Changes in Conidia of *Monilinia fructicola* in Response to Incubation Temperature

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ABSTRACT

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Incubation at 15, 20, or 25 C caused differences in the size, percentage germination, and aggressiveness of 2-wk-old conidia from two isolates of *Monilinia fructicola* cultured on potato-dextrose agar. The volumes of *M. fructicola* conidia were largest at 15 C and smallest at 25 C. The percentage germination was lower on water agar for spores produced at 25 C than for those produced at 15 C but were approximately equal on peach agar. The temperature at which spores were produced influenced their capacity to initiate rot on peach fruit. Three days after inoculation the size of lesions on wounded fruit was largest for conidia from 15 C, intermediate for conidia from 20 C, and smallest for conidia from 25 C. Similar results were found when fruit was inoculated without wounding.

Additional key words: brown rot, germination, infectivity, peach.

The conidia of *Monilinia fructicola* (Wint.) Honey have been reported to be in the range of 6–19 × 19–28 μm (15); other reports indicate average sizes of 10.2 × 15.9 μm (9), 9.9 × 14.7 μm (12), 11 × 15 μm (11), and 14 × 21 μm (1). The sizes of conidia of *M. fructicola* vary greatly in response to the environment (1,2,10–12), but how this variation may influence the quality of macroconidia as inoculum has not been examined.

Temperature has been implicated as a factor affecting size changes of conidia of *Monilinia laxa* (Aderh. and Ruhl.) Honey (2,19). The effect on size may be due to a direct effect of temperature on the pathogen or temperature may alter the growth substrate. A physical or chemical change of the substrate on which a fungal pathogen grows may affect inoculum by changing the endogenous nutrient (13,17) or alter the synthesis of a specific substance that affects growth (3). Substrate influence may explain the difference in size between those conidia found on peach blossoms (16.24 × 10.46 μm) and on potato-dextrose agar (PDA) (9.09 × 14.24 μm) (10). After the observation that spore size was influenced by temperature, a laboratory study was initiated on the effects of incubation temperatures on the quality of *M. fructicola* conidia as inoculum.

MATERIALS AND METHODS

Two isolates of *M. fructicola*, A (ATCC 32670) and B (ATCC 44557), were grown in 90-mm-diameter plastic petri dishes containing 20 ml of PDA in unlighted incubators at 15, 20, or 25 C ± 1 C. Conidia were washed from the surface of 2-wk-old agar cultures, filtered through four layers of cheesecloth, washed twice in deionized water by centrifugation and resuspended in water. The conidia were then counted and sized with a particle counter (Electro Zone/Celloscope, model 112LTH, Particle Data, Inc., Elmhurst, IL 60126) or with a microscope equipped with an ocular micrometer.

Germination was determined by placing washed conidia on water agar (20 g agar per liter of water) or peach agar (128 g of Gerber [Gerber Products Co., Fremont, MI 49412] strained peaches and 20 g agar per liter of water) at 25 C and by microscopically examining one plate per hr (100 spores) for 5 hr, and again after 24 hr. A spore was considered germinated when a germ tube was longer than the length of the spore. For inoculation, a washed suspension of conidia was counted with the particle counter and diluted to 30 or 300 conidia per 0.03 ml. Fruit placed in a plastic fruit tray were inoculated by placing a 0.03-ml drop of conidial suspension onto an uninjured surface of a peach, or on a wound made by puncturing the surface 2 mm deep with a glass tube 2 mm in diameter. The liquid in the drop of inoculum suspension was allowed to evaporate to near dryness, which required about 2 hr at 20 C. The fruit was then covered with an inverted plastic fruit tray that did not touch the inoculated surface of the fruit. In all tests the inoculated fruit was placed in four random blocks and held at 20 C. The diameters of the lesions (wounded fruit) or the numbers of infected fruit (unwounded fruit) were recorded after 3–4 days.

Tests with noninjured fruit had four replicates of 25 fruit per treatment, whereas tests with wounded fruit had four replicates of 10 fruit per treatment. Treatments of both noninjured and wounded fruit included a noninoculated control (water only placed on fruit) and fruit inoculated at one site with 30 or 300 conidia produced at 15, 20, or 25 C. Tests with both isolates of *M. fructicola* were repeated three times with similar results.

RESULTS

Spore production and size. Generally more conidia were produced at 20 C than at 15 or 25 C, but their average size was greatest at 15 C (Tables 1 and 2). In a typical test, isolate A produced conidia that measured 14 × 10 μm, 13 × 10 μm, and 12 × 9 μm at 15, 20, and 25 C, respectively (Figs. 1 and 2). The volume of spores produced at 15 C consistently was significantly different from those produced at 25 C. Spores from 20 C were intermediate in size and often were not significantly different from those produced at 25 C (Table 2). The differences in the volume of conidia were similar whether measured by microscopic means or by the electronic particle counter (Table 1).

Spore germination. On water and peach agar the conidia produced at 15 C germinated first and were followed by those from cultures grown at 20 and 25 C. Germination of spores was nearly complete in 5 hr on water agar when the spores were produced at 15 C, and on peach agar for conidia from all temperature regimes. After 24 hr on water agar, total germination was as much as 20% lower when the conidia were produced at 25 C than when they were produced at 15 C. On peach agar, all or nearly all conidia germinated within 24 hr, regardless of the temperature at which they were produced (Table 1). Differences in the germination of the

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TABLE 1. Size and germination of conidia produced by two isolates of *Monilinia fructicola* grown on potato-dextrose agar for 14 days at 15, 20, or 25 C

Isolate and temperature (C)	Conidia per plate (no.)[a]	Size and S.E.				Germination (%):[d]			
						after 5 hr		after 24 hr	
		Length[b] (μm)[e]	Width[b] (μm)[e]	Volume[b,c] (μm^3)[e]	Volume[a] (μm^3)	Water[f] agar	Peach[g] agar	Water[f] agar	Peach[g] agar
Isolate A									
15	940,000	14 (0.27)	10 (0.24)	784 (44)	743	95	98	98	99
20	3,230,800	13 (0.33)	10 (0.26)	644 (40)	635	92	100	100	100
25	2,077,600	12 (0.29)	9 (0.23)	581 (29)	581	79	97	99	97
Isolate B									
15	747,600	15 (0.53)	11 (0.26)	911 (59)	852	80	99	100	100
20	9,686,000	13 (0.38)	9 (0.16)	625 (28)	581	8	94	87	100
25	8,186,800	12 (0.49)	9 (0.22)	498 (36)	526	22	90	81	96

[a] Measured by an electronic particle counter.
[b] Measured microscopically (25 conidia).
[c] Volume = (π length/6) $\times$ (width)^2.
[d] Based on 100 conidia.
[e] Standard error in parentheses.
[f] Water agar contains 20 g agar per liter of water.
[g] Peach agar contains 20 g agar, 128 g of strained peaches per liter of water.

TABLE 2. Average volume of conidia measured for two isolates of *Monilinia fructicola* grown on potato-dextrose agar 14 days at 15, 20, or 25 C[a]

Incubation temperature (C)	Isolate A (μm^3)	Isolate B (μm^3)
15	824 ± 86[b]	967 ± 157[b]
20	702 ± 50	682 ± 156
25	616 ± 46	548 ± 61

[a] Spore volumes measured by an electronic particle counter.
[b] $P = 0.05$ confidence limit, data based on six tests on 5,000–10,000 conidia at each temperature.

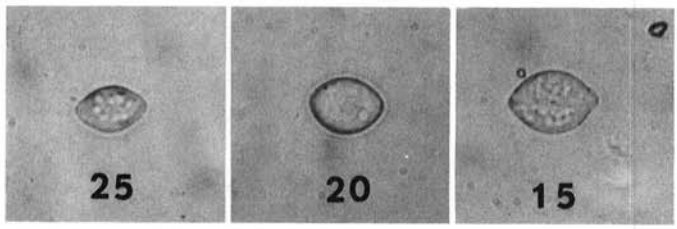

Fig. 2. Average size conidia from *Monilinia fructicola* ATCC 44557, grown 14 days on potato-dextrose agar at 25, 20, or 15 C. The sizes are: 12 $\times$ 9 μm for 25 C, 13.2 $\times$ 9.9 μm for 20 C, or 15.5 $\times$ 10.8 μm for 15 C.

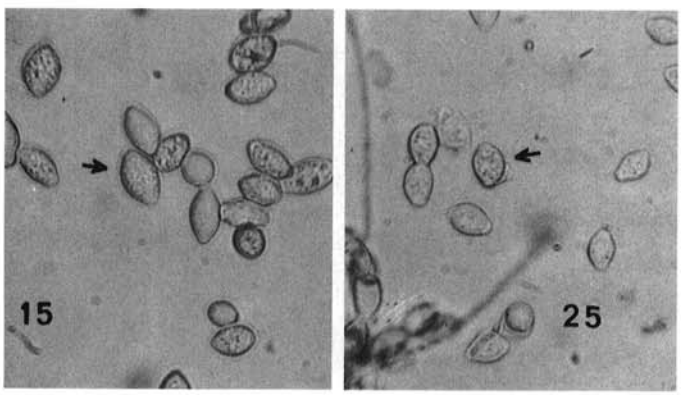

Fig. 1. Conidia from *Monilinia fructicola* ATCC 32670, grown for 14 days on potato-dextrose agar at 15 or 25 C. The arrows point to a spore of average size, 14 $\times$ 10 μm for 15 C and 12 $\times$ 9 μm for 25 C.

spores produced at the three temperatures were consistent in all tests, but differences between the isolates were not.

Pathogen aggressiveness. The 15 C conidia produced at 15 C consistently induced larger lesions than those obtained from cultures grown at 25 C when placed on wounded fruit (Fig. 3). Also, conidia produced at 15 C on sound fruit induced a higher level of infection than did the conidia produced at 25 C (Fig. 4). Applying 300 conidia to the fruit consistently increased the lesion size and frequency of infection as compared to 30 conidia per inoculation. Increasing the number of conidia on the fruit does not increase the severity of rot linearly (14); consequently, this makes it difficult to obtain the relative aggressiveness of the conidia when only two inoculum densities are used. However, because the severity of rot, as measured by lesion expansion or frequency of infection, was nearly equal for 30 conidia per inoculation produced at 15 C or 300 conidia produced at 25 C (Figs. 3 and 4), a comparison here is valid. The conidia grown at 15 C on PDA appear to be nearly 10 times as aggressive as conidia grown at 25 C on this medium.

Differences in the severity of rot caused by the two isolates were not consistent in all tests (Fig. 3).

DISCUSSION

The conidia of *M. fructicola* varied in size as a result of incubation temperature. The effect of temperature on the size and aggressiveness of naturally occurring inoculum is not known, but data presented herein suggest that a relatively cool environment influences the biosynthetic process for production of conidia by increasing their size and thus the energy available for infection, inoculum potential sensu Garrett (5). There are a number of infection courts on the peach fruit (6,14). The faster germination of conidia produced at 15 C when compared with those from cultures grown at 25 C indicates that the former are more vigorous and would therefore have an increased probability of locating a suitable infection court and penetrating the host. Germination alone, however, may not explain the aggressiveness of the spores grown at 15 C. A cool environment may induce additional characteristics not found in spores produced at a warm temperature. Greater vegetative vigor of an isolate of *M. fructicola* also has been associated with increased lesion expansion (7). Thus, temperature may change both the rate of infection by changing the germination of the spore and lesion development by changing some other factor.

Various critical temperatures are associated with the activity of fungal pathogens. The optimum temperature for growth of *M. fructicola* is between 20 and 25 C (4,16), the maximum between 30 and 33 C, and the minimum between 1 and 3 C (4,8,16). Sporulation occurs between 5 and 30 C, with the optimum between 15 and 25 C (8,16); germination of the conidia occurs within the same range (16,18). The critical temperatures associated with aggressiveness, although not definitely determined by this study,

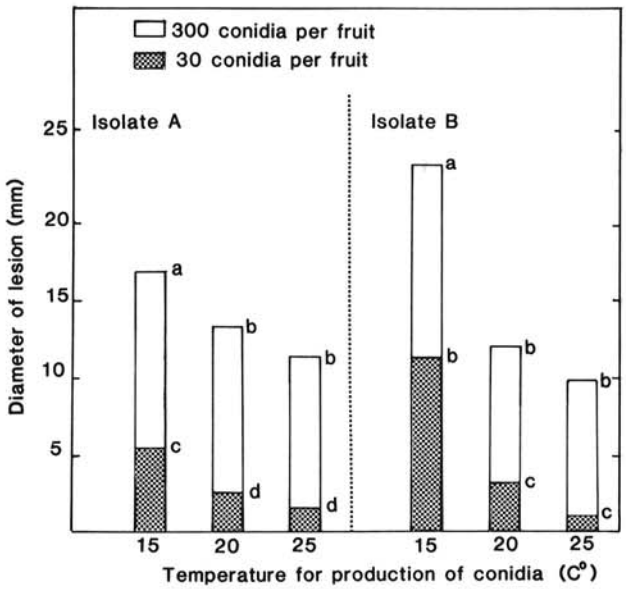

Fig. 3. Brown rot lesion diameter after 3 days at 20 C on 'Windsor' peach fruit inoculated at a wound with conidia grown at 15, 20, or 25 C. The fruit were inoculated with 30 or 300 conidia per fruit wound from two isolates of *Monilinia fructicola* (**A,** ATCC 32670 or **B,** ATCC 44557). Means shown for one isolate followed by the same letter are not significantly different according to Duncan's multiple range test, $P = 0.05$.

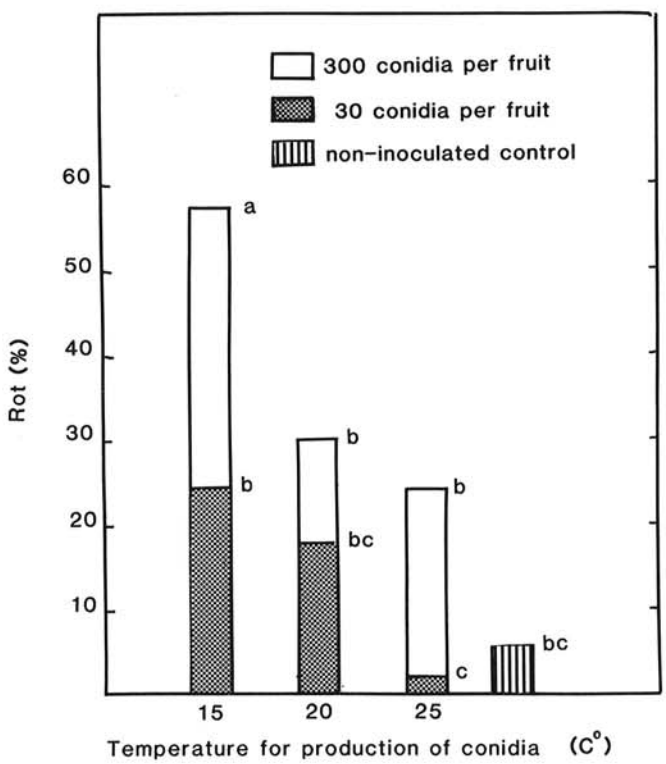

Fig. 4. Percent of fruit with brown rot after 4 days at 20 C on sound 'Autumn Gem' peach fruit inoculated with conidia grown at 15, 20, or 25 C. Fruits were inoculated with 30 or 300 conidia per fruit from *Monilinia fructicola* ATCC 44557. Means followed by the same letter are not significantly different according to Duncan's multiple range test, $P = 0.05$.

appear to differ from those associated with simple growth or reproduction in that moderately low temperatures (ie, 15 C) result in the production of more aggressive conidia than production at higher temperatures.

The manipulation of conidium size may be a useful tool for studying the physiology of brown rot. Further, knowing the effects of temperature on the quality and quantity of inoculum would improve our ability to predict the occurrence and severity of brown rot. Finally, low temperatures may increase the aggressiveness of other pathogens causing diseases in fruit and vegetables.

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