Annual Report - 2010

Prepared for the Prune Board of California

Title:	Epidemiology and management of brown rot and rust of prune – Development of an integrated program with new fungicides and optimal timing
Status:	Third Year of Four
Principal Investigator:	J. E. Adaskaveg Department of Plant Pathology, University of California, Riverside 92521
Cooperating:	D. Thompson, H. Förster, R. Buchner (UCCE-Tehama Co.), J. Connell (Butte Co.), and F. Niederholzer (UCCE-Sutter-Yuba Co.)

SUMMARY OF RESEARCH ACCOMPLISHMENTS DURING 2010

1. ***Blossom blight*:** In laboratory studies using detached prune blossoms, all fungicides evaluated, including Quash, Luna Privilege, and several new pre-mixtures (Luna Sensation, Inspire Super, Quilt Xcel, Inspire XT, BAS703) were highly effective in reducing the incidence of blight when used as pre- or post-infection treatments. As in 2009, the biocontrol Actinovate as a pre-infection treatment also significantly reduced the incidence of infections from that of the control, but was less effective than the fungicides.
2. ***Fruit brown rot:*** Fungicides of the DMI class (i.e., Indar, Tilt) or pre-mixtures containing a DMI fungicide (Quilt Xcel, Quadris Top) were very effective on wound- and non-wound-inoculated fruit. Luna Privilege (fluopyram) was also highly effective on wound-inoculated fruit, whereas Luna Sensation that has a fluopyram component was less effective. Treatments with only Ph-D numerically reduced the incidence of decay from that of the control. Treatments applied at 5 days before harvest were more effective than when applied 12 days before harvest. In another trial, applications at 160 gal/A were generally beneficial for reducing decay of wound-inoculated fruit harvested from inside clusters. Thus, pre-harvest application are best applied in combination with a spray oil at higher gallonage
3. ***Rust***: In a fall-season trial using a single application of each treatment, none of the fungicides evaluated in our field plot was highly effective in reducing the incidence or severity of prune rust. Still, all fungicides evaluated significantly reduced disease levels. The fungicide Tilt had the lowest levels after 4 weeks.
4. ***In vitro fungicide sensitivity tests.*** No shifts in sensitivity of *Monilinia* spp. populations to DMI or AP fungicides was detected at nine locations in Butte Co. Sites were selected with high brown rot incidence and where apparently preharvest fungicide treatments did not provide satisfactory decay control. Thus, based on these data (with additional data pending, however), we conclude that brown rot that developed at these locations after fungicide treatments was not due to resistance in the pathogen populations. AP fungicide resistance has not persisted or spread within the brown rot pathogen populations.
5. ***Identification of* Aspergillus *species on dried plums.*** A molecular method was developed for the differentiation of species of *Aspergillus* occurring on dried and fresh prune fruit. Most species identified in 2009 and 2010 samplings belong to the Section Nigri of the genus *Aspergillus*. A single isolate that we obtained in 2008 was identified as *A. flavus*, but we did not test if this isolate is an aflatoxin producer. Molecular identification of *Aspergillus* species is possible in one to a few days as compared to 1 to 2 weeks when cultural characteristics are used.

INTRODUCTION

Brown rot, caused by *Monilinia* species is the most important blossom and preharvest disease of prune in California. In the main growing areas of the state, *M. laxa* is the primary pathogen on blossoms, whereas *M. fructico*la is the main pathogen on fruit. Still, both species can be found causing blossom blight and fruit rot in California. Currently, fungicide treatments that are properly timed are the most effective method to control this disease. Among the fungicides registered on fresh and dried prune, the DMI fungicides Orbit and Indar, the anilinopyrimidines Vangard and Scala, the dicarboximide Rovral, and the QoI-SDHI (succinate dehydrogenase

inhibitor) pre-mixture Pristine are most effective against blossom blight. In addition, the DMI Elite and the DMI-QoI premixture Adament are registered on fresh prunes. The pre- and post-infection activity of these fungicides on prune blossoms was characterized previously by us. In 2010 we continued to evaluate several new fungicides (Quash, Luna Privilege - fluopyram, and pre-mixtures: Luna Sensation - fluopyram + trifloxystrobin, Inspire Super – difenoconazole + cyprodinil, Quilt Xcel - propiconazole + azoxystrobin, Inspire XT – difenoconazole + propiconazole, BAS703) and results are presented in this report. A current trend in fungicide registrations for tree fruit crops is the use of pre-mixtures. This is done to reduce the risk of resistance development to any single class of fungicides. In blossom studies we also evaluated the biocontrol Actinovate (*Streptomyces lydicus*) that was also tested in 2009. The information we are providing is helping to identify new effective materials and treatment strategies. For example, fungicides with a good post-infection activity (i.e., 'kick-back action') in addition to pre-infection activity could be applied as a single, delayed bloom application instead of a standard two- or -three spray program described for preventing infections of sepal (green tip), petals (white tip), and stamen/pistils (full bloom) of prune blossoms under conditions that are less favorable for disease.

Preharvest applications with fungicides to prevent losses from fruit brown rot are generally not as highly effective on prune as on other stone fruit crops such as peaches and nectarines. This is because the waxy bloom on the prune fruit prevents sufficient coverage of the fruit surface by most formulated fungicide products. In our previous trials on preharvest treatments, we demonstrated that when fungicides were mixed with spray adjuvants (e.g., summer oils), the efficacy of most treatments was significantly improved. In 2010, all treatments were applied in combination with a spray oil. Because prune fruit developing in clusters are often not well protected by spray applications and are more likely to develop brown rot, as in 2009, we compared two application volumes in one of the studies to potentially increase the efficacy of the treatments. New materials evaluated in 2010 included Luna Privilege, Luna Sensation, Inspire Super, Quilt Xcel, Quadris Top (difenoconazole + azoxystrobin), BAS703, and polyoxin-D (Ph-D) that was provided in a formulation that potentially could be approved for organic crop production.

Prune rust did not develop at our orchard locations in the past two years. In 2010, the disease started to develop in early September at one site. Although late in the season, fungicides were applied to obtain efficacy data on new treatments that could be of value in high-disease seasons. New materials evaluated included Luna Sensation, Inspire Super, Quadris Top, BAS703, and Ph-D.

Rotations and mixtures of fungicides with different modes of action are critical to prevent the overuse of any one class of fungicide that may lead to the selection of resistant pathogen populations. In previous years, we had detected a low incidence of cyprodinil resistance in *Monilinia* sp. populations in some prune orchards in California. In 2010 there were several reports where treatments with Vangard or Tilt (Orbit) did not provide satisfactory fruit brown rot control. To find out if fungicide resistance was involved in this lack of adequate efficacy we sampled diseased fruit from several affected fruit lots and evaluated the recovered isolates of *Monilinia fructicola* and *M. laxa* for their in vitro sensitivity against cyprodinil and propiconazole.

Another objective of our research was the isolation and identification of molds on dried plums that was pursued at the request of farm advisors. Isolates were collected from stored fruit of the 2009 crop and from fresh fruit of the 2010 crop. We developed a molecular characterization method for isolates of *Aspergillus* species that we collected in our samplings. The goal is to have a simple, rapid method available to differentiate between harmless saprobes and potentially harmful mycotoxin-producing species.

OBJECTIVES

1. Evaluate the efficacy of new fungicides and pre-mixtures representing different chemical classes in laboratory and field trials.
   - **a.** Evaluation of fungicides for control of brown rot blossom blight and brown rot of fruit.
     - i. Pre- and post-infection activity of selected fungicides against blossom blight.
     - ii. Evaluation of preharvest fungicides in combination with selected spray adjuvants (laboratory inoculations of field-treated, harvested fruit)
   - **b.** Evaluation of fungicide efficacy against rust (If rust does not develop in spring and summer, fall trials will be initiated in selected areas prone to rust disease).
2. Epidemiological studies with prune rust (*pending disease development in the field*).

a. Inoculation studies in the greenhouse using potted plants (peach, prune, and selected roostocks) and inoculation of different tissues (i.e., leaves and stems) at different stages of development. For inoculation, different sources of rust (i.e., peach, prune, etc.) will be used.

3. Monitoring of *Monilinia* spp. populations obtained from decaying fruit for their in vitro sensitivities against commonly used fungicides (i.e., cyprodonil, propiconazole, etc.)

4. Molecular identification of *Aspergillus* species on dried plums using rDNA sequence data.

MATERIALS AND METHODS

Evaluation of fungicides for management of brown rot blossom blight. Laboratory studies were done using French prune blossoms obtained from the UC Davis, Plant Pathology field station. For post-infection studies, blossoms at popcorn stage were collected and allowed to open in the laboratory. They were then inoculated with a conidial suspension of *M. laxa* (20K conidia/ml), treated after 24 h using a hand sprayer, and incubated at 20C. For pre-infection studies, blossoms were first treated and then inoculated. Three replications of eight blossoms were used for each fungicide. Treatments were applied using rates suggested by the fungicide manufacturers. Data were analyzed using analysis of variance and least significant difference (LSD) mean separation procedures of SAS 9.1.

Evaluation of fungicides for management of prune rust. A field trial was established in a commercial orchard to evaluate the efficacy of new fungicides. Fungicides were applied on Sept. 10 at the onset of rust symptoms in the test portion of the orchard. Disease was evaluated on Oct. 20-2010. For this, 30-40 leaves from each of the 5 single-tree replications were evaluated using a scale for 0 = no disease, 1 = <25%, 2 = 26-50%, 3 = 51-75%, and 4 = 76-100% of leaf area affected.

Evaluation of fungicides for management of preharvest fruit decay. Field plots to evaluate preharvest fungicide applications for control of fruit brown rot were established at UC Davis and in a commercial orchard in Sutter-Yuba Co. All treatments were in combination with a spray oil (Omni Supreme) used at 1.5% v/v. In the Sutter-Yuba plot, treatments were applied on 8-23-10 and fruit were harvested randomly from each of the four single-tree replications 5 and 12 days after treatment. Ten fruit from each tree were either wound- or non-wound-inoculated with droplets of conidial suspensions of *M. fructicola* (30,000 conidia/ml). For the wound-inoculations, drops of inoculum were placed on wounds of fruit (ca. 1 mm x 2 mm x 2 mm deep). Fruit were then incubated at 20C for 7 days. In the UC Davis plot, 12 and 7 day PHI treatments were applied in the field using an air-blast sprayer at 80 or 160 gal/A. At harvest, either single fruit from the tree perimeter (exposed fruit) or fruit from clusters were collected and wound-inoculated or non-wound-inoculated with conidia of *M. fructicola* (30,000 conidia/ml) on the unexposed side of the fruit. Fruit from inside clusters were inoculated on the inside facing side. Fruit were then incubated for 7 days at 20 C. Data were analyzed using analysis of variance and least significant difference (LSD) mean separation procedures of SAS 9.1.

In vitro fungicide sensitivity tests. Isolates of *M. fructicola* and *M. laxa* were obtained from nine fruit lots originating in Butte Co. The sensitivity of isolates against cyprodinil and propiconazole was assessed using the spiral gradient dilution method. Conidia were used to inoculate amended PDA agar plates along the fungicide concentration gradient and plates were evaluated for fungal growth after 3 days of incubation at 20C. EC_{50} values of isolates were compared to isolates from the baseline population.

Identification of* Aspergillus *species on fresh and dried plums. Fungal isolates were identified as species of *Aspergillus* based on morphological characteristics. A total of 86 isolates were obtained from 8 lots fruit of dried fruit from the 2009 crop and 47 isolates were obtained from 5 lots of fresh fruit from the 2010 crop. The ITS1 region of rDNA was amplified from representative isolates (based on colony morphology) using universal primers ITS1 and ITS2. Amplification products were digested with restriction enzymes *Alu*I, *Hin*fI, *Mbo*I, *Rsa*I, and *Taq*I. DNA fragments were separated in agarose gels, fragment patterns were analyzed visually, and isolates were grouped according to their fragment patterns. Subsequently, ITS1 sequences were obtained from 2 isolates from each restriction pattern group and sequences were aligned using ClustalW and compared to those of isolates of *Aspergillus* deposited in Genbank. A dendrogram with sequences from representative prune isolates and from reference sequences was constructed by the Neighbor-Joining method using PAUP.

RESULTS AND DISCUSSION

Evaluation of fungicides for management of brown rot blossom blight. In laboratory studies using detached prune blossoms, all fungicides evaluated, including several new pre-mixtures (Luna Sensation, Inspire Super, Quilt Xcel, Inspire XT, BAS703) were highly effective in reducing the incidence of blossom blight when used as pre- or post-infection treatments (Fig. 1). The post-infection activity was evaluated in these experiments to assess the potential efficacy of the treatments as a single application in a delayed bloom application when recent blossom infections need to be controlled. Actinovate that was only used as a pre-infection treatment also significantly reduced the incidence of infections from that of the control, but was less effective than the fungicides. This biocontrol treatment was also effective in our 2009 studies, thus, although less effective than any of the fungicides, it may still have a potential to be useful in organic dried plum production.

Evaluation of fungicides for management of prune rust. Four weeks after each treatment in the fall season, none of the fungicides evaluated in our field plot was highly effective in reducing the incidence or severity of prune rust (Fig. 2). This is because treatments were applied only once, rust symptoms were already present at application time and many infections had already occurred, and because environmental conditions (leaf wetness, conducive temperatures) in this late season trial were highly conducive. Still, all fungicides evaluated significantly reduced disease levels. Propiconazole (i.e., Tilt) resulted in the lowest levels of disease (Fig. 2). Disease incidence on the Tilt-treated trees was reduced by more than 50% as compared to the control and severity (based on a rating scale) was reduced by approximately 80%.

Evaluation of fungicides for management of fruit brown rot. The efficacy of preharvest fungicides applied in combination with a spray oil for control of fruit brown rot decay was evaluated in two field trials. In the Sutter-Yuba trial, most of the fungicides were highly effective and most were more effective on non-wound inoculated fruit (Fig. 3). As previously shown, fungicides of the DMI class (i.e., Indar, Tilt) or pre-mixtures containing a DMI fungicide (Quilt Xcel, Quadris Top) were also very effective when fruit were wound-inoculated after treatment and harvest. Luna Privilege (fluopyram) was also highly effective on wound-inoculated fruit, but Luna Sensation that has a fluopyram component was less effective. Ph-D only numerically reduced the incidence of decay from that of the control. Treatments applied at 5 days before harvest were more effective than when applied 12 days before harvest (Fig. 3). These comparative evaluations show that among the most effective DMI alternatives are the SDHIs (e.g., fluopyram) and QoIs (e.g., Abound, Gem – see 2009 data). As an anti-resistance strategy, these classes should best be used in pre-mixtures (e.g., Luna Sensation, Quadris Top, Quilt Xcel) to reduced the risk of selection of fungicide resistant isolates. As previously shown, Elevate also shows good efficacy as a preharvest treatment.

In the UC Davis trial, the efficacy of preharvest applications with five fungicides was evaluated using two application volumes (80 and 160 gal/A). Exposed fruit collected from the outer canopy and fruit inside clusters were used. With most fungicides, more decay developed on fruit collected inside clusters as compared to exposed fruit indicating that fungicide residues on these fruit were lower on the clustered fruit than on the exposed fruit (Fig. 4A,B); this trend was more evident in the 7-day PHI applications. Applications at higher gallonage were beneficial for wound-inoculated fruit harvested from inside clusters for Indar and Quadris Top at the 7-day PHI treatments and for Tilt, Pristine, and Luna Sensation for the 12-day PHI applications. Thus, not all treatments at both timings benefited from the higher gallonage. However, for none of the fungicides and timings, was the higher gallonage application significantly less effective than the lower gallonage treatment. Thus, higher-gallonage treatments are recommended to obtain the best treatment efficacy. Overall, the incidence of decay was still relatively high for most treatments in the fruit cluster evaluation using wound-inoculation because fruit were inoculated on the side facing away from the spray direction. Thus, in this assay, the efficacy of the fungicides was highly challenged. On non-wound-inoculated fruit, in contrast, many treatments resulted in low decay incidences.

To summarize pre-harvest application methods, the highest treatment efficacy is obtained when fungicide-oil mixtures are applied at higher volumes. The spray oil is either providing better coverage or may result in better penetration of the fungicide into the fruit. Not all fungicides, however, are compatible with oils and a comparison between different spray adjuvants is warranted.

In vitro fungicide sensitivity tests. Another objective of our prune research project was to evaluate pathogen isolates for their in vitro fungicide efficacy against important fungicide classes (i.e., the DMIs and anilinopyrimidines). We focused our samplings from locations where fungicide treatments did not provide satisfactory decay control. We obtained fruit with brown rot decay from nine orchard locations that were

treated with propiconazole (Tilt, Orbit) and obtained isolates of *M. fructicola* and *M. laxa*. Some results of these samplings are still pending. To date, up to 22 brown rot isolates were obtained from a single location. From three locations only *M. fructicola* was isolated, from another three locations only *M. laxa* was isolated, and from another three locations both species of *Monilinia* were recovered (Table 1). Fungicide sensitivity assays indicated that the total of 34 isolates of *M. fructicola* and 94 isolates of *M. laxa* evaluated to date were all highly sensitive against propiconazole with an EC_{50} range for mycelial growth of 0.002 to 0.019 ppm for *M. fructicola* and of 0.003 to 0.032 ppm for *M. laxa* (Table 1). Sensitivity against cyprodinil (Vangard) was only evaluated for *M. laxa* and all isolates were determined to be sensitive with an EC_{50} range for mycelial growth of 0.018 to 0.160 ppm. Thus, based on these data (with additional data pending), we conclude that brown rot that developed at these locations after fungicide treatments was not due to resistance in the pathogen populations. Highly conducive environmental conditions in the late season with rainfall, as well as inadequate application methods (e.g., alternate row spraying, spraying at low gallonage) probably attributed to the development of decay. Thus, AP-fungicide resistance that we reported on previously has not persisted or spread within the brown rot pathogen population. Because in previous years we were able to detect isolates of both *Monilinia* spp. resistant to cyprodinil at a few locations, the risk of resistance development does exist if anti-resistance guidelines are not followed.

Table 1. In vitro fungicide sensitivities (EC_{50} values) of isolates of *Monilinia fructicola* and *M. laxa* from prune fruit collected in Butte Co. in 2010

Lot No.	Species	No. isolates	EC_{50} range propiconazole (mg/L)*	EC_{50} range cyprodinil (mg/L)
1	*M. fructicola*	2	0.002-0.003	---
2	*M. fructicola*	3	0.002-0.005	---
3	*M. fructicola*	11	0.002-0.019	---
4	*M. fructicola*	8	0.002-0.013	---
	M. laxa	11	0.003-0.024	0.023-0.050
5	*M. fructicola*	3	0.003-0.005	---
	M. laxa	19	0.004-0.032	0.018-0.066
6	*M. fructicola*	7	0.002-0.012	---
	M. laxa	16	0.010-0.030	0.029-0.160
7	*M. laxa*	18	0.008-0.032	0.022-0.070
8	*M. laxa*	16	0.009-0.024	0.025-0.063
9	*M. laxa*	14	0.005-0.030	0.025-0.053

* -Sensitivity against mycelial growth was determined using the spiral gradient dilution method.

* - Fungicide sensitivity assays indicated that the total of 34 isolates of *M. fructicola* and 94 isolates of *M. laxa* evaluated to date were all highly sensitive against propiconazole with an EC_{50} range for mycelial growth of 0.002 to 0.019 ppm for *M. fructicola* and of 0.003 to 0.032 ppm for *M. laxa*. Cyprodonil sensitivity data for *M. fructicola* is pending.

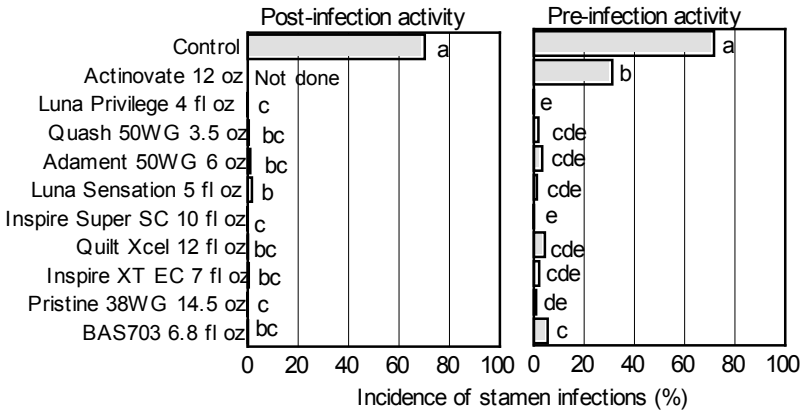
Identification of* Aspergillus *species on fresh and dried plums. Species of *Aspergillus* are known to produce several toxins. For example, others have reported that some strains of *A. flavus* produce aflatoxin, some strains of *A. niger* produce ochratoxin, and some strains of *A. terreus* produce citrinin. Thus, the proper identification of *Aspergillus* isolates is important. As in previous reports by us and others, we found *Aspergillus*

spp. colonizing dried and fresh prune fruit. The incidence of colonization was up to 50% on damaged or injured fresh fruit. Most of the isolates were pigmented black to dark gray. Using five restriction enzymes, sequence polymorphisms were detected in the amplified ITS1 DNA regions among isolates and isolates could be grouped accordingly (Fig. 5). For example, in Fig. 5, the ITS1 region of six species was digested with two restriction enzymes. Selected isolates from each restriction fragment group were sequenced and sequences were found to be identical or almost identical within each restriction pattern group.

DNA sequences from *Aspergillus* spp. isolates from prune were compared to those of reference sequences in Genbank including *A. niger, A. brasiliensis, A. phoenicis, A. tubingensis, A. japonicus, A. flavus*, and *A. chevalieri*. *A. japonicus* and *A. chevalieri* were included because these species were reported from prune fruit previously. Most of the reference sequences came from cultures that were well characterized previously by others (Parenicova et al. 2000. *Pages* 413-424, in: Integration of Modern Taxonomic Methods for *Penicillium* and *Aspergillus*, R.A. Samson and J.I. Pitt, eds.). *A. niger, A. brasiliensis, A. phoenicis, A. foetidus, A. tubingensis,* and *A. japonicus* are members of the black Aspergilli (i.e., *Aspergillus* Section *Nigri*). The taxonomy of this section has been controversial, and currently six to eight species are recognized. These species are very closely related and can only be separated by extensive sequence analysis of rDNA, but not by morphological features. Still, *A. phoenicis, A. niger, A. tubingensis*, and *A. foetidus* are not clearly separated and some taxonomists do not consider *A. foetidus* and *A. tubingensis* valid species. A phylogenetic analysis of selected prune isolates and reference sequences is presented in Fig. 6. This dendrogram confirms the close similarity of most species within Section Nigri; *A. japonicus*, however, is clearly separated. Of the total of 70 isolates of *Aspergillus* collected in 2009 and 2010 and analyzed to date (additional results for 2010 isolations are still pending) all except one isolate were found to be part of the *Aspergillus* Section *Nigri*. The majority of isolates were identified as *A. phoenicis/A.foetidus* and *A. tubingensis*, two isolates were identified as *A. brasiliensis*. One isolate with yellow to brown colony pigmentation is pending identification. A single isolate that we obtained in 2008 was identified as *A. flavus* (yellow-green Aspergilli, *Aspergillus* Section *Flavi*), but we did not test if this isolate is an aflatoxin producer.

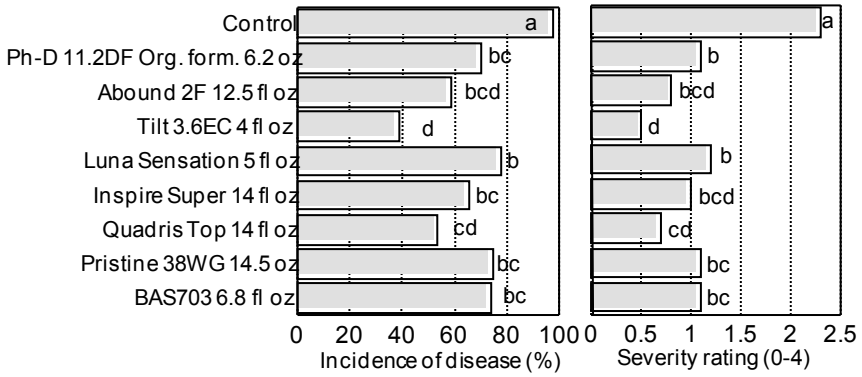
In our molecular species identification, restriction fragment pattern groups correlated closely with DNA sequence data. In addition, all species of *Aspergillus* identified from prune to date, with the exception of *A. phoenicis* and *A. tubingensis* (that again cannot be easily differentiated by other molecular methods) could be separated by restriction analysis. Using this technique, the rapid identification of common species of *Aspergillus* on dried plum can be done within 1 to a few days rather than weeks based on morphological cultural characteristics.

Fig. 1. Efficacy of post-infection treatments with a bioncontrol or fungicides for management of blossom blight of French prune



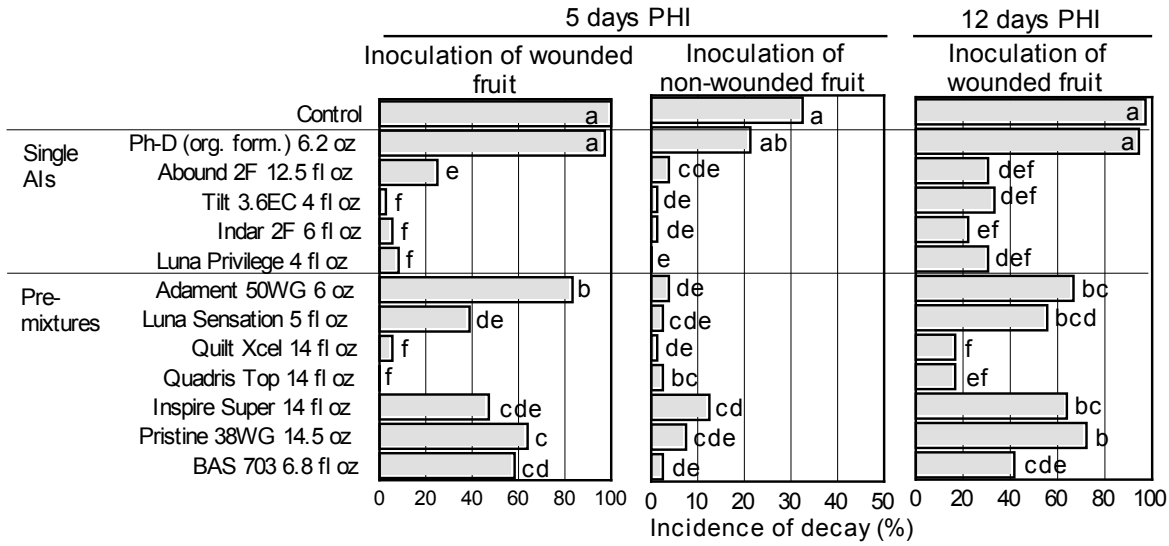
For evaluation of the pre-infection acticity, blossoms were collected in the field and treated in the laboratory using a hand sprayer. After 12 h blossoms were inoculated with a spore suspension of *M. laxa* (15K/ml). For evaluation of the post-infection activity, blossoms were collected and inoculated and were treated with a hand-sprayer after 24 h. Blossoms were evaluated for stamen infections after 4-5 days of incubation at 20 C.

Fig. 2. Efficacy of fungicide applications for management of rust of French prune in Butte Co. 2010 - Late season after harvest evaluations -



Treatments were applied in the field on 9-10-10 using an air-blast sprayer at 100 gal/A. For evaluations on 10-20-10, 30-40 leaves from each of the 5 single-tree replications were evaluated using a scale for 0 = no disease, 1 = <25%, 2 = 26-50%, 3 = 51-75%, and 4 = 76-100% of leaf area affected.

Fig. 3. Efficacy of 5- and 12-day PHI fungicide applications for management of postharvest brown rot in a field trial in Sutter-Yuba Co. 2010

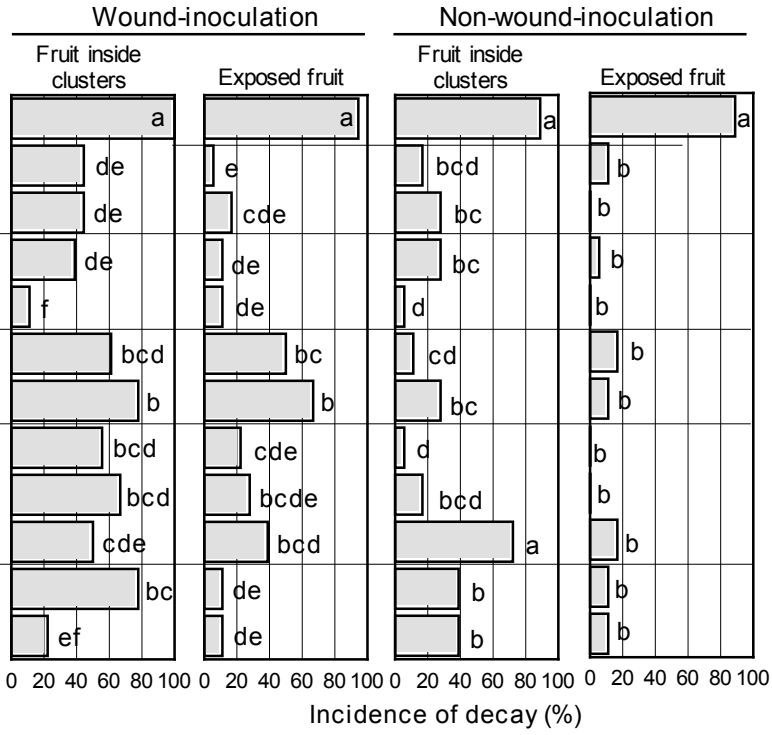


Treatments were applied in the field in combination with Omni Supreme Spray oil (1.5%) on 8-27-10 using an air-blast sprayer (100 gal/A). After harvest, fruit were either wound- or non-wound inoculated with conidia (30,000 conidia/ml) of the pathogen *Monilinia fructicola*. Fruit were then incubated for 7 days at 20 C.

Fig. 4. Efficacy of 14+0-day PHI fungicide applications for management of postharvest brown rot of French prune at UC Davis 2010.
- Evaluation of application volumes in preventing decay of exposed fruit and fruit inside clusters -

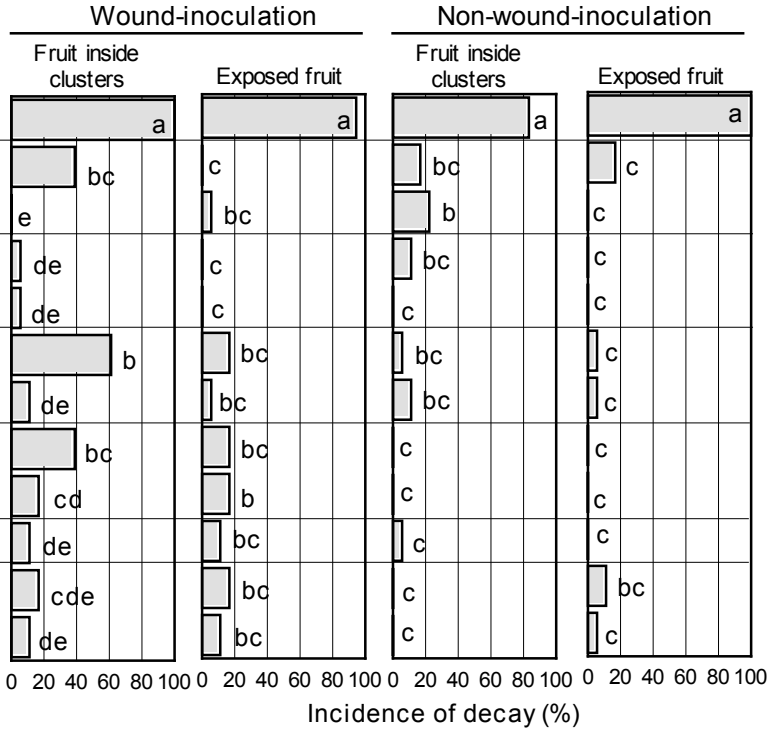
A. 7-days PHI

Treatment	Application volume/A
Control	---
Tilt 3.6EC 4 fl oz	80 gal
Tilt 3.6EC 4 fl oz	160 gal
Indar 2F 6 fl oz	80 gal
Indar 2F 6 fl oz	160 gal
Pristine 38WG 14.5 oz	80 gal
Pristine 38WG 14.5 oz	160 gal
Luna Sensation 5 fl oz	80 gal
Luna Sensation 5 fl oz	160 gal
Inspire Super 14 fl oz	160 gal
Quadris Top 14 fl oz	80 gal
Quadris Top 14 fl oz	160 gal



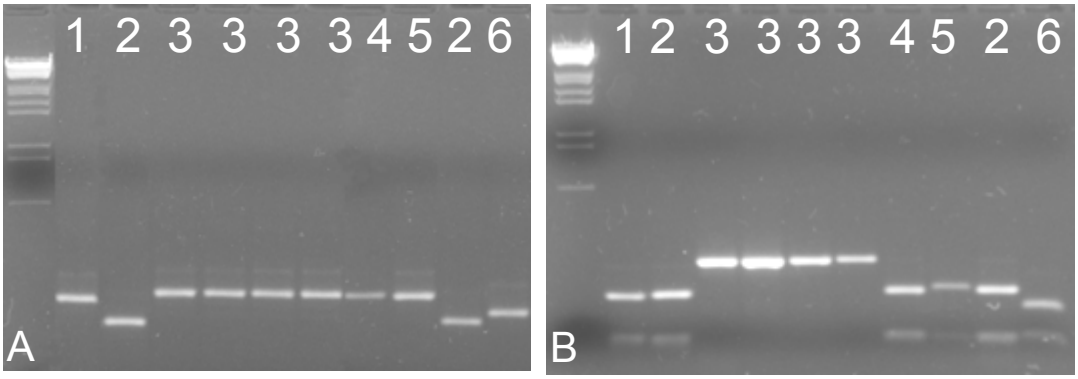
B. 12-days PHI

Treatment	Application volume/A
Control	---
Tilt 3.6EC 4 fl oz	80 gal
Tilt 3.6EC 4 fl oz	160 gal
Indar 2F 6 fl oz	80 gal
Indar 2F 6 fl oz	160 gal
Pristine 38WG 14.5 oz	80 gal
Pristine 38WG 14.5 oz	160 gal
Luna Sensation 5 fl oz	80 gal
Luna Sensation 5 fl oz	160 gal
Inspire Super 14 fl oz	160 gal
Quadris Top 14 fl oz	80 gal
Quadris Top 14 fl oz	160 gal



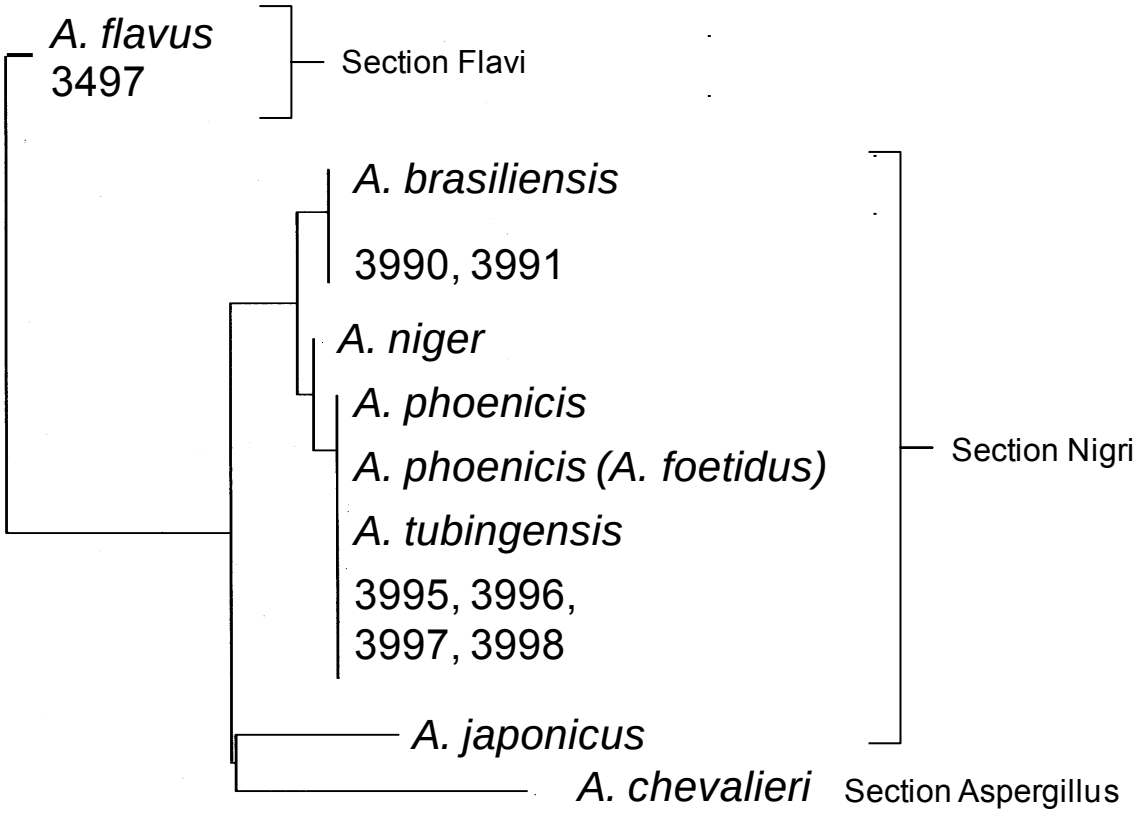
Treatments were applied in the field in combination with Omni supreme Spray Oil (1.5%) using an air-blast sprayer at 80 or 160 gal/A. Omni Supreme Spray oil was used. At harvest, either single fruit from the tree perimeter (exposed fruit) or fruit from clusters were collected and wound-inoculated with conidia of *M. fructicola*(30,000 conidia/ml) on the unexposed side of the fruit. Fruit from inside clusters were inoculated on the inside facing side. Fruit were then incubated for 7 days at 20 C.

Fig 5. Restriction analysis of the amplified ITS1 region of rDNA from selected reference isolates and *Aspergillus* spp. isolated from dried plum fruit



ITS1 sequences were amplified using universal primers and amplification products were restricted with **A.** *Taq*I or **B.** *Rsa*I. Isolates in each gel are the same and are in the same order. Polymorphisms can be detected among isolates using the two restriction enzymes. Lanes with the same number are the same species.

Fig 6. Phylogenetic analysis of ITS1 sequence data from isolates of *Aspergillus* spp. from dried plum fruit and selected reference cultures



Sequences with number assignments were obtained from cultures from prune fruit collected in 2009 and 2010. Sequences of *Aspergillus brasiliensis, A. chevalieri, A. flavus, A. japonicus, A. niger, A. phoenicis,* and *A. tubingensis* were obtained from Genbank. Sequences were aligned using ClustalW and a Neighbor-Joining tree was constructed using PAUP.