Temperature, Free Moisture, and Inoculum Concentration Effects on the Incidence and Development of White Rot of Apple

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ABSTRACT

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The greatest incidence of infection of cultivar Top Red Delicious apples inoculated with *Botryosphaeria dothidea* conidia occurred at 30 and 35 C. Optimum rot development occurred at temperatures between 26 and 32 C. The temperatures at which lesion size increased most rapidly were in the range of optimum growth of the pathogen in culture. On unwounded apples, inoculum concentrations as high as 3×10^6 *B. dothidea* conidia per milliliter failed to produce infection, while concentrations as low as 5×10^3 conidia per milliliter caused as much infection on wounded apples as the maximum inoculum rate tested. When apples were wounded prior to inoculation, there was no significant difference in the infection rate between apples incubated for 0, 4, 8, 12, and 24 hr under a free moisture regime.

The ascomycetous fungus *Botryosphaeria dothidea* (Moug. ex. Fr.) Ces. & de Not. (=*B. ribis* Gross. & Dug.) causes a serious fruit rot and canker on apple trees (*Malus pumila* Miller) in the midwestern and southeastern United States. *B. dothidea* produces a soft, light colored rot on apple fruit, which is referred to as white rot or bot rot. This rot is sometimes visually indistinguishable from black rot, which is caused by *B. obtusa* (Schw.) Shoemaker, making isolation of the pathogen necessary for correct identification of the causal organism (8).

The effect of temperature on the growth of *B. dothidea* in vitro has been reported by several workers (1,14,15,18) who found that growth occurs between 10 and 35 C, with optimum growth at about 30 C. Fulkerson (6) reported that rot symptoms developed most rapidly at 18–24 C (65–75 F) while temperatures of 29 and 13 C (85 and 55 F) were unfavorable for rot development. Data on the effect of temperature on the incidence of rot infections are absent from the literature.

A minimum period of free moisture or high relative humidity is a common requirement for germination and infection by fungal spores (2). Brown (1) found that 97% of the conidia of *B. dothidea* germinated on water agar after 225 min, and that spores placed on wounded apple stems had penetrated them after 6 hr in a humidity chamber. However, he did not establish the minimum period of free moisture or inoculum level necessary to establish infection. He also reported that germ tubes consistently grew toward wounded areas of the stems. Several other workers (3,9,16–18) have reported that *B. dothidea* is primarily a wound pathogen, although the possibility of direct penetration under high inoculum concentrations has not been studied.

If environmental factors affecting the epidemiology of white rot of apple could be determined, this information could be utilized by growers to change from a conventional calendar-based spray program (10) to a spray-as-needed program. The benefits that

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could be realized would be savings to growers in spray application costs and less contamination of the environment by pesticides.

The objective of this study was to determine the effect of temperature, free moisture, inoculum concentration, and wounding on white rot of apple.

MATERIALS AND METHODS

Unless otherwise noted, the inoculum for these studies was prepared from 3-wk-old petri dish cultures of *B. dothidea* grown on acidified (pH 4) potato-dextrose agar (APDA) prepared from fresh potatoes at 30 C under continuous fluorescent light. The plates were flooded with a 0.01% Tween-20 solution and a spatula was used to scrape the surface of the culture to obtain a conidial/mycelial suspension. The resulting suspension was filtered through four layers of cheesecloth to remove mycelial fragments. The spore concentration of the suspension was determined with a hemacytometer and adjusted to the desired concentration with 0.01% Tween solution. Mature cultivar Top Red Delicious apples were used in all tests.

Apples for inoculation were washed in a mild detergent solution, rinsed with distilled water, and allowed to dry.

TABLE 1. Effect of inoculum concentration of *Botryosphaeria dothidea* and wounding on the incidence of rot lesions on apples[x]

Inoculum concentration (conidia/ml)	Wounded: Number apples infected[y]	Wounded: Mean number lesions per apple	Unwounded: Number apples infected[y]	Unwounded: Mean number lesions per apple
5×10^3	8/9	1.78 a	...[z]	...
10^4	9/9	2.11 a	...	...
5×10^4	9/9	3.00 a	...	...
10^5	9/9	2.44 a	1/9	0.11 b
5×10^5	...	...	0/9	0.00 b
10^6	...	...	1/9	0.11 b
3×10^6	...	...	1/9	0.11 b

[x]Number per total number observed.
[y]Inoculum levels not tested.
[z]Values followed by the same letter are not significantly different ($P=0.05$).

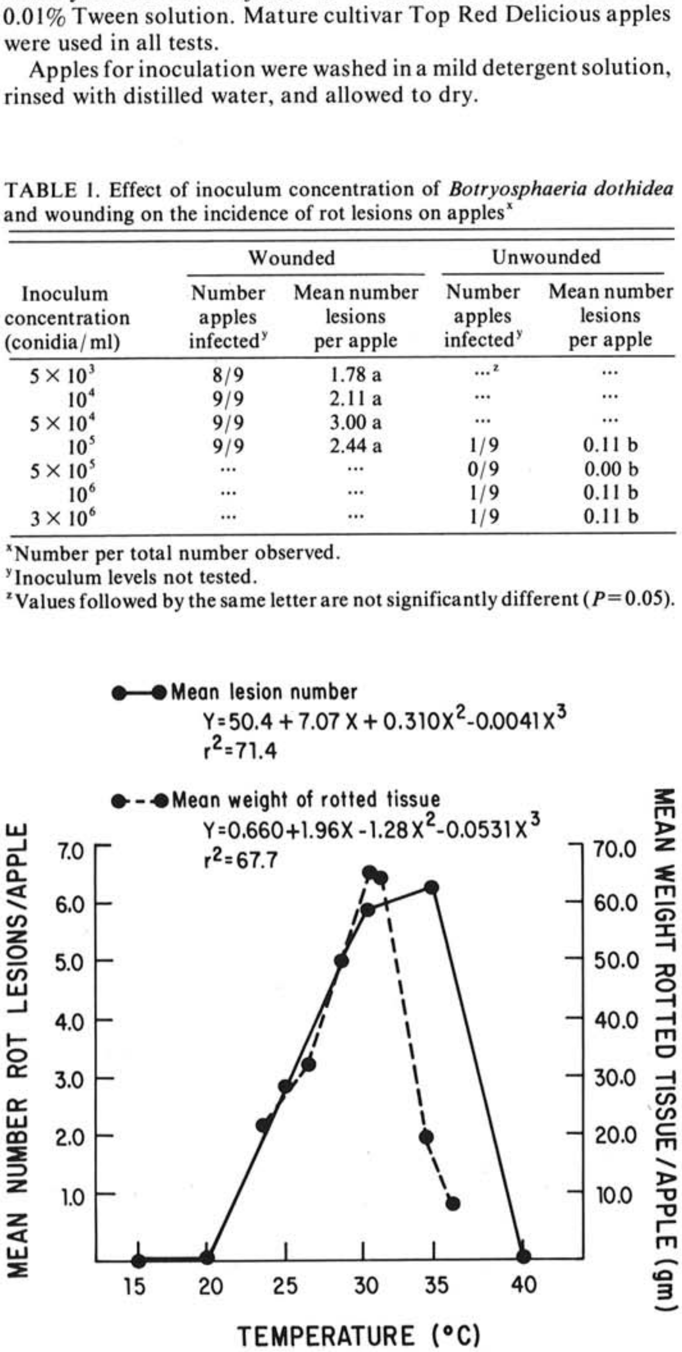

Fig. 1. Infection incidence and rot development after 6 and 4 days, respectively, on apples at various temperatures after inoculation with *Botryosphaeria dothidea*.

There were eight treatments, with nine replicate apples per treatment. Four of the treatments consisted of spraying wounded apples until runoff with suspensions containing 5×10^3, 10^4, 5×10^4, or 10^5 conidia per milliliter. The apples were wounded over their entire surface by a puncturing tool consisting of five dissecting needles taped into a bundle. Wounds were about 5 mm deep. The other four treatments consisted of spraying unwounded apples until runoff with conidial suspensions of 10^5, 5×10^5, 10^6, or 3×10^6 conidia per milliliter. The inoculated apples and appropriate controls were placed in damp chambers on wire mesh screens with wet absorbent cotton lining the bottom of the chambers, and incubated at 30 C. After 8 days, the number of apples that developed lesions, and number of lesions per apple in each treatment, were recorded.

The incidence of infection at various temperatures was determined by inoculating apples with a spray suspension containing 10^5 conidia per milliliter. The inoculum and the apples to be inoculated were prepared as described above, except that all treated apples were wounded prior to inoculation. The inoculated apples were placed in damp chambers and subjected to temperature regimes of 15, 20, 25, 30, 35, and 40 C. The apples were placed in incubators at the appropriate temperatures 12 hr before inoculation to allow the internal temperature of the apples to equilibrate at the treatment level. After 6 days, the number of apples infected and number of lesions per apple at each temperature were recorded.

To assess the effects of temperature on the rate of lesion development, apples were washed, rinsed, and dried as described earlier, and each apple was weighed and numbered. Inoculum was prepared by cutting agar plugs with a 4.0-mm-diameter cork borer from the advancing margins of 2-day-old cultures of *B. dothidea* on water agar. A 125-mm-deep section of the apples was removed with an 8.0-mm-diameter cork borer, and a water agar plug containing mycelium of *B. dothidea* was placed at the bottom of the hole in each apple. The apple section was then replaced and the cut area was sealed with tape to prevent drying. The apples were placed in damp chambers and incubated for 4 days at treatment temperature of 24, 26, 28, 30, 32, 34, and 36 C. Each treatment consisted of three apples with three replications per treatment plus controls. After 4 days, the apples were removed from the damp chambers and the rotted tissue was excised from each apple. The weight of rotted tissue was determined by substracting the weight of the apples after removal of the rotten tissues from the initial weight of the apples. This method of assessment was employed because superficial measurement of lesion diameter did not reliably indicate the extent of the rot.

The effect of temperature on the in vitro growth of *B. dothidea* was measured for comparison with other workers' results and with our results on infection and lesion development on apples. APDA was seeded with agar plugs cut with a 6.0-mm-diameter cork borer from the advancing margins of cultures of two single conidial isolates that had been growing for 2 days on APDA. The seeded

TABLE 2. Effect of temperature on growth rate on PDA of two isolates of *Botryosphaeria dothidea*[y]

Temperature (C)	Mean colony diameter after 2 days (mm)[z]: Isolate I	Isolate II
15	13.33 a	18.67 a
20	14.00 a	25.33 b
23	35.67 b	53.33 c
25	42.00 c	50.33 c
28	57.00 d	69.00 d
30	64.67 e	79.33 e
33	30.33 b	54.33 c
35	11.00 a	12.67 a
40	0.00 f	0.00 f
FLSD ($P=0.05$) Type I vs Type II	5.9	

[y]($P=0.05$) according to Duncan's multiple range test. Data are the mean of three replications.
[z]Values followed by the same letter are not significantly different.

plates were incubated at 15, 20, 23, 25, 28, 30, 33, 35, and 40 C. Each treatment was replicated three times. The plates were incubated for 2 days in the dark, after which growth was determined by measuring the colony diameter.

A dew chamber (Percival Model I-35 DL) was used to determine the length of time free moisture was required for apples to be infected. Apples were washed, rinsed, puncture wounded, and inoculated with a spray suspension containing 10^5 conidia per milliliter. The apples were allowed to dry and one group was immediately moved to a 30 C incubator at 37% relative humidity to provide a free moisture treatment of 0 hours. The remaining apples were placed in the dew chamber, which was calibrated to provide dew at an air temperature of 30 C, where they remained for 4, 8, 12, or 24 hr before being moved to the 30 C incubator at 37% relative humidity. After 5 days, the number of apples infected and number of infections per apple were recorded for each treatment.

RESULTS

Wounding and inoculum concentration. Apples wounded before inoculation developed rot at all inoculum levels tested. The difference in infection rate between these treatments was not significant (Table 1). The mean number of lesions per apple was 2.44 at the highest inoculum level (10^5 conidia per milliliter) and 1.78 at the lowest level (5×10^3 conidia per milliliter). In contrast, apples that had not been wounded before inoculation had very few infections and almost all of those were centered around lenticels and stem openings of the apple. These infections occurred through natural openings and were not considered to be instances of direct penetration.

Temperature studies. Infection incidence and rot development on apples were influenced by temperature. After 6 days at 30 and 35 C, all apples incubated were infected, and at 25 C for the same period all but one were infected. Also, the mean number of lesions per apple was significantly greater at 30 C (5.8 lesions per apple) and 35 C (6.2 lesions per apple) than at 25 C (2.8 lesions per apple) (Fig. 1). No visible infections developed at 15, 20, or 40 C after 6 days, but when apples incubated at 15 and 20 C were transferred to 30 C, rot lesions were visible after 2 days at the higher temperature.

Rot developed most rapidly at 26–32 C, less rapidly at 24 and 34 C, and slowly at 36 C (Fig. 1).

The effect of temperature on the growth rate of *B. dothidea* in vitro was similar to that for rot development on apples. The optimum temperature for growth of each isolate was at 30 C, and the organism grew almost as well at 28 C (Table 2). Growth of isolate 1 was less at 25 C and much less at temperatures below the 23 or above 33 C. Isolate 2 grew equally well at 23, 25, and 33 C. As with isolate 1, growth was slow at 15, 20, and 35 C. Neither isolate grew at 40 C. Also, isolate II grew significantly faster ($P = 0.05$) than isolate 1 at temperatures between 15 and 33 C.

In the free-moisture study, all of the apples developed numerous infections whether incubated for 0, 4, 8, 12, or 24 hr with free moisture.

DISCUSSION

No previous studies are available on the effect of the inoculum level of *B. dothidea* on infection rates. These results indicate that when wounded apples are present, low inoculum levels will suffice to cause infections. The lowest level of inoculum tested in our study (5×10^3 conidia per milliliter) was sufficient for inducing significant disease levels in vitro. Conidia of *B. dothidea* are exuded from pycnidia in a compact, gelatinous mass and are dispersed by splashing rain or water and possibly by insects. In a field situation, splash droplets that have contacted these spore masses probably contain spore concentrations in excess of that required to cause infections on wounded apples. The fact that we were unable to induce infection on unwounded apples with a high level of inoculum supports previous reports that *B. dothidea* is primarily a wound pathogen (1,3,9,17–19). Some infections may occur through unwounded tissues, but it is doubtful that this manner of penetration is of much importance in field situations. The likelihood exists that some type of wounding agent, possibly insects, may play an important role in the epidemiology of bot rot. Also, the effect of wounding on infection rates may be more complex than that of simply providing a means of entrance for the pathogen. Brown (1) noted that germ tubes of *B. dothidea* consistently grew toward wounded areas of apple stems, which suggests the possibility of a chemotactic interaction between host and pathogen, which could play an important role in the infection process.

The fact that *B. dothidea* was successful in infecting apples at 37% relative humidity (RH) presents an interesting dilemma. A study on the germination of fungus spores in relation to RH (2) has shown that conidia of certain genera of powdery mildews are the only fungal spores that can successfully germinate and cause infections at RH below 75%. The most likely explanation of our results is that during inoculation, spores were deposited directly within wounds in the apples. Because of exudations from the wounded tissues, free moisture or an RH close to 100% is probably present in the wound punctures, which enables the conidia to germinate and infect normally. In order to accurately assess the relation of RH and free moisture to infection by *B. dothidea*, the degree of wounding necessary for infection to occur should be defined and moisture levels manipulated in the wound area to determine the inoculum levels necessary for infection.

Temperature requirements for different aspects of white rot development show that the pathogen functions best under warm conditions (Fig. 1). The optimum temperature range for infection, 30–35 C, is slightly higher than the 28–32 C optimum range for rot development. Both infection and rot development slow rapidly outside these ranges. Infection does not occur at 40 C and rot development is severely curtailed at 36 C. That apples incubated at 15 and 20 C did not develop rot lesions until they were transferred to 30 C, indicates that although conidia will remain viable at lower temperatures, infection either does not occur or it occurs only slowly. The disease progressed to some extent even at temperatures as low as 24 C and as high as 34 C.

Our observations that the effect of temperature on growth of *B. dothidea* in culture is similar to its effect on growth in apples generally agrees with those of other workers' results (1,15,16,19). However, Fulkerson (6) reported that temperatures of 30 C (85 F) were unfavorable for rot development. Our results do not support his observations.

The possibility that multiple strains or races of *B. dothidea* exist has been raised by other workers (4–6,12–14). Drake (4) found that isolates of *B. dothidea* from hosts other than apple were pathogenic on apples, but that the symptoms varied with different isolates. The two isolates used in our studies showed substantial differences in growth rates; isolate I grew significantly more slowly in culture than did isolate II. Our Type I isolate was recovered from lesions on apples that expanded slowly, were rather firm to the touch, and had a dark brown coloration. In contrast, the Type II isolate was recovered from lesions that expanded rapidly, were soft to the touch, and had a light brown to buttery yellow coloration. This raises the possibility that there are two or more strains of *B. dothidea* pathogenic on apples in Georgia.

The results of these studies indicate that *B. dothidea* causes rot of apples under a fairly wide range of environmental conditions. However, with careful monitoring of weather conditions, it may be possible to use this information in disease forecasting, thereby making control of white rot more effective and economical for apple growers.

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