Thermolability of Dormant and Germinated Monilinia fructicola and Rhizopus stolonifer Spores

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ABSTRACT

Exposure of dormant *Monilinia fructicola* spores to 52-C broth for 120 sec prevented subsequent germination of these spores at 21 C. Shorter exposures to 52 C or exposures in 49 C broth for 60 and 120 sec greatly reduced subsequent germination. *Rhizopus* spores survived these temperatures better than *Monilinia* spores. Germinated spores of both genera were killed more quickly and at lower temperatures than the dormant spores. Hot broth treatments caused changes in the morphological characteristics of germinated *Rhizopus*, but not those of germinated *Monilinia* spores. Phytopathology 60: 866-868.

In recent years, interest in thermotherapy to control the development of decay of produce after harvest has revived. Exposure of peaches to 52 C water for 2 to 3 min effectively controls development of decay caused by either *Monilinia fructicola* (Wint.) Honey or *Rhizopus stolonifer* (Ehr. ex Fr) Lind. (3). Because the control of development of decay of peaches caused by *Monilinia* or *Rhizopus* by heat treatments has not been fully explained, further studies were conducted on the effect of heat on spores of these organisms. In these studies, both dormant and germinated spores were treated.

MATERIALS AND METHODS.—*Monilinia* or *Rhizopus* isolates obtained from decayed peaches, then purified and maintained on potato-dextrose agar (Difco), were used as a source of spores. In these tests, 5 ml of broth (0.5% Bactopeptone, 0.3% beef-extract, pH about 7.0) was heated in 25-ml flasks in a constant-temp shake culture bath. Broth temp were maintained at 43, 46, 49, and 52 C. Then 0.5 ml of a suspension of *Monilinia* or *Rhizopus* spores (obtained from week-old cultures) was pipetted into each flask for each temp and exposure time (ranging from 10 to 120 sec). Spore concentrations had been previously adjusted so the concentration in the heated broth was about 300 spores/ml. After each exposure at a given temp, 1 ml of the heated spore suspension was cooled almost immediately when the suspension was pipetted onto previously cooled potato-dextrose agar in petri dishes. Then the dishes were placed at 21 C for 24 hr and the number of germinated spores counted under a microscope (magnification about ×150).

For studies with germinated spores, suspensions of dormant *Monilinia* or *Rhizopus* spores were incubated in nutrient broth at 21 C until about 90% of the spores had germ tubes 2-3 times the diam of the spores. *Monilinia* spores germinated without perceptible swelling in 4 to 5 hr. *Rhizopus* spores swelled appreciably in 2-3 hr, and continued to swell until germinated in 5 to 6 hr. The germinated spores were added to and then removed from the heated broth in the same fashion as the dormant spores. The number of these spores with germ-tube elongation after heating was counted after the petri dishes were placed for 24 hr at 21 C.

RESULTS.—*Dormant spores.*—Exposure of dormant spores (*Monilinia* or *Rhizopus*) to broth temp of 43 or 46 C for 30-120 sec had little or no effect upon the number of surviving spores (Table 1). Each exposure to broth at 49 or 52 C reduced the number of spores that survived. After the 120-sec exposure to 49 or 52 C broth, almost all or all of the *Monilinia* spores were killed, and the number of *Rhizopus* spores surviving was reduced greatly. More *Rhizopus* than *Monilinia* spores survived exposure to 49 or 52 C broth.

Germinated spores.—Exposure of germinated spores to heated broth usually reduced greatly the number of spores with continued germ-tube elongation (Table 1). After exposure to 43 or 46 C broth, usually fewer *Rhizopus* than *Monilinia* spores survived; after exposures to 49 or 52 C broth, none of the *Monilinia* spores survived; after most exposures, a low per cent of the *Rhizopus* spores survived. The greater susceptibility of *Rhizopus* than *Monilinia* spores to the lower-temp broth may be related to the swollen condition of the *Rhizopus* spores.

The survival of dormant and germinated *Monilinia* and *Rhizopus* spores after exposure to 52 C broth is shown in Fig. 1. Fewer dormant *Monilinia* than *Rhizopus* spores germinated after each exposure. The dormant *Monilinia* spores, however, needed an exposure of at least 30 sec at 52 C to reduce drastically subsequent germination. When the germinated spores were heated for only 10 sec in 52 C broth, the number of both *Monilinia* and *Rhizopus* spores with continued germ-tube elongation was reduced drastically. At this exposure, the number of surviving *Rhizopus* spores was somewhat less than of the *Monilinia* spores. Elongation of germ tubes of *Monilinia* spores was prevented after a 30-sec exposure, and after 60 sec, elongation of germ tubes from most of the *Rhizopus* spores was prevented.

Microscopic examination.—When examined microscopically at ×650, nonheated germinated *Rhizopus* spores differed markedly from heated spores. Nonheated spores contained a large vacuolate body covered with many striations that apparently arose from the irregular outer wall. Within the spores, cytoplasm and definite but unidentified bodies could be seen. In some cases, the inner wall appeared to be connected with or to form the germ tube. The germ tubes were mostly hyaline, but contained a continuous stream of cytoplasm and unidentified bodies, some hyaline and some dark. When the spores were heated at 52 C for 120 sec,

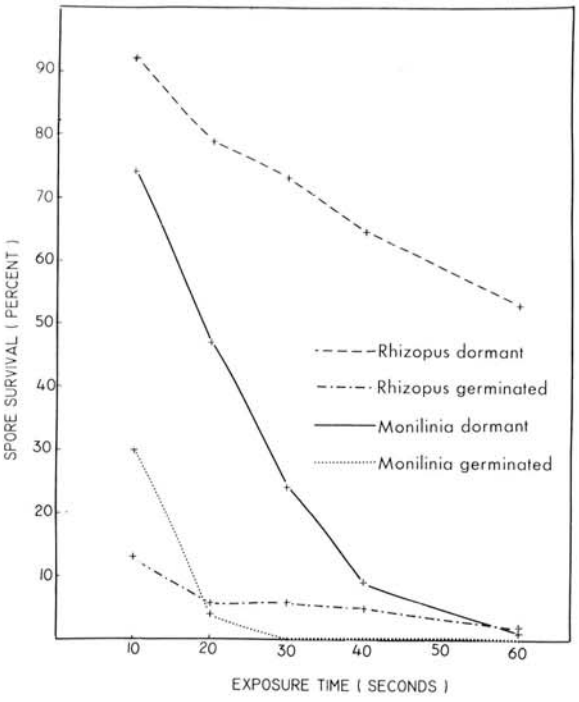

Fig. 1. Per cent survival of *Monilinia fructicola* and *Rhizopus stolonifer* spores after exposure to nutrient broth at 52 C. Nonheated dormant *Monilinia* and *Rhizopus* spores averaged 95% germination; about 95% of the nonheated germinated spores of each genera had continued elongation of the germ tube.

the vacuolate body was less distinct or disappeared completely. Usually the internal contents of both the spores and germ tube were intensely granular. No definite shrinking of the spore or germ tube occurred, but sometimes the germ tube was abnormally twisted. The outer walls of the spore and germ tube were intact. The granular condition of *Rhizopus* spores often was more apparent when they were heated at 46 or 49 C than when heated in 52 C broth. Changes in the internal contents of heated *Monilinia* spores were not apparent. The internal contents of germinated *Rhizopus* and *Monilinia* spores have been studied more thoroughly with electron and light microscopes, and results will be reported later.

DISCUSSION.—Other reports state that dormant *Monilinia* and *Rhizopus* spores can be killed by relatively short exposure to moderately high temp, and that *Monilinia* spores are killed by lower temp or shorter exposures than *Rhizopus* spores (1, 2, 4). The present work compares the survival of dormant and germinated *Monilinia* or *Rhizopus* spores after exposures to heated broth. It shows that germinated spores of both genera are killed more quickly and at lower temp than the dormant spores.

Although data from the treatment of organisms on artificial media cannot be extrapolated to predict results on living material, they might help to explain the effects of a treatment when the organisms are on the host. Thus, the differences in sensitivity of dormant and germinated spores to heat may explain the effectiveness of heat treatments in reducing decay development of peaches.

TABLE 1. Survival of *Monilinia fructicola* and *Rhizopus stolonifer* spores after exposure to nutrient broth at various temp[a]

Broth temp	Exposure time	Survival			
		Monilinia		*Rhizopus*	
		Dormant	Germinated	Dormant	Germinated
C	*sec*	%	%	%	%
43	30	95.0	94.6	95.0	23.9
	60	95.0	94.3	95.0	15.3
	120	92.0	67.3	95.0	12.2
46	30	88.6	83.8	95.0	9.5
	60	78.0	18.8	94.2	9.2
	120		1.3	77.5	7.9
49	30	56.6	0	53.6	6.8
	60	2.8	0	34.2	3.7
	120	1.1	0	7.4	4.5
52	30	24.0	0	72.9	5.9
	60	1.2	0	53.0	1.6
	120	0	0	15.4	0

[a] Three replications of 200 spores each for the 43- and 46-C treatments and 6 replications of 200 spores each for the 49- and 52-C treatments were counted. Nonheated dormant *Monilinia* or *Rhizopus* spores averaged 95% germination; about 95% of the nonheated germinated spores of each genera had continued elongation of the germ tubes.

Peaches may be inoculated with *Monilinia* spores during the growing season, and with both *Monilinia* and *Rhizopus* spores during the harvesting operations. Normally there is a delay of several hr from the time of harvest until the fruit can be given any postharvest treatments. During this period, the fruit is usually held at temp favorable for the growth of these organisms. Consequently, by the time of treatment dormant spores may have germinated and may have penetrated the fruit. Protective coatings of approved postharvest chemicals usually do not prevent development of such established infections. On the other hand, treatment in 52-C water for 2-3 min has prevented decay development of peaches with established infections (3). Since the delay between harvest and treatments will allow time for spores to germinate, and since germinated *Monilinia* and *Rhizopus* spores are killed rapidly at 52 C, it appears that the effectiveness of the heat treatment in reducing decay development of peaches may be due more to the destruction of germinated than dormant spores.

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