Prediction of Brown Rot of Dried Plum: Spore Inoculum Potential and Threshold Conditions Leading Latent Infection to Fruit Rot Caused by *Monilinia fructicola*

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ABSTRACT

We performed two studies in 2001 towards the general goal to predict brown rot of prune. In the first study, we have conducted two major experiments to investigate the dynamics of inoculum potential of *Monilinia fructicola* in relation to cultural practices in prune orchards and to determine the threshold conditions that trigger latent infection of *M. fructicola* to become fruit rot. In the first study, spore density (ascospores and conidia) of *M. fructicola* in the air was investigated daily from mid March to mid August using spore traps in two prune (*Prunus domestica)* orchards located in Glenn (Orchard 1) and Tehama (Orchard 2) Counties. In Orchard 1, infections of blossoms and fruit were also determined at different blossom and fruit developmental stages. Effects of fruit thinning and irrigation on sporulation of thinned fruit were studied. Fungicide treatment on sporulating thinned fruit on the orchard floor was also conducted. The spore densities in the air were at a low level in early bloom, increased to a high level at full bloom, and decreased to a lowest level at the end of bloom period. The spore densities ranged from 0 to 32 and from 0 to 25 spores per liter per minute during this period. The minimal spore density was observed at the beginning of May. The timing of fruit thinning and irrigation in Orchard 1 significantly promoted sporulation on thinned fruit, as well as increased fruit infection late in the season. This orchard had high inoculum potential and blossom infections, and an irrigation immediately after fruit thinning created favorable conditions for sporulation on thinned fruit. Spore density in the air increased during a 7 – 10 day period after each irrigation, and reached a maximal about 60 spores per liter per minute. Spore density decreased along with the drying process of thinned fruit, and increased again following a next irrigation. Fruit infection was significantly increased due to cultural practices in this orchard. Prolonging the drying process of thinned fruit significantly decreased risk of fruit infection, shortened the sporulation period of the pathogen on thinned fruit, and obviously became an important criterion for disease control. Spraying thinned fruit on the orchard floor with iprodione at recommended dosage significantly reduced latent infections of fruit on trees. The significance of sources of secondary infection of fruit from conidia of *M. fructicola* produced on thinned fruit, especially in the orchards which had high inoculum potentials, was confirmed.

In the second study, inoculations were performed in ten prune orchards located in nine counties of California. Inoculations were conducted eight times from the full bloom to the first harvest stages in most of these orchards. In each inoculation, branches bearing 40 – 50 blossoms or 30 – 40 fruit were inoculated with conidia suspension of *Monilinia fructicola* by hand spraying. Three inoculum concentrations, 5,000, 20,000, and 50,000 conidia/ml were applied. Each inoculated branch was covered with a plastic bag to keep high humidity for about 14 - 16 hours. All inoculated fruit were maintained on trees, and were harvested separately 2 weeks before commercial harvest. The overnight freezing incubation technique (ONFIT) was used to determine the incidence of latent infection (ILI). Branches with fruit rot were recorded at harvest, and the

percentage of branches with fruit rot (PBFR) was determined. The average ILI and PBFR were significantly changed at different fruit developmental stages over the season, and a linear correlation between ILI and PBFR was obtained. Four conditions leading latent infection to fruit rot, including latent infection level, fruit development stage, inoculum concentration, and total hours of relative humidity (RH) greater than 90% (hRH) and dew period (hDEW) from mid-July to mid-August, were determined. Three levels of PBFR, 1, 5, and 10%, were assigned, and threshold conditions leading to these levels of PBFR were determined based on the experimental results. Generally, infections late in the season were related to greater PBFR than those occurred early in the season. The inoculum concentration of 5,000 conidia/ml did not cause PBFR greater than 10% at any stage, while the concentration of 50,000 conidia/ml caused PBFR greater than 1% at all stages over the growing season. The thresholds of hRH leading to PBFR of 1, 5, and 10% were 24, 104 and 204 hours, respectively, and the threshold of hDEW leading to PBFR of 5 and 10% were 76 and 176 hours, respectively.

INTRODUCTION

Monilinia fructicola is the causal pathogen of brown rot of stone fruit. The disease mainly expresses as two phases, blossom blight and fruit rot. Under favorable conditions, ascospores or conidia produced from mummies infected by *M. fructicola* on the orchard floor or on the trees could serve as inoculum sources that cause blossom blight in the spring. These primary infections could be latent until favorable conditions are encountered and become fruit rot (Cruickshank & Wade, 1992; Wade & Cruickshank, 1992). After bloom, infections of fruit could continue as secondary infections which are affected by microclimatic conditions in the orchards. The main inoculum sources for secondary infection in California nectarine orchards were determined to be the thinned fruit with sporulation (Hong *et al.*, 1997). Therefore, infection of blossoms in the spring and fruit during the growing season could relate to the severity of fruit rot late in the season.

Inoculum potential in orchards has been identified as an important factor, affecting both blossom blight (Luo *et al.*, 2001b) and latent infection of fruit (Luo & Michailides, 2001a). Thus, estimation of inoculum potential can be critical in disease control. Since ascospores and conidia of *M. fructicola* are airborne, inoculum potential of the pathogen could be estimated as spore density in the air over the season. Additionally, infection levels of blossoms could be useful in estimating the initial infection level early in the season and possible fruit rot severity depending on microclimatic conditions in orchards late in the season. Although infection of fruit could be most likely latent (without expressing symptoms) under unfavorable conditions, an overnight freezing incubation technique (Michailides *et al.*, 1997; Michailides *et al.*, 2000) has been used to effectively determine the levels of fruit latent infection from early to late season (Luo & Michailides, 2001a), as well as helps find possible relationships between latent infection and environments (Luo & Michailides, 2001b).

In addition to inoculum potential, development of brown rot of stone fruit also depends on blossom and fruit susceptibility to infection (Luo & Michailides, 2001a; Luo *et al.*, 2001b). The risk of infection level could be high when a high inoculum potential and the most susceptible blossom or fruit developmental stages coincide. Although the changes in blossom and fruit

susceptibility to infection over the season are basically clear (Luo & Michailides, 2001a; Luo *et al.*, 2001b), dynamics of inoculum potentials in orchards with different practices still need further investigation. Obviously, this information could be important to determine the possible risk of infection at different stages and to design effective disease control strategies. We need to answer questions on how spore density fluctuates over the season in different orchards, and how these changes are affected by orchard cultural practices, including fruit thinning, irrigation, and fungicide application.

Apparently, not all latent infections could induce fruit rot before harvest (Cruickshank and Wade, 1992; Northover and Cerkauskas, 1994). In most cases of different stone fruits, infections could be continuously latent even after harvest, which are the sources for the postharvest brown rot during the period of the fruit cold storage (Byrde and Willetts, 1977). Therefore, estimation of latent infection during the growing season is meaningful for both pre- and post-harvest disease management, and reducing latent infection can become important in disease management.

Differing from other stone fruit, prunes are usually dehydrated immediately after harvest, and there is generally no postharvest brown rot problem during storage for the majority of prune production in California. Therefore, the possibility of latent infections leading 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 could lead what level of latent infection to what level of fruit rot before harvest. This information could be obtained from experiments in orchards at different locations representing various environments.

Decision support for disease management requires knowledge of the threshold conditions that trigger latent infection to prune fruit rot expression. These conditions may include fruit developmental stage, level of latent infection, inoculum potential, and environment.

OBJECTIVES

1) Determine the dynamics of spore density in the air over the growing season,
2) Study the effects of orchard cultural practices on dynamics of inoculum potential and fruit latent infection,
3) Determine the relationships among latent infection, inoculum concentration, environment, and fruit rot, and
4) Determine the threshold conditions leading latent infection to fruit rot of prune,

PROCEDURES

Study 1. Dynamics of inoculum potential of *Monilinia fructicola* in relation to cultural practices in prune orchards

Two prune orchards, one in Glenn County (Orchard 1) and one in Tehama County (Orchard 2), California, were selected. Both orchards showed a history of severe brown rot and high inoculum potentials. A Burkard spore trap was located in each orchard. The spore traps were placed at 1.5 m height from the ground. Tapes used in the spore traps to catch spores were replaced weekly. The whole area of each tape was examined with a microscope at 250 magnification, and the total number of spores was counted daily. The daily spore density in the air was determined from

March 6 to August 15 in Orchard 1, however, only from March 5 to April 20 in Orchard 2.

In addition to the size and shape that were used to identify the conidia and ascospores of *M. fructicola* from spore trap tape samples, identification was confirmed by culturing spores from the tapes on acidified potato dextrose agar. The spore density was calculated as spores per liter per minute (Tate & Wood, 2000) in the air, and the curves of spore density versus dates were obtained.

Infection of blossoms was determined at bloom. Blossoms samples were taken on 6 and 26 March in Orchard 1 and 22 March in the Orchard 2. For each sampling, 30 branches were randomly selected, and all blossoms of each branch were detached. The flowers were soaked into 300 ml of 10% commercial bleach for 5 min, and washed with sterile distilled water 5 times. The blossoms of each sample were separately placed on top of two layers of sterile wet paper towels in a sterile plastic container. The containers were incubated at room temperature (23 ± 2° C) for 7 days, the blossoms that showed sporulation of *M. fructicola* on stems were counted, and the incidence of blossom infection as percentage of flowers with sporulation was calculated for each branch.

Latent infection was also determined after bloom in Orchard 1. At each sampling time, twenty branches were randomly selected and all fruit of each branch were collected separately. Fruit samples were taken on May 11 and 29, June 20, and July 12, and July 29, at pit hardening, late pit hardening, early embryo growth, late embryo growth, and before first harvest (Polito, 1981), respectively. In each sampling, 15 to 20 branches were randomly selected, and all fruit of each branch were collected and processed to determine the incidence of latent infection. The incidence as percentage of brown rot of fruit was calculated for each sample.

In Orchard 1, the grower thinned fruit from trees on June 1 and 2 at the rate of 800 to 1,500 fruit per tree. Irrigation was conducted by using sprinklers on a movable pipe system. Irrigations begun on June 4 and performed every 2 weeks with a general schedule of one week irrigation followed by one week drying. The last irrigation was applied in mid-August.

The thinned fruit with sporulation was observed several times in this orchard. On June 25, a plot with 25 trees (5 trees for each of 5 rows) was marked. Percentage of thinned fruit with sporulation was recorded on each of west and east site of each tree. This recording was continued in the same plot on July 11 and 30. Five more trees at outside of this plot were randomly selected, and all fruit from each of west- and east-site were collected from each tree separately on July 11. To determine speed of drying, individual thinned fruit was weighed in g, and average fruit weight and standard deviation for each site of each tree were calculated as g/ tree. On August 15, the fruit from three branches of each tree in this plot were randomly selected and harvested separately. The ONFIT was used to determine the average incidence of latent infection of fruit for each tree.

An additional plot containing 100 trees (10 trees for each of 10 rows) was marked on 11 July. Four situations of thinned fruit were investigated in this study: 1) thinned fruit on west site of the tree were completely dry while those on east side still had at least 20% water content; 2) there was no difference in water content of thinned fruit between the two sites of the tree; 3) thinned fruit on east site of the tree were completely dry while those on west side still had at least 20% water content (opposite to the situation 1); and 4) trees were without any thinned fruit under their canopy. The percentage of thinned fruit in each category was calculated.

On July 11, three 25-tree plots (5 trees for each of 5 rows) were randomly selected in Orchard

1. The fungicide iprodione (Rovral®) was used to spray the thinned fruit at a rate of 2 liters per tree (0.05%) with a back pack gas sprayer. On July 30, any sporulation that developed on the thinned fruit was recorded. On 15 August, three branches of each tree of each of the three plots were randomly selected and fruit in each branch were separately harvested. The fruit were processed with the ONFIT described above to determine the average incidence of latent infection for each tree.

Daily spore density in average number of spores per liter per minute versus date was used to obtain the curves of dynamics of spore density over the season. Average incidence of latent infection on different blossom and fruit developmental stages from Orchard 1 were used to obtain the infection curve over the season. The average incidences of latent infection at different developmental stages were also compared among each other with LSD. Incidences of thinned fruit bearing sporulation per tree of the 25-tree plot at different observation times were used to obtain the plot maps to determine how sporulation developed on thinned fruit. Comparisons in average incidence of latent infection between plots with the fungicide-treatment and the plot without a fungicide treatment were conduced with LSD.

Study 2. Threshold conditions leading latent infection of *M. fructicola* to fruit rot expression.

An isolate of *M. fructicola* collected from a prune orchard and stored at the Kearney Agricultural Center was used in this study. Spore inoculum was prepared by flooding with 3 milliliters of sterile distilled water easch Petri dish with sporulating colonies of *M. fructicola* and adjusting to different concentrations microscopically.

Ten prune orchards were selected in nine counties located in Central and Sacramento Valleys of California, including Tulare, Fresno, Madera, Yolo, Sutter, Colusa, Butte, Glenn, and Tehama. The orchards showed different microclimatic conditions. In most 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 (Table 1). In each inoculation, three concentrations of *M. fructicola*, 5,000, 20,000 and 50,000 conidia per ml, were used. Bloom inoculations were conducted from March 19 to 23 at full bloom stage. Branches bearing 40 to 60 blossoms were selected. Each branch was covered with a plastic bag and sprayed uniformly inside the bag with 50 ml conidia suspension for each inoculum concentration by using a hand-held sprayer. Immediately after inoculation, the branch was closed tightly in the plastic bag to create high humidity. For each experiment, inoculations were completed at about 17:00 to 19:00 PST. A 14-hour wetness duration was accomplished by uncovering the plastic bags in 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 non-inoculated tree each were sprayed with 50 ml water and maintained wet for 16-hour wetness duration to serve as the non-inoculated control for each inoculation experiment in each location.

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

Location inoc.date	Bloom and fruit developmental stage	Fruit size (mm) Length/width[c]	Ave. fruit weight (g)[c]
Tulare[d]			
19-Mar	Full bloom		
2-Apr	Fruit set		
18-Apr	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-Jun	Embryo growth	33.7/24.2	10.3
9-Jul	Before first harvest	...	...
Fresno			
19-Mar	Full bloom		
2-Apr	Fruit set		
18-Apr	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-Jun	Late embryo growth	31.8/22.2	8.5
16-Jul	Before first harvest	...	...
27-Jul	First harvest	...	...
Madera			
20-Mar	Full bloom		
3-Apr	Fruit set		
17-Apr	Early pit hardening	17.3/9.1	0.94
15-May	Late pit hardening	29.7/19.6	6
6-Jun	Embryo growth	34/20.5	9.2
6-Jul	Before first harvest	40.5/30.0	18.3
26-Jul	First harvest	40.9/32.7	22.6
Yolo[d]			
21-Mar	Full bloom		
4-Apr	Fruit set		
14-Apr	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-Jul	Before first harvest	...	...
28-Jul	First harvest	...	...
Colusa			
21-Mar	Full bloom		
4-Apr	Fruit set		
14-Apr	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-Jun	Late embryo growth	32.7/22.9	9.2
10-Jul	Before first harvest	38.8/29.9	16.7
28-Jul	First harvest	38.3/29.6	17.7
Sutter			
23-Mar	Full bloom		
5-Apr	Fruit set		
15-Apr	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-Jun	Late embryo growth	35.2/23.0	12.7
12-Jul	Before first harvest	42.3/33.1	26.4
30-Jul	First harvest	42.9/33.9	26.8
Butte			
23-Mar	Full bloom		
5-Apr	Fruit set		
15-Apr	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-Jun	Late embryo growth	37.1/26.4	14.2
12-Jul	Before first harvest	44.0/34.3	25.7
30-Jul	First harvest	44.0/34.6	29.2
Glenn-1			
22-Mar	Full bloom		
5-Apr	Fruit set		
15-Apr	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-Jun	Late embryo growth	32.4/21.9	8.4
11-Jul	Before first harvest	38.1/29.2	17
29-Jul	First harvest	27.7/29.1	16.9
Glenn-2[d]			
22-Mar	Full bloom		
5-Apr	Fruit set		
15-Apr	Early pit hardening	16.1/8.7	0.9
20-Jun	Late embryo growth	32.1/22.2	8.4
11-Jul	Before first harvest	35.9/27.4	14.1
29-Jul	First harvest	37.6/29.9	18.5
Tehama[d]			
20-Mar	Full bloom		
3-Apr	Fruit set		
17-Apr	Early pit hardening	25.2/15.0	2.9
15-May	Late pit hardening	...	...
6-Jun	Embryo growth	33.3/23.9	10.3
6-Jul	Before first harvest	42.7/32.8	23.2
26-Jul	First harvest	...	...

[a] Six branches of prune trees were inoculated with *M. fructicola*, and artificial dew was generated to obtain 16-hour wetness durations on inoculated branches and 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] Less than eight times of inoculation were conducted in some orchards due to adverse field conditions.

Inoculations continued at fruit set and further fruit developmental stages (Table 1) using the same inoculation methods described above. In each inoculation, six repeated branches each bearing 30 to 40 fruit for each tree were used for each inoculum concentration and maintained wet for 16 hours described above. A data logger (ONSET Company, Pocasset, MA) was used in each orchard to record hourly temperature, humidity, and dew temperature during the experiments.

Fruit on all inoculated trees were maintained until harvest. Depending on the location of the orchard, the collection of these fruit was 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 of each replicated branch were placed in a paper bag and stored at 4°C until processed with the ONFIT for detection of latent infection by *M. fructicola*. Fruit covered with sporulation of *M. fructicola* was recorded, and incidence as percentage of brown rot of fruit was calculated for each sample.

A split-plot design was applied in this study. Location was treated as replication, inoculation date was the main-plot treatment, and inoculum concentration was the subplot treatment. Incidence of latent infection (ILI) for each inoculated branch was used in analysis. Additionally, percentage of branches with fruit rot (PBFR) was also used for the treatments of inoculation date, 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 of SAS to determine the significance of variances of both ILI and PBFR from replication, main-plot, and sub-plot treatments. The corresponding errors applied in the split-plot design were used in comparison of the means of ILI and PBFR among inoculation dates by using 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: total number of hours when RH $\geq$ 90% (hRH) and total number of hours of dew period (hDEW) from 15 July to 15 August. The values of these two variables were calculated from data obtain from data logger for each orchard. Linear regressions between PBFR and hRH and between PBFR and hDEW were conducted by using the REG procedure of SAS.

Four threshold conditions leading latent infection to fruit rot expression before harvest were considered: threshold of latent infection levels, threshold of fruit developmental stages, threshold of inoculum concentrations, and threshold of environments. Three levels of PBFR were arbitrarily assigned: low level when PBFR equal or less than 1, moderate level when PBFR was greater than 1, and equal or less than 5, and high level when PBFR greater than 5, and equal or less than 10. The average values of ILI and PBFR from all ten locations for each inoculation date were used in a linear regression between incidence of latent infection (ILI) and PBFR ($ILI = \alpha + \beta \times PBFR$). 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 ten locations over the growing season, the corresponding dates were determined for the threshold growth stages when PBFR values were 1, 5 and 10, respectively. The threshold of inoculum concentrations for each of three PBFR levels were determined from the dynamic curves of ILI over the growing season for three inoculum concentrations by using the threshold of latent infection. The environmental thresholds were assigned as thresholds of hRH and hDEW, and the threshold values for the three PBFR levels were obtained from the regressions between PBFR and hRH and between PBFR and hDEW, when PBFR = 1, 5, and 10, respectively.

RESULTS

Study 1. Dynamics of inoculum potential of *M. fructicola* in relation to cultural practices in prune orchards

Similar trends of dynamics of spore density in the air were observed in the two orchards in the spring (Fig. 1). In early March, about 5 spores (both ascospores and conidia) per liter air per min were recorded in each orchard. Populations of the pathogen's spores increased gradually along with time, and the peaks of the curves were from about late March to beginning of April, which coincided with full bloom (Polito, 1981). Spores of the pathogen populations decreased after that period until May. The spore densities in the air were very low at the end of May. In Orchard 1, thinning of fruit was performed on 1 to 2 June and irrigation started on June 4. After 5 to 7 days, spore density in the air started to increase, and the first peak of spore density appeared on June 10 to 12 after first irrigation. The spore density decreased dramatically to a very low level on June 20 to 26. However, the second irrigation started on June 22, and the spore density in the air increased again 8 days after this irrigation. The second peak of the spore population curve appeared on June 30 to July 5, and after that date, the pathogen populations generally fluctuated and decreased gradually with two small peaks down to a very low level on about July 18. The third irrigation started on July 14, and the pathogen populations started to increase 7 days after this irrigation with the third peak on about July 26 to 28.

The average incidence of blossom infection at the popcorn stage was about 3.16% (Fig. 2). The infections increased to 11.8% at the full bloom stage. The average incidence of latent infection of fruit was 5.5% at the pit hardening stage and slightly increased to about 8% at the late pit hardening and the early embryo growth stages. The incidence of latent infection increased to 14% at the late embryo growth stage and 38.1% at the before first harvest stage. Statistics demonstrated that there was no significant difference in average incidence of latent infection between bloom stages (March 6 and 26) and fruit developmental stages from pit hardening to early embryo growth (from May 11 May to 20).

In the 25-tree plot in Orchard 1, fifteen trees had underneath thinned fruit with sporulation on June 25, but the incidence was less than 5% (Fig. 3). However, the incidence of thinned fruit with sporulation dramatically increased after about 2 weeks. The data on July 11 showed that the incidence of thinned fruit with sporulation ranged from 0 to 90%. Of 20 trees, 11 trees showed greater incidence of thinned fruit with sporulation in the east site than in the west site, and in 6 trees, the incidence of thinned fruit with sporulation in the west site of the trees was greater than those in the east site (Fig. 3). On July 30, the incidence of thinned fruit with sporulation decreased to less than 5%. However, there were still 5 trees with greater incidence of thinned fruit with sporulation in the east site than in the west site. From the 100-tree plot on 11 July, there were 56 trees that had underneath thinned fruit with greater water content in the east site than in the west site (Fig. 4). The opposite situation was observed only on 9 trees, and there were 23 trees that had underneath thinned fruit with same water content in the west and east site of the trees.

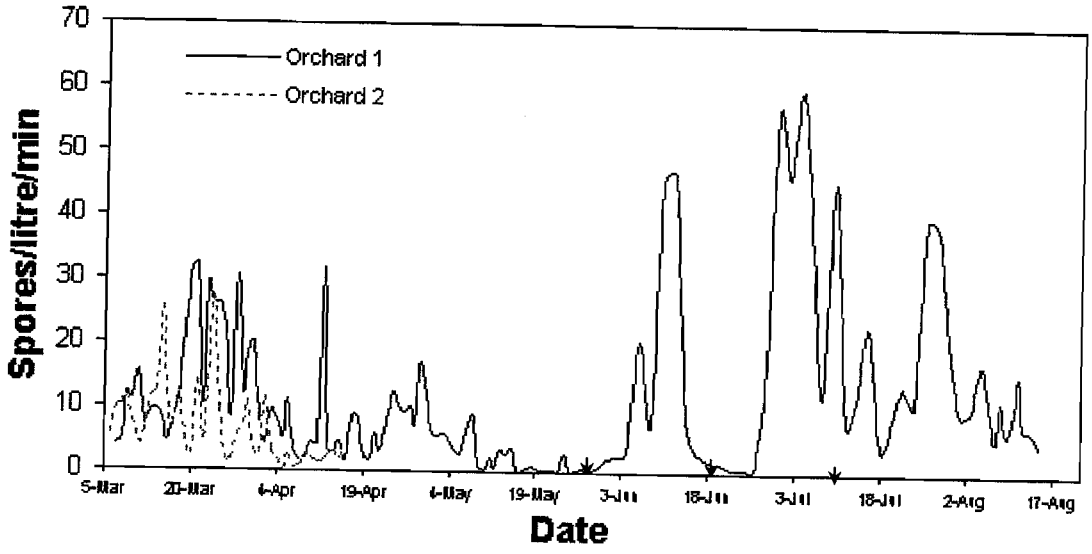

Fig. 1. Dynamics of daily spore density of *Monilinia fructicola* in the air determined from samples using Burkard spore trap over the growing season in two prune orchards located in Glenn County (Orchard 1) and Tehama County (Orchard 2). The arrows indicate the irrigation dates in Orchard 1. Fruit thinning was performed in this orchard on June 1 and 2 followed by the first irrigation on June 4, 2001.

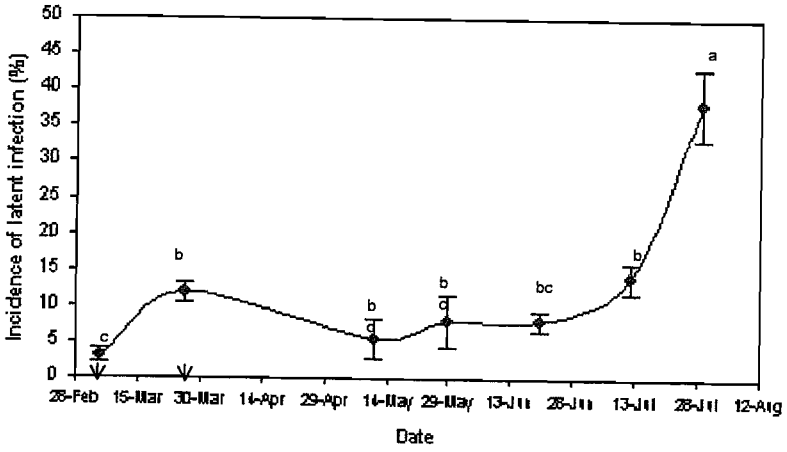

Fig. 2. Incidence of latent infection by *Monilinia fructicola* at different bloom and fruit developmental stages. The data are from prune Orchard 1, where blossoms and fruit from multiple branches were sampled at each sampling time and processed with the overnight freezing incubation technique to determine the incidence of blossoms and fruit with latent infection. The LSD was used to compare the incidence of latent infection between any two stages. The arrows indicate the two bloom stages.

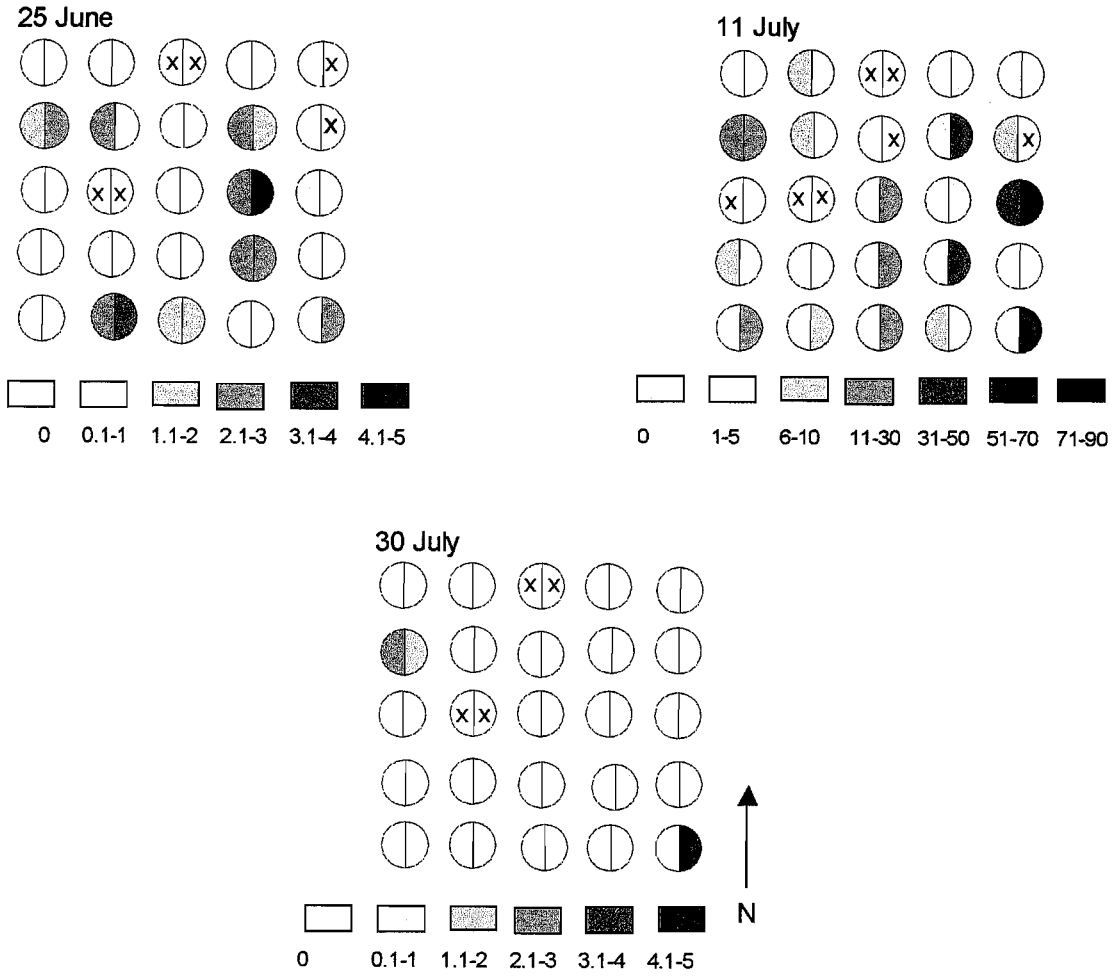

Fig. 3. Distributions of incidence of thinned fruit showing sporulation of *Monilinia fructicola* in the 25-tree plot for different sampling times in Orchard 1. The incidence (%) was classified into different levels at different sampling times, and was recorded at the west and east sites of each

Analysis of weight of thinned fruit from east and west sites of 5 trees sampled on July 11 demonstrated that there were significant differences in fruit weight between trees and between orchard floor site of trees. The interaction between trees and sites was not significant. The average fruit weight on the east site of trees was significantly greater (2.85 ± 0.95g, n=128) than those on the west site of trees (1.76 ± 0.65g, n=123).

The average incidence of latent infection of fruit in each of the three fungicide-treatment plots was significantly less than that of the non-fungicide-treatment plot (Fig. 5). The average incidence of latent infection from the 25 trees in the untreated plot was 25.8%, while the incidence of latent infection in the fungicide-treated plots ranged from 4.6 to 6.4 %. No differences in average latent infection among the fungicide-treated plots were found.

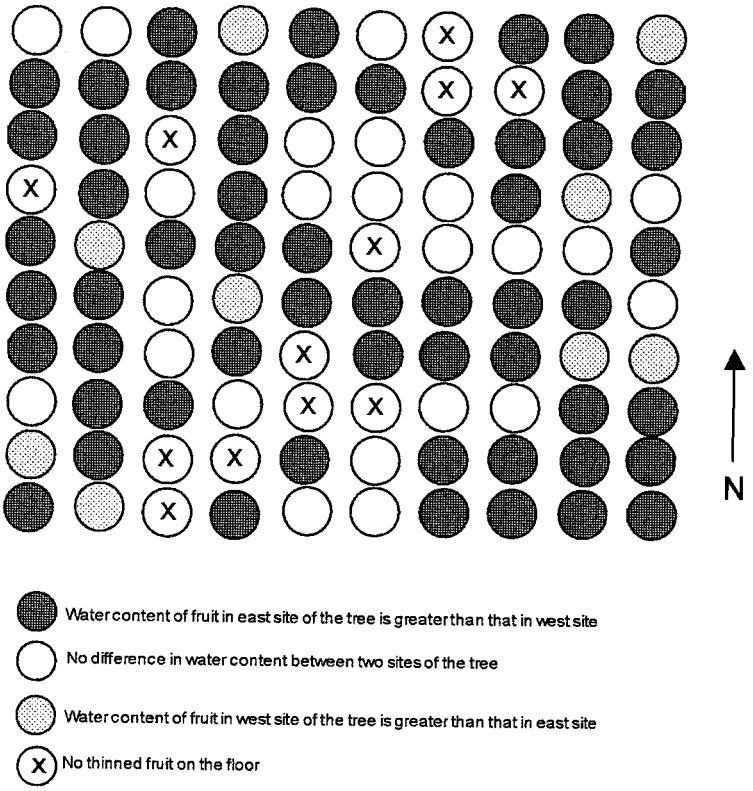

Fig. 4. Distribution of four different dry situations of thinned fruit in a 100-tree plot in prune Orchard 1. The data were from investigations on July 11, 2001.

Study 2. Threshold conditions leading latent infection of *M. fructicola* to fruit rot expression.

The ANOVA demonstrated that both inoculation date and inoculum concentration were all significant at $P < 0.0001$ for both ILI and PBFR. 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. 6). The curve of ILI showed that the average ILI at the full bloom stage (around March 23) was less than 10% and reached about 10% at the fruit set stage (around April 5). However, no significant difference between these two stages was found. The average ILI increased to about 27.2% at the pit hardening stage (around May 10), which was significantly higher than those at the three precedent stages (Fig. 6). After this stage, the

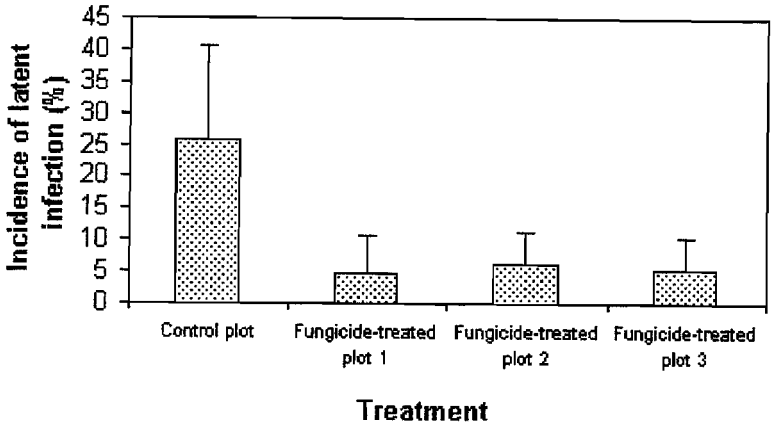

Fig. 5. Comparisons in average incidence of latent infection by *Monilinia fructicola* between the fungicide-treatment plots and the non-fungicide plot. Each bar represents the average incidence from a 25-tree plot with 3 replicated branches for each tree.

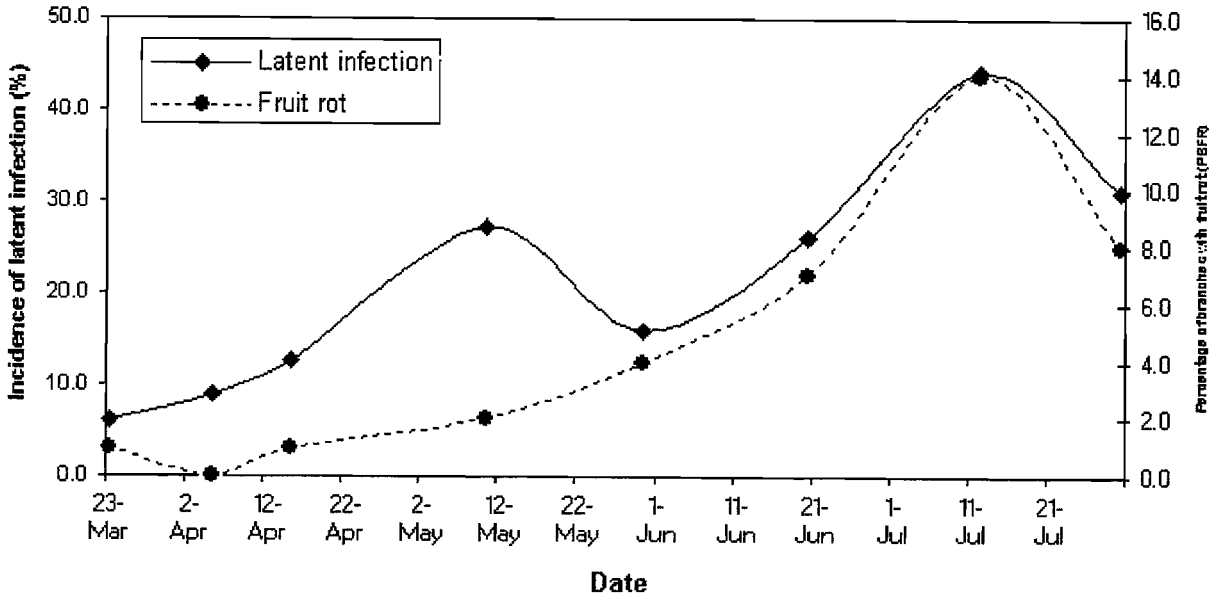

Fig. 6. Incidence of latent infection (ILI) of prune and percent branches with fruit rot (PBFR) induced by inoculation with *Monilinia fructicola* at different dates over the growing season of prune. The inoculations were conducted in 10 orchards in California. Each point in each line represents an average value from multi-location and three inoculum concentrations. Each point of dotted line represents the PBFR induced by the infections occurred in the corresponding date.

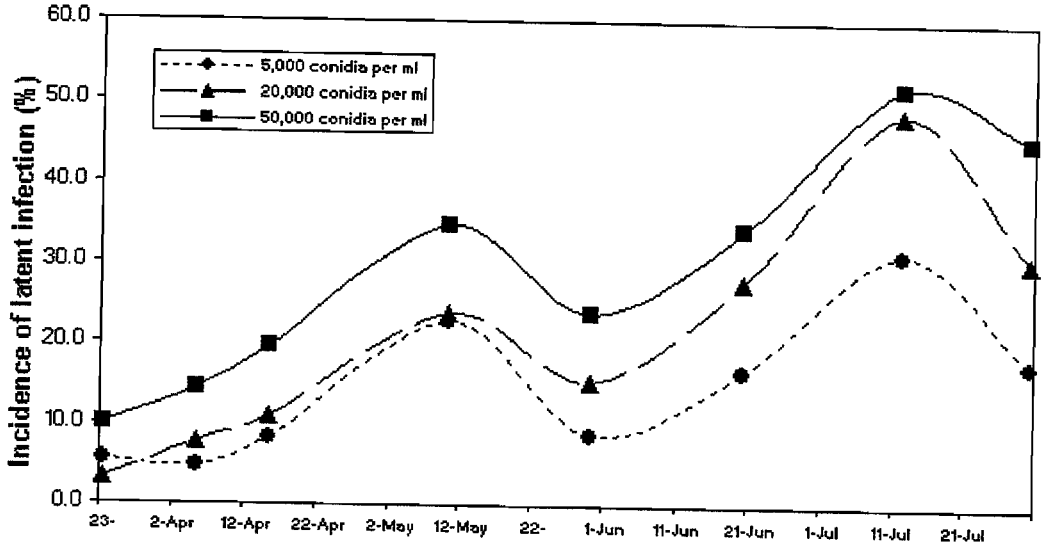

Fig. 7. Incidence of latent infection (ILI) of prune fruit caused by *Monilinia fructicola* with three different inoculum concentrations at different dates over the growing season. Each point represents an average from multiple locations in California. Data are from inoculation experiments conducted in different prune orchards.

average ILI decreased to a significantly lower level at 15.8% at the embryo growth stage (around May 30), but increased again reaching 26.2% at the late embryo growth stage (around June 20). The maximum average ILI in the season was 44.1% at the before first harvest (around July 12), which was significantly higher than those at all other stages (Fig. 6). The average ILI decreased at the first harvest since the last time of inoculation was close to fruit harvest.

A similar trend was found for the dynamics of percentage of branches with fruit rot (PBFR) (Fig. 6). The infections occurred at early stages from the bloom to the embryo growth stages led to a low level of PBFR, and there was no significant difference in PBFR among the fruit developmental stages. After the embryo growth stage, the chance of infections leading to PBFR increased, and the PBFR reached 7% at the late embryo stage, which was significantly higher than those at the first four stages. The PBFR continuously increased, reaching a maximum of 14% at the before first harvest stage, and was significantly higher than that at the previous stage. Thus, latent infections led higher levels of PBFR at late stages than at early stages.

Three near-parallel dynamic curves of incidence of latent infection (ILI) were determined for the three inoculum concentrations (Fig. 7). The basic trends of curves of ILI were similar to those in Figure 1. The curves of ILI were visually proportionally increased along with the increase in inoculum concentration. Comparisons in ILI among inoculum concentrations for each inoculation date demonstrated that the ILIs for the inoculum concentration of 50,000 conidia/ml were significantly higher than those for 5,000 conidia/ml at all stages except at the full bloom and the pit hardening stages (Fig. 7). The ILIs for the concentration of 20,000 conidia/ml at the late

stages (after late embryo) were significantly higher than those of 5,000 conidia/ml. The ILIs for the concentration of 20,000 conidia/ml at the last stages (after late embryo) were also significantly higher than those at the early stages (before late embryo) (Fig. 7).

Two linear regressions describing the relationships between PBFR and hRH and PBFR and hDEW were obtained (Fig. 8). Along with increasing hRH and hDEW, PBFR increased at a rate of 0.5% per hour.

A linear regression between incidence of latent infection (ILI) and percentage of branches with fruit rot (PBFR) was obtained (Fig. 9) as PBFR = -2.6 + 0.3355 ILI ($r^2 = 0.82$ and $P = 0.002$) by using average data for inoculation dates. Therefore, calculated with this equation when PBFR equaled to 1, 5, and 10, respectively, the thresholds of ILI leading to the corresponding levels of PBFR were 10.7, 22.7, and 37.6% (Table 2).

The thresholds of fruit developmental stages relating to corresponding three levels of PBFR, 1, 5, and 10%, were determined directly from the PBFR dynamic curve (Fig. 10). The general trend showed that late season was related to greater PBFR than early season. Along with the season, the chance that latent infections could become fruit rot increased. Under the conditions of inoculum concentrations used in this study, the date which related to low (1%) PBFR was around April 12 at the late fruit set stage, namely, the latent infections that occurred before or at this stage could lead to a low level of PBFR. However, the latent infections that occurred around June 8, at about the late embryo growth stage, were related to a moderate level (5%) of PBFR. Therefore, the growing season during late fruit set and late embryo growth were related to the PBFR from 1 to 5%. The PBFR increased along with the season, and reached 10% at the end of June when fruit were at the late embryo growth stage. Therefore, the PBFR increased from 5 to10% during the period of the embryo growth and the late embryo growth stages, which was usually in June and July. Obviously, the risk of latent infection leading to fruit rot increased after the late embryo growth stage and reached a maximum level at the before first harvest stage (Fig. 10).

The thresholds of inoculum concentration causing three levels of PBFR were determined by using the three thresholds of ILI (10.7, 22.7, and 37.6%) (Fig. 11). The concentration of 5,000 and 20,000 conidia/ml of *M. fructicola* could not result in low level (1%) of PBFR before April 16, at about the pit hardening stage. This concentration could result a PBFR greater than 5% after June 25 and the late embryo growth stage (Fig. 11), and could not result in a PBFR greater than 10% at during the entire season. After this stage, all concentrations of *M. fructicola* could result in a PBFR greater than 1 (Fig. 11), except for the 5,000 conidia/ml concentration during the period from May 26 to June 11 (Fig. 11). However, the concentration of 20,000 conidia/ml of *M. fructicola* could result in a PBFR greater than 5% only during the pit hardening stage (May 2 to 18) and after the embryo growth stage (after 11 June), which was susceptible to latent infection (Fig. 11). This concentration could also result in a PFBR greater than 10 at about the end of June after the late embryo growth stage. The concentration of 50,000 conidia/ml of *M. fructicola* could result in a PFBR greater than 1 during the entire season. After about April 18 before the pit hardening stage, this concentration could result in a PBFR greater than 5.0, and after about June 25 (during the period from late embryo growth stage to harvest) in a PBFR greater than 10% (Fig. 11).

Thresholds of environment in terms of total hours of RH greater than 90% (hRH) and total hours of dew (hDEW) from July 15 to August 15 were determined from the regressions between

PBFR and these two variables (Fig. 12). The 24.2 hours was a threshold hRH that led latent infection to a PBFR equal to or greater than 1%. The corresponding thresholds of hRH leading

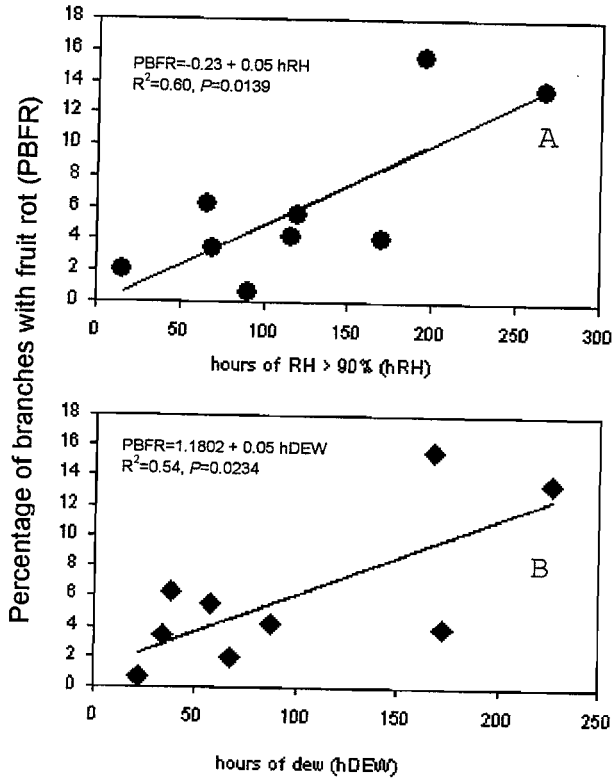

Fig. 8. Linear regressions between percent branches with fruit rot (PBFR) and A, total number of hours of RH greater than 90%, and B, total number of hours of dew from 15 July to 15 August. Each point is an average PBFR from each location. The data of the Glenn-2 orchard were not included because no fruit rot was observed in this orchard.

latent infection to 5% and 10% of PBFR were 104.6 and 204.6 hours, respectively, under the conditions of this study (Fig. 12A). Although the threshold of hDEW leading latent infection to 1% of PBFR was not available from the calculation, the thresholds of hDEW for 5% and 10% of PBFR were 76.4 and 176.4 hours, respectively (Fig. 12B).

All threshold conditions that led latent infection of fruit to the three levels of percentage of branches with fruit rot (PBFR) are summarized in Table 2.

CONCLUSIONS

1) Similar levels of spore density occurred in the two orchards in spring, and a higher density was observed at about the full bloom stage. After that period, pathogen population density decreased to a low level in April and May.

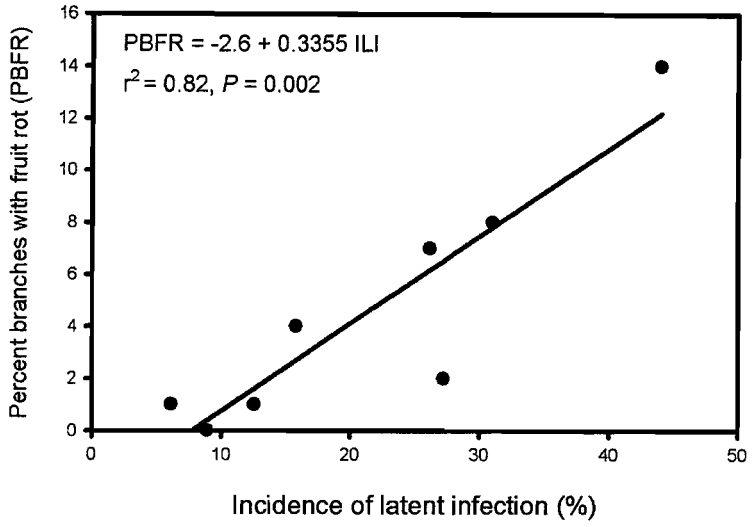

Fig. 9. Linear regression between incidence of latent infection (ILI) and percentage of branches with fruit rot (PBFR) caused by *Monilinia fructicola*. Each dot represents an average value of multiple locations for each inoculation.

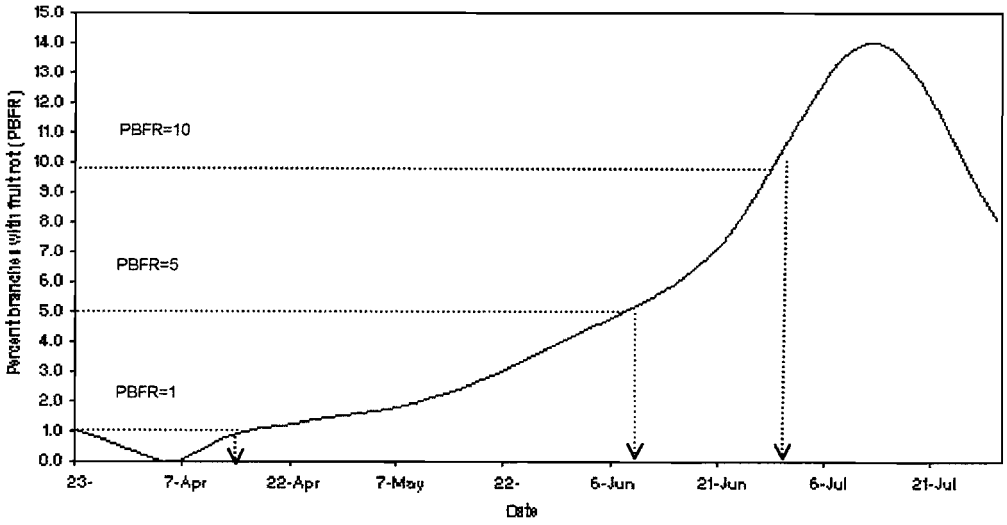

Fig. 10. Threshold fruit developmental stages related to three levels of percentage of branches with fruit rot (PBFR) caused by *Monilinia fructicola*. The dotted lines indicate the corresponding threshold dates. The solid curve is the developmental curve of fruit rot from Fig. 6.

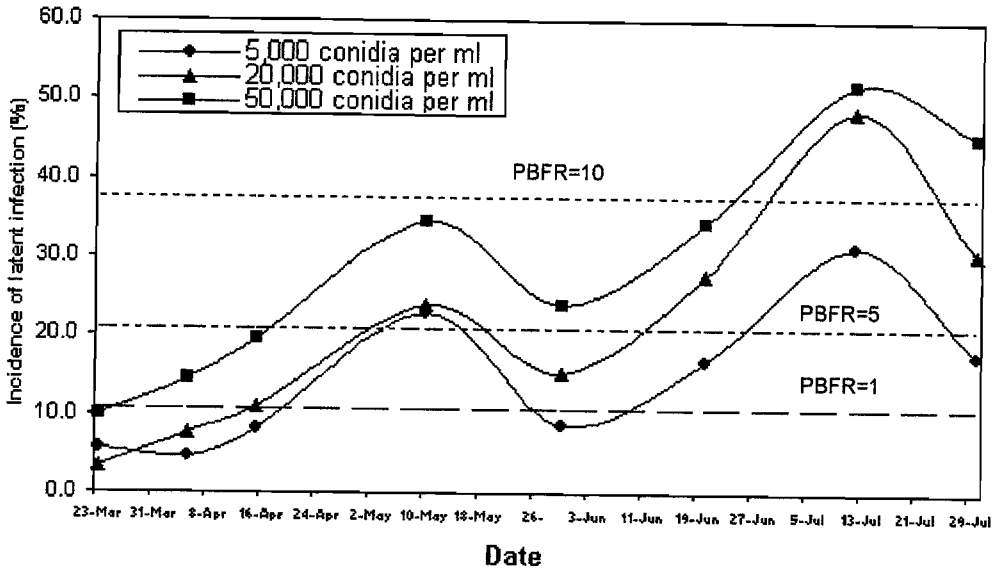

Fig. 11. Development of fruit latent infection over the growing season induced by three inoculum concentrations of *Monilinia fructicola*. The three dotted lines represent the three corresponding levels of percentge of branches with fruit rot (PBFR). The thresholds of different inoculum concentrations leading latent infection to different levels of PBFR could be determined.

2) The incidences of latent infection did not change significantly from the bloom stage to the mid embryo growth stage. However after that period, the incidence of latent infection of fruit significantly increased and reached the highest level before harvest. This increase was related to the secondary infection of fruit late in the season, which may occur during and after each irrigation.

3) The incidence of latent infection was significantly reduced by spraying fungicide on thinned fruit on the orchard floor.

4) Timing of fruit thinning and irrigation is an important factor for disease management. Cultural practices of orchards that promote a fast drying of thinned fruit could significantly shorten sporulation period and reduce inoculum potential, as well as the risk of latent infection of fruit by *M. fructicola*.

5) Conditions leading latent infection to prune fruit rot included a) incidence of latent infection, b) fruit developmental stage, c) inoculum concentration, and d) microclimatic environment.

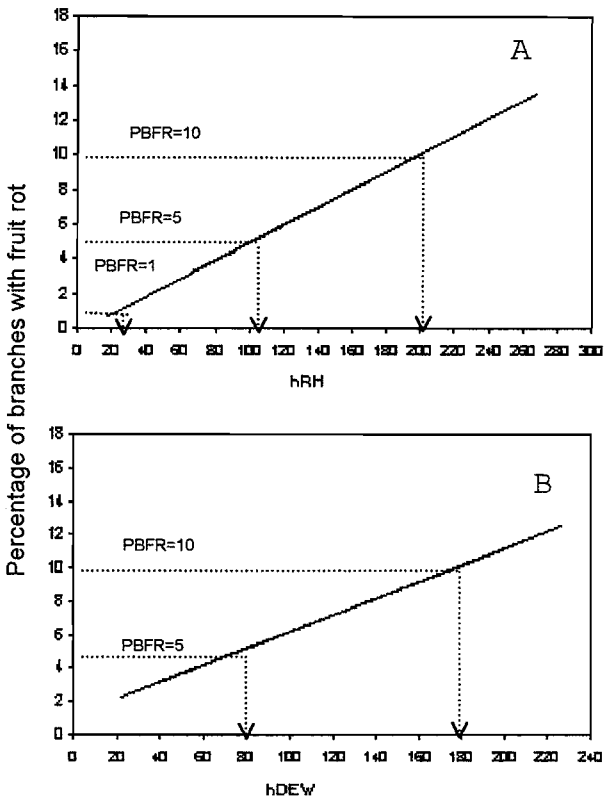

Fig. 12. Thresholds of (A) total hours of relative humidity (RH) greater than 90% (hRH), and (B) total hours of dew (hDEW) from 15 July to 15 August leading latent infection to different levels of percent branches with fruit rot (PBFR) caused by *Monilinia fructicola*, as indicated by the dotted lines. The linear lines are obtained from Fig. 8.

6) When incidence of latent infection (ILI) is greater than 10.7%, the percentage of branches with fruit rot (PBFR) could possibly be greater than 1%. When the ILI is less than 37.6%, the PBFR can reach up to 10%.

7) The concentration of 20,000 conidia/ml of *M. fructicola* could result in a PBFR greater than 5% only during the pit hardening stage and after the embryo growth stage. The concentration of 50,000 conidia/ml of *M. fructicola* could result in a PFBR greater than 1 during the entire season.

8) The 24.2 hours was a threshold of total hours from July 15 to August 15 when RH was greater than 90% (hRH) that led latent infection to a PBFR equal to or greater than 1. The corresponding thresholds of hRH leading latent infection to 5 and 10% of PBFR were 104.6 and 204.6 hours, respectively.

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

Threshold condition	PBFR=1%	PBFR=5%	PBFR=10%
Incidence of latent infection (%)	10.7	22.7	37.6
Fruit developmental stage	late fruit set	embryo growth	late embryo growth
Inoculum concentration:			
5,000 conidia/ml	during pit hardening and after late embryo growth	after late embryo growth	not at all
Period	20 April – 28 May, after 11 June	1 May - 16 May, after 27 June	not at all
20,000 conidia/ml	After fruit set	during pit hardening and after embryo growth	after late embryo growth
Period	after 15 April	2 May - 20 May, after 12 June	after 30 June
50,000 conidia/ml	full bloom	before pit hardening	late embryo growth
Period	after 25 March	after 18 April	after 25 June
Environment (hRH) (hours)	24.2	104.6	204.6
Environment (hDEW) (hours)	not available	76.4	176.4

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