Effect of Temperature on *Monilinia fructicola* Conidia Produced on Fresh Stone Fruits

D. J. PHILLIPS, Plant Pathologist, Quality Maintenance and Transportation Research Unit, Horticultural Crops Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Fresno, CA 93747

ABSTRACT

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Incubation at 15, 20, or 25 C caused differences in the size and aggressiveness of conidia from *Monilinia fructicola* cultured on peach or nectarine fruit. The volume and aggressiveness of the conidia grown in incubators were greatest at 15 C and least at 25 C. Temperature had less effect on the size of spores produced on nectarines than on peaches. Spores produced on potato-dextrose agar were smaller and less aggressive than those produced on fresh peaches. Spores collected from fruit rotting in orchards near Fresno, CA, became smaller as the average 3-day maximum temperature increased.

Additional key words: brown rot, germination

The size of conidia of *Monilinia fructicola* (Wint.) Honey varies greatly in response to the environment (1,4,6). *M. fructicola* conidia produced on potato-dextrose agar (PDA) at a moderately low temperature (15 C) are larger and more aggressive than conidia produced at a higher temperature (25 C) (6). Substrate has been shown to influence aggressiveness in some fungi (5,7) and may influence the size of conidia produced by *M. fructicola* (4). This paper reports results of laboratory and orchard studies on the effect of temperature on size, germination, and aggressiveness of conidia of *M. fructicola* produced on peach and nectarine fruit.

MATERIALS AND METHODS

Laboratory studies. Spores were grown in temperature-controlled (±0.5 C) incubators during 1981 and 1982 in 15 tests with peaches (eight cultivars) and 25 tests with nectarines (16 cultivars) grown in the vicinity of Fresno, CA. When possible, the tests used paired samples consisting of one sample from a peach and one from a nectarine. Sound fruit were selected soon after harvest. Fifteen fruits were selected from each test cultivar and inoculated with an uncounted suspension of conidia obtained from a 2-wk-old culture of *M. fructicola* (ATCC 44557) that was grown at 20 C on PDA. The inoculation was made by placing a drop of the conidial suspension onto a puncture (2–4 mm) made with a sterile dissecting needle on the surface of the test fruit. Inoculated fruit were placed on a tray, covered loosely with a flexible plastic sheet, and incubated at 15, 20, or 25 C (five fruits per temperature per test cultivar). The size and percent germination of spores were determined after 5 days.

In two separate laboratory tests, the aggressiveness of the spores was determined in addition to their size and germination. For these tests, conidia were grown at 15, 20, or 25 C on peach fruit (cultivars Red Top or Suncrest) and on PDA. Samples of the conidia grown on fruit at each temperature (5-day-old cultures) and on PDA (2-wk-old cultures) were measured for spore volume and for germination on water agar (20 g of agar per liter of water) or peach agar (128 g of Gerber strained peaches and 20 g agar per liter of water) and inoculated into fresh peaches to test the aggressiveness of the conidia.

Field studies. Conidia of *M. fructicola* (wild isolates) were collected from peach and nectarine orchards in the California counties of Fresno and Tulare in 1981 and 1982. The volume and germination (1982 only) of these conidia were tested for possible correlations with the 3-day running average (RA) of the maximum, minimum, and average temperature data taken from a weather station in Fresno, CA. The RA was employed because the temperature 3 days before the collection date was an estimate of the conditions during spore formation. Spores for this phase of the study were obtained from 76 peach-growing sites and 81 nectarine-growing sites.

Spore aggressiveness. To test conidia for aggressiveness, suspensions of conidia were washed twice with distilled water, counted, and diluted to 35 or 350 conidia per 0.03 ml. Peach fruit placed in a plastic fruit tray were inoculated by placing a 0.03-ml drop of conidial suspension onto a wound made by puncturing the surface 2 mm deep with a glass tube 2 mm in diameter. The drop was allowed to evaporate to near dryness, which required about 2 hr at 20 C. The fruit were then covered with an inverted plastic fruit tray that did not touch the inoculated surface of the fruit. Inoculated fruit were placed in four randomized complete blocks and held at 20 C. Diameters of lesions formed at the inoculation site were measured after 3 or 4 days. Each of the two tests of aggressiveness had four replicates of five fruits per treatment. Treatments included an uninoculated control (water only placed on the fruit) and fruit inoculated at one site with 35 or 350 conidia produced on PDA or peach at 15, 20, or 25 C.

Spore size. Spore sizes were determined by measuring the volume of individual conidia with a particle counter (Electro Zone/Celloscope, model 112LTH, Particle Data, Inc., Elmhurst, IL). Conidia were suspended in distilled water, filtered through cheesecloth, and measured with the counter.

Spore germination. For germination tests, conidia were taken from the sporulating surface of the fungus colony with a glass rod and transferred directly onto water agar plates, and in some tests, onto peach agar. Germination was determined by microscopically examining one plate from each sample at 3, 5, and 24 hr. A spore was considered germinated when a germ tube was longer than the length of the spore.

RESULTS

Laboratory studies. The size of spores produced on peaches and nectarines in laboratory incubators was significantly influenced by the temperature during spore production. The average volumes of spores produced at 15, 20, or 25 C were 1,002, 862, and 697 μ^3, respectively. When temperature was treated as a continuous variable in the statistical analysis, the effect of temperature was significantly greater in peaches (-39 ± 5 μ^3 change per degree [C]) than in nectarines (-22 ± 4 μ^3 change per degree [C]) (Table 1). Of the nectarine cultivars tested in 1981 and 1982, nine of 18 did not produce spores that changed consistently with the temperature, whereas all peach cultivars produced spores that were smaller as the temperature increased. The

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Table 1. Size of conidia produced by *Monilinia fructicola* (ATCC 44557) grown on nectarines and peaches for 5 days at 15, 20, or 25 C (1982)

Inoculation date	Spore volume (μ^3)[a]						
	Nectarine				Peach		
		Production temperature (C)[b]				Production temperature (C)[b]	
	Cultivar	15	20	25	Cultivar	15	20	25
27 May	Aurielo Grand[c]	850	960	850	...	...	...	...
1 June	Aurielo Grand[c]	900	960	525	Springcrest	1,175	745	635
2 June	Armking	960	745	580	Springcrest	1,175	745	635
4 June	May Grand	1,015	800	690	...	...	...	...
8 June	May Grand	905	745	420	Red Babcock	1,070	960	635
10 June	...	...	...	...	Gemfree	1,070	960	635
17 June	...	...	...	...	Gemfree	1,070	850	745
17 June	...	...	...	...	June Lady	1,125	1,015	850
21 June	Spring Red	1,070	850	1,070	...	...	...	...
21 June	Early Sungrand	1,070	960	745	...	...	...	...
30 June	Independence[c]	1,015	1,070	850	...	...	...	...
1 July	Moon Grand	1,070	960	745	Red Top	1,070	850	850
14 July	Red Grand[c]	905	960	690	...	...	...	...
21 July	...	...	...	...	Sparkle	1,015	800	525
23 July	Red Free	850	850	635	...	...	...	...
28 July	Red Free[c]	690	800	635	...	...	...	...
5 August	Fantasia[c]	905	1,070	960	Sparkle	960	800	745
12 August	Flamekist	800	635	420	...	...	...	...
12 August	Royal Giant	905	580	470	...	...	...	...
27 August	Autumn Grand[c]	850	960	905	...	...	...	...

[a] Measured by an electronic particle counter. The midpoint of the mode interval from a histogram with 110-μ^3 intervals is reported.
[b] When temperature was analyzed as a continuous effect, the volume estimates and standard errors (in parentheses) for nectarine spores at 15, 20, and 25 C were 941 (24), 830 (15), and 718 (24), respectively, and for peach spores, 1,070 (33), 877 (21), and 684 (33).
[c] Nectarine cultivars where the largest spores were produced at 20 C.

effects of cultivar, although significant, are not reported because careful tests under various cultural conditions are needed to establish a consistent cultivar reponse.

Germination after 24 hr for spores produced on peaches and nectarines did not vary consistently with the temperature during spore production. Germination was greater after 3 hr for spores produced at 15 C than at 25 C.

In the two tests in which spore aggressiveness was evaluated, the spores produced on peaches were larger and more aggressive than spores produced on PDA (Table 2). Spores produced on both peaches or PDA were larger and more aggressive when produced at 15 C than when produced at 20 or 25 C (Table 2). Germination was greater at 15 than at 25 C on both growth substrates; however, germination was affected significantly by temperature only when the spores were produced on PDA.

Field studies. The volumes of spores produced on fruit in an orchard averaged 1,475 μ^3 in 1981 and 1,221 μ^3 in 1982 (Table 3). The volume of the spores was significantly and negatively correlated with the RA of the maximum, minimum, and average temperature in 1981 but not with the average in 1982 (Table 4). The RA of the maximum air temperature yielded a consistent correlation with spore volume. In 1982, when 127 orchards were sampled, there was a decrease of 25 μ^3 for each increase of 1 degree in the 3-day average of the maximum temperature in the range of 25 and 40 C. The volumes of spores produced on peaches or nectarines were similar.

Table 2. Spore volume, spore germination and aggressiveness of spores produced at 15, 20, or 25 C on potato-dextrose agar (PDA) or on peach fruit

Fungus[a] growth substrate	Growth temperature (C)[b]	Spore volume (μ^3)[c]	Spore germination (%)[d]				Inoculum density (spores/ puncture)	Spore aggressiveness (lesion diam. [mm])[e]
			On water agar			On PDA		
			3 Hr	5 Hr	24 Hr	24 Hr		
PDA	15	740	20	70	99	100	35	9.6
							350	30.3
	20	530	0	38	98	98	35	11.9
							350	27.0
	25	530	2	10	90	94	35	4.6
							350	9.9
Peaches[f]	15	1,015	93	100	98	98	35	26.7
							350	27.9
	20	1,015	83	99	98	98	35	22.5
							350	26.6
	25	850	89	95	95	97	35	14.2
							350	21.1

Comparisons of group means	Lesion diameter (mm)[g]
PDA	15.5 b
Peaches	21.8 a
35 Spores/puncture	13.6 b
350 Spores/puncture	23.7 a
15 C	22.1 a
20 C	21.9 a
25 C	11.9 b

[a] *Monilinia fructicola* (ATCC 44557).
[b] Temperature maintained at ±0.5 C.
[c] Measured by an electronic particle counter. The midpoint of the mode interval from a histogram with 110-μ^3 intervals.
[d] Each datum represents the germination of 100 spores after 24 hr on water agar (20 g agar/L) at 20 C.
[e] Each figure represents the mean from four replicates of five peaches, cultivar Sparkle.
[f] Cultivar Suncrest.
[g] All treatments were analyzed as noncontinuous variables. Means not followed by the same letter differ according to Duncan's multiple range test ($P = 0.05$).

Germination of the spores produced on peaches and nectarines in 1982 and measured after 24 hr was significantly (127 samples, $P = 0.01$) and negatively correlated with this average maximum temperature (Table 4). However, when the data for spores from nectarines and peaches were analyzed separately, an increase in temperature significantly

Table 3. Size and germination of *Monilinia fructicola* spores collected in 1981 and 1982 from orchards in the San Joaquin Valley of California

Sampling year	Fruit type	Number of samples	Spore volume (μ^3)				Spore germination (%)			
			Mean	Standard deviation	Maximum	Minimum	Mean	Standard deviation	Maximum	Minimum
1981	All	24	1,475	395	2,152	806	...	...	...	...
1982	All	127	1,221	218	2,066	689	76	20	100	12
	Peach	58	1,213	240	2,066	689	77	21	100	12
	Nectarine	69	1,228	199	1,862	718	75	20	100	18

Table 4. Correlation of 3-day average, maximum, and minimum air temperatures with the volume and germination of spores of *Monilinia fructicola* collected from peach and nectarine orchards in 1981 and 1982

Daily temperatures (3-day running average)	Correlation coefficients (fruit type and year)						
	Spore volume				Spore germination		
	All (1981)	All (1982)	Peach (1982)	Nectarine (1982)	All (1982)	Peach (1982)	Nectarine (1982)
Average	−0.65*[a]	−0.20	−0.21	−0.27	−0.04	−0.02	−0.04
Maximum	−0.49*	−0.43*	−0.44*	−0.44*	−0.19	−0.04	−0.34*
Minimum	−0.67*	−0.29*	−0.26	−0.33*	−0.19	−0.08	−0.28*

[a] * = Significance at $P = 0.01$ or less.

reduced the germination of the spores produced on nectarines but not of those produced on peaches.

DISCUSSION

Results indicate that environmental conditions during spore production of *M. fructicola* on host fruits influence the inoculum potential of the propagules. The spore physiology can be especially altered by changes in the fungal growth substrate (3). Spore size and germination of spores may influence pathogenicity (2). In these and earlier studies (6), large spores produced by an isolate of *M. fructicola* were more aggressive than small spores. Spore size may be controlled by unknown factors, such as growth-regulating metabolites, which can be modified by changing the growth temperature and substrate. An interaction between temperature and substrate was shown by the greater influence of temperature on the size of spores produced on peaches than of spores produced on nectarines.

The exogenous nutrients needed for germination may be less for a large than for a small spore (2), and more energy may be available in large than in small spores for successful colonization of a substrate. Our study demonstrated that the proportion of spores that germinate, although altered by the temperature during spore production, did not always follow the changes in spore size. Thus, germination, although a prerequisite for colonization, may be only one of several growth processes that contribute to the aggressiveness of the spores.

We do not know the mechanism causing temperature-related changes in the spores. The composition of the spores of *M. fructicola* produced in different temperatures should be defined.

It is recognized that a single weather station, as used in this study, to estimate conditions in a large area ignores a wide variation in temperature and moisture found among and within the orchards. A direct estimate of the microclimate of the spores should provide better information. However, the size of the spores on fruit in the orchards sampled correlated with data from this weather station, which indicates that temperature influences the size of naturally occurring spores. Air temperature prior to the infection should be considered when estimating the disease potential or developing models of brown rot development in an orchard.

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