Threshold Conditions That Lead Latent Infection to Prune Fruit Rot Caused by *Monilinia fructicola*

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ABSTRACT

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Inoculations were performed six to eight times in each of 10 prune orchards located in nine counties of California. In each inoculation, branches that bore 40 to 60 blossoms or 30 to 40 fruit were inoculated with conidial suspensions of *Monilinia fructicola*. Three inoculum concentrations and 14 to 16 h of humidity were used for each inoculation. All inoculated fruit were maintained on trees and harvested separately 2 weeks before commercial harvest. The incidence of latent infection (ILI) and percentage of branches with fruit rot (PBFR) were determined for each inoculation in each orchard. As the ILI increased, the PBFR also increased linearly. Five conditions that lead latent infection to fruit rot include (i) latent infection level; (ii) fruit developmental stage; (iii) inoculum concentration; (iv) total hours of relative humidity greater than 90% (hRH); and (v) total hours of dew period (hDEW) from mid-July to mid-August. Three levels of PBFR, 1, 5, and 10% were assigned, and threshold conditions that lead to these levels were determined based on the experimental results. The relative probabilities that lead latent infection to fruit rot (r_PBFR) at different fruit developmental stages were calculated. A preliminary decision support model to guide fungicide application was developed based on the above results. One of the four recommendations, safe, wait, check historical weather as a reference, and apply a fungicide immediately, could be provided based on the level of latent infection and the decision process developed through this study.

Additional keywords: dried plum, *Prunus domestica*, stone fruit.

Brown rot of stone fruit (*Prunus* spp.) is caused by the fungal pathogen *Monilinia fructicola* (G. Wint.) Honey (2,3). Main disease symptoms include blossom blight and fruit rot (3). Ascospores or conidia produced from mummies infected by *M. fructicola* on the orchard floor serve as sources of inoculum that cause blossom blight in the spring under favorable conditions (3,11,12,22). When microclimatic conditions are unfavorable, these primary infections can remain latent until conditions become favorable for disease development that leads to fruit rot (4,10,24). The main inoculum sources for secondary infection were determined to be conidia produced on the thinned infected fruit on the orchard floor (8), and the latent infection could occur over the whole season under favorable conditions. The level of latent infection in fruit is influenced by both primary and secondary infection (14,23,24). Additionally, even when the inoculum potential level in orchards is consistently similar during the growing season, the level of latent infection at different fruit developmental stages could be different. In our previous study (14), a seasonal pattern of bloom and fruit susceptibility to infection over the season was determined, which demonstrated that susceptibility of both bloom and fruit significantly affected latent infections. Favorable microclimatic conditions in orchards are undoubtedly critical during the infection process when proper inoculum and high fruit susceptibility coincide. In addition to temperature, wetness duration (WD) was considered to be an important microclimatic factor that affects infection of fruit. Based on experimental results, we determined the seasonal risky patterns of wetness duration that led to different levels of latent infection and established a risk analysis to estimate the level of latent infection at different fruit developmental stages (15).

Different from the foliar diseases of row crops, although infections could occur over the season, not all latent infections induce fruit rot before harvest (4,20). In most cases of different stone fruits, infections can be continuously latent even after harvest and act as sources for postharvest brown rot during fruit cold storage (3). Therefore, to estimate latent infection during the growing season is meaningful for pre and postharvest disease management, and the reduction of latent infections can become important in disease management.

Differing from other stone fruits, prunes are usually dehydrated immediately after harvest in commercial productions in California. There is generally no postharvest brown rot problem during storage for the majority of prune production in California. Therefore, the probability of latent infections that lead to fruit rot before harvest is more important information for disease management in prunes than in other stone fruits. It is still unknown what conditions result in different levels of latent infection and fruit rot before harvest. This information can be obtained from experiments in orchards that represent various environments.

It is already clear that susceptibility of bloom and fruit to infection could change in the season (14), and environments could affect this susceptibility (13,14,15,17). Decision support for disease management requires the information relevant to threshold conditions that trigger latent infection to expression of fruit rot, and a decision model is helpful to guide growers for fungicide application to reduce risk of fruit rot. These conditions may include fruit developmental stage, level of latent infection, inoculum potential, and environment. The objectives of this study were to (i) determine the quantitative relationships among latent infection, inoculum concentration, environment, and prune fruit rot; (ii) determine the threshold conditions that lead latent infection to cause fruit rot; and (iii) develop a decision model for fungicide application during the growing season by use of the experimental results.

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MATERIALS AND METHODS

Preparation of inoculum. An isolate of *M. fructicola* collected from a prune orchard and stored at the Kearney Agricultural Center, University of California in Parlier, was used in this study. This isolate was cultured in petri dishes (10 × 150 mm) that contained potato dextrose agar amended with 25% lactic acid (2.6 ml/liter) and incubated at 23 ± 2°C for 5 days in the dark. The pathogen was subcultured on new dishes under the same conditions for 5 days. Spores of *M. fructicola* were harvested by pouring 3 ml of sterile distilled water in each petri dish and the different concentrations were determined with a hemacytometer.

Experimental design. Ten prune orchards were selected in nine counties located in the Central and Sacramento valleys of California. The orchards had different microclimatic conditions. In six orchards, eight inoculations were conducted at stages of full bloom, fruit set, early pit hardening, pit hardening, embryo growth, late embryo growth, before first harvest, and first harvest (21) (Table 1). Seven inoculations were conducted in three orchards, and six inoculations were conducted in one orchard (Table 1). In each inoculation, three concentrations, 5,000, 20,000, and 50,000 conidia per ml of *M. fructicola*, were used. Inoculations of bloom were conducted from 19 to 23 March 2001 at the full bloom stage (21). Branches that bore 40 to 60 flowers were selected. Each branch was covered with a plastic bag (30.4 × 20.3 × 76.2 cm; gauge 0.002) and sprayed uniformly inside the bag with 50 ml of the conidial suspensions for each inoculum concentration by using a hand-held sprayer (ACE Hardware Corporation, Oak Brook, IL). Immediately after inoculation of each branch, the plastic bag was closed tightly to create high humidity. For each experiment, inoculations were done from 1700 to 1900 h. Fourteen- to sixteen-hour durations of wetness were accomplished by uncovering the plastic bags the following morning. In each inoculation, three inoculum concentrations were separated on three trees, and six replicated branches of each tree were randomly chosen for each inoculation concentration. Five branches of a noninoculated tree were sprayed with 50 ml of water and maintained wet for a 16-h wetness duration to serve as noninoculation control for each experiment in each location.

Inoculations continued at fruit set and further fruit developmental stages (Table 1) with the same inoculation methods described above. In all inoculations, six replicate branches each bearing 30 to 40 fruit for each tree were used for each inoculum concentration, and a 16-h wetness duration was created for all

TABLE 1. Summary of inoculation experiments with *Monilinia fructicola* in 10 prune orchards located in nine counties of California in 2001[a]

Location and inoculation date	Bloom and fruit developmental stage[b]	Fruit size (mm) length/width[c]	Ave. fruit weight (g)[c]
Tulare[d]			
19 March	Full bloom		
2 April	Fruit set		
18 April	Early pit hardening	20.4/13.3	1.5
7 May	Pit hardening	31.1/19.2	6.3
25 May	Early embryo growth	32.7/20.9	7.6
18 June	Embryo growth	33.7/24.2	10.3
9 July	Before first harvest	…	…
Fresno[d]			
19 March	Full bloom		
2 April	Fruit set		
18 April	Early pit hardening	23.6/12.1	2.5
7 May	Pit hardening	26.2/15.7	3.9
15 May	Early embryo growth	30.4/19.2	5.6
18 June	Late embryo growth	31.8/22.2	8.5
16 July	Before first harvest	…	…
27 July	First harvest	…	…
Glenn-1			
22 March	Full bloom		
5 April	Fruit set		
15 April	Early pit hardening	18.5/9.1	1.2
10 May	Pit hardening	27.2/17.3	4.7
29 May	Embryo growth	27.3/16.9	4.6
20 June	Late embryo growth	32.4/21.9	8.4
11 July	Before first harvest	38.1/29.2	17
29 July	First harvest	27.7/29.1	16.9
Madera[d]			
20 March	Full bloom		
3 April	Fruit set		
17 April	Early pit hardening	17.3/9.1	0.94
15 May	Late pit hardening	29.7/19.6	6
6 June	Embryo growth	34/20.5	9.2
6 July	Before first harvest	40.5/30.0	18.3
26 July	First harvest	40.9/32.7	22.6
Tehama[d]			
20 March	Full bloom		
3 April	Fruit set		
17 April	Early pit hardening	25.2/15.0	2.9
15 May	Late pit hardening	…	…
6 June	Embryo growth	33.3/23.9	10.3
6 July	Before first harvest	42.7/32.8	23.2
26 July	First harvest	…	…
Colusa			
21 March	Full bloom		
4 April	Fruit set		
14 April	Early pit hardening	14.3/6.9	0.8
9 May	Pit hardening	30.3/18.8	5.5
31 May	Embryo growth	32.7/21.3	8.9
21 June	Late embryo growth	32.7/22.9	9.2
10 July	Before first harvest	38.8/29.9	16.7
28 July	First harvest	38.3/29.6	17.7
Sutter			
23 March	Full bloom		
5 April	Fruit set		
15 April	Early pit hardening	13.7/7.1	0.6
10 May	Pit hardening	29.5/19.2	6.4
30 May	Embryo growth	29.6/21.1	14.4
20 June	Late embryo growth	35.2/23.0	12.7
12 July	Before first harvest	42.3/33.1	26.4
30 July	First harvest	42.9/33.9	26.8
Glenn-2[d]			
22 March	Full bloom		
5 April	Fruit set		
15 April	Early pit hardening	16.1/8.7	0.9
20 June	Late embryo growth	32.1/22.2	8.4
11 July	Before first harvest	35.9/27.4	14.1
29 July	First harvest	37.6/29.9	18.5
Butte			
23 March	Full bloom		
5 April	Fruit set		
15 April	Early pit hardening	15.0/7.3	0.7
10 May	Pit hardening	26.8/17.7	4.7
30 May	Embryo growth	33.1/23	9.7
20 June	Late embryo growth	37.1/26.4	14.2
12 July	Before first harvest	44.0/34.3	25.7
30 July	First harvest	44.0/34.6	29.2
Yolo[d]			
21 March	Full bloom		
4 April	Fruit set		
14 April	Early pit hardening	15.1/7.4	0.63
9 May	Pit hardening	26.6/15.2	3.2
31 May	Embryo growth	27.6/17.7	4.72
10 July	Before first harvest	…	…
28 July	First harvest	…	…

[a] Six branches of prune trees were inoculated with *M. fructicola*, and artificial dew was generated to obtain 16-h wetness durations on inoculated branches to induce latent infection.
[b] Inoculations were conducted at different bloom and fruit developmental stages with three inoculum concentrations.
[c] Fruit size and weight are averages of 30 fruit samples on corresponding date of inoculation.
[d] Fewer than eight times of inoculation were conducted due to the adverse field conditions.

inoculations. A data logger (ONSET Company, Pocasset, MA) was used in each orchard to record hourly temperature, humidity, and dew point temperature, which were used to determine the daily dew period during the experiments.

Fruit on all inoculated trees were maintained on the trees until harvest. Depending on the location of the orchard, the harvest dates were from 10 to 15 August (about 2 weeks before commercial harvest). During harvest, branches with fruit showing sporulation or appearing as mummified fruit (mummies) were recorded. Fruit without any symptoms of brown rot from each replicated branch were placed in a paper bag and stored at 4°C until processed to detect latent infection by *M. fructicola*.

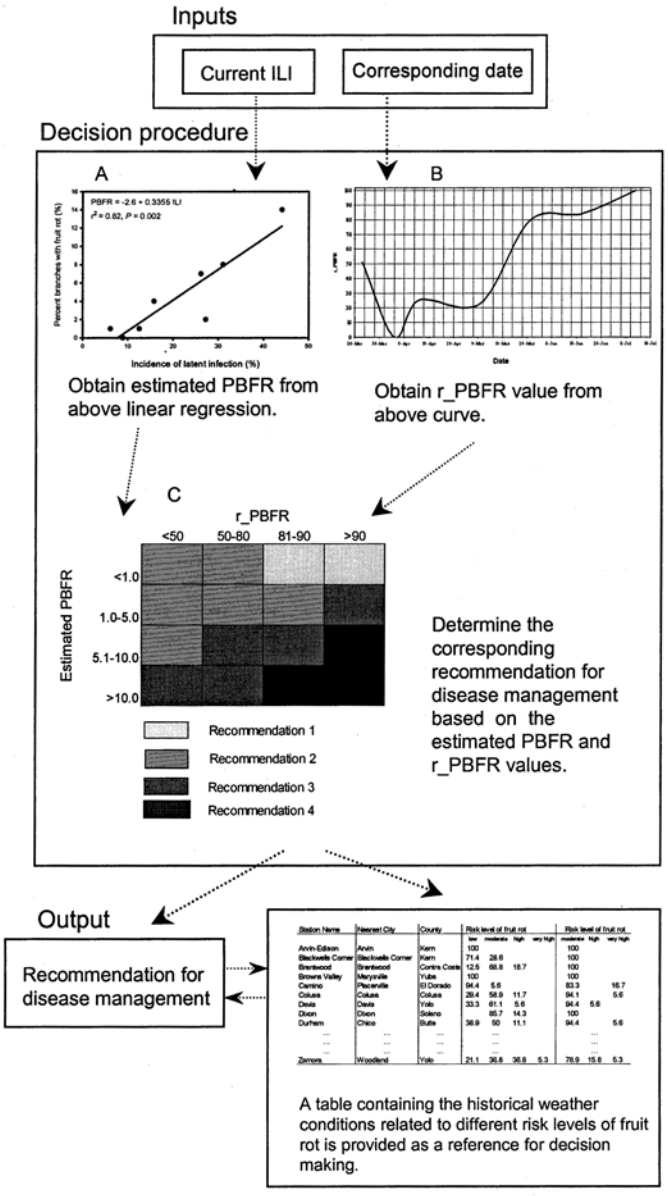

Fig. 1. Schematic diagram of the decision support model for fungicide application to reduce risk of prune fruit rot caused by *Monilinia fructicola*. The user is required to input the incidence of latent infection (ILI) and the corresponding date (ILI can be determined by using the overnight freezing incubation technique (18,19). The model determined **A,** the possible percentage of branches with fruit rot (PBFR) by using a linear regression between ILI and PBFR and **B,** a curve of relative probability of leading latent infection to fruit rot (r_PBFR) over the growing season. **C,** Recommendation of fungicide application was provided based on the decision process that considered both PBFR and r_PBFR. One of four recommendations was provided as an output of the model. Historical weather conditions relating to different risk levels of fruit rot for different locations of California also were provided in the model.

Determination of latent infection of fruit. The overnight freezing incubation technique (18,19) was used to determine the incidence of fruit with latent infection. To process the samples of harvested fruit, plastic containers (40 × 4 × 2 cm) and screens were sterilized by soaking in 10% commercial bleach for at least 8 h. For each sample, fruit were surface sterilized in a chlorine solution (32 ml of 0.525% sodium hypochlorite, 32 ml of 95% ethyl alcohol, and 0.01 ml surfactant Tween 20 in 2 liters of water) for approximately 15 to 20 min. The fruit were washed with sterile distilled water 10 times and placed on a sterilized plastic screen in a container with 150 ml of water at the bottom. The containers were placed in a freezer at –16°C for 10 h initially and on a laboratory bench at 23 ± 2°C for 5 days. By this time, the number of fruit covered with sporulation of *M. fructicola* was recorded, and incidence as percentage of fruit with brown rot was calculated for each sample.

Data analysis. A split-plot design was applied in this study (7). Location was treated as replication, inoculation date was the main-plot treatment, and inoculum concentrations were the subplot treatments. Incidence of fruit latent infection (ILI) for each inoculated branch was used in the analysis. Additionally, percentage of branches with fruit rot (PBFR) was also used for the treatments of inoculation date and inoculum concentration, as well as a combination of location and inoculum concentration for each inoculation date. Analysis of variance was applied by using the GLM procedure (SAS Institute, Cary, NC) to determine the significance of variances of both ILI and PBFR from main-plot and subplot treatments. Corresponding errors applied in the split-plot design (7) were used in comparison of means of ILI and PBFR among inoculation dates by using the least significant difference (LSD). The means of ILI among the three inoculum concentrations were also compared for each inoculation date. The dynamics of average ILI from all locations over the growing season were determined. Since most fruit rot appeared after mid-July, two environmental variables were used in the analysis: (i) total number of hours when relative humidity was ≥90% (hRH); and (ii) total number of hours of dew period (hDEW) from 15 July to 15 August. The values of these two variables were calculated from data obtained from the data logger installed in each orchard. Linear regressions between PBFR and hRH and between PBFR and hDEW were conducted by using the REG procedure of SAS.

Determination of threshold conditions that lead latent infection to fruit rot. Four threshold conditions that lead latent infection to fruit rot expression before harvest were considered: (i) threshold of latent infection; (ii) threshold of fruit developmental stage; (iii) threshold of inoculum concentration; and (iv) threshold of environments. Four levels of PBFR were arbitrarily assigned: (i) low level when PBFR ≤ 1%; (ii) moderate level when 1% < PBFR ≤ 5%; (iii) high level when 5% < PBFR ≤ 10%; and (iv) very high level when PBFR > 10%. The average values of ILI and PBFR from all 10 locations for each inoculation date were used in a linear regression between ILI and PBFR (PBFR = $\alpha + \beta \times$ ILI). From this regression, the ILI values were calculated when values of PBFR were 1, 5, and 10%, respectively. These three ILI values were used as thresholds of ILI. On the dynamic curve of mean PBFR from all 10 locations over the growing season, the corresponding dates were determined and showed the threshold growth stages when PBFR values were 1, 5, and 10%, respectively. The thresholds of inoculum concentrations for each of the three PBFR levels were determined from the dynamic curves of ILI over the growing season for the corresponding three inoculum concentrations by using the thresholds of ILI. The environmental thresholds were assigned as thresholds of hRH and hDEW, and the threshold values for the three PBFR levels were obtained from regressions between PBFR and hRH and between PBFR and hDEW, when PBFR was 1, 5, and 10%, respectively.

The rate of PBFR per ILI at the ith sampling (R_i) was calculated by $R_i = PBFR_i/ILI_i$, where $PBFR_i$ is the PBFR at the ith sampling

time, and ILI_i is the ILI at the ith sampling time. The relative R value for the ith sampling time (r_PBFR_i) was calculated by $r\_PBFR_i = R_i/R_{max} \times 100$, where R_{max} is the maximal R among the R values during the growing season. The r_PBFR_i could imply the relative probability of ILI that leads to PBFR at the sampling time i, and these values were used to draw the r_PBFR curve over the growing season from 20 March to 10 July by using the computer software Microsoft Excel (Microsoft Corp., Bothell, WA). A table containing each date and the corresponding r_PBFR for this time period was also established from this curve.

Development of a decision model for fungicide application. Using the above threshold conditions that trigger latent infection to fruit rot developed a preliminary decision model. The purpose of the model was to guide growers in deciding on fungicide application during the growing season to reduce risk of latent infection and fruit rot. The principle of the model was to determine whether fungicide application is necessary based on consideration of the dynamics of latent infection over the growing season. To use the model, a continued investigation of latent infection over the season in the orchard was suggested. As the model's inputs, the incidence of ILI and the investigation date were used to run the decision procedure (Fig. 1). The ILI was used to calculate the possible PBFR by using the linear regression between ILI and PBFR (Fig. 1A). The investigation date was used to determine the r_PBFR value (Fig. 1B). To determine whether fungicide application is necessary, a decision process was developed by using these two values (Fig. 1C). Four situations were considered as recommendation for disease management: (i) safe, no need to apply fungicide; (ii) wait for results of further investigation of latent infection; (iii) refer to reference of historical weather conditions; and (iv) apply fungicide immediately. One of the four recommendations for disease management was provided as the model output (Fig. 1). The model was written with Microsoft Visual Basic 6.0 (Microsoft Corp.) for a PC version, and with Active Server Pages (ASP) (Microsoft Corp.) for an Internet version.

To obtain the information on historical weather conditions that could be used as a reference for disease management, 32 locations in stone fruit growing areas of California were selected. Hourly weather data from 1983 to 2001, including air temperature, dew point temperature, and relative humidity in each location were collected from the California Irrigation Management Information System (CIMIS). For each location, hRH and hDEW from 15 July to 15 August were calculated for each year, and the percentages of yearly hRH and hDEW that lead to low, moderate, and high PBFR, respectively, were produced for each location based on thresholds of hRH and hDEW. All calculations were performed with SAS (SAS Institute).

RESULTS

The inoculation date and inoculum concentration for ILI and PBFR were significant at $P < 0.0001$. The interaction between inoculation date and inoculum concentration was also significant at $P = 0.0003$. By using average ILI and PBFR from all locations and inoculation concentrations for each inoculation date, the respective dynamic curves over the growing season were obtained (Fig. 2). For the curve of ILI, the average ILI at the full bloom stage (around 23 March) was less than 10% and reached approximately 10% at the fruit set stage (around 5 April). However, no significant difference between these two stages was found. The average ILI increased to approximately 27.2% at the pit hardening stage (around 10 May), which was significantly higher than those at three precedent stages (Fig. 2). After this stage, the average ILI decreased to a significantly lower level at 15.8% at the embryo growth stage (around 30 May), but increased again reaching 26.2% at the late embryo growth stage (around 20 June). The maximum average ILI in the season was 44.1% at the before first harvest stage (around 12 July), which was significantly higher than those at all other stages (Fig. 2). The average ILI decreased at first harvest since the last time of inoculation was close to fruit harvest.

A parallel trend was found for the dynamics of PBFR, particularly for the inoculations at the last four developmental stages (Fig. 2). Infections that occurred from bloom to the embryo growth stages lead to a low level of PBFR, and there was no significant difference in PBFR among the fruit developmental stages. After the embryo growth stage, chance of infections that lead to PBFR increased, and the PBFR reached 7% at the late embryo stage, which was significantly higher than those at the first five stages. The PBFR continuously increased, reaching a maximum of 14% at the before first harvest stage, which was

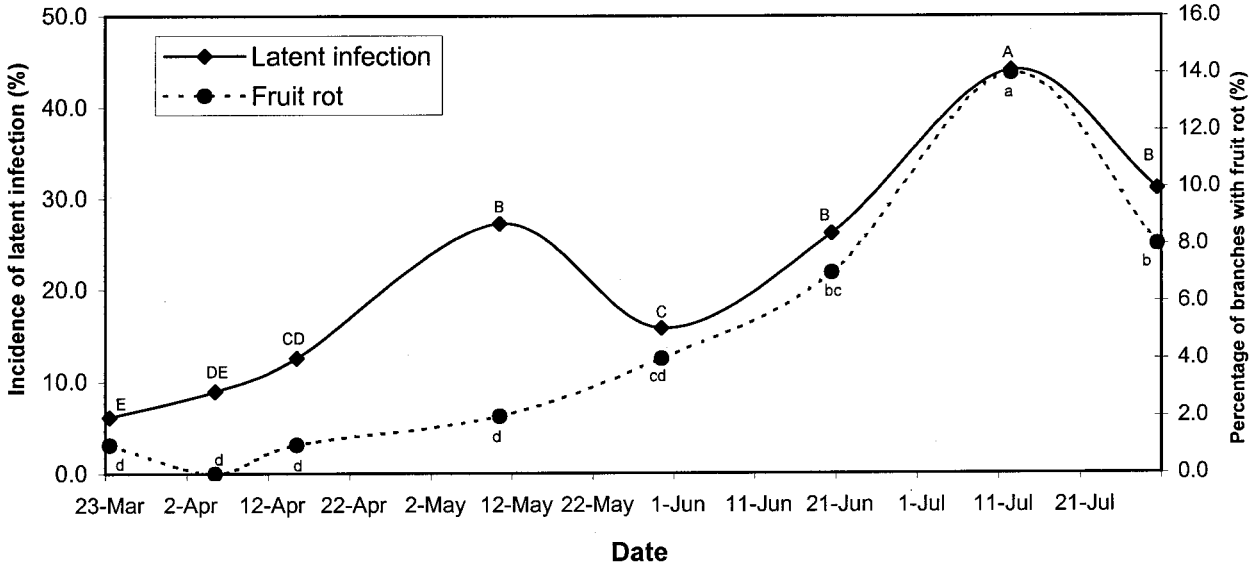

Fig. 2. Incidence of latent infection (ILI) of prune and percentage of branches with fruit rot (PBFR) induced by inoculation with *Monilinia fructicola* at different dates over the growing season. The inoculations were conducted in 10 orchards in California. Each point in each line represents an average value from multi-location and three inoculum concentrations. Each point of the dotted line represents the PBFR recorded at harvest but induced by the infections that occurred on the corresponding date. Upper case letters are used in comparisons among ILIs and lower case letters are used in comparisons among PBFRs. The values of points with a letter in common are not significantly different at $P \leq 0.05$.

significantly higher than that at the previous stage. Thus, latent infections at late stages lead to higher levels of PBFR than those at early stages.

Three near-parallel dynamic curves of ILI were determined for the three inoculum concentrations (Fig. 3). The basic trends of curves of ILI were similar to those in Figure 2. The curves of ILI almost proportionally increased with increase in inoculum concentration. Comparisons in ILI among inoculum concentrations for each inoculation date revealed that the ILIs for the inoculum concentration of 50,000 conidia per ml were significantly higher ($P \leq 0.05$) than those for 5,000 conidia per ml at all stages except full bloom and pit hardening stages (Fig. 3). The ILIs for the concentration of 20,000 conidia per ml at the late stages (after late embryo growth) were significantly higher ($P \leq 0.05$) than those of 5,000 conidia per ml. The ILIs for the concentration of 20,000 conidia per ml at the last stages (after late embryo growth) were also significantly higher ($P \leq 0.05$) than at the early stages (before late embryo growth) (Fig. 3).

Two linear regressions to describe the relationships between PBFR and hRH and between PBFR and hDEW were obtained (Fig. 4). Along with increasing hRH and hDEW, PBFR increased at a rate of 0.5 per h.

A linear regression between ILI and PBFR was obtained (Fig. 5) by using averages of each inoculation date. Therefore, calculated with this equation when PBFR equaled 1, 5, and 10%, respectively, the thresholds of ILI that lead to the corresponding levels of PBFR were 10.7, 22.7, and 37.6%, respectively (Table 2).

Thresholds of fruit developmental stages relating to the corresponding three levels of PBFR, 1, 5, and 10%, were determined directly from the PBFR dynamic curve (Fig. 6). Late season was related to greater PBFR than early season under the same inoculum conditions. Along with the season, the chance that latent infection could become an active lesion to induce fruit rot increased. Under the conditions of inoculum concentrations used in this study, the date that related to low PBFR (1%) was around 12 April at the stage of late fruit set, namely, latent infections that occurred before or at this stage could lead to a low level of PBFR under the inoculum conditions used in the study. However, latent infections that occurred around 8 June at approximately the late embryo growth stage were related to a moderate level of PBFR (5%). Therefore, the growing season during late fruit set and late embryo growth were related to 1 to 5% of PBFR. The PBFR reached 10% at the end of June when fruit were at the late embryo growth stage. Therefore, the PBFR increased from 5 to 10% during the period from the embryo growth to the late embryo growth stages, which was usually June to July. Therefore, the risk of latent infection that led to fruit rot increased after the late embryo growth stage and reached a maximum level at the before first harvest stage (Fig. 6).

Thresholds of inoculum concentration that caused three levels of PBFR were determined by using the corresponding three thresholds of ILI (10.7, 22.7, and 37.6%) (Fig. 7). Concentrations of 5,000 and 20,000 conidia per ml of *M. fructicola* did not result in a low level of PBFR (1%) before 16 April at approximately the pit hardening stage. The concentration of 5,000 conidia per ml of *M. fructicola* resulted in a PBFR greater than 5% during the period of 2 May and 16 May at the late pit hardening stage and after 25 June at the late embryo growth stage (Fig. 7). This concentration did not result in a PBFR greater than 10% throughout the season. After the pit hardening stage, all concentrations of *M. fructicola* resulted in a PBFR greater than 1% (Fig. 7), except for the 5,000 conidia per ml during the period from 26 May to 11 June (Fig. 7). However, the concentration of 20,000 conidia per ml of *M. fructicola* resulted in a PBFR greater than 5% only during the pit hardening stage (2 May to 18 May) and after the embryo growth stage (after 11 June), which was a susceptible stage to latent infection (Fig. 7). This concentration also resulted in a PFBR greater than 10% at the end of June after the late embryo growth stage. The concentration of 50,000 conidia per ml of *M. fructicola* could result in a PFBR greater than 1% during the entire season. After approximately 18 April before the pit hardening stage, this concentration could result in a PBFR greater than 5%, and also bring about a PBFR greater than 10% after approximately 25 June during the period from late embryo growth to harvest (Fig. 7).

Thresholds of environment in terms of hRH and hDEW from 15 July to 15 August were determined from regressions between PBFR and these two variables (Fig. 8). The hRH threshold that leads latent infection to a PBFR equal to or greater than 1% was 24.2 h. The corresponding thresholds of hRH that lead latent infection to 5 and 10% of PBFR were 104.6 and 204.6 h, respectively, under the conditions of this study (Fig. 8A). Although the threshold of hDEW that leads latent infection to 1% of PBFR was not available from the regression (Fig. 8B), the thresholds of

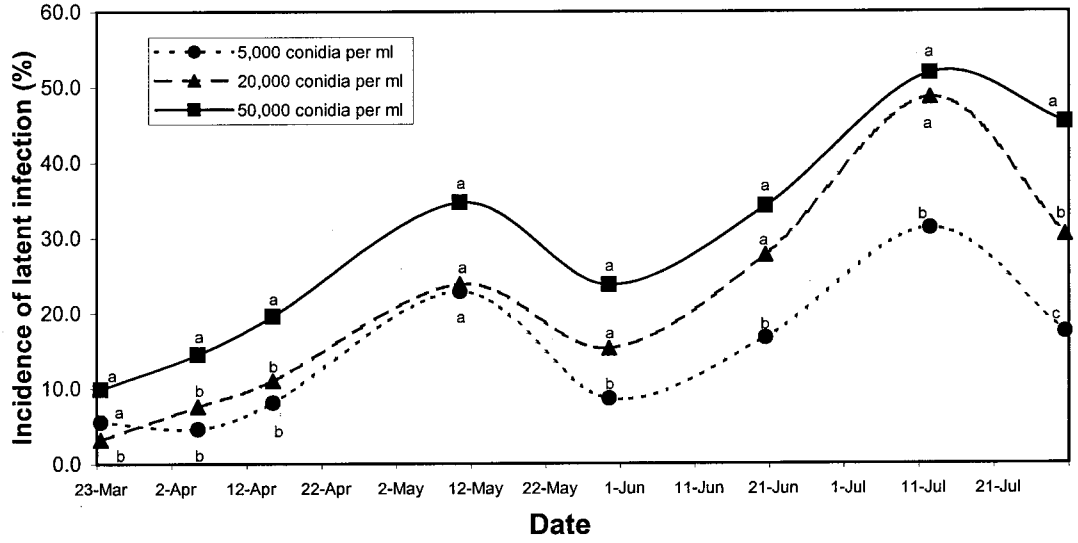

Fig. 3. Incidence of latent infection (ILI) of prune fruit caused by *Monilinia fructicola* with three inoculum concentrations at different dates over the growing season. Each point represents an average from multiple locations in California. Data are from inoculation experiments conducted in 10 different prune orchards. The ILIs among three inoculum concentrations were compared for each sampling date by using the least significance difference (LSD), and the values in the curves indicated with a common letter are not significantly different at $P \leq 0.05$.

hDEW for 5 and 10% of PBFR were 76.4 and 176.4 h, respectively (Fig. 8B). All threshold conditions that lead latent infection of fruit to the three levels of PBFR are summarized in Table 2.

The r_PBFR curve (Fig. 9) showed the changes in relative probability that lead latent infection to fruit rot over the growing season. The r_PBFR values were less than 50% before the late pit hardening stage (19 May) and increased to 80% after approximately 10 days. The r_PBFR values remained between 80 and 90% until the end of June during the embryo growth stages and increased to greater than 90% until 10 July at the before first harvest stage. Thus, the decision for disease management considered the changes in the probability of leading latent infection to fruit rot over the growing season, and four r_PBFR values, less than 50%, 50 to 80%, 81 to 90%, and greater than 90%, were used to represent various possibilities of leading latent infection to fruit rot at the corresponding fruit developmental stages.

The estimated PBFR calculated with ILI by the regression in Figure 5 and r_PBFR determined by the corresponding date from Figure 9 were used to develop the decision process (Fig. 10). Four recommendations for disease management were used: (i) safe, no need of fungicide application in the season; ii) wait, continue to investigate latent infection; (iii) check reference of historical weather to decide if fungicide application is needed; and (iv) spray fungicide immediately.

The multi-year hourly weather data from 15 July to 15 August for each location were used to calculate the percentages of hRH and hDEW relating to low, moderate, and high levels of PBFR. Four situations of hRH relating to different risks of fruit rot were determined as: (i) low risk when hRH ≤ 24.2; (ii) moderate risk when 24.2 < hRH ≤ 104.6; (iii) high risk when 104.6 < hRH ≤ 204.6; and (iv) very high risk when hRH > 204.6. Three situations of hDEW relating to different risk levels of fruit rot were also determined as: (i) moderate risk when hDEW ≤ 76.4; (ii) high risk when 76.4 < hDEW ≤ 176.4; and (iii) very high risk when hDEW > 176.4. The percentages of each risk situation that occurred historically in each location are listed in Table 3. This table can be used as a reference in decision making for fungicide application. When recommendation 3 is encountered, the user needs to check the historical weather at the nearest location from this table. However, users need to make a decision on fungicide application themselves based on their attitude toward possible risk to fruit rot with a reference of historical weather conditions.

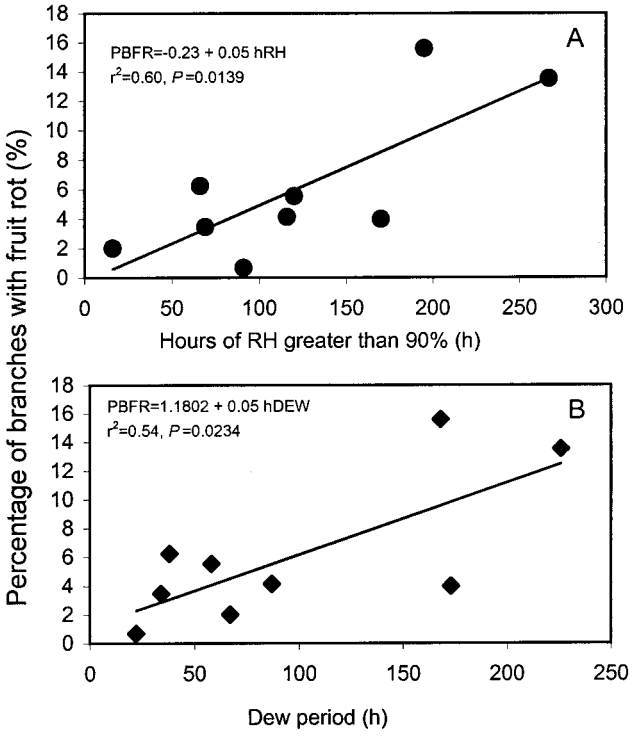

Fig. 4. Linear regressions between percentage of prune branches with fruit rot (PBFR) caused by *Monilinia fructicola* and **A,** total number of hours of relative humidity greater than 90% (hRH), and **B,** total number of hours of dew (hDEW) from 15 July to 15 August. Each point is an average of PBFR from each location. The data of the Glenn-2 orchard were not included because no fruit rot was observed in this orchard.

DISCUSSION

Through experiments in multiple locations, we determined that conditions that lead latent infection to fruit rot of prune caused by *M. fructicola* included the incidence of latent infection, fruit developmental stage, inoculum concentration, and microclimatic environment. Three percentages of PBFR were generally assigned as low, moderate, and high level of fruit rot, and the corresponding conditions that lead latent infection of fruit to these levels of PBFR could be used as thresholds to predict the different risk levels of fruit rot.

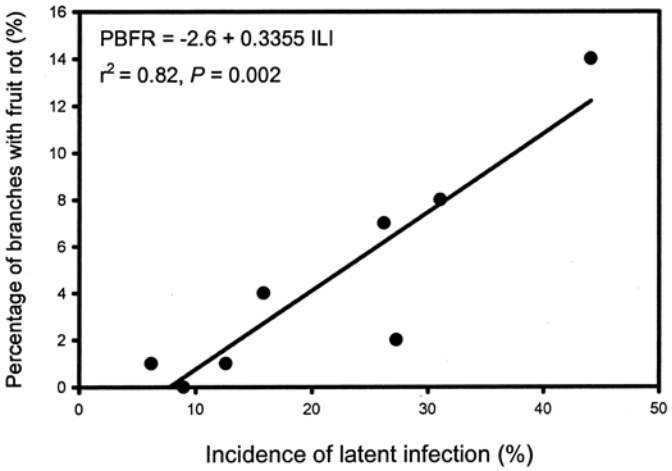

Fig. 5. Linear regression between incidence of latent infection (ILI) and percentage of branches with fruit rot (PBFR) caused by *Monilinia fructicola* on prune. Each dot represents an average value of multiple locations for each inoculation.

TABLE 2. Summary of threshold conditions that lead latent infection to fruit rot caused by *Monilinia fructicola*. The information was obtained from inoculation experiments conducted in 10 prune orchards located in nine counties of California

Threshold condition	PBFR[a] = 1.0	PBFR = 5.0	PBFR = 10.0
Latent infection (ILI)	10.7	22.7	37.6
Fruit developmental stage	Late fruit set	Embryo growth	Late embryo growth
Inoculum concentration (conidia per ml)			
Full bloom	>50,000	NA[b]	NA[b]
Fruit set	>5,000 and <20,000	>20,000	>50,000
Pit hardening	NA[b]	>5,000	>50,000
Early embryo growth	>5,000	>20,000	>50,000
Late embryo growth	≥5,000	>5,000 and <20,000	>50,000
Before first harvest	NA[b]	≥5,000	≥20,000
First harvest	≥5,000	>5,000 and <20,000	>20,000 and <50,000
Environment (hRH)[c]	24.2	104.6	204.6
Environment (hDEW)[d]	NA[b]	76.4	176.4

[a] PBFR = percentage of branches with fruit rot.
[b] Cannot be calculated from the results in Figure 8.
[c] hRH = total hours of relative humidity greater than 90%.
[d] hDEW = total hours of dew period from mid-July to mid-August.

Only when incidence of ILI is greater than 10.7% will the PBFR possibly be greater than 1%. If the ILI is less than 37.6%, the PBFR will most likely be less than 10%. The regression between ILI and PBFR (Fig. 5) by using data from different fruit developmental stages implies that this relationship can be used at any developmental stage during the growing season. Therefore, since differences in susceptibility to latent infection at different fruit developmental stages exist, a low level of latent infection may occur at a resistant stage to trigger a low level of PBFR. Similarly, a high level of latent infection may occur at a susceptible stage to induce a high level of PBFR. A similar finding on peach fruit in Georgia was from Emery et al. (5). They determined significant correlations between final incidence of latent infection (12 days before harvest) and incidence of fruit rot and between incidence of latent infection at onset of pit hardening and incidence of fruit rot in naturally infected orchards. The incidences of blossom blight and fruit rot at harvest were also significantly correlated. Ibbotson-Darhower et al. (9) also drew similar conclusions.

We determined the thresholds of fruit developmental stages that related to different levels of PBFR. However, the conclusions were based on the inoculum concentrations used in these experiments. For these inoculum concentrations, we concluded that later fruit developmental stages were related to a higher level of PBFR than earlier stages, namely, the risk of latent infection that leads to fruit rot is higher later than earlier in the season. Therefore, secondary infections that occur late in the season should be emphasized in disease management. Zehr (25) also concluded that infection of peach blossoms may not be important for fruit rot of late-maturing cultivars, although blossom infection was an important source of inoculum for preharvest fruit infection in early- and mid-season cultivars. That may be because conidia from infected mummies in mid- and late-season add to the inoculum load and may enhance the infections of fruit. We also observed in a prune orchard (16) that improper timing of fruit thinning and orchard irrigation significantly promoted sporulation on infected thinned fruit and increased level of latent infections late in the season.

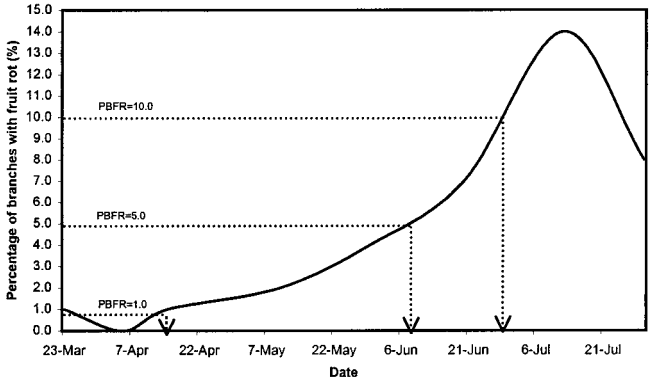

Fig. 6. Threshold fruit developmental stages related to three levels of percentage of branches with fruit rot (PBFR) caused by *Monilinia fructicola* on prune. The dotted lines indicate the corresponding threshold dates. The solid curve is the fruit rot developmental curve from Figure 2.

The same inoculum concentrations of *M. fructicola* can cause different levels of latent infection at different fruit developmental stages (14). Therefore, the risk of fruit rot can be different depending on infection level and the corresponding developmental stage. The information in Figure 7 is useful in determining what level of inoculum concentration and which fruit developmental stage can bring about a certain level of fruit rot. The quantitative relationships between inoculum concentration and level of latent infection of blossoms and fruit were determined in earlier studies (14,15,17). A combination of these two series of results provides a better understanding of disease development expressed as both latent infection and fruit rot.

The thresholds of hRH and hDEW can be useful for disease prediction, especially when the level of latent infection late in the season is known. We found that humidity in orchards from mid-July to mid-August is critical for expression of fruit rot, since the total hours of high humidity and dew period during this period significantly promoted expression of fruit rot before harvest and could be used as environmental factors to predict the possible fruit rot level. It is common knowledge that brown rot of stone fruits occurs late in the season, especially near harvest (3). The possible reasons are: (i) resistance to fruit rot development is higher earlier in the season than later in the season; and (ii) the humidity in late season is higher than in early season. Differences in concentration of sugars and/or acids might be a reason for inducing fruit rot late in the season. For example, Bostock et al. (1) found that a high level of phenolic acid on peach fruit surface could suppress cutinase production by *M. fructicola*. The concentrations of phenolic acid in immature and mature fruit might be different. This study showed that only when high humidity late in the season

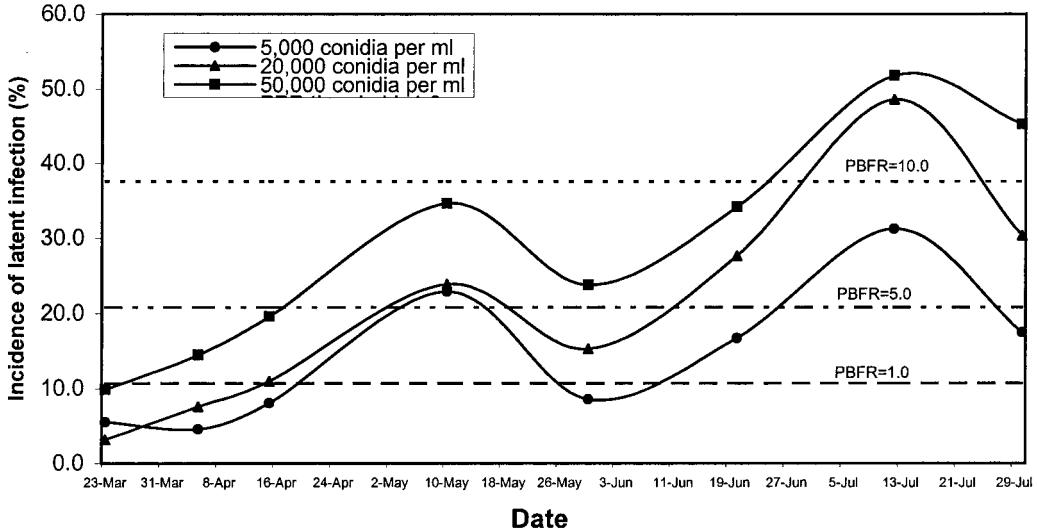

Fig. 7. Development of fruit latent infection of prune over the growing season induced by three inoculum concentrations of *Monilinia fructicola.* The three dotted lines represent the three corresponding levels of percentage of branches with fruit rot (PBFR). The thresholds of different inoculum concentrations that lead latent infection to different levels of PBFR could be determined.

was encountered that latent infections developed into fruit rot. This conclusion also implies that proper timing of irrigation in orchards late in the season could be important in disease control.

In a previous study (15), we provided information about critical environments relating to different risk levels of latent infection over the growing season. In this study, we obtained thresholds of hRH and hDEW in late season that lead to different levels of fruit rot. A combination of these two series of studies can be applied in disease prediction and to guide disease management. For example, a risk level of latent infection could be estimated based on predicted wetness duration at a certain fruit developmental stage (15), and a probability of these latent infections becoming fruit rot could be estimated by use of thresholds of hRH and hDEW late in the season provided in this study. Microclimatic conditions in orchards at different fruit developmental stages can be adjusted by manipulation of cultural practices, and by using our risk assessment approach as a reference (15), the possible risk of latent infection could be reduced.

By using the thresholds of PBFR, we established a preliminary decision support model for fungicide application to reduce risk of fruit rot. This model is now available to use on our web site containing the Decision Support System for IPM of Prune Brown Rot (DSS-PBR). Model testing is now a part of our ongoing research projects. In addition to conventional fungicide application at bloom, some growers spray fungicide on trees in mid-season to reduce latent infection and risk of fruit rot. However, the decision making for this mid-season application based on investigation of real level of latent infection in orchards is rarely used. A continuation of investigating latent infection in orchards is required when this model is used, and the simple method of the overnight freezing incubation technique (18,19) to determine the level of latent infections in orchards is introduced on our web site. The investigation during the season could be at 2 to 3 week intervals until 10 July. When latent infection level is less than the threshold of fruit rot at early fruit developmental stages, the model recommends "wait and continue determining latent infection" (Fig. 10). Only until latent infections are much greater than the threshold of fruit rot in the late season and a moderate to high level of PBFR is predicted, an immediate spray of fungicide is recommended without checking weather condition (Fig. 10).

When the level of latent infection is greater than the threshold of fruit rot in the mid-season, recommendation 3 is provided (Fig. 10), and the necessity of fungicide application is dependent upon weather or microclimatic conditions in the orchards from mid-July to mid-August. This model provides information on historical weather conditions relating to possible risk levels of fruit rot for 32 stone fruit-grown locations in California. When the level of latent infection in an orchard is greater than the threshold of fruit rot at the susceptible fruit developmental stages, the occurrence of severe fruit rot depends on microclimatic conditions late in the season in the orchard. The probability of occurrence of disease-

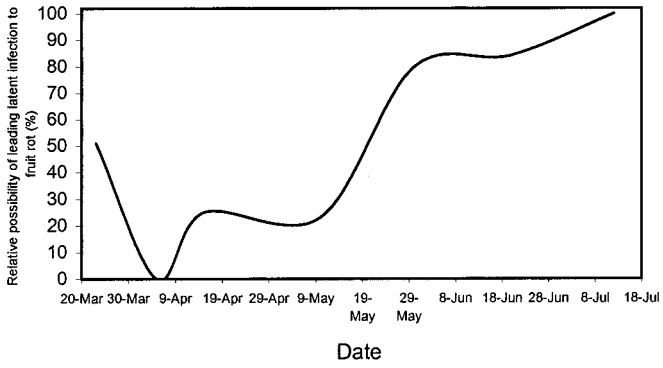

Fig. 9. Changes in the relative probability (r_PBFR) of leading latent infection to fruit rot caused by *Monilinia fructicola* on prune over the growing season. The values were calculated with the incidence of latent infection (ILI) and percentage of branches with fruit rot (PBFR) obtained from experimental results in 10 prune orchards.

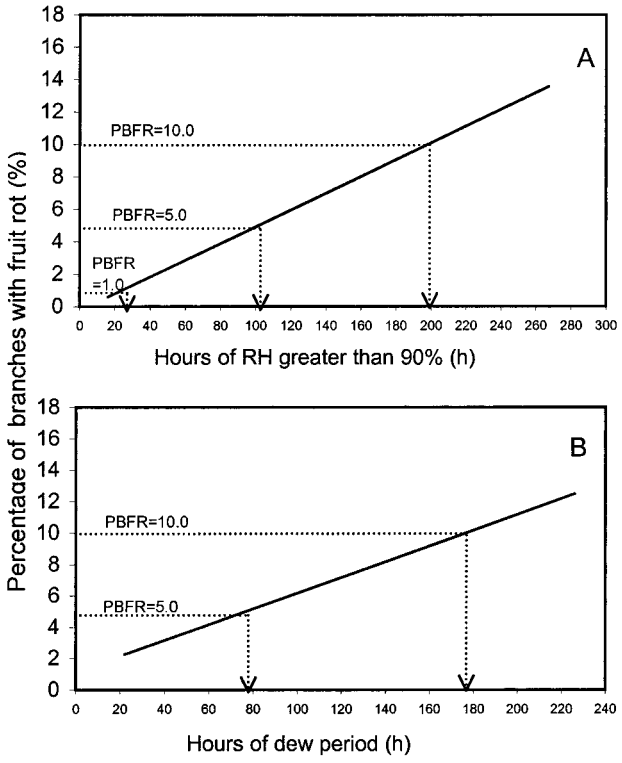

Fig. 8. Thresholds of **A,** total hours of relative humidity greater than 90% (hRH) and **B,** total hours of dew (hDEW) from 15 July to 15 August that lead latent infection to different levels of percentage of branches with fruit rot (PBFR) caused by *Monilinia fructicola*, as indicated by the dotted lines. The linear lines are obtained from Figure 4.

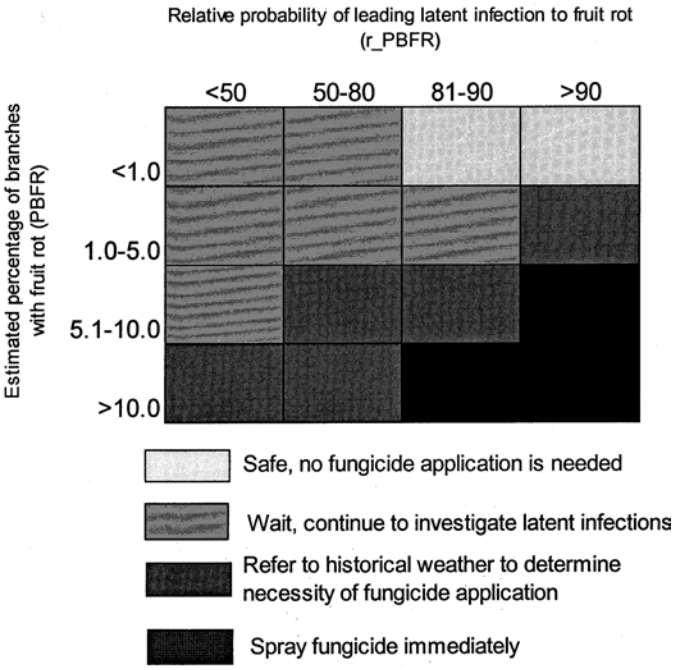

Fig. 10. Diagram of decision process used in the decision support model for fungicide application to reduce risk of prune fruit rot caused by *Monilinia fructicola*. Each of the four recommendations for disease management is given based on estimated percentage of branches with fruit rot (PBFR) and relative probability of leading latent infection to fruit rot (r_PBFR).

TABLE 3. Percentages of total hours of each relative humidity greater than 90% (hRH) and dew period (hDEW) from 15 July to 15 August relating to different risk levels of fruit rot caused by *Monilinia fructicola* for each location in different counties in California

			hRH				hDEW		
Station name	Nearest city	County	Low	Moderate	High	Very high	Moderate	High	Very high
Arvin-Edison	Arvin	Kern	100[a]	…	…	…	100	…	…
Blackwells Corner	Blackwells Corner	Kern	71.4	28.6	…	…	100	…	…
Brentwood	Brentwood	Contra Costa	12.5	68.8	18.7	…	100	…	…
Browns Valley	Marysville	Yuba	100	…	…	…	100	…	…
Camino	Placerville	El Dorado	94.4	5.6	…	…	83.3	…	16.7
Colusa	Colusa	Colusa	29.4	58.8	11.8	…	94.1	…	5.9
Davis	Davis	Yolo	33.3	61.1	5.6	…	94.4	5.6	…
Dixon	Dixon	Solano	…	85.7	14.3	…	100	…	…
Durham	Chico	Butte	38.9	50	11.1	…	94.4	…	5.6
Firebaugh/Telles	Firebaugh	Fresno	89.5	10.5	…	…	52.6	15.8	31.6
FivePoints/WSFS USDA	Five Points	Fresno	94.74	5.26	…	…	89.47	89.47	…
Fresno State	Fresno	Fresno	100	…	…	…	100	…	…
Gerber	Gerber	Tehama	62.5	37.5	…	…	87.5	6.3	6.2
Gerber Dryland	Gerber	Tehama	100	…	…	…	60	40	…
Hastings Tract	Dixon	Solano	16.7	83.4	…	…	100	…	…
Kesterson	Gustine	Merced	100	…	…	…	90.9	9.1	…
Kettleman	Kettleman City	Kings	94.7	5.3	…	…	89.5	…	10.5
Lindcove	Lindcove	Tulare	91.7	8.3	…	…	100	…	…
Lodi	Lodi	San Joaquin	18.8	37.5	37.5	6.3	81.2	6.3	12.5
Los Banos	Los Banos	Merced	50	41.7	8.3	…	100	…	…
Manteca	Manteca	San Joaquin	14.3	31.4	14.3	…	92.9	7.1	…
Modesto	Modesto	Stanislaus	…	30.8	61.5	7.7	84.6	15.3	…
Nicolaus	Nicolaus	Sutter	100	…	…	…	100	…	…
Orland	Orland	Glenn	8.4	58.3	25	8.3	91.7	8.3	…
Panoche	Firebaugh	Fresno	33.3	66.7	…	…	100	…	…
Parlier	Parlier	Fresno	35.3	35.3	29.4	…	100	…	…
San Luis Obispo	San Luis Obispo	San Luis Obispo	46.7	…	33.3	20	93.3	6.7	…
Shafter/USDA	Shafter	Kern	66.2	11.9	21.9	…	82.5	17.5	…
Stratford	Stratford	Kings	94.7	5.3	…	…	100	…	…
Visalia/ICI Americas	Visalia	Tulare	27.8	44.4	27.8	…	94.4	5.6	…
Walnut Creek	Walnut Creek	Contra Costa	21.4	7.1	42.9	28.6	100	…	…
Zamora	Woodland	Yolo	21.1	36.8	36.8	5.3	78.9	15.8	5.3

[a] Historical weather data from 1983 to 2001 were collected from the California Irrigation Management Information System (CIMIS). Results of field experiments were used to calculate thresholds of hRH and hDEW relating to different risk levels of fruit rot. The information in this table was used as a reference of historical weather conditions in a specific location for decision making on a fungicide application to reduce the risk of prune fruit rot.

favorable historical weather conditions in a specific location could be a reference to estimate possible future weather relating to disease development. To make a decision on fungicide application in such cases depends on the grower's attitude toward risks. If microclimatic conditions in late season are known for an orchard based on orchard-surrounding environments and ongoing cultural practices, the grower can easily decide if a fungicide application is needed. For example, if the orchard is located in a relatively dry area but irrigation in the late season is frequently applied, high humidity in the orchard in late season will be steady, and the grower may need to decide on a fungicide application in mid-season to reduce latent infection and risk of fruit rot in the late season.

The reduction in latent infection is not only critical in the growing season, but also important for the postharvest period. Even though fruit rot may not be severe at harvest, a high incidence of latent infection of fruit could be a high potential of postharvest fruit rot. This fact especially applies to the stone fruits that are stored and marketed fresh. Moreover, in the last several years, fresh market of prunes has increased (6), and brown rot in postharvest storage may become a concern to growers and packinghouse owners. The results of this study are useful not only for prune, but also for other stone fruits grown in California.

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