Selective Medium for Detection of Inoculum of Monilinia spp. on Stone Fruits

Douglas J. Phillips and John M. Harvey

Research Plant Pathologist and Research Leader, Agricultural Research Service, U.S. Department of Agriculture, Market Quality and Transportation Research Laboratory, 2021 South Peach Avenue, Fresno, California 93727.
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ABSTRACT

Stone fruits from California orchards were washed and the wash water plated on a medium selective for *Monilinia* spp. Inoculum on the surface of sound stone fruits ranged from 0 to 170,000 propagules per fruit when measured at or near harvest. Propagules believed to be ungerminated conidia of *M. fructicola* commonly were found covering sound fruit in orchards where sporulation had occurred on nearby infected fruit. There was a detectable increase in the amount of inoculum present on the fruit late in the harvest season.

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Additional key words: brown rot.

Brown rot of stone fruit is caused in California by *Monilinia fructicola* (Wint.) Honey and *M. laxa* (Alderh. & Ruhl.) Honey (5, 14). Disease development depends on a susceptible state of the host, the presence of the fungus, an environment favorable to the growth of and penetration by the fungus, and infection of the host. Conidia, shed from infected areas remaining on the tree from a previous year, or ascospores produced from mummies on the soil, provide inoculum for primary (current-year) infections. Primary infections occur on blossoms or on fruit, particularly on fruit injured by insects, and lead to secondary infections and further dissemination of conidia (3, 6, 17).

We report on estimates (determined by using a selective medium) of the density of propagules of *Monilinia* spp. which occurred on stone fruits in California at harvest. An abstract on the selective medium has been published (15).

MATERIALS AND METHODS.—*Monilinia-selective medium (MSM).*—Our medium was derived from that of Nash (13), but contains only the following: 1,000 μg/ml pentachloronitrobenzene (PCNB) (from a 75% wettable powder) 4% canned Gerber strained peaches (Gerber Products Co., Fremont, Mich.), 20 μg/ml neomycin, 1,000 μg/ml streptomycin, and 2% agar in distilled water. The agar and water were mixed, autoclaved at 121 C for 5 minutes, and cooled to 80 C. Other components were then added and 20-25 ml of the mixture was poured into sterile 100-mm diameter petri dishes. The antibiotics were added from stock solutions in water. Free water on the surface of the agar medium was reduced by holding the cooled plates for 48 hours before use.

Fruit sample preparation and spore plating.—Freshly picked fruits were washed vigorously for 15 minutes in 100 ml of water per fruit containing .005% Tween-20 wetting agent (Atlas Chemical Industries, Wilmington, Delaware). One milliliter of the wash water was spread over the surface of a plate of MSM. Plates were incubated in diffused light and at room temperature for 3-7 days before *Monilinia* colonies were counted. We will refer to the propagules found by plating as "conidia", even though other forms of propagules such as mycelial fragments may have been washed from the fruit.

Most colonies of *M. fructicola* or *M. laxa* growing on MSM began as white irregular colonies that developed dark centers and edges with age (Fig. 1), although some variation was common. Characteristic monilioid chains of conidia arose from the center of the colonies.

It should be noted that the amount of medium in the petri dish influenced the growth of *Monilinia* spp. on the plate. Colonies of *Monilinia* spp. were darker and more distinct on 100 mm plates containing 20 ml or more MSM than on plates containing less than 20 ml (Fig. 2).

RESULTS.—*Counts of conidia from pure culture or from inoculated peaches.*—Conidia from 1-week-old pure cultures of *M. fructicola* and *M. laxa* were suspended in tap water and counted with a hemacytometer. The suspensions of conidia were serially diluted to 0.1, 0.01, 0.002, 0.001, and 0.0001 in 0.1% water agar and plated (five plates per dilution) on MSM. Counts by the two methods were similar, regularly decreased with the dilution, and generally indicated no interaction between propagules (12) (Fig. 3). This experiment was repeated with similar results.

Conidia from 1-week-old pure cultures of *M. fructicola* were suspended in water and counted with a hemacytometer, diluted in water and applied to fruits by dipping or spraying. The number of spores on each fruit

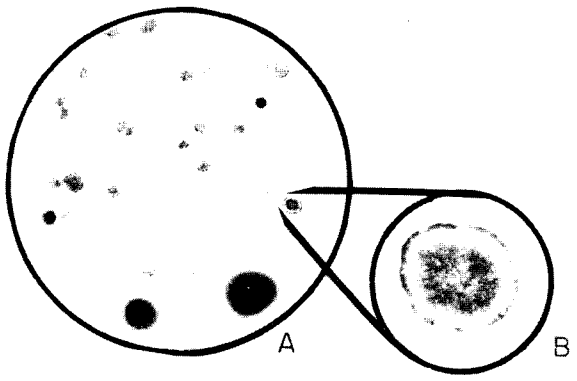

Fig. 1-(A, B). A) Plate of Monilinia-selective medium with 23 colonies of *Monilinia fructicola* and four other fungal colonies. **B)** Note the pattern of growth and pigmentation of *M fructicola*.

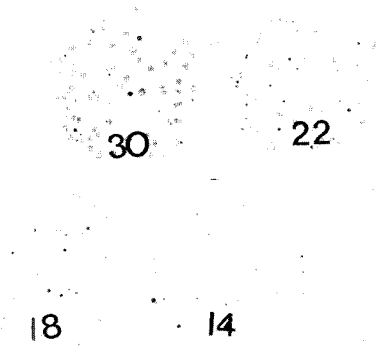

Fig. 2. Colonies of *Monilinia fructicola* on plates containing 10, 14, 18, 22, and 30 ml of Monilinia-selective medium.

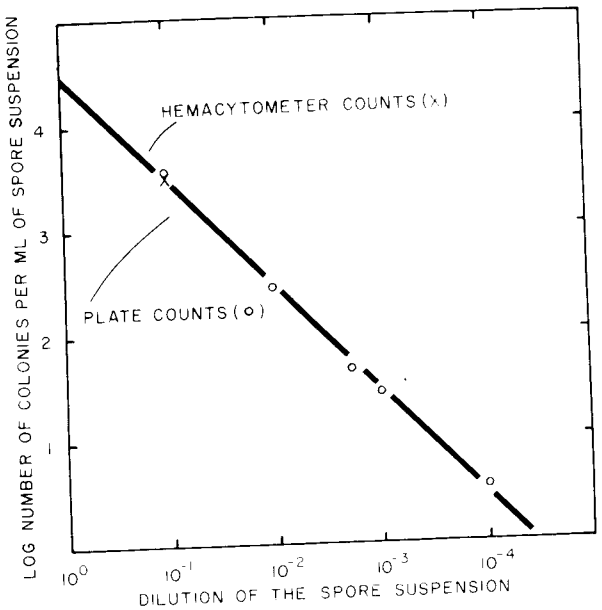

Fig. 3. Dilution-plate counts of *Monilinia fructicola* on Monilinia-selective medium showing two hemacytometer counts (×) and five plate counts (O). The highest plate count was estimated by counting colonies in five 3.2-cm^2 areas on each of five plates. Colonies at these high densities were small, but usually discreet.

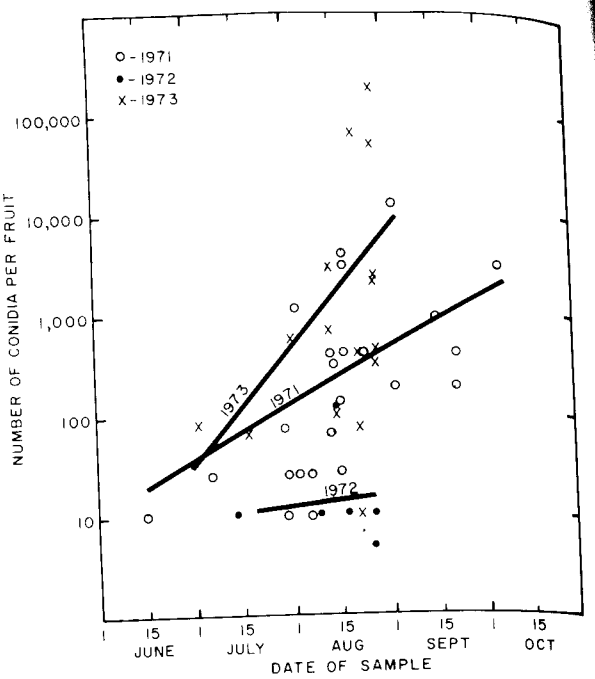

Fig. 4. The number of conidia per fruit on 10-fruit composite samples collected in various orchards during 1971-73 where brown rot was found in the orchard. The lines shown are linear regressions of the data from each sample year and the length generally indicates the relative number of samples each year.

was estimated by measuring the amount of liquid retained by each fruit. Plate counts showed that 85-90% of the conidia placed on the fruits could be recovered in one 15-minute wash. However, if the spore suspension was allowed to dry on the fruits, the plate counts were reduced 25% or more. In eight tests during 2 years, recoveries from plums, nectarines, and peaches that were inoculated and allowed to dry showed a mean of 50% recovery and a standard error of 16%.

Colonies of bacteria, yeast, or other fungi could obscure *Monilinia* spp. growing on MSM. Growth of these competing organisms was dramatically increased if free water was present on the agar surface prior to plating the sample. Platings of suspensions containing 30-100 conidia/ml added to the water in which the fruit was washed showed that the *Monilinia* count decreased an average of 75% when non-*Monilinia* colonies exceeded about 400 per plate.

Plate counts of Monilinia conidia from fruit in orchards.—Beginning in April 1971, and continuing through the harvest period, samples of 10 sound fruit were taken each month from 22 commercial orchards near Fresno, California. The date of harvest varied from July through September, depending on the cultivar. Fruits were selected from trees throughout the orchard; sampling sites were within 8 feet of the ground and at least several feet from any visibly infected fruits. The samples were washed in 1 liter of water and 1-ml aliquots of the wash water were plated on each of four-to-eight MSM plates.

Using this sampling technique, we found *Monilinia* propagules only on fruits from orchards where visible sporulation was seen on rotting fruits. Most isolates were *Monilinia fructicola* (when identified on PDA) and the numbers of colonies generally increased during the growing season (Fig. 4), as did the number of infected fruits in the orchards. Five *M. fructicola* isolates from MSM plates we tested were pathogenic to peach fruits.

When four composite 10-fruit samples and four-to-six single-fruit samples (each fruit washed separately) were

TABLE 1. Counts of *Monilinia* spp. propagules on composite 10-fruit samples and on single fruit samples from five orchards near Fresno, California

Orchard and stone fruit host	Number of *Monilinia* spp. propagules per fruit[a]			
	Composite 10-fruit samples[b]		Single fruit samples[c]	
	Average	Range	Average	Range
Casselman Plum	180	(100-300)	150	(25-375)
Gold King Nectarine	1,890	(175-6,000)	1,310	(50-5,650)
Sept. Grand Nectarine	13,460	(8,100-24,100)	10,850	(0-52,030)
Sept. Grand Nectarine	210	(50-350)	590	(166-1,500)
Late Santa Rosa Plum	863	(275-1,450)	120	(0-425)

[a]Single or 10-fruit samples were taken from an orchard on the same day. The number of propagules per fruit was estimated from counts of colonies growing on four MSM plates with 1-ml aliquots of the wash water used for each sample.
[b]Based on 10-fruit samples washed together and replicated four times.
[c]Based on four to six single fruits washed individually in 100-ml of water.

taken from four different orchards, the average number of spores per fruit in the four composite samples were similar to the average number in the single-fruit samples (Table 1). Although the range in the counts was greater in the single-fruit than in the composite samples, spore distribution in the orchard was uniform enough for a single composite sample to be fairly representative of the inoculum density.

Comparison of the number of propagules on the upper sides (stem ends) of six fruits from each of the four orchards with the number of propagules on the lower sides (blossom ends) showed no significant difference in counts (Table 2).

Additional sampling from orchards where brown rot occurred in 1972 and 1973 showed low spore counts in 1972, but higher counts in 1973, particularly late in the season (Fig. 4). The highest number of spores found in any one sample was 170,000 spores per fruit when a plum orchard (cultivar Casselman) was sampled in August 1973. Harvesting operations also can contribute to the late-season increase in spores on unpicked fruit in an orchard (Table 3).

DISCUSSION.—Although not totally selective, MSM can be used to estimate spore density of *Monilinia* spp. on the surface of fruit. The counts of spores washed from fruit represent a minimum estimate of the average number of propagules on the fruit in the sample. Recovery of conidia suspended in water and allowed to dry on the fruit were quite low. The proportion of the inoculum recovered by washing an orchard sample may differ from that recovered by washing samples inoculated with spores in water because much of the fruit in the orchard was not thoroughly wet prior to sampling. By using the surface of the fruit as the spore collection site, the count may be related to the potential incidence of disease, since the inoculum is at a potential infection court (17). Other workers have shown that the quantity of sporulation at harvest is important in determining postharvest losses (4); therefore, our plate counts could serve as an estimate of postharvest brown rot development.

Conidia can be disseminated from infection sites during warm summer or fall weather, but the conidia may not remain viable on the fruit for extended periods (2, 3, 7, 10). Spore densities, ranging from 100-170,000 spores per fruit, are probably not the result of long-term build-up of inoculum, but rather the result of current sporulation from infected fruit in the orchard.

Spore counts from fruits may generally predict potential losses, but latent infection (8, 11, 16) or latent contamination (9) may provide inoculum not measured

TABLE 2. Counts of *Monilinia* spp. propagules on the blossom and stem end of fruit from four orchards near Fresno, California

Orchard and stone fruit host	Number of *Monilinia* spp. propagules[a] per half fruit[b] (avg)			
	Blossom end half		Stem end half	
Casselman Plum	60	(25-100)	25	(5-40)
Sept. Grand Nectarine	3,500	(0-8,900)	3,090	(0-7,020)
Sept. Grand Nectarine	1,400	(0-3,910)	640	(0-1,460)
Late Santa Rosa Plum	67	(0-145)	92	(5-175)

[a]Mean and 90% confidence interval (in parentheses).
[b]Each datum is based on six-to-eight 1-ml samples of wash water (100 ml per fruit) from each of six fruits harvested from an orchard on the same day.

TABLE 3. Counts of *Monilinia* spp. propagules on fruit collected before and after the first commercial harvest in three orchards

	Number of *Monilinia* spp. propagules per fruit[a]	
	Before harvest	After harvest
Casselman plum[a]	400	2,700
Parade peach[a]	100	1,400
Casselman plum[b]	63,100	110,000

[a]Each datum is based on four 1-ml samples of wash water (100 ml per fruit) from two 10-fruit samples.
[b]Each datum is based on four 1-ml samples of wash water (100 ml per fruit) diluted to 10,000 ml per fruit from two 10-fruit samples.

by these spore counts. The relationship between spore density on the fruit and postharvest brown rot development may be influenced by variations in the condition of the fruit, such as ripeness, mechanical injury, and fungicide residues (1). Moreover, estimates of loss also must consider that increasing the inoculum density may decrease the incubation period (1).

This study demonstrates a range in inoculum occurring on stone fruits at harvest in California and may help explain the variable results obtained from some standard disease control measures. High inoculum densities may be of particular importance if fruit is mechanically harvested.

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Phoma glomerata, a New Pathogen of Wheat and Triticales, Cultivar Resistance Related to Wet Period

R. M. Hosford, Jr.

Associate Professor, Department of Plant Pathology, North Dakota State University, Fargo, North Dakota 58102.

Cooperators: H. B. Caldwell, J. C. Craddock, R. L. Haspel, R. A. MacArthur, P. E. Reynolds, F. J. Zillinsky.

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ABSTRACT

Phoma glomerata caused no leaf spotting on any of the tested Gramineae following a postinoculation wet (mist) period of 6 or 12 hours. Following a wet period of 24 hours it caused severe leaf spotting on spring wheat cultivars Red River 68, Taichung 2, Ring, Inrat 69, and Zafrani and on a triticale with Armadillo parentage. It caused slight spotting on Waldron spring wheat and Hercules durum. Only after a postinoculation wet period of 48 hours did it cause severe spotting on Waldron and moderate spotting on Hercules. The triticales Fasgro 418, Fasgro 419, NDT 24, and 209 were not spotted. After 72 hours in mist the fungus caused severe spotting on Tobari 66 spring wheat and Leeds durum, and slight spotting on Marquis spring wheat. It did not cause spotting on Chris, C306, ND495, and ND487 spring wheats, Wells durum, Larker barley, Caribou rye, or Lodi oats. Apparently the fungus required a postinoculation wet period to cause leaf spotting, and expression of susceptibility or resistance to leaf spotting was associated with the duration of the wet period.

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Additional key words: Peyronellaea glomerata, disease resistance.