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OCCURRENCE AND SIGNIFICANCE OF BROWN ROT FUNGI (MONILINIA SPECIES) IN BLOSSOMS AND FRUITS OF FRENCH PRUNE

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ABSTRACT

A survey was completed in 34 commercial prune orchards in the spring and summer of 1992. Although both species of *Monilinia* were present during spring in blighted blossoms and latent-infected green prunes, later in season during commercial harvest *M. fructicola* was predominant. The 1992 survey also indicated that the shift of *Monilinia* species is still in progress since higher percentage of orchards than a decade ago now show decay of prune fruit exclusively by *M. fructicola*. In the 1982-1983 survey about 70% of the orchards surveyed had both *M. fructicola* and *M. laxa* but in 1992 we found only about 30% of the orchards surveyed with both species, suggesting that *M. laxa* isolates have somehow been excluded from a number of prune orchards. Furthermore, resistance to benomyl of *M. fructicola* predominated in prune orchards by 1992 (91% of the orchards surveyed) compared to 64% in 1982 and 71% in 1983. Occasionally, both *M. fructicola* and *M. laxa* infected the same blossom or fruit and infections by *Botrytis cinerea* and either *M. fructicola* or *M. laxa*, or by all three organisms were more frequent than infections by both *Monilinia* species alone.

INTRODUCTION

Since 1972, brown rot disease (caused by *Monilinia fructicola* or *M. laxa*) in prune orchards has been controlled successfully with sprays of benomyl used alone or combined with other fungicides, but in recent years, severe outbreaks of brown rot have occurred in these orchards. Multiple applications of benomyl result in the selection of *Monilinia* species resistant to this fungicide (Gilpatrick 1982), and we suspected such selectivity might have also occurred in the populations of *Monilinia* species in California prune orchards.

In 1987, based on data from extensive surveys done in 1982 and 1983, Michailides et al. (1987) showed a shift in the *Monilinia* species in prune orchards with *M. fructicola* becoming the predominant species. Earlier surveys, however, in the forties or fifties (Hewitt & Leach 1939, Barnett & Bodine 1944) revealed that *M. laxa* was the predominant species. Because these shifts in the two species are of importance in the epidemiology and the control methods for the disease, we initiated a survey in certain prune areas to determine the distribution of *Monilinia* species and to ascertain the percentage of isolates resistant to benomyl.

OBJECTIVE

Survey *Monilinia fructicola/M. laxa* in commercial prune orchards to determine population dynamics of benomyl-resistant and -sensitive isolates (ten years after the 1982-1983 survey).

PROCEDURES

Isolation of Monilinia isolates. Blighted twigs and/or blossoms were collected from certain orchards showing high incidence of brown rot in April and then at the beginning of May 1992. At commercial harvest (July 30), mummified prunes with sporulating brown rot were collected from bins delivered to several different commercial dehydrators. Twigs and/or blighted blossoms or fruit samples were placed individually in plastic bags and brought to the laboratory in ice chests. About 20-30 samples were taken from each orchard. Spore masses or plant tissues were plated on acidified potato-dextrose agar plates (APDA). Three isolations per sample were made. After 5-7 days at room temperature (72±1 F), the isolates were identified to species on the basis of morphological characteristics.

Determination of benomyl resistance. Two to three mycelial plugs (5 mm in diameter) per isolate were transferred on PDA amended with 1 μg benomyl (Benlate 50W). Colony diameter (resistant isolates) or absence of growth (sensitive isolates) was recorded after 4 days (for *M. fructicola*) or 7 days (for *M. laxa*). All isolates that grew on the fungicide-amended medium were transferred to APDA plates. After 4 days, two 5-mm mycelial plugs were placed on PDA amended with 5 μg benomyl. Isolates that grew at 5 μg benomyl were tested further on PDA amended with 10 μg benomyl.

Infection of green prune fruit. In three orchards in Glenn County where blossom blight was very common, green fruit with latent (=incipient) infections (small black specks on the fruit surface) were collected at random on 8 and 26 May. Fruits were incubated over wire screens in plastic containers that had water on the bottom to increase relative humidity. Infected fruit were recorded after 7, 10, 15, and 30 days incubation at 73 F. Spore masses from sporulating *Monilinia* species on prunes were transferred to APDA plates to determine the species of *Monilinia* and then to PDA plates amended with 1 or 5 μg benomyl to determine the percentage of resistant isolates that developed on green fruits after expansion of latent infections.

RESULTS

Isolation efficiency, measured as the percentage of successful over attempted isolations, was high (90-100%) because most of the samples had abundant sporulation of *Monilinia* species, and isolation was relatively easy by plating spore masses directly in the agar media.

Distribution of Monilinia species. All six orchards sampled in the spring had both *M. fructicola* and *M. laxa*. However, only two of the orchards had a majority of *M. fructicola* while in four of the orchards *M. laxa* predominated in blossom samples (Table 1). Infections of blossoms and/or fruit by both *M. fructicola* and *M. laxa* ranged from 2-21%. In four of the orchards *B. cinerea* was present with either *M. laxa* or *M. fructicola* in 4-26.5% of the isolations (Table 1).

Isolations from mummified fruit collected from certain fields and bins delivered to dehydrators revealed an abundance of *M. fructicola* isolates; three of the samples had only 2.5-9.5% *M. laxa* (Table 2). Mummified fruit collected from the trees revealed the same proportions. All eight samples contained isolates resistant to 1 μg benomyl; the samples from Madera had

highly resistant isolates (5.4% of the isolates grew in media amended with 10 μg benomyl) (Table 2).

In about 70% of the growers' samples, only *M. fructicola* was isolated (Table 3). In orchards with a mixture of the two species, *M. fructicola* was isolated more often than *M. laxa* (Table 3). Although the percentage of samples with *M. laxa* ranged from 5-22%, in only one orchard the ratio of the two species was about 1:1. None of the samples taken from the Corning and Hamilton City dryers contained any *M. laxa* (Table 3). From a total of 838 isolations made from fruits (Tables 2 & 3) only 5 prunes (0.6%) yielded both *Monilinia* species.

Detection of benomyl-resistant isolates. Benomyl-resistant isolates of *M. fructicola* were recovered from the spring samples in all six orchards. About 6 to 67% of the recovered isolates were resistant to 1 μg benomyl (Table 1). Resistant isolates were recovered from both blighted prune blossoms and infected green fruit. None of these isolates grew in media amended with 5 μg benomyl. Resistant isolates of *M. fructicola* were also recovered in both orchards sampled on 8 May 1992. In the east side of this orchard where there were a lot of fruit with latent infections, 23.5% of *M. fructicola* isolates were resistant to 1 μg benomyl. Of these resistant isolates, 17.4% (4.1% of the total isolates) were resistant to 5 μg benomyl. In the west side of this orchard where blossom blight predominated, 25% of the total isolates were resistant to 1 μg benomyl and 50% of these isolates (12.5% of the total isolates) were resistant to 5 μg. In the second orchard where the incidence of fruit with latent infections was less frequent, only 10% of *M. fructicola* isolates were resistant to 1 μg benomyl. Mummified fruit from the east and west sides of the orchard in Glenn County at harvest had 15 and 33% of the *M. fructicola* isolates resistant to 1 μg benomyl (Table 2).

Among the 34 growers sampled on 30 July 1992, 31 (91%) had samples with *M. fructicola* resistant to 1 μg benomyl (Table 3). The percentage of isolates with resistance to 1 μg benomyl was variable from sample to sample and ranged from 5.5 to 86%. Only two samples (~6%) had isolates that were resistant to 5 μg benomyl (Table 3).

Latent infections of prune fruit by Monilinia species. Isolations from green fruit revealed both *Monilinia* species, *M. fructicola* and/or *M. laxa*, or *Botrytis cinerea*. *B. cinerea* has been reported previously in causing decay of green fruit. In some instances, *M. fructicola* and *M. laxa*, or *M. fructicola* and *B. cinerea*, or *M. laxa* and *B. cinerea* were isolated from the same fruit or developed on the same fruit after incubation. In the Butte County orchard where *M. laxa* was the predominant species (Table 1), *M. laxa* was isolated exclusively from all sampled green fruits. In addition, in this orchard we observed an unusual partial blight of young leaves in the spring. Sporulation on the blighted lesions of leaves was of the *Monilinia* type. Isolations from the margins of the blighted areas revealed only *M. laxa*.

For green fruit with latent infections collected on 8 May, it took 15 days under favorable conditions (high relative humidity) in plastic containers to reach levels of decay up to 40-60% (Fig. 1). However, 30 days incubation was necessary for green fruit with latent infections collected on 26 May to reach about the same levels of decay (35-57%) (Fig. 2).

DISCUSSION

A comparison of this study's results with those of a previous study done by Michailides et al. (1987) based on surveys completed in 1982 and 1983, indicates that 10 years after the last survey the populations of the *Monilinia* species in prune orchards have changed. We found a smaller percentage of orchards with both species than about 70% recorded in 1982 and 1983. About 70% of the orchards sampled in 1992 had fruit decayed exclusively by *M. fructicola*.

In the 1987 study, comparisons of the survey data with older surveys strongly suggested a shift in populations of *Monilinia* species in prune orchards. The results of the present survey support the suggestion that this shift is still in progress, since fewer orchards contained both *Monilinia* species (Fig. 3), with *M. laxa* somehow being excluded from a number of prune orchards.

Isolation of both *Monilinia* species from individual fruits suggests that simultaneous infection and development of the two pathogenic species is possible. The reason why *M. fructicola* predominated in mummified fruit is unknown. Although *M. fructicola* and *M. laxa* can cause latent infections on green fruit, because these infections require very extended favorable conditions for development, it is unknown at the present how much they can contribute to decay during maturation of the fruit. For instance, we observed that decay of mature fruit was about the same in an orchard with fruit showing abundant latent infections during spring and in a second orchard where latent infections were not as common. Furthermore, the majority of the latent infections do not survive until harvest. In addition, in another study we showed that infection of mature prunes can occur by conidia of *M. fructicola* deposited (or trapped) between contact surfaces of prunes (Michailides 1993). This may explain why brown rot on prune fruit usually results in "mummified fruit clusters" of more than two and up to 10 fruit attached. Fruit contact surfaces have been reported in favoring disease development caused by *B. cinerea* in grapes (Marois et al. 1986). In a study with prune fruit collected from a commercial orchard, we showed that brown rot decay occurs more frequently and faster on contact surfaces than in contact free surfaces of mature prune fruit.

CONCLUSIONS

1) Shift of *Monilinia* species is still in progress with *M. fructicola* becoming the predominant species since a higher percentage of orchards than a decade ago now show decay of prune fruit exclusively by *M. fructicola*.

2) *Monilinia fructicola* isolates resistant to 1 μg benomyl were found in 91% of the prune orchards surveyed. (64-71% of the orchards surveyed in 1982-83 had *M. fructicola* resistant to benomyl.)

3) Although occasionally both *M. fructicola* and *M. laxa* can infect the same blossom or fruit, infections by *Botrytis cinerea* and either *M. fructicola* or *M. laxa*, or by all three organisms are more frequent.

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Table 1. Incidence of *Monilinia* species and *Botrytis cinerea* in blighted blossoms, and green prune fruit collected on 28 April 1992.

Orchard	County	*Monilinia* isolates (No.)	*M. fructicola* (%)	*M. laxa* (%)	*M. fructicola* and *M. laxa* (%)	*B. cinerea* (%)[1]	*M. fruct.* resistant to 1 µg benomyl (%)[2]
1	Butte	49	5	65	2	$27^{L,F}$	6
2	Butte	38	16	45	21	$8^{F,L}$	67
3	Butte	115	76	12	12	0	19
4	Butte	52	31	50	15	4^{L}	15
5	Glenn	30	13	70	7	10^{L}	10
6	Glenn	30	80	17	3	-	20

[1] *B. cinerea* growing with *M. laxa* (L) or with *M. fructicola* (F) or with both species (F,L).

[2] None of the isolates that grew in PDA plates amended with 1 µg benomyl grew in plates amended with 5 µg benomyl.

Table 2. Frequency of *Monilinia* species in various fruit samples collected on 24 July 1992 from the field and bins at dehydrators.

Sample/grower	*Monilinia* isolates (%)	*M. fructicola* (%)	*M. laxa* (%)	*M. fructicola* and *M. laxa* (%)	*M. fructicola* resistant to benomyl 1 µg	5 µg	10 µg
Madera 20438*	38	97.4	2.6	0	75.7	5.4	5.4
Madera 20439*	37	100.0	0.0	0	70.0	8.1	8.1
Colusa, #1*	21	90.5	9.5	0	16.0	0.0	0.0
Colusa, #2*	16	100.0	0.0	0	18.8	0.0	0.0
Colusa, #3*	40	90.0	2.5	8	38.9	0.0	0.0
Z-Y East**	40	100.0	0.0	0	15.0	0.0	0.0
Z-Y West**	19	94.7	5.3	0	33.0	0.0	0.0
Princeton**	38	100.0	0.0	0	16.0	0.0	0.0

* Samples collected from bins at the dehydrators.

** Collected from trees.

Table 3. Frequency of *Monilinia* species in prune fruit surveyed during commercial harvest in 1992.

Dryer	Grower[1]	*Monilinia* isolates (%)	*M. fructicola* (%)	*M. laxa* (%)	*M. fructicola* and *M. laxa* (%)	*M. fructicola* resistant to 1 µg benomyl
Corning	1	17	100	0	0	5.8
	2	19	100	0	0	21.0
	3	20	100	0	0	20.0
	4	20	100	0	0	85.0
	5	20	100	0	0	15.0
Hamilton City	1	20	100	0	0	10.0
	2	20	100	0	0	80.0
	3	20	100	0	0	20.0
	4	19	100	0	0	10.5
	5	20	100	0	0	60.0
Red Bluff	1	17	94	6	0	18.0
	2	17	88	22	0	33.0
	3	18	100	0	0	0.0
	4	18	100	0	0	5.5
	5	19	100	0	0	21.0
Gridley	1	18	95	5	0	5.8
	2	17	82	18	0	14.3
	3	18	100	0	0	0.0
	4	18	100	0	0	16.7
	5	19	48	42	10	5.3
Live Oak	1	15	93	7	0	13.0
	2	15	100	0	0	20.0
	3	19	89	11	0	26.0[2]
	4	18	100	0	0	16.6
	5	13	100	0	0	23.0
Marysville	1	16	94	6	0	86.0
	2	15	100	0	0	20.0
	3	11	100	0	0	9.0
	4	14	100	0	0	7.0
	5	18	100	0	0	5.5
Yuba City	1	20	95	5	0	37
	2	9	100	0	0	0
	3	20	100	0	0	50[2]
	4	12	92	8	0	50

[1] All fruit samples were collected from bins delivered to the dryers on 30 July 1992. Isolations were made on 11 August.

[2] Five percent of these isolates grew at 5 µg benomyl.

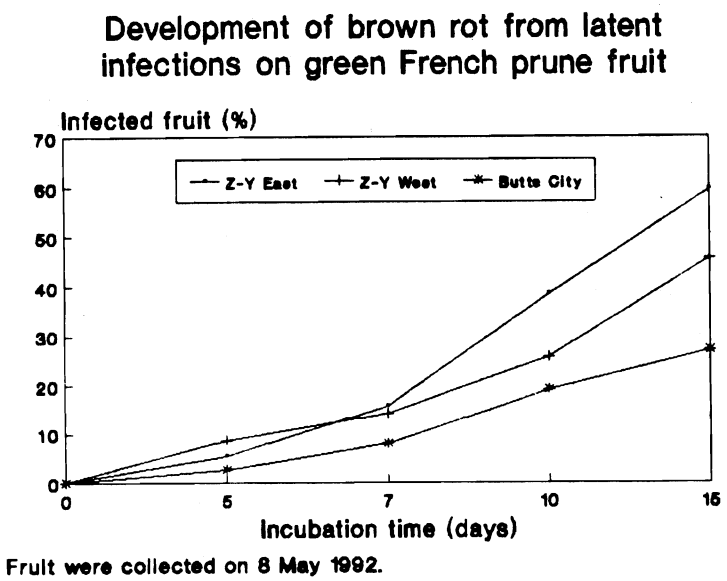

Figure 1. Development of brown rot decay on green French prunes with latent infections that had been collected from three commercial orchards on 8 May 1992 (after incubation under ~100% relative humidity for 15 days).

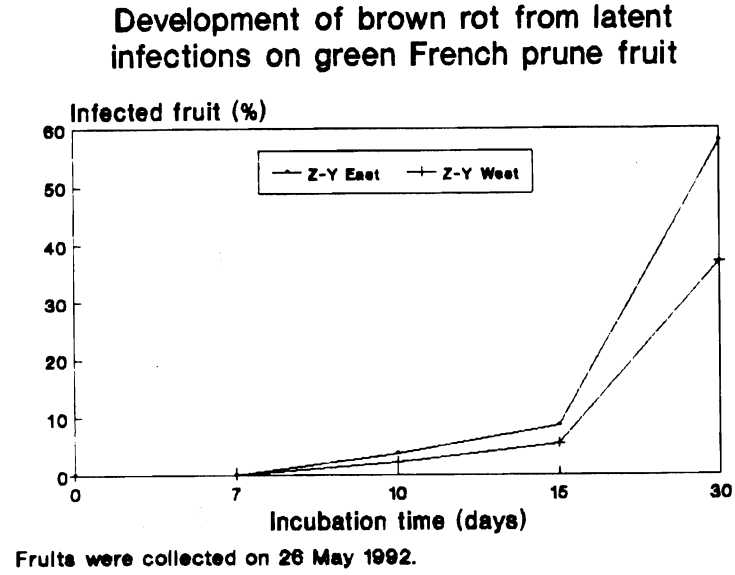

Figure 2. Development of brown rot decay of green French prunes with latent infections harvested on 26 May (after incubation under ~100% relative humidity for 30 days).

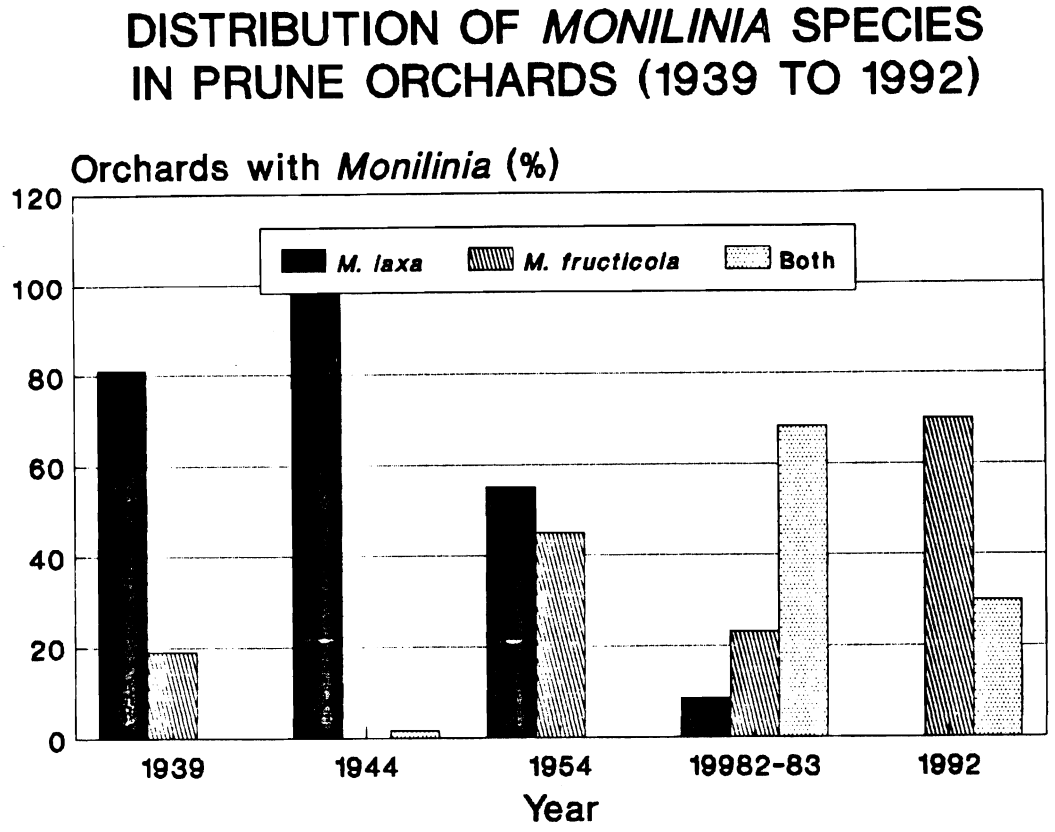

Figure 3. Distribution of *Monilinia* species in prune orchards determined from surveys in 1939 through 1992.