Shift of *Monilinia* spp. and Distribution of Isolates Sensitive and Resistant to Benomyl in California Prune and Apricot Orchards

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ABSTRACT

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Monilinia fructicola and *M. laxa* are the causal agents of brown rot blossom and twig blights and fruit rot of prunes (*Prunus domestica*) and apricots (*P. armeniaca*) in California. In contrast to 1974, *M. fructicola* was more commonly isolated than *M. laxa* from diseased prunes and apricots in 1982 and 1983. All isolates of *M. laxa* were sensitive to benomyl. Isolates of *M. fructicola* resistant to 1 μg/ml benomyl were detected in prune orchards in 10 of 12 counties surveyed and in apricot orchards in all six counties surveyed. In addition, in three counties, *M. fructicola* isolates resistant to 4 μg/ml benomyl were found in both prune and apricot orchards. Reasons for the shift in *Monilinia* spp. are discussed.

Production of prune (*Prunus domestica* L.) and apricot (*Prunus armeniaca* L.) is an intensive, specialized industry in California with 28,300 and 8,600 ha, respectively (1,3). The most serious disease attacking aerial plant parts of prune and apricot trees is brown rot, caused by *Monilinia fructicola* (Wint.) Honey and *M. laxa* (Aderh. & Ruhl.) Honey (7,12), which accounted for annual losses and expenses for control of $2.82 million in 1963 (15).

Since 1972, brown rot disease in prune and apricot 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.

Continuous use of pesticides can disturb the balance of pathogen populations of foliar (17) and soilborne diseases (16). Multiple applications of dodine and benomyl resulted in the selection of *Venturia* and *Monilinia* spp. resistant to these fungicides, respectively (5), and we suspected that selectivity might also have occurred in the populations of *Monilinia* spp. in California orchards. We therefore initiated a survey of the major prune and apricot areas in central and northern California to determine the distribution of *Monilinia* spp. and to ascertain the percentage of fungal isolates resistant to benomyl. An abstract of this work was published (7). Two representative isolates of *M. fructicola*, one benomyl-resistant (ATCC 62879) and one benomyl-sensitive (ATCC 62880), and an isolate of *M. laxa* (ATCC 62881), all from prune, were deposited with the American Type Culture Collection.

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MATERIALS AND METHODS

Sampling method and isolation of *Monilinia* spp. Isolates of *Monilinia* spp. were collected from 34 prune and 11 apricot orchards in 1982 and from 74 prune and 22 apricot orchards in 1983. Blighted twigs and/or blossoms were collected from all orchards from May through June of these years. At harvest (15 August to 10 September), prunes with sporulating brown rot were collected from bins delivered to different commercial and private dehydrators. Diseased apricot fruits were taken off trees. Each twig, blossom, or fruit sample was placed in an individual plastic bag and brought to the laboratory in an ice chest. About 15–25 samples were taken from each orchard. Fungal isolations were made in a day, mainly by sampling from sporodochia on blossoms, twigs, or fruits. When sporodochia were absent, isolations were made from pieces of infected tissues. A dissecting microscope was used for viewing and selecting clean and intact sporodochia such as those found between cracks of blighted twigs or at the base of flower penduncle, or the lower part of the calyx, and in deep areas of wrinkled surfaces of decayed fruit. Spore masses or plant tissues were plated on acidified potato-dextrose agar plates (APDA). Two isolations per sample were made. After 4–5 days at room temperature (23 ± 1 C), the isolates were identified to species on the basis of colony morphology (6).

Determination of benomyl resistance. Two mycelial plugs (5 mm diameter) per isolate were removed from the margins of 5-day-old cultures and placed on PDA amended with 1 μg benomyl (Benlate 50W). Inoculated plates were held at room temperature, and colony growth or absence of growth was recorded after 4 days (*M. fructicola*) or 7 days (*M. laxa*). All isolates producing growth on the fungicide-amended medium were transferred to APDA plates. After 4 days at room temperature, two 5-mm mycelial plugs were placed on PDA amended with

Table 1. Distribution of *Monilinia* spp. in prune and apricot orchards in California in 1982 and 1983

Sample source (county)	Sample tissue[a]	Number of orchards sampled in		Number of orchards with: Only *M. fructicola*		Only *M. laxa*		*M. fructicola* and *M. laxa*		*M. fructicola* resistant to 1 μg benomyl	
		1982	1983	1982	1983	1982	1983	1982	1983	1982	1983
Prune											
Butte, Sutter	Twigs/blossoms	3	6	0	0	0	3	3	3	3 (1)[b]	2 (1)
Butte	Fruit	–	5	–	3	–	0	–	2	–	4
Sutter	Fruit	–	10	–	6	–	0	–	4	–	9
Colusa	Fruit	–	8	–	2	–	0	–	6	–	6
Tehama	Fruit	–	10	–	1	–	0	–	9	–	10
Yuba	Fruit	14	7	5	1	0	0	9	6	12 (8)	5
Yolo	Twigs/fruit	4	–	0	–	4	–	0	–	0	–
Sonoma	Fruit	–	3	–	1	–	0	–	2	–	2
Solano	Fruit	2	3	0	0	1	1	1	2	0	1
Madera	Fruit	1	5	0	0	0	0	1	5	1	4 (2)
Merced	Fruit	1	–	0	–	0	–	1	–	1	–
Fresno	Fruit	–	1	–	0	–	0	–	1	–	0
Tulare	Fruit	9	16	4	3	0	0	5	14	1 (1)	8
Subtotal		34	74	9	16	5	4	20	54	18 (10)	51 (3)
Apricot											
Merced	Twigs/blossoms	–	3	–	1	–	2	–	0	–	1
San Joaquin	Twigs/blossoms	–	5	–	2	–	0	–	3	–	5
Santa Clara	Twigs/blossoms	–	1	–	0	–	0	–	1	–	1
Stanislaus	Twigs/blossoms	–	8	–	5	–	0	–	3	–	8 (2)
Yolo	Twigs/blossoms	–	5	–	2	–	3	–	0	–	2
Contra Costa	Fruit	11	–	5	–	0	–	6	–	11 (7)	–
Subtotal		11	22	5	10	0	5	6	7	11 (7)	17 (2)
Total		45	96	14	26	5	9	26	61	29 (17)	68 (5)

[a] Blighted twigs and blossoms were collected from prune orchards from May through June, and prune fruits were collected from harvest bins at the dryers from August through September. Apricot twigs and blossoms were collected from April through May and, fruits, in July.
[b] Number of orchards from which isolates of *M. fructicola* grew on medium amended with 4 μg benomyl per milliliter.

Table 2. Frequency of *Monilinia* spp. in prune and apricot orchards surveyed in 1982 and 1983

Location (county)[a]	*Monilinia* isolates (no.) in		*M. fructicola* (%)[b]		*M. laxa* (%)[b]	
	1982	1983	1982	1983	1982	1983
Prune						
Butte, Sutter	66	92	78.8	23.9	21.2	76.1
Butte	–	114	–	94.7	–	5.3
Sutter	–	220	–	90.0	–	10.0
Colusa	–	154	–	87.0	–	13.0
Tehama	–	232	–	71.6	–	28.4
Yuba	222	149	82.4	79.2	17.6	20.8
Yolo	77	–	0.0	–	100.0	–
Sonoma	–	59	–	54.2	–	45.8
Solano	27	69	7.4	18.8	92.6	81.2
Madera	20	116	60.0	81.0	40.0	19.0
Merced	22	–	5.0	–	95.0	–
Fresno	–	12	–	66.7	–	33.3
Tulare	153	351	90.8	88.3	9.2	11.7
Subtotal	587	1,568	66.3	76.7	33.7	23.3
Apricot[c]						
Merced	–	39	–	51.3	–	48.7
San Joaquin	–	84	–	65.5	–	34.5
Santa Clara	–	6	–	66.7	–	33.3
Stanislaus	–	149	–	95.3	–	4.7
Yolo	–	96	–	62.5	–	37.5
Contra Costa	186	–	95.7	–	4.3	–
Subtotal	186	374	95.7	75.1	4.3	24.9
Total	773	1,942	73.3	76.4	26.7	23.6

[a] Prune samples from Butte and Sutter counties were blighted twigs and blossoms; from Yolo, twigs and fruit; and samples from the other locations were decayed fruit collected from harvest bins at different dehydrators.
[b] Percentages of *Monilinia* spp. are based on the total isolates for all orchards surveyed in each location.
[c] Apricot samples were blighted twigs and blossoms except those from Contra Costa County, which were from infected fruits.

4 μg benomyl. Colony growth or absence of growth was recorded after 4 days of incubation at room temperature. Isolates that grew at 4 μg benomyl were tested further on PDA amended with 8 μg benomyl.

RESULTS

Isolation efficiency. Isolation efficiency was measured as the percentage of successful over attempted isolations. In 1982, 91% of the isolations yielded brown rot fungi (efficiency from blighted prune twigs or blossoms and fruit was 97 and 89%, respectively, and from apricot fruit, 94%). In 1983, when more than twice as many isolations were made as in 1982, the overall success efficiency was 92% (efficiency for blighted prune twigs or blossoms and fruit was 83 and 95%, respectively, and from apricot twigs or blossoms, 85%). The remaining 6–10% of the isolations made from prune and apricot yielded *Botrytis cinerea* Pers.: Fr., *Aspergillus niger* v. Tieghem, *A. ochraceous* Wilhelm, *Cladosporium herbarum* (Pers.) Link ex Gray, *Mucor hiemalis* Wehmer, *M. racemosus* Bonorden, *Penicillium* spp., *Rhizopus stolonifer* (Ehrenb.:Fr.) Lind, and a yeast.

Distribution of *Monilinia* spp. Fifty-nine percent (20 of 34) and 73% (54 of 74) of prune orchards had both *Monilinia* spp. in 1982 and 1983, respectively (Table 1). In addition, 58% of apricot orchards

(26 of 45) in 1982 and 63% (61 of 96) in 1983 contained both *Monilinia* spp. (Table 1). When only a single species was present in an orchard, more prune and apricot orchards were detected with *M. fructicola* than with *M. laxa* in both 1982 and 1983 (Table 1). In orchards with a mixture of the two species, *M. fructicola* was isolated more often than *M. laxa* (Table 2). Only a few orchards in Solano County and one in Merced County had more *M. laxa* than *M. fructicola* isolates (Table 2). Overall, either *M. fructicola* or *M. laxa* alone were isolated more often than both together from fruit and twig/blossom tissues. Among 2,715 isolations, only two prune fruit samples yielded both *Monilinia* spp. in the same petri plate.

Detection of benomyl-resistant isolates. Benomyl-resistant isolates of *M. fructicola* occurred in prune and apricot orchards (Table 1). More than 50% of the prune and apricot orchards surveyed yielded isolates of *M. fructicola* resistant to at least 1 μg benomyl (Table 1). In several orchards, as much as 4 μg benomyl was tolerated (Table 1). In addition, isolates resistant up to 8 μg benomyl were detected in a few apricot orchards. In our tests, all isolates of *M. laxa*, regardless of host origin or sample source, were sensitive to benomyl.

DISCUSSION

Hewitt and Leach (6) indicated that *M. laxa* was widespread in all stone fruit-growing areas, whereas *M. fructicola* was usually more localized in the peach-producing areas of the Sacramento Valley (Table 3). Results of our surveys indicated that both *Monilinia* spp. were widespread in the prune- and apricot-growing areas (Table 1). More than 60% of the orchards sampled contained both fungal species. In orchards with a single species, those with *M. fructicola* were prevalent (28%, i.e., 40/141) over *M. laxa* (10%, i.e., 14/141) in 1982 and 1983. These findings contrast sharply with previous reports (Table 3) in which 81% of isolates from stone fruits, in general, were *M. laxa* (6), 91–100% of isolates from apricots were *M. laxa* (2,8,14), and in plum and prune orchards, 33% (14), 55% (8), or 95% (2) of isolates were *M. laxa*.

The data of Table 3 indicate a shift in populations of *Monilinia* spp. in prune and apricot orchards. This shift may have been induced in the populations by extensive use of benzimidazoles, particularly benomyl, which was registered in 1972 and has been used exclusively in repeated applications on peaches and nectarines. By 1977, in peach and nectarine orchards (13) and in 1982 and 1983 in prune and apricot orchards, *M. fructicola* showed widespread resistance to benomyl. Possible reasons for the delay in detecting resistance to benomyl in apricot and prune orchards could be: 1) the abundance of isolates of *M. laxa* in prune and apricot orchards; 2) the limited use (one blossom spray [for prunes] or two sprays [for apricots]) of benomyl alone, which helps reduce selection pressure: 3) the low disease incidence, and 4) the lack of yearly monitoring of orchards to detect resistant species of *Monilinia*. The higher levels (tolerating up to 8 μg) of benomyl resistance of *M. fructicola* isolates from apricots may be related to the fact that apricot growers apply at least two sprays of benomyl during bloom (9) and prune growers apply only one spray during early bloom or a combination of benomyl with captan at full bloom to control both brown rot and prune russet scab (J. M. Ogawa and T. J. Michailides, *unpublished*). Most of the prune and apricot orchards in 1982 and 1983 and in at least one year preceding the two sampling years were sprayed with benomyl.

Although all isolates of *M. laxa* in our study were sensitive to benomyl, benomyl resistance in *M. laxa* was reported in apricot orchards in 1980 (10,11) and in almond orchards in 1981 (4). Our inability to find benomyl resistance in *M. laxa* in prune orchards may have been due to insufficient isolates, the overabundance of *M. fructicola* (Table 2), or the reduced capacity of benomyl-resistant isolates of *M. laxa* to infect almond and prune blossoms (4).

Although changes in climatic conditions, cultural practices, and relative acreage in production may have contributed somewhat to the shift of populations of *Monilinia* spp. in prune and apricot orchards in California, the use of benomyl may also have played a major role in this shift. Supporting evidence is that all isolates of *M. laxa* collected were still sensitive to benomyl, whereas a large percentage of *M. fructicola* isolates were found resistant. Benomyl sprays applied for several years have reduced the level of plant parts infected by *M. laxa* and increased the freedom for infection by benomyl-resistant *M. fructicola*, thus making isolates of *M. laxa* more difficult to find. Yet, the increased populations of benomyl-resistant *M. fructicola* in the orchard, especially during the 1982 and 1983 seasons, forced abandonment of the crop in certain apricot orchards.

Our survey confirms that shifts in *Monilinia* spp. can occur when one species is benomyl-sensitive while the other species shows predominantly resistant lines. Furthermore, the limited spray applications in apricot and prune orchards may have delayed the development of populations of *M. laxa* resistant to benomyl. However, possible movement of abundant benomyl-resistant populations of *M. fructicola* from peach or nectarine orchards to prune and apricot provided the high inoculum level that caused the epidemics of brown rot blossom blight and fruit rot in those crops during 1982 and 1983.

Our findings strongly suggest the need for geographic monitoring programs that identify and assess benomyl-resistant populations of *Monilinia* spp. and alert growers to other brown rot fungicides that effectively control the disease for a time but that also select resistant pathogens. Monitoring programs will also help determine if resistant isolates of a pathogen have moved between orchards. We feel that such monitoring would provide an indication of the potential for fungicides under conditions conducive to brown rot epidemics.

Table 3. Distribution of *Monilinia* spp. on stone fruit, particularly in plum, prune, and apricot orchards from 1939 to 1983

Reference	Year	Hosts	Number of orchards surveyed	Percentage of orchards with		
				M. laxa	*M. fructicola*	Both species
Hewitt & Leach (6)	1939	Stone fruits (in general)	178	81.0 (144)[a]	19.0 (34)	–
Barnett & Bodine (2)	1944	Apricots	67	98.5 (66)	0.0 (0)	1.5 (1)
		Plums and prunes	127	95.3 (121)	1.6 (2)	3.1 (4)
Ogawa et al (8)	1954	Apricots	53	91.0 (48)	9.0 (5)	–
		Plums and prunes	20	55.0 (11)	45.0 (9)	–
Tate et al (14)	1974	Apricots	18	100.0 (18)	0.0 (0)	–
		Plums	3	33.3 (1)	67.7 (2)	–
This report	1982, 1983	Apricots	33[b]	15.2 (5)	45.5 (15)	39.4 (13)
		Prunes	108[b]	8.3 (9)	23.1 (25)	68.5 (74)

[a] Figures in parentheses indicate orchard numbers.
[b] Data of 2 yr.

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