Effects of Hull Abscission and Inoculum Concentration on Severity of Leaf Death Associated with Hull Rot of Almond

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ABSTRACT

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Almond fruit of cv. Nonpareil with slightly (1 to 10%), partially (15 to 40%) or almost completely (70 to 95%) abscised hulls were inoculated with *Rhizopus stolonifer* in 1991 and with *R. stolonifer* or *Monilinia fructicola* in 1992. More leaf death occurred both years near inoculated fruits with slightly or partially abscised hulls than near fruits with almost completely abscised hulls. Leaf death was greater near hulls inoculated with *M. fructicola* than with *R. stolonifer* in 1992. Disease severity did not differ among fruit with partially abscised hulls inoculated with 10^3, 10^4, or 10^5 spores of *R. stolonifer* per ml in 1991, and with the same concentrations of *R. stolonifer, M. fructicola,* or *M. laxa* in 1992. More leaf death occurred near fruit inoculated with *M. laxa* than with *R. stolonifer* and *M. fructicola* in 1992.

Additional keywords: *Prunus dulcis, R. arrhizus, R. circinans*

Hull rot disease of almond, *Prunus dulcis* (Mill.) D. Webb, is generally caused by *Rhizopus stolonifer* (Ehrenb.: Fr.) Vuill. or *Monilinia fructicola* (G. Wint.) Honey and infrequently by *M. laxa* (Aderhold & Ruhland) Honey, *R. circinans* Tiegh., and *R. arrhizus* A. Fischer (3,6). These fungi produce gray to brown lesions on the mesocarp (hull) of maturing almond fruit. Necrosis and death of nearby leaves and part or all of the attached spur or shoot ensues. The seed is not damaged but destruction of fruit-bearing spurs affects yield. The vascular tissues connecting the dead leaves and infected fruit become brown to black. Dense, black sporulation of *Rhizopus* spp. usually forms between the hull and shell, and buff-colored conidia of *M. fructicola* often develop on exterior and interior surfaces of infected hulls. Fumaric acid, or a derivative, produced in the hull by the pathogens and transported to the leaves and shoots, has been implicated as a toxin responsible for the death of these tissues (5). Some infected fruit and dead leaves remain attached to the tree after harvest.

Not all fruit infections lead to leaf and shoot death even though hull lesions and fungal sporulation are alike for all infections. Either or both of the principal pathogens, *R. stolonifer* and *M. fructicola*, may be present in orchards having the disease. Investigations on the comparative severity of hull rot caused by *M. fructicola, M. laxa,* or *R. stolonifer* have been inconclusive (1,6).

The hull of maturing almond fruit dehisces along the ventral suture (4) and detaches from the pedicel. The opening (split) widens over several days and the hull loses moisture. By harvest, the hulls of most fruit are quite dry and somewhat hard. A few vascular elements keep healthy fruit attached to the tree, and fruit readily drop when the tree is shaken (2). Hull rot infections occur during hull split because the pathogens cannot invade the exterior hull surface (6).

Hull rot is most common on the cultivar Nonpareil and is associated with vigorous trees supplied with abundant nitrogen and water (7). Early harvest provides some control, whereas irrigations close to harvest exacerbate hull rot damage (7). In an experiment in Kern County, CA, termination of irrigation 2 weeks preharvest reduced hull rot by 400 to 500% in two consecutive years (9). Chemical controls are not available for this disease (10).

The severity of hull rot may be the result of many interacting factors that have not been described. We report here the effects of hull abscission on hull rot caused by *R. stolonifer* and *M. fructicola* and the relative severity of hull rot caused by *M. fructicola, M. laxa,* and *R. stolonifer* at three inoculum concentrations.

MATERIALS AND METHODS

Orchards. Experiments were conducted at two locations. Orchard 1 was a commercial orchard in Kern County, CA, planted in 1981 with cultivars Nonpareil and Carmel in a 2 row by 2 row pattern on a 7.6 × 7.6 m spacing. Trees were irrigated with microsprinklers and harvested on 16 August 1991. Experiments described here were located in the normal-irrigation control trees of an irrigation experiment performed in this orchard and reported elsewhere (9). The normal-irrigation control trees received 787 mm of water from early bloom to a week before harvest. Orchard 2 was a 15-year-old orchard, planted with cultivars Nonpareil and Mission in alternating rows with tree-to-tree spacing of 6.1 × 6.1 m, and located at the Kearney Agricultural Center, Fresno County, CA. The orchard was flood-furrow irrigated, received 762 mm of water between bloom and harvest, the normal amount for the area, and was harvested on 10 August 1992.

Inoculum preparation. Isolates of *R. stolonifer* and *M. fructicola* were obtained from infected almond hulls and *M. laxa* from almond twig cankers. *R. stolonifer* and *M. fructicola* were cultured on acidified (2.5 ml of 25% lactic acid [vol/vol] per liter of medium) potato dextrose agar (APDA) for 7 to 14 days at room temperature (20 to 22°C) under diurnal laboratory light conditions. *M. laxa* was grown on oatmeal agar in alternating 48-h periods of light and dark for 14 days to stimulate sporulation. Sporangiospores of *R. stolonifer* and conidia of *Monilinia* spp. were washed from 7- to 14-day-old cultures with sterile, deionized water, passed through three layers of cheesecloth to remove mycelial fragments and clumped spores, counted with a hemacytometer, and adjusted to desired concentrations with sterile, deionized water. Spore suspensions were prepared immediately before inoculations and stored in an ice chest while in the field. Germination was determined by counting 100 spores in each of two APDA culture plates seeded with 0.1 ml suspension after incubation at 20 to 22°C for 6 h (*R. stolonifer*) or 24 h (*M. fructicola* and *M. laxa*). Germination ranged from 82 to 98%.

Hull abscission. To determine the relationship of the amount of hull abscission at the time of inoculation to hull infection and leaf death, three categories of hull abscission were identified by visual estimation of the percent separation of the hull from the

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pedicel as follows: slight (1 to 10%), partial (15 to 40%), or almost complete (70 to 95%). A hand-pump atomizer was used to inoculate 25 fruit per replication in each category through the open sutures with 0.1 ml of a suspension of 10^4 spores of *R. stolonifer* per ml on 24 and 30 July and 4 August 1991 in orchard 1, and *R. stolonifer* or *M. fructicola* on 8 and 15 July 1992 in orchard 2. Similar, noninoculated fruit were used as controls, and inoculated and control fruit were marked for later identification. Each test fruit was situated next to a cluster of three to six healthy leaves. The experiments were evaluated on 13 August 1991 in orchard 1 and 27 July 1992 in orchard 2.

Inoculum concentration and pathogen comparison. Suspensions containing 10^3, 10^4, or 10^5 spores of *R. stolonifer* per ml were introduced into 25 fruit per replication having partially abscised hulls on 24 and 30 July and 4 August 1991 in orchard 1. The same inoculum concentrations of *R. stolonifer, M. fructicola*, and *M. laxa* were similarly tested in orchard 2 on 9 and 16 July 1992. Experiments were evaluated on 14 August 1991 in orchard 1 and 29 July 1992 in orchard 2.

Disease evaluation. Two hull rot symptoms, leaf death and hull infection, were considered separately. Many almond leaves tend to be clustered near fruit that are located on short spurs or shoots. Most or all the leaves in such clusters wither and die if affected by the hull rot toxin. The designation "leaf strike" used here refers to the cluster of dead leaves associated with a diseased fruit. At the end of each experiment, the condition of leaves near each inoculated and control fruit was recorded, and fruit were removed and examined in the laboratory for presence of hull rot lesions and external fungal growth. *R. stolonifer* was identified directly by the characteristic growth and sporulation on the hulls. Hulls inoculated with *M. fructicola* or *M. laxa* and that had lesions were removed from the nut and incubated in moist chambers at 22 ± 1°C for 5 days to promote sporulation. Conidia or mycelium from five to 10 subsamples of hulls were transferred to oatmeal agar and APDA to distinguish *M. fructicola* from *M. laxa* (3,8).

Hull moisture content. On each inoculation date, 20 fruit that represented each hull abscission category of inoculated fruit were collected from among the trees used for the inoculation experiments in each replication in both orchards. Fruit were sealed in plastic Ziploc bags, stored in an ice chest, and returned to the laboratory. Hulls were separated from the nuts, weighed, and air dried in a forced air oven (model SA-550, Grieve Corp., Round Lake, IL) at 65°C for 72 h to determine dry weights. Percent hull moisture was calculated from these values.

Design and analysis. The experimental design in both orchards was a randomized complete block with three (orchard 1) or four (orchard 2) replications. In orchard 1, each block consisted of three adjacent trees, and blocks were 16 rows apart. Treatments were located on one to three trees per block, depending upon availability of fruit at the appropriate stages of hull abscission. Blocks were adjacent in orchard 2 and each block consisted of one tree. The treatment design for experiments in both locations was a two-way factorial with data combined over date. An arcsine transformation was performed on data for percent leaf strikes and infected hulls before analysis of variance and mean separation by Duncan's multiple range test using MSTATC statistics (Michigan State University, East Lansing).

Table 1. Effects of hull abscission on hull rot of almond fruit (cv. Nonpareil) inoculated with *Rhizopus stolonifer* (1991)

Treatment	Hull rot symptoms[w]		Hull moisture content (%)[x]
	Leaf strikes (%)	Hull infection (%)	
Hull abscission			
Slight (1 to 10%)	76.3 a[y]	80.0 a	79.6
Partial (15 to 40%)	43.6 b	66.8 ab	79.2
Almost complete (70 to 95%)	28.2 c	54.5 b	78.8
Date inoculated			
24 July	43.1	73.9	80.2
30 July	57.6	60.0	79.9
4 August	47.5	67.3	77.8
Significance of *F*, *P* = [z]			
Hull abscission	0.003	0.025	NS
Date inoculated	NS	NS	NS
Abscission × date	NS	NS	NS

[w] Twenty-five fruit, each situated next to healthy leaves, of each hull abscission category per replication were inoculated with 0.1 ml of a suspension of 10^4 spores of *R. stolonifer* per ml on each date. Inoculated fruit were collected and the condition of associated leaves recorded on 13 August 1991. Trees were commercially harvested 16 August 1991.

[x] Percent hull moisture content was determined on each inoculation date from 20 fruit of each hull abscission category per replication.

[y] Three replications of each treatment were arranged in a randomized complete block design. Means are for main effects and those followed by the same letter do not differ significantly according to Duncan's multiple range test ($P = 0.05$).

[z] Overall significances of *F* are for main effects and their interactions. NS = not significant, $P \geq 0.10$.

Table 2. Effects of hull abscission and pathogen on hull rot of almond fruit (cv. Nonpareil) inoculated with *Monilinia fructicola* or *Rhizopus stolonifer* (1992)

Treatment	Hull rot symptoms[w]		Hull moisture content (%)[x]
	Leaf strikes (%)	Hull infection (%)	
Hull abscission			
Slight (1 to 10%)	47.7 a[y]	65.1	78.4 a
Partial (15 to 40%)	34.4 b	71.0	78.3 a
Almost complete (70 to 95%)	17.8 c	60.1	77.5 b
Pathogen			
M. fructicola	47.8 a	73.3 b	...
R. stolonifer	32.3 b	83.7 a	...
Noninoculated control	19.8 c	29.2 c	...
Date inoculated			
8 July	34.8	68.1	78.2
15 July	31.8	62.7	77.4
Significance of *F*, *P* = [z]			
Hull abscission	0.001	NS	0.022
Pathogen	0.001	0.001	...
Date inoculated	NS	0.076	NS
Interactions			
Abscission × pathogen	0.001	NS	...
Abscission × date	0.072	NS	NS
Date × pathogen	0.002	NS	...
Abscission × date × pathogen	NS	NS	...

[w] Twenty-five fruit, each situated next to healthy leaves, per replication of each hull abscission category were inoculated with 0.1 ml of a suspension of 10^4 spores of *M. fructicola* or *R. stolonifer* per ml. Inoculated fruit were collected and the condition of associated leaves recorded on 27 July 1992. Trees were harvested 10 August 1992.

[x] Percent hull moisture content was determined on each inoculation date from 20 fruit of each hull abscission category per replication.

[y] Four single-tree replications of each treatment were arranged in a randomized complete block design. Means are for main effects and those followed by the same letter do not differ according to Duncan's multiple range test ($P = 0.05$).

[z] Overall significances of *F* are for main effects and their interactions. NS = not significant, $P \geq 0.10$.

RESULTS

Effects of hull abscission. Leaf strikes in orchard 1 were more abundant near hulls in the early stages of abscission, and the percent leaf strikes in each hull abscission category differed significantly from the others (Table 1). Hull infection was greater in fruit with slightly than with almost completely abscised hulls but hull infection of partially abscised hulls did not differ from the other two categories. Percent leaf strikes or hull infection were not affected by inoculation date, and hull moisture content did not differ among hull abscission categories or inoculation dates. The interaction of hull abscission and inoculation date was not significant.

In orchard 2, the relationship of percent leaf strikes and hull abscission was similar to that found in orchard 1, but percent hull infection was not significantly affected (Table 2). Hull moisture content was significantly greater in slightly and partially abscised hulls than almost completely abscised hulls. *M. fructicola* caused more leaf strikes than *R. stolonifer*, but the reverse was true for percent hull infection. No significant differences in percent leaf strikes, hull infection, or hull moisture content were found between inoculation dates.

There was a greater decrease in percent leaf strikes as the degree of hull abscission increased in inoculated than in noninoculated treatments, resulting in a significant interaction between pathogen and hull abscission (Fig. 1A). Similarly, the percent leaf strikes in the noninoculated control increased with respect to the inoculated treatments on the second inoculation date, resulting in a significant interaction between pathogen and inoculation date (Fig. 1B).

Inoculum concentration and pathogen comparison. Inoculum concentration, exclusive of the control, did not significantly affect percent leaf strikes or infected hulls in orchard 1 (Table 3) or orchard 2 (Table 4). Hull moisture content, 77.2 and 78.3% in orchards 1 and 2, respectively, was the same for all inoculum concentration treatments because all were represented by the same samples of fruit with partially abscised hulls. Inoculation date had no significant effect on percent leaf strikes or hull infection in either orchard (Tables 3 and 4). In orchard 2, *M. laxa* caused more leaf strikes than *M. fructicola* or *R. stolonifer*, and *M. fructicola* and *R. stolonifer* did not differ.

The infected noninoculated control fruit represented natural infection. Only *R. stolonifer* was observed in control fruit in orchard 1. In orchard 2, *M. fructicola* and *R. stolonifer* caused 99 and 1%, respectively, of the natural infections of control fruit. Among inoculated fruit in orchard 2, *M. laxa* was not found among fruit inoculated with *M. fructicola* but *M. fructicola* infected 1 to 5% of fruit inoculated with *M. laxa*. Inoculated fruit that exhibited growth of both *Rhizopus* and *Monilinia* spp. never exceeded 1%. Consequently the species used as inoculum was credited with hull infection and associated leaf death.

DISCUSSION

In this study, several factors appeared to influence the amount and severity of hull rot disease of almond. For instance, fruit maturity at the time of inoculation influenced leaf death; leaf death was more likely near hulls at early stages of abscission. Hull infection generally declined with advancing fruit age, but not as much as leaf death. The trend toward overall lower levels of leaf death and hull infection as harvest neared may have resulted from a general hastening of fruit maturation at the end

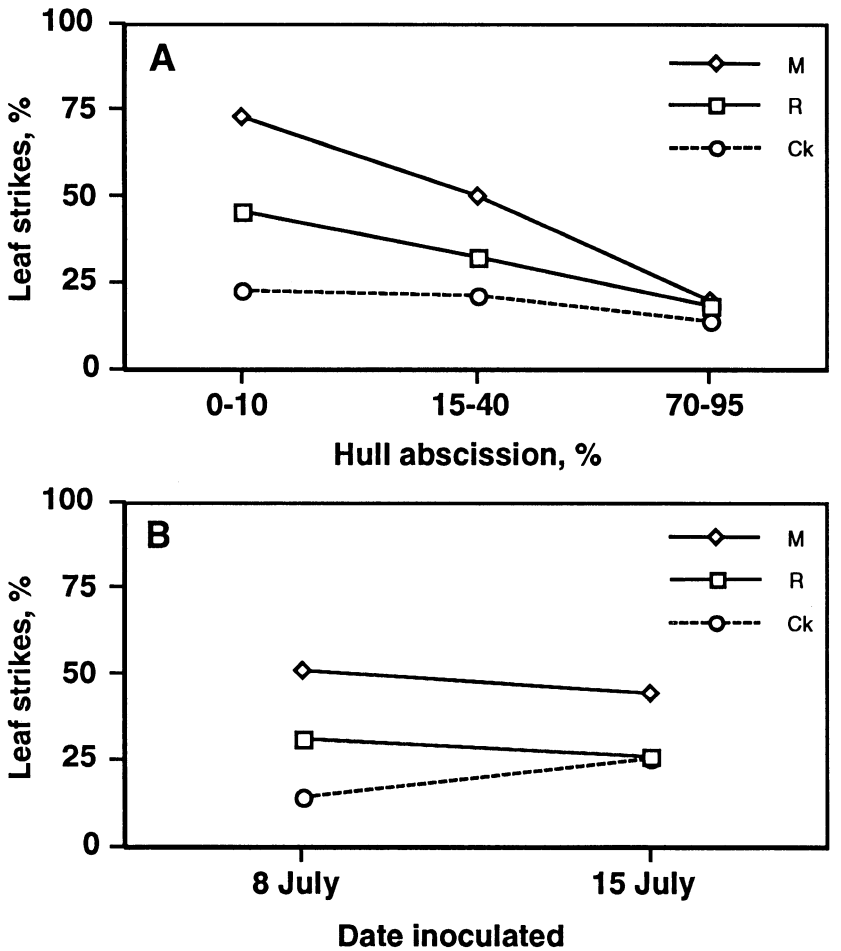

Fig. 1. Effects of hull abscission levels (**A**) and date of inoculation (**B**) of almond fruit (cv. Nonpareil) on the incidence of leaf strikes were recorded on 27 July 1992. Twenty-five fruit, each situated next to healthy leaves, per replication of each of three hull abscission percentages were inoculated with a suspension of *M. fructicola* (M) or *R. stolonifer* (R) on each date. CK = noninoculated control fruit. Trees were harvested 10 August 1992.

Table 3. Effect of inoculum concentration on hull rot of almond fruit (cv. Nonpareil) inoculated with *Rhizopus stolonifer* (1991)

Treatment	Hull rot symptoms[x]	
	Leaf strikes (%)	Hull infection (%)
Inoculum concentration (spores/ml)		
10^5	67.1 a[y]	76.7 a
10^4	54.2 a	67.8 a
10^3	58.7 a	68.2 a
Noninoculated control	10.9 b	17.8 b
Date inoculated		
24 July	40.8	43.4
30 July	50.3	65.7
4 August	55.9	56.7
Significance of F, P = [z]		
Inoculum concentration	0.001	0.001
Date inoculated	0.059	NS
Inoculum × date	NS	NS

[x] Twenty-five fruit, each situated next to healthy leaves, per replication were inoculated with 0.1 ml of a spore suspension of each inoculum concentration on each date. Fruit were collected and condition of associated leaves recorded 14 August 1991. Trees were commercially harvested 16 August 1991.

[y] Three replications of eight-tree plots of each treatment were arranged in a randomized complete block design. Means are for main effects and those followed by the same letter do not differ significantly according to Duncan's multiple range test ($P = 0.05$).

[z] Overall significances of F are for main effects and their interactions. NS = not significant, $P \geq 0.10$.

Table 4. Effects of inoculum concentration on hull rot of almond fruit (cv. Nonpareil) inoculated with *Monilinia fructicola*, *M. laxa*, or *Rhizopus stolonifer* (1992)

Treatment	Hull rot symptoms[x]	
	Leaf strikes (%)	Hull infection (%)
Inoculum (spores/ml)		
10^5	51.7 a[y]	83.1 a
10^4	45.3 a	75.2 a
10^3	46.8 a	76.3 a
Noninoculated control	20.1 b	33.6 b
Pathogens		
M. laxa	59.7 a	82.5
M. fructicola	45.8 b	76.1
R. stolonifer	38.3 b	76.8
Date inoculated		
17 July	49.0	83.7
21 July	46.9	74.3
Significance of F, P = [z]		
Inoculum concentration	0.001	0.001
Pathogen	0.001	NS
Date inoculated	NS	NS
Interactions		
Inoculum × pathogen	NS	NS
Inoculum × date	NS	NS
Pathogen × date	NS	NS
Inoculum × pathogen × date	NS	NS

[x] Twenty-five fruit, each situated next to healthy leaves, per replication were inoculated with 0.1 ml of a spore suspension of each pathogen at each inoculum concentration on each date. Inoculated fruit were collected and the condition of associated leaves recorded on 29 July 1992. Trees were harvested 10 August 1992.

[y] Four single-tree replications of each treatment were arranged in a randomized complete block design. Means are for main effects and those followed by the same letter do not differ significantly according to Duncan's multiple range test ($P = 0.05$).

[z] Overall significances of F are for main effects and their interactions. NS = not significant, $P \geq 0.10$.

of the season. On later inoculation dates, we noticed that hulls of fruit at early stages of dehiscence or abscission often were lighter green and appeared older than hulls of similar fruit earlier in the season. Also, hull moisture content, regardless of hull dehiscence, tended to decrease (though not significantly) later in the season. If the toxin theory of pathogenesis (5) is correct, hull abscission would interrupt transport and thus reduce the amount of toxin sent to leaves and shoots, which also could contribute to the lower incidence of leaf strikes associated with more mature fruit. Orchard management practices, such as reduced irrigation and fertilization, that encourage rapid and early fruit maturation may reduce damage by hull rot disease.

Fruit maturity perhaps affected the responses of the pathogens tested in orchard 2. In the hull abscission experiment, *M. fructicola* caused significantly more leaf death than *R. stolonifer*. The interaction between hull abscission and pathogen (Fig. 1A) indicates that, with respect to leaf strikes, greater differences occurred between the fungi when slightly as opposed to partially abscised fruit were inoculated. In the inoculum concentration experiment, partially abscised hulls were selected because more were available, and the amount of leaf death associated with inoculations using *M. fructicola* and *R. stolonifer* did not differ significantly. The combined effect of the pathogen involved and the stage of maturity at which the fruit were inoculated may have influenced the amount of leaf death.

The role of hull moisture content is unclear. The magnitude of differences in hull moisture content was small in comparison with the differences in leaf strikes or hull infection. If hull moisture content is important, whatever governs leaf death would have to be sensitive to small changes in hull moisture content to account for these disparities. The relative humidity in the space between the hull and shell probably decreased as hulls opened, creating an internal climate less favorable to fungal germination and infection in widely opened hulls.

Sources of inoculum for these pathogens have not been identified. *R. stolonifer* likely is present in soil and debris in most orchards. *M. fructicola* seldom attacks blossoms of almond and thus would rarely survive in infected blossoms or twig cankers. *R. stolonifer* and *M. fructicola* are common on mature stone fruits in California during the time when almond trees in neighboring orchards approach harvest (hull abscission). Thus, these pathogens might be introduced from nearby peach and nectarine orchards via insect vectors or air currents. In addition, spores produced in almond fruit infected with hull rot can provide spore inoculum for later infections. The usual absence of *M. laxa* in the hull rot complex is of interest because this fungus is the almost exclusive cause of brown rot blossom and twig blight of almond in spring and is indigenous in many almond orchards (10). That *M. laxa* caused more hull rot than the other two pathogens when introduced into fruit suggests that natural inoculum of this fungus may not be present in almond orchards in summer when fruit are susceptible to hull rot.

Formal surveys to identify the distribution and frequency of the hull rot pathogens in commercial orchards have not been conducted, but *M. laxa* has not been encountered in routine specimens submitted to this laboratory or in any of the many naturally infected hulls we have processed during the course of these experiments. In the past, *Rhizopus* was present more frequently than *Monilinia* (1). Recently, Cooperative Extension agents report that Rhizopus hull rot is common in southern San Joaquin Valley orchards, where few commercial stone fruit orchards are present, and Monilinia or both Monilinia and Rhizopus hull rot occur in more northerly located orchards, where extensive acreage of stone fruits exist.

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