Annual Report - 2008

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:	First-Year of Three
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SUMMARY OF RESEARCH ACCOMPLISHMENTS DURING 2008

1. In laboratory studies on the pre- and post-infection activity of fungicides for managing brown rot blossom blight, all fungicides evaluated were highly effective and significantly reduced the incidence of disease. The new SBIs Quash and Inspire were the most effective treatments when applied either before (pre-infection activity) or after (post-infection activity) inoculation with *Monilinia laxa*. Two of the three natural products evaluated (MOI 104 and Cerebrocide) also significantly reduced the incidence of infections and efficacy was higher in the post-infection as compared to the pre-infection treatments. Although the natural products were less effective than any of the fungicides, they still have a potential to be useful in organic production.
2. In two field trials, the efficacy of preharvest applications against brown rot decay of French prune fruit inoculated with *M. fructicola* was evaluated. We confirmed last year's studies that a significant increase in fungicide efficacy can be obtained when applications are done in combination with a spray oil. No highly effective single-active-ingredient alternatives to the SBIs were identified that provided a high reduction of decay incidence of treated fruit in wound- and non-wound-inoculation studies. The numbered compound USF2015 (belonging to a different, undisclosed class), however, was very effective on non-wound inoculated fruit. Furthermore, the new pre-mixture Adament proved to be highly efficacious using both inoculation methods, and the numbered pre-mixtures A13703 and A15909 demonstrated good activity on non-wound inoculated fruit.
3. Preharvest applications with the three natural products evaluated in the blossom studies were ineffective in reducing the incidence of postharvest brown rot decay.
4. Late season identification of *Aspergillus* species of dried plums was done using morphological methods. Two species groups were identified: *A. niger* (non-toxin-forming *A. niger* group) and *A. chevalieri* (mycotoxin – producing – *A. glaucus* group). Molecular identification procedures are being evaluated. Dehydration does not eliminate these fungi and the latter species has been described as the most common dehydrated fruit in bulk bins.

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. fructicola* is the main pathogen on fruit. Currently, fungicide treatments that are properly timed are the most effective method to control this disease. Among the registered fungicides, the SBI fungicide Orbit, the anilinopyrimidines Vangard and Scala, the dicarboximide Rovral, and the strobilurin-carboxamide premixture Pristine are most effective against blossom blight. The pre- and post-infection activity of these fungicides on prune blossoms was characterized in our 2007 studies. This was followed in 2008 by studies with newly registered and experimental fungicides (e.g., USF2016, A13703, etc.), as well as three new natural products (e.g., MOI-104, MOI-107, Cerebrocide) that have not been evaluated previously. This information will help to identify new effective materials and will help in making decisions on treatment timing. 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 two-spray program.

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 a sufficient coverage of the fruit surface by most formulated fungicide products. In our field trials in 2007 on preharvest treatments, fungicides were mixed with spray adjuvants and this significantly improved fungicide efficacy. These studies were repeated in 2008 using a range of registered and new fungicides, as well as natural products. Thus, the goals for our blossom and preharvest fungicide research are to develop alternative chemistries (e.g., new single fungicides such as V-10135 and USF2015, as well as new pre-mixtures containing two active ingredients from different classes) to the SBI and anilinopyrimidine fungicides for blossom blight and brown rot management so that preharvest rotation programs can be designed that prevent the overuse of any one class of fungicide. Without the development of new classes of fungicides, the potential of resistant populations to develop against new single-site mode-of-action fungicides is high. Additionally, a goal of this research is to optimize the effectiveness of fungicides and this is being accomplished with the use of oil as a treatment adjuvant.

Another objective of our prune research project was the monitoring of *M. fructicola* populations for their in vitro fungicide efficacy against important fungicide classes (i.e., the SBIs and anilinopyrimidines). This was done because treatment failures after anilinopyrimidine applications were reported by some prune growers in 2007 and additionally, to detect any resistance development at an early stage before it becomes widespread. It is important to stay ahead of any spread of resistant populations that would eventually result in the loss of fungicide efficacy. In these surveys in 2007 we detected one isolate of *M. fructicola* that was resistant to the anilinopyrimidine cyprodinil. Due to the very dry spring in 2008, disease pressure at most locations was very low and no failures of fungicide applications were reported. Thus, we were not able to collect fungal isolates for this research objective.

Studies had also been planned on the epidemiology and management of prune rust. These studies were to focus on the host specificity of isolates of stone fruit rust, sources of spring inoculum in orchards, and on disease management. Because, as in 2007, the incidence of rust was very low again in 2008, these studies could not be conducted. Isolation and identification of molds on dried plums was pursued at the request of farm advisors. Two species groups were identified morphologically as *Aspergillus niger* (non-toxin-forming *A. niger* group) and *A. chevalieri* (mycotoxin – producing – *A. glaucus* group).

Objectives

1. Evaluate the efficacy of new fungicides representing different chemical classes in 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.
2. Epidemiological studies with prune rust.
   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.
   b. Spring surveys for sources of inoculum in orchards that had outbreaks in the previous growing season.
3. Monitoring of *Monilinia* spp. populations obtained from decaying fruit for their in vitro sensitivities against commonly used fungicides.

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 this, blossoms at popcorn stage were collected, allowed to open in the laboratory, and were either inoculated with a conidial suspension of *M. laxa* (20K conidia/ml) and treated after 24 h using a hand sprayer, or treated and inoculated after 24 h of incubation at 20C. Three replications of eight blossoms were used for each fungicide. Treatments included the SBIs Indar, Quash, and Inspire as well as the pre-mixtures Inspire Super (SBI + AP), Adament (SBI + QoI), Distinguish (AP + QoI), and the numbered compounds USF2016 and A13703. In another test, three natural products (i.e., MOI 104, MOI 107, and Cerebrocide) were evaluated. Treatments were applied using rates suggested by the treatment 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 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. In the UC Davis plot, treatments of Orbit, Elevate, USF2015 (a new active ingredient), and Pristine were applied at 14+7, 7, and 14 days PHI using an air-blast sprayer calibrated to 100 gal/A. These treatments were done without or with the addition of Omni Supreme Spray oil (2%). In the Sutter-Yuba Co. trial, all treatments (i.e., the SBIs Elite, Orbit, Indar, Quash, Inspire; the new-class fungicide V-10135; the pre-mixtures Adament, A13703, and A15909; as well as the natural products Cerebrocide, MOI 104, and MOI 107) were done in combination with a spray oil and were applied 10 or 14 days PHI. There were four single-tree replications for each treatment in each plot. After harvest, ten fruit from each tree replication were either spray- or wound-inoculated with conidia of *M. fructicola* (30,000 conidia/ml). Spray-inoculations were done on non-wounded fruit, whereas for the wound-inoculations, drops of inoculum were placed on wounds of fruit (ca. 2 mm x 2 mm x 2 mm deep). Fruit were then incubated at 20C for 7 days. Data were analyzed using analysis of variance and least significant difference (LSD) mean separation procedures of SAS 9.1.

Identification of molds on dried plums. Isolation and identification of molds on dried plums was pursued at the request of farm advisors. Species groups were identified based on spore morphology, conidiogenous cell apparatus (size and number of sterigmata), and on cultural characteristics on standardized media.

RESULTS AND DISCUSSION

Evaluation of fungicides for management of brown rot blossom blight. In a laboratory study, the pre- and post-infection activity (treatments 24 h before or after inoculation) of fungicides was compared. In the first study on the evaluation of new fungicides, using both inoculation-treatment schedules, all fungicides were highly effective and significantly reduced the incidence of stamen infections from that of the control (Fig. 1A). Numerically, the new SBI fungicides Quash and Inspire were slightly more effective than the other treatments when applied either before (pre-infection activity) or after (post-infection activity) inoculation with *M. laxa*. Thus, because selected fungicides have protective and post-infection (curative) activity and are also very persistent, single, carefully timed (e.g., late bloom) applications, can be highly effective when environmental conditions for disease are not highly conducive. This information supports a single fungicide application following our ‘delayed-bloom model’ for blossom blight management that is being followed for other stone fruit crops.

In a comparison of three natural products, MOI 104 and Cerebrocide at selected rates also significantly reduced the incidence of infections when treatments were applied 24 h before inoculation with the pathogen (Fig. 1B). The efficacy of both of these products, however, was higher as post-infection as compared to pre-infection treatments. This is in contrast to what is usually observed in the evaluation of synthetic fungicides where the pre-infection activity is generally higher. This indicates that the natural products are not very persistent and rapidly loose their activity or volatize off. This property may limit the use of the products in the field although potentially, they could be applied as soon as possible after an infection period. Thus, although the natural products were less effective than any of the fungicides, they still have a potential to be useful in organic production.

Evaluation of fungicides for management of fruit brown rot. The efficacy of preharvest fungicides for control of fruit brown rot decay was evaluated in two field trials. In the UC Davis trial, applications with selected fungicides (i.e., Orbit, Elevate, USF2015, Pristine) were done without or with the addition of a spray oil. All treatments were generally significantly more effective in combination with the spray oil when harvested, wounded or non-wounded fruit were inoculated with *M. fructicola* (Fig. 2A,B). This is in agreement with our 2007 studies and indicates that the addition of the spray oil improves penetration and coverage of the treatments.

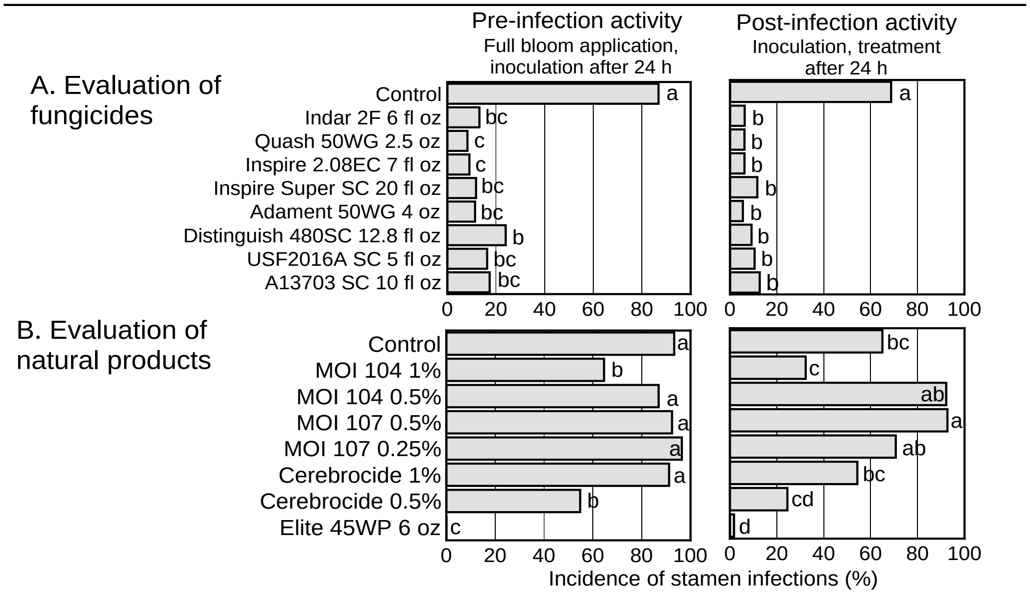
After wound-inoculation, the 14-day PHI treatments were less effective than the 7-day PHI treatments, and two applications at 14 and 7 days before harvest did not result in an increased efficacy as compared to the 7-day PHI applications (Fig. 2A). Overall, Orbit had the highest efficacy and this fungicide was also still very effective in the 14-day PHI treatments when mixed with spray oil. On non-wound inoculated fruit, all treatments were more effective than on wound-inoculated fruit. The highest overall efficacy of the fungicide-oil applications was observed in the 14+7 day PHI applications and decay using the four fungicides evaluated was reduced to very low levels (Fig. 2B). Applications at only 7 days PHI were generally somewhat less effective and all four fungicides performed similarly. In the 14-day PHI applications, Elevate was the least effective material. Otherwise, the four fungicides evaluated, including USF2015 that belongs to a new class, performed very similar.

In the Sutter-Yuba Co. trial, applications with selected fungicides and natural products were all done in combination with a spray oil to compare a range of single active ingredients and pre-mixtures. In comparing 10-day and 14-day PHI treatments, there was no consistent trend as to which one was the better timing (Fig. 3A). In the wound-inoculation studies, the new SBI-QoI pre-mixture Adament was the most effective treatment at both application timings, whereas the new-class fungicide V-10135 was among the least effective. Thus, in combination with oil, V-10135 still lacks sufficient penetration to inhibit decay from wounds occurring after treatment. On non-wound inoculated fruit, all fungicides were very effective with Elite, Quash, and the numbered pre-mixture A15909 resulting in the lowest decay incidence at both timings (Fig. 3B). The three natural products that were evaluated had no effect in reducing brown rot decay of harvested, non-wound inoculated fruit.

In summary, one important finding of our pre-harvest treatment studies was that a significant increase in fungicide efficacy can be obtained when applications are done in combination with a spray oil. The 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. Additionally, no highly effective single-active-ingredient alternatives to the SBIs were identified that provided a high reduction of decay incidence of treated fruit in wound- and non-wound-inoculation studies. Still, the numbered compound USF2015 (belonging to a different, undisclosed class) was very effective on non-wound-inoculated fruit. Furthermore, the new pre-mixture Adament proved to be highly efficacious using both inoculation methods, and the numbered pre-mixtures A13703 and A15909 demonstrated good activity on non-wound inoculated fruit.

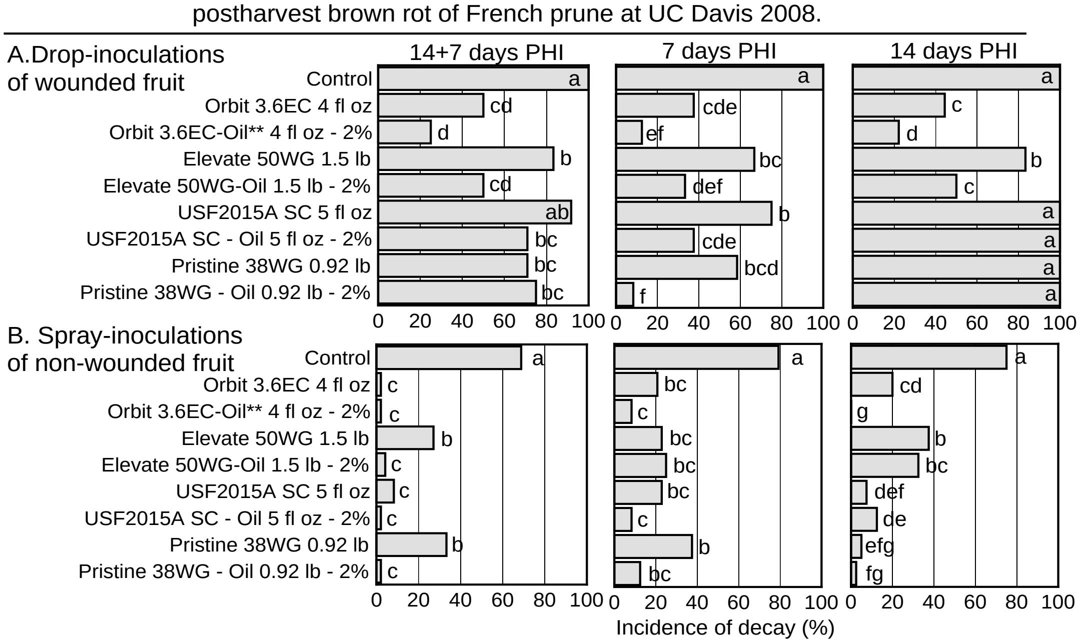
Identification of molds on dried plums. Late season identification of *Aspergillus* species of dried plums was done using morphological methods. Two species groups were identified: *Aspergillus niger* (non-toxin-forming group) and *A. chevalieri* (mycotoxin – producing – *A. glaucus* group). Cultures identified as *A. chevalieri* had a single row of sterigmata (5-7 μm in length) and globose spinulose spores (ca. 5.0 μm in diam.). Cultures identified as *A. niger* had two rows of sterigmata with the first row >20 μm in length and globose ornamented (spinulose) spores (ca. 3-4 μm in diam.). Molecular identification procedures are being evaluated. Dehydration does not eliminate these fungi and the latter species is the most common on dehydrated plums in bulk bins.

Fig. 1. Efficacy of pre- and post-infection treatments with selected fungicides or natural products for management of blossom blight of French prune



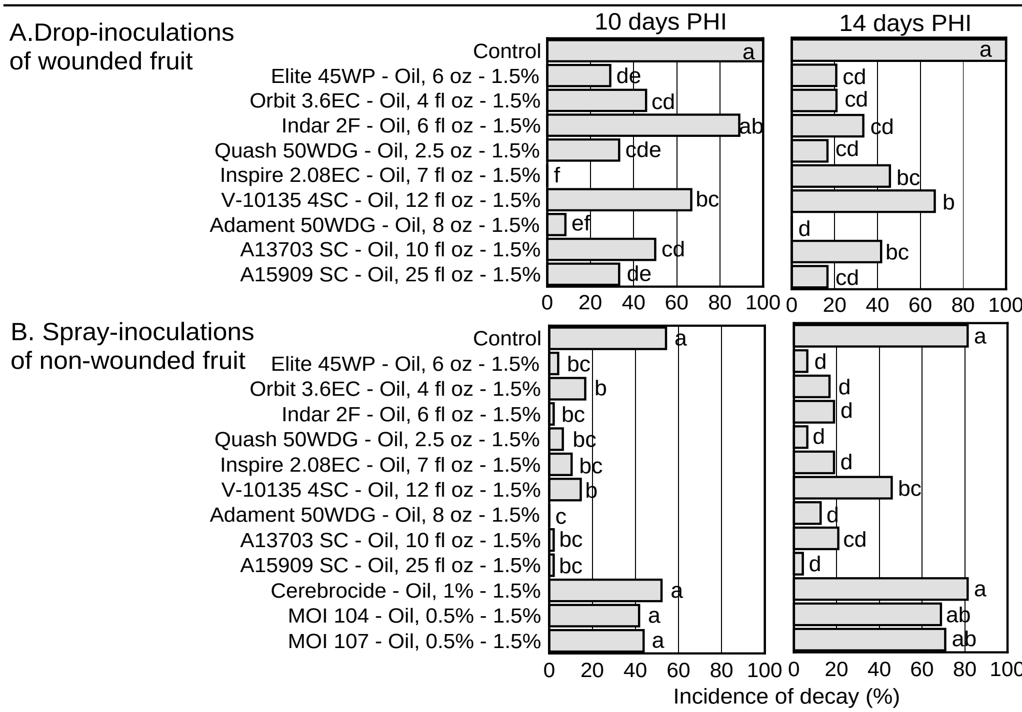
For evaluation of the pre-infection activity, blossoms were collected at popcorn stage, allowed to open in the laboratory. and treated using a hand sprayer. After 24 h blossoms were inoculated with a spore suspension of *M. laxa* (20K/ml). For evaluation of the post-infection activity, blossoms were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

Fig. 2. Efficacy of 14+7-, 7-, and 14-day PHI fungicide applications for management of postharvest brown rot of French prune at UC Davis 2008.



Treatments were applied in the field on 8-14 and 8-21-08 using an air-blast sprayer (100 gal/A). Omni Supreme Spray oil was used. After harvest, fruit were either spray- or wound-inoculated with conidia of *M. fructicola* (30,000 conidia/ml). Fruit were then incubated for 7 days at 20 C.

Fig. 3. Efficacy of 10- and 14-day PHI fungicide applications for management of postharvest brown rot in a field trial in Sutter-Yuba Co. 2008.



Treatments were applied in the field on 8-18-08 using an air-blast sprayer (100 gal/A). Omni Supreme Spray oil was used. After harvest, fruit were either spray- or wound-inoculated with conidia of *M. fructicola* (30,000 conidia/ml). Fruit were then incubated for 7 days at 20 C.