and pycnidia in sterile distilled water in a commercial blender. The resulting suspension was strained through three layers of cheesecloth, and the conidial suspension was standardized to 120,000 conidia per milliliter.

The agar dish isopiestic equilibration technique, described by Harris et al (8)

Present address of second author: Escuela de Fitotecnia, Universidad de Costa Rica, Costa Rica.

Use of trade names does not imply endorsement by the North Carolina Agricultural Research Service of the products used nor criticism of similar ones not mentioned.

Accepted for publication 22 April 1991 (submitted for electronic processing).

and modified by Alderman and Beute (1), was used to control relative humidity (RH). Details of the technique are described in Arauz and Sutton (2). Relative humidities used in this study were 100, 99, 98, 95, 92, and 88.5%. Two free-water treatments were also included—one in which the conidial suspension was not allowed to dry and one in which the conidial suspension was allowed to air dry (approximately 20 min) and was then rewetted. Germination was evaluated at 4, 8, 12, 16, 20, 24, 28, and 32 C, maintained in controlled environmental chambers (Percival Manufacturing Co., Boone, IA). Humidity chambers were preconditioned overnight at the desired temperature before conidia were placed into them.

Drops (2 μl) of conidial suspension (containing approximately 100 conidia) were placed in the centers of microscope cover glasses (18 × 18 mm), and four cover glasses were placed in each humidity chamber. After 4, 8, 12, or 24 hr in environmental chambers, one cover glass was removed from each relative humidity chamber and inverted on top of a drop of cotton blue in lactophenol. Relative humidity chambers were resealed and returned to environmental chambers. Percent spore germination was determined by observing 50 conidia selected arbitrarily on each cover glass. A conidium was considered germinated if the germ tube was at least one-half the length of the spore.

The experiment was replicated three times on different days. The experimental design was a split-split-plot with temperature as the whole plot, isolates as the subplot, and relative humidity as the sub-subplot. Because isolates 2 and 3 did not germinate in the absence of free water, the ANOVA was conducted as a split plot. A separate analysis was conducted for each assessment time.

Ascospore germination. Ascospores were obtained from naturally infected apple prunings from an orchard. Prunings, approximately 0.5–1.0 cm diameter, were cut into 15-cm segments, washed under tap water to remove superficial saprophytes, immersed in distilled water for 20 min, and blotted dry. Prunings were placed in a spore tower (7) and ascospores were collected for 5–20 min on microscope cover glasses. Cover glasses were placed in humidity chambers on removal from the spore tower. Temperature and relative humidity treatments were the same as those for conidia except no rewetted treatment was used because no spore suspension was prepared. Percent ascospore germination was determined by observing 50 ascospores selected arbitrarily on each cover glass. An ascospore was considered germinated if the germ tube was at least one-half the length of the spore. Germ tube length of ascospores was determined for the 12-hr treatments by measuring 10 germ tubes selected arbitrarily on each coverslip.

The experimental design was a split plot with temperatures as whole plots and relative humidities as subplots. Because greater variability in ascospore numbers on the coverslips was anticipated than in the experiment with conidia, the experiment was run four times on different dates.

Regression analysis. Linear, quadratic, and cubic effects of temperature and relative humidity and their interactions on spore germination and germ tube length (ascospores only) were tested for the 12-hr readings. Adjusted R^2s were obtained for each regression equation by dropping regression parameters not significantly different from zero ($P < 0.05$), unless higher degree terms of the corresponding variable were associated with significant parameters, and conducting the regression again.

RESULTS

Conidial germination in free water. Isolates 1 and 2 germinated at lower temperatures than isolate 3 after 4, 8, or 12 hr (Fig. 1A–C). After 24 hr, 13 and 21% of the conidia of isolates 1 and 2, respectively, had germinated at 8 C but none of the conidia from isolate 3 had germinated (*data not shown*). Percent germination of isolate 3 was 13.9% at 12 C after 24 hr compared with 74.5 and 76.4% for isolates 1 and 2, respectively; however, all isolates achieved 80% or greater germination after 12 hr at 16 C or higher. The predicted optimum temperatures for isolates 1, 2, and 3 were 29.5, 27.8, and 26.7 C, respectively (Fig. 1D). No

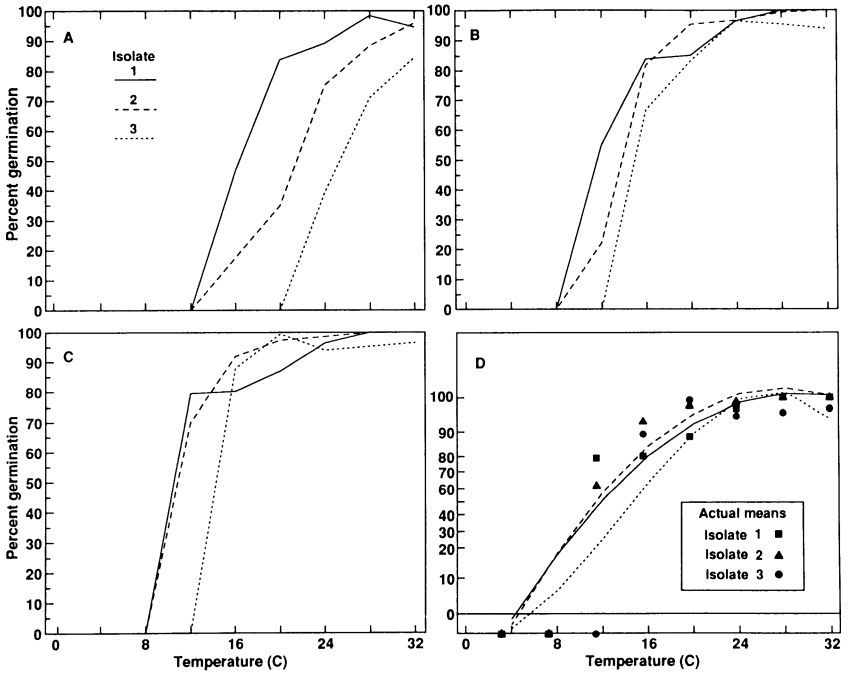

Fig. 1. Influence of temperature on conidial germination of three isolates of *Botryosphaeria dothidea* in free water after **(A)** 4 hr, **(B)** 8 hr, **(C)** 12 hr, and **(D)** germination after 12 hr predicted by the following equations: Y (isolate 1) = $0.5866 + 0.1464T - 0.0025T^2$ ($R^2_{adj} = 0.87$); Y (isolate 2) = $-0.6968 + 0.1653T - 0.0030T^2$ ($R^2_{adj} = 0.92$); and Y (isolate 3 = $0.2197 + 0.0074T^2 - 0.0018T^3$ ($R^2_{adj} = 0.84$), where Y = arcsine (in radians) $\sqrt{X}$, X = proportion of spore germinated and T = temperature (C). Nonlinearity of scale on vertical axis in **D** results from back transforming to percentage the arcsine $\sqrt{X}$ scale used in the data analysis.

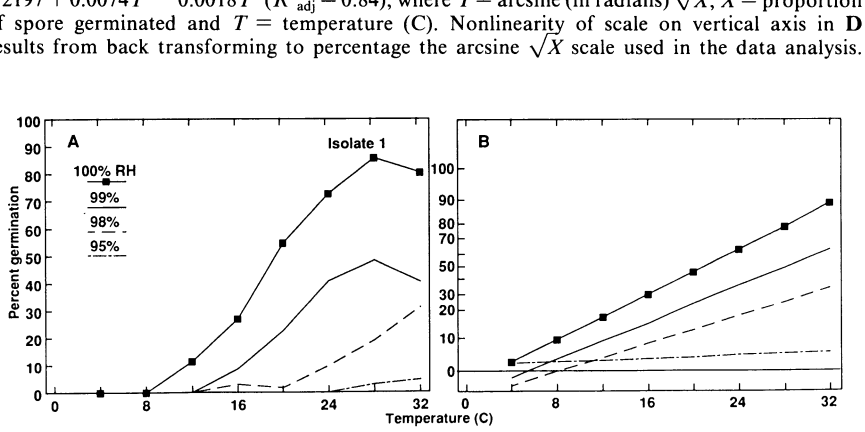

Fig. 2. Effect of relative humidity on conidial germination of isolate 1 of *Botryosphaeria obtusa* 12 hr after treatments were imposed. **(A)** Actual data and **(B)** predicted values from regression equations (equation 1 in text). Only isolate 1 germinated in the absence of free water.

germination was observed at 4 C after 24 hr for any isolates.

Effect of temperature and relative humidity on conidial germination. Less than 5% of the conidia of isolate 2 germinated at 100% RH and virtually none germinated at 99% RH or lower. No conidia of isolate 3 germinated in any RH treatment. Conidia of isolate 1 germinated from 100 to 95% RH, although the percent germination decreased with RH (Fig. 2). There were no significant differences ($P = 0.05$) among the percent germination at 24, 28, and 32 C for 100, 99, or 98% RH, respectively. Less than 5% of the conidia germinated at 95% RH. The combined effect of temperature and moisture on germination of conidia of isolate 1 after 12 hr was described by the following equation: $Y = 289.078 - 0.764T - 5.903H + 0.030H^2 + 0.008TH$ ($R^2_{adj} = 0.70$) (equation 1), where Y = arcsine (in radians) $\sqrt{X}$, X = proportion of germinated conidia, T = temperature (C), and H = percent RH.

Ascospores generally germinated over a wider RH range than conidia (Fig. 3). Germination was greater than 85% at 98–100% RH for temperatures of 20 C or greater after 4 hr. After 8 hr, 41 to 82% germination was achieved at 95% RH at temperatures ranging from 20 to 32 C. One percent and 36% germination were recorded at 92% RH for 28 and 32 C, respectively, after 12 hr. Germination of ascospores was generally lower in the free-water treatment than in the 100% RH treatment after 4, 8, or 12 hr. The predicted optimum temperature for ascospore germination in free water was 24.6 C. The combined effects of temperature and RH on ascospore germ tube elongation are presented in Figure 4. Ascospore germ tube length generally decreased linearly with decreasing RH. Germ tubes of ascospores were significantly ($P = 0.05$) longer in the free water treatment than in the 100% RH treatment at 28 and 32 C. The combined effect of temperature and RH on ascospore germination and germ tube length after 12 hr was described by the following equations: Y (germination) = $3798.905 - 23.522T - 114.409H + 0.4598TH + 0.00024T^3 - 0.00383H^3$ ($R^2_{adj} = 0.86$) (equation 2), where Y = arcsine (in radians) $\sqrt{X}$, X = proportion of spores germinated, T = temperature (C), and H = percent RH; and Y (germ tube length) = $- 723425.557 + 74.305T - 5.297T^2 + 0.537T^3 + 22606.751H - 235.590H^2 + 0.818H^3 + 0.0188T^2H$ ($R^2_{adj} = 0.77$) (equation 3), where Y = germ tube length (μm), T = temperature (C), and H = percent RH.

Effect of air drying. Germination of conidia in free water was greater than germination when conidia were dried and rewetted or maintained at 100% RH (Fig. 5). Only 13.3 and 2.8% of the conidia of isolates 2 and 3, respectively, ger-

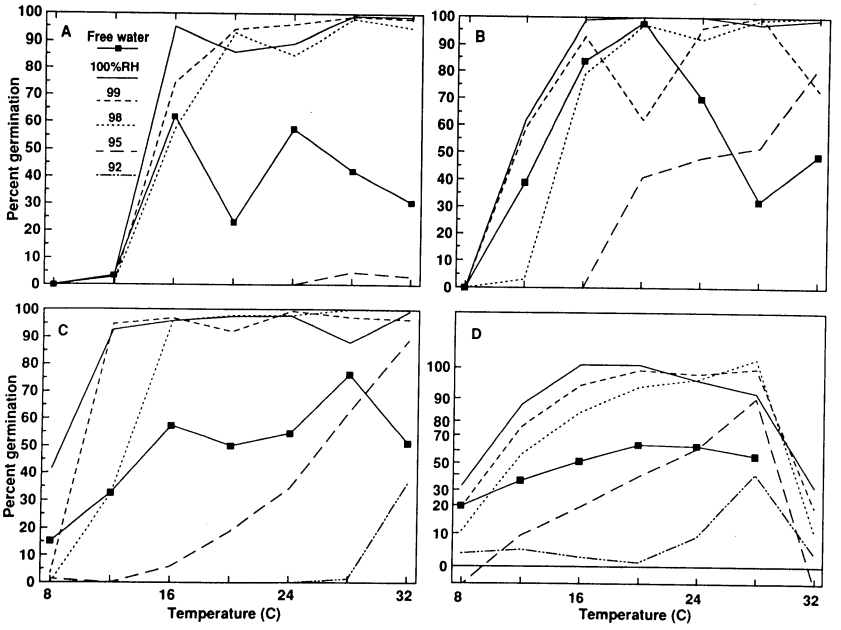

Fig. 3. Effect of temperature and relative humidity on the germination of ascospores of *Botryosphaeria dothidea* after **(A)** 4 hr, **(B)** 8 hr, **(C)** 12 hr, and **(D)** predicted values from regression equation (equation 2 in text). For free water treatment, $Y = 0.1188 + 0.0807T - 0.00176T^2$ ($R^2_{adj} = 0.26$, $P = 0.09$), where Y = arcsine (in radians) $\sqrt{X}$, X = proportion of germinated ascospores, T = temperature (C). The nonlinearity of the scale on the vertical axis results from back transforming to percentage the arcsine $\sqrt{X}$ scale used in the analysis.

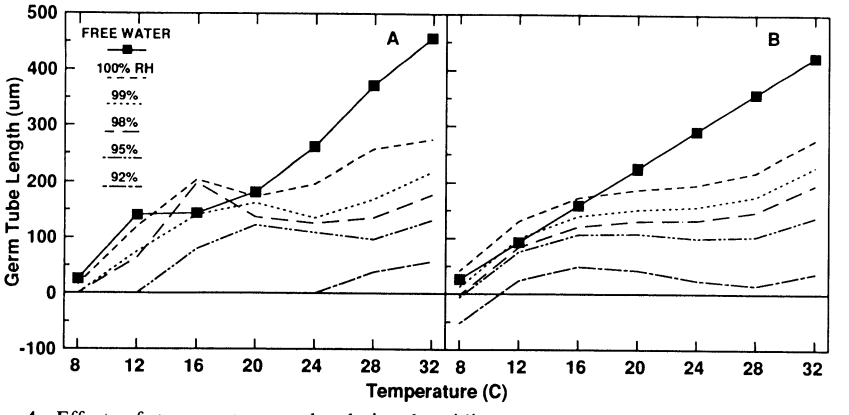

Fig. 4. Effect of temperature and relative humidity on ascospore germ tube length of *Botryosphaeria dothidea* 12 hr after treatments were imposed. **(A)** Actual data and **(B)** predicted values from regression equation (equation 3 in text). For free-water treatment, germ tube length was predicted from the following equation: $Y = -105.8441 + 16.5584T$ ($R^2_{adj} = 0.79$), where Y = germ tube length (μm) and T = temperature (C).

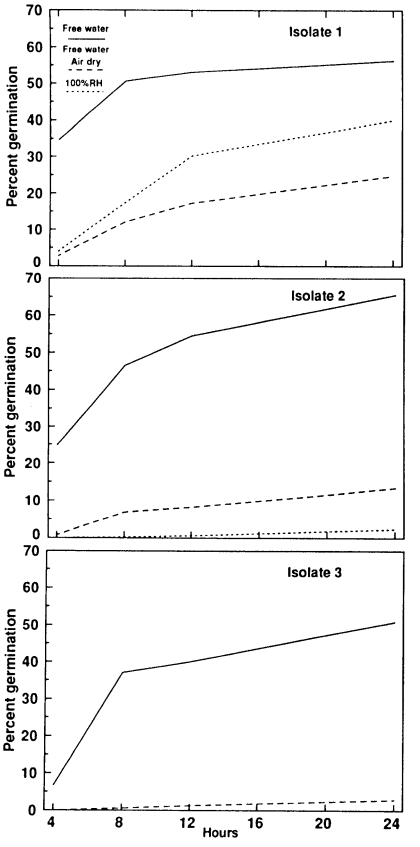

Fig. 5. Effects of water availability on the mean percent germination of conidia of three isolates of *Botryosphaeria dothidea* after 4, 8, 12, and 24 hr incubation. Average of eight temperatures (4, 8, 12, 16, 20, 24, 28, and 32 C).

minated after 24 hr following air drying and rewetting. Seventeen percent of the conidia of isolate 1 that were air dried germinated after 12 hr; germination increased to 24.5% after 24 hr. Germination of conidia of isolate 1 at 100% RH was greater than the rewetted treatment, but less than the free-water treatment, and ranged from 50 to 55% from 8 to 24 hr. Germination of conidia of isolates 2 and 3 was only 2.3 and 0%, respectively, after 24 hr at 100% RH.

DISCUSSION

Our results confirm those of Eid (5) who found that the optimum temperature for germination of conidia of *B. dothidea* was 25–30 C in free water. He reported no germination at 8.8 C after 25 hr; however, we found that 13.4–20.8% of the conidia of isolates 1 and 2 germinated at 8 C after 24 hr. The predicted optimum temperatures for germination of the three isolates we tested were similar. The predicted optimum temperature for ascospore germination was less than for conidia, although few ascospores germinated at 8 C after 12 or 24 hr. The optimum temperature for germ tube growth appears higher than the optimum for germination. Although we did not evaluate germ tube elongation above 32 C, germ tube length was greatest at that temperature. Kohn and Hendrix (9) reported that the optimum temperature for mycelial growth was 30 C.

Conidia of only one of the three isolates tested germinated well in the absence of free water. Germination of isolate 1 at 99% RH was about one-half that of 100% RH; less than 5% of the conidia germinated at 95% RH after 24 hr. This is in contrast to *B. obtusa* (Schwein.) Shoemaker, where some conidial germination occurred at RH as low as 92% (2).

Ascospores germinated over a wider RH range than conidia; approximately 30% germination was recorded at 92% RH after 12 hr. Germination was equally good at 98–99% RH. The results are similar to those reported for ascospores of *B. obtusa* (2). The ability of ascospores to germinate at less than 100% RH would function to extend the length of the germination period after the end of a wetting period and needs to be taken into account in determining whether or not infection criteria have been met. This ability may be highly significant after rains in the evening during the summer when RH may remain close to 100% through the night.

Germination of conidia of *B. dothidea* was greatly reduced when conidia were allowed to air dry and then were rewetted. Germination and germ tube growth of conidia of *B. obtusa* are irreversibly stopped by a 1-hr dry period after 4 hr of wetting (3). It appears that *B. dothidea* is equally sensitive to drying, but further studies are needed for confirmation. If ascospores and conidia of *B. dothidea* are sensitive to drying, then interrupted wetting periods would not be additive as with other pathogens such as *Venturia inaequalis* (Cooke) G. Wint. (12).

Germination of ascospores and conidia of *B. dothidea* occurred rapidly at favorable temperatures as evidenced by nearly 100% germination after 4 hr. This suggests that infection through wounds in the apple cuticle can occur quickly under favorable temperature and moisture conditions and may support the rationale for the use of a protectant as opposed to an after-infection control program for white rot. An after-infection program for white rot is predicated on the availability of a suitable eradicant fungicide and satisfactory control of other summer diseases of apples within the context of the white rot program.

The three isolates of *B. dothidea* used in this study varied in their responses to temperature and RH. Isolates 1 and 2 germinated at cooler temperatures than isolate 3, although isolate 3 had a lower (predicted) optimum for germination. Furthermore, isolates 2 and 3 had higher moisture requirements for germination than isolate 1. Other researchers have found isolates of *B. dothidea* to be highly variable in cultural characteristics and pathogenicity (6,14). This suggests that in determining criteria for fruit infection by *B. dothidea*, multiple isolates should be used to account for this variation.

ACKNOWLEDGMENTS

We thank L. R. Pope and Rae Cobb for technical assistance.

LITERATURE CITED

1. Alderman, S. C., and Beute, M. K. 1986. Influence of temperature and moisture on germination and germ tube elongation of *Cercospora arachidicola*. Phytopathology 76:715-719.
2. Arauz, L. F., and Sutton, T. B. 1989. Influence of temperature and moisture on germination of ascospores and conidia of *Botryosphaeria obtusa*. Phytopathology 79:667-674.
3. Arauz, L. F., and Sutton, T. B. 1990. Effect of interrupted wetness periods on spore germination and apple infection by *Botryosphaeria obtusa*. Phytopathology 80:1218-1220.
4. Brown, E. A., II, and Britton, K. O. 1986. *Botryosphaeria* diseases of apple and peach in the southeastern United States. Plant Dis. 70:480-484.
5. Eid, R. F. 1959. Etiology and control of *Botryosphaeria ribis* on apple. M.S. thesis. University of Delaware, Dover. 78 pp.
6. Fulkerson, J. F. 1960. *Botryosphaeria ribis* and its relation to a rot of apples. Phytopathology 50:394-398.
7. Gilpatrick, J. D., Smith, C. A., and Bowers, D. R. 1972. A method for collecting ascospores of *Venturia inaequalis* for spore germination studies. Plant Dis. Rep. 56:39-42.
8. Harris, R. F., Gardner, W. R., Adebayo, A. A., and Sommers, L. E. 1970. Agar dish isopiestic equilibration method for controlling the water potential of solid substrates. Appl. Microbiol. 19:536-637.
9. Kohn, F. C., Jr., and Hendrix, F. F. 1982. Temperature, free moisture, and inoculum concentration effects on the incidence and development of white rot of apple. Phytopathology 72:313-316.
10. 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.
11. 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.
12. Schwabe, W. F. S. 1980. Wetting and temperature requirements for apple leaf infection by *Venturia inaequalis* in South Africa. Phytophylactica 12:69-80.
13. Sutton, T. B. 1981. Production and dispersal of conidia by *Physalospora obtusa* and *Botryosphaeria dothidea* in apple orchards. Phytopathology 71:584-589.
14. 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.