

Annual Report - 2015

Prepared for the Dried Plum 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:	Second Year
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Acknowledgement:	SunSweet Growers Cooperative

SUMMARY OF RESEARCH ACCOMPLISHMENTS DURING 2015

1. **Brown rot blossom blight.** Natural incidence of blossom blight in 2015 was very low, and data on fungicide efficacy could only be obtained from laboratory studies using detached blossoms. Among conventional fungicides, single-active-ingredients (Rhyme, Quash, Fontelis, Kenja, and EXP-1), tank mixtures (Ph-D+Tebucon and Kenja+ a QoI or a DMI fungicide), as well pre-mixtures (Luna Experience, Luna Sensation, Merivon, and experimentals EXP-2 and -3) demonstrated excellent activity. The biocontrol Botector and the natural product Fracture were less effective but still significantly reduced the incidence of infection from that of the control.
2. **Bacterial blossom blast.** Due to unfavorable environmental conditions in the spring of 2015 (warm temperatures and low rainfall), no disease developed.
3. **Fruit brown rot.** In applications done at 130 gal/A in combination with 1.5% oil, all fungicides evaluated significantly reduced the incidence of brown rot when harvested fruit were non-wound-inoculated. After wound-inoculation, only treatments containing FRAC group 3 (i.e., a DMI that has locally systemic activity) resulted in very low levels of brown rot, whereas the contact fungicides Fontelis, Luna Sensation, and Merivon, as well as the fermentation product polyoxin-D were less effective. Additionally, the DMI fungicides provided excellent control of brown rot of exposed fruit and of fruit in clusters on the tree.
4. In **baseline sensitivity** studies for *M. fructicola*, the sensitivity range for polyoxin-D among 32 isolates was determined to be 0.08 to 3.6 ppm (mean 1.34 ppm). Ranges for the SDHIs boscalid, fluxapyroxad, penthiopyrad, and fluopyram were 0.024 to 0.386 ppm (mean 0.118 ppm), 0.004 to 0.050 ppm (mean 0.019 ppm), 0.004 to 0.076 ppm (mean 0.023 ppm), and 0.004 to 0.132 ppm (mean 0.021 ppm), respectively.
5. **Rust.** In a late-season study on management of rust, most fungicides were highly effective. Ph-D was also effective and highly efficacious when mixed with a FRAC Group 3 fungicide. Protexio was the least effective.
6. **Contamination of dried plums with *Aspergillus* species.** When dried fruit from lots from the 2015 harvest were re-hydrated and incubated at high relative humidity, growth of xerotolerant *Aspergillus* spp. occurred at high incidence on all samples currently evaluated. The origin of *Aspergillus* spp. contamination on dried plum fruit is still unclear. These fungi only develop when fruit are improperly dried, not stored under proper environmental conditions, or when fruit are re-hydrated improperly during storage.

INTRODUCTION

Brown rot, caused by *Monilinia* species is the most important blossom and preharvest disease of prune in California. In many growing areas of the state, *M. laxa* is the primary pathogen on blossoms, whereas *M. fructicola* is the main pathogen on fruit. Still, both species can be found causing blossom blight and fruit rot depending on the geographical production areas in California. Currently, fungicide treatments that are properly timed are the most effective method to control this disease. Highly effective fungicides of different classes are being evaluated: the currently registered FRAC group (FG) 2 Rovral/Nevado/Iprodione; FG 3 Tebucon/ Toledo, Indar, Tilt/Bumper, Quash, and Rhyme; the recently registered FG 7 Fontelis; FG 9 Scala and Vanguard; FG 11 Abound and Gem; and FG 17 Elevate and Protexio (pending) (Fig. 1). Pre-mixtures have also provided excellent control, and products evaluated include: FG 3/9 Inspire Super; FG 3/11 (Quadris Top, Quilt

Excel); and FG 7/11 (Pristine and the recently registered Merivon). Pending registrations on dried plum include FG 7 Kenja, and the pre-mixtures Luna Sensation (FG 7/11- fluopyram + trifloxystrobin), Luna Experience (FG 3/7 - fluopyram + tebuconazole), and Viathon (FG 3/33 – tebuconazole + potassium phosphite). Pre-mixtures are highly effective, consistent, and provide resistance management on stone fruit crops because they have two modes of action.

We also continued our evaluations of the newly registered FG 19 polyoxin-D (Ph-D; Oso) the natural product Fracture (active ingredient is an extract of *Lupinus alba*), and the biocontrol Botector (*Aureobasidium pullulans*). Results obtained in 2013-2015 demonstrated good to intermediate brown rot blossom blight control in our trials. Polyoxin-D was also effective against fruit brown rot. The active ingredients of these products have exempt status in the United States. Potentially, the National Organic Materials Review Institute (OMRI) could certify some formulations for use in the organic production of stone fruit including prune. Thus, these products could be critical developments for the organic production segment of the dried plum industry, as well as to conventional growers because preharvest rotation programs need to be designed that prevent the overuse of any one fungicide mode of action (FG). Therefore, evaluation of polyoxin-D, Botector, and possibly Fracture for management of brown rot and other diseases of prune was a focus of our field trials in 2015.

Comparative evaluations of treatments can be helpful in making decisions on selection of materials and treatment timing. For example, fungicides with a good post-infection activity (i.e., ‘kick-back action’) could be applied as a single, delayed bloom application instead of the common two-spray program when disease pressure is low. This information can also be applied to preharvest treatments when unexpected rains delay fungicide applications for 1-2 days and materials with post-infection activity are needed. Several highly effective fungicides are necessary for managing any particular disease of a crop. This allows for rotations and reduces the risk of selecting for resistance. Tank- or pre-mixtures with older multi-site mode of action fungicides such as captan and chlorothalonil (e.g., Bravo/Echo/Equus) for russet scab also need to be evaluated alone and in combination with newer fungicides as bloom treatments.

In our fungicide field programs we are also demonstrating how to improve the efficacy of preharvest fungicide treatments. The addition of a summer spray-oil significantly increases the efficacy of most fungicides in reducing brown rot. These studies need to be continued with other adjuvants. We also demonstrated that preharvest fungicide applications at higher water volumes (i.e., 160 vs. 80 gal/A) in most cases significantly improved fungicide efficacy on fruit developing in clusters inside the tree canopy. The overall objective is to rotate products representing different FGs and using any one of the FGs only once (or twice) per season. Rotations of pre-mixtures that alternate at least one of the FGs in the mixture are part of resistance management strategies.

In some years with spring and summer rainfall, early season (e.g., early summer) epidemics of prune rust caused by the fungus *Tranzschelia discolor* can cause defoliation and subsequent direct (e.g., sunburn) and indirect (e.g., re-foliation of trees and reduced bloom in the subsequent season) crop losses. In the last few years, we have identified new effective materials in FGs 3, 7, 11 and 19, as well as pre-mixtures such as 3/11 and 7/11 products. Fungicides and integrated approaches need to be evaluated in season-long disease management programs that take into account the control of multiple diseases such as brown rot and prune rust.

Another disease that we are studying is bacterial blast of blossoms and bacterial canker of woody tissues of prune and other stone fruit crops caused by *Pseudomonas syringae* pv. *syringae* and other pathovars. Bacterial blast and canker are associated with nematode root damage and cold, wet environments. Blossom blast is associated with cold injury. With bacterial infection, blossoms become dark to black in color, wilt, and die. Copper treatments have been used with inconsistent results for years. Copper can be phytotoxic to blossoms, and we have shown that pathogen populations have developed moderate copper resistance. We will continue experiments to validate or provide “proof of concept” that the new antibiotic kasugamycin is effective on prune. This product was federally registered in September 2014 on pome fruit for fire blight management, and registration is pending on almond, cherry, and walnut. Unfortunately, no disease was detected in our field studies last year. Thus, we are still defining experimental conditions to obtain efficacy data on prune. The industry has never had a highly effective material available for management of bacterial blossom blast and our studies could potentially lead to a major advancement for the dried plum industry.

Isolation and identification of molds on dried plums were pursued at the request of farm advisors. Our

emphasis has been on species of the genus *Aspergillus*, which are the most commonly found fungi on dried plums. Of these, *A. flavus*, *A. niger*, and *A. carbonarius* are of major concern because some strains can produce aflatoxins (*A. flavus*) and ochratoxins (*A. niger*, *A. carbonarius*) that are highly toxic. During five years of samplings, nine species of *Aspergillus* were obtained from fresh and dry prune fruit and characterized using molecular and cultural characteristics. These species were shown to be xerophilic and thermophilic. Additionally, a xerotolerant, toxin-producing Basidiomycota fungus, *Wallemia* sp., was found in 2014. These fungi only develop on fruit that are improperly dried, not stored under proper conditions, or re-hydrated improperly during storage.

By evaluating surface sterilized and non-sterilized fruit, we showed that most of the molds are surface contaminants. In 2013-14, temperature studies demonstrated that most species of *Aspergillus* could not survive 18 h at 75C (167F). In 2014 and 2015, however, these fungi survived commercial drying processes and were thought to contaminate the fruit before drying and most likely during harvest. Thus, in 2015, we continued with our sampling of dried plums and we continued evaluations of surface sterilization methods in preventing contamination and storage decay of dried plums.

OBJECTIVES

1. Evaluate the efficacy of new fungicides, pre-mixtures, polyoxin-D, and biocontrols representing different modes of action for brown rot blossom blight and brown rot fruit rot in laboratory and field trials, as well as rust in field trials.
 - a. Pre- and post-infection activity of selected fungicides against brown rot blossom blight and fruit rot.
 - b. Evaluation of preharvest fungicides in combination with selected spray adjuvants
 - c. Evaluation of fungicide efficacy against prune rust.
2. Evaluate the efficacy of new products against bacterial blast in flower inoculation studies and/or canker in stem inoculation studies.
 - a. Biologicals/natural products (e.g., Actinovate, polyoxin-D, Blossom Protect).
 - b. Antibiotics – kasugamycin and other antibiotics.
 - c. Systemic acquired resistance (SAR) compounds – Actigard, PM-1, and possibly others.
3. Continue to develop baseline sensitivity data for SDHI and new fungicides (e.g., polyoxin-D).
4. Survey of *Aspergillus* species on dried plum, evaluate heat tolerance of these fungi, and surface sterilization of harvested fruit prior to drying plums.

MATERIALS AND METHODS

Evaluation of fungicides for management of brown rot blossom blight. Fungicide pre- and post-infection activity was evaluated in laboratory studies. For post-infection activity, blossoms at popcorn stage were collected and allowed to open. They were then inoculated with a conidial suspension of *M. fructicola* (2×10^4 conidia/ml), treated with selected fungicides after 19 h using a hand sprayer, and incubated at 20C. For pre-infection activity, blossoms were first treated with a fungicide 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 9.4.

Evaluation of bactericides, biocontrols and SARs for management of bacterial blast. Blossoms at popcorn stage were treated with selected treatments (the antibiotic Kasumin, the biologicals Actinovate, Double Nickel 55, Blossom Protect, and the SARs – Actigard and PM-1) using a hand sprayer, inoculated with a suspension of *P. syringae* (1×10^7 cfu/ml), and covered with a plastic bag overnight. Bags were removed the next morning after 16 h of wetness and blossoms were evaluated for disease 7 to 10 days after inoculation. Three replications of eight blossoms were used for each treatment. Data were analyzed using analysis of variance and least significant difference (LSD) mean separation procedures of SAS 9.4.

Evaluation of fungicides for management of preharvest fruit decay. Field trials to evaluate preharvest fungicide applications for control of fruit brown rot were done in a commercial orchard in Yuba Co. Treatments were applied 14 days before harvest using an air-blast sprayer calibrated at 130 gal/A. All fungicides were applied in combination with 1.5% of a spray oil (i.e., Gavicide). Single fruit from the tree perimeter (exposed

fruit) or fruit from inside clusters (10 fruit each from each of four single-tree replications) were collected at harvest and wound-inoculated (wounds ca. 1 mm x 2 mm x 2 mm deep) with conidia of *M. fructicola* (3×10^4 conidia/ml) or non-wound inoculated (5×10^5 conidia/ml). Outside-canopy fruit were inoculated on the exposed side of the fruit, whereas fruit from inside clusters were inoculated at sites where fruit touched each other. After inoculation, fruit were incubated for 7-10 days at 20 C. Data were analyzed using analysis of variance and least significant difference (LSD) mean separation procedures of SAS 9.4.

Evaluation of fungicides for management of prune rust. A field trial was established in a commercial orchard in Yuba Co. to evaluate the efficacy of new fungicides. Fungicides were applied on 7-30-15 (as a preharvest application for management of fruit brown rot) and on 9-3-15 specifically for fall season rust management. Disease was evaluated on 10-29-15. Two disease severity readings were done on each of four quadrants of each tree using a scale from 0 (= no disease) to 5. Data were analyzed using analysis of variance and least significant difference (LSD) mean separation procedures of SAS 9.4.

Evaluation of baseline sensitivities of *M. fructicola* isolates against polyoxin-D and SDHI fungicides. Thirty-nine isolates of *M. fructicola* from our fungal collection obtained between 1992 and 2005 were cultured on V8 agar, and conidia were used in spiral gradient dilution assays with polyoxin-D, boscalid, fluopyram, fluxapyroxad, and penthiopyrad. EC_{50} values were determined for mycelial growth after 3 days of incubation of the fungicide-amended plates as described previously. Data were summarized graphically.

Contamination of dried plums in storage with *Aspergillus* species. Dried fruit from prune lots of the 2015 harvest were obtained in November and December from a processing plant. Samples of 130-160 fruit were re-hydrated in sterile distilled water for 1 h, placed into plastic trays in plastic boxes, bagged, and incubated at 25 C, >95% RH for up to 4 weeks. Fruit were misted periodically with water to maintain high humidity. Fruit were evaluated weekly for fungal surface growth and fungi were identified by genus.

RESULTS AND DISCUSSION

Overview. Due to a very dry spring in 2015, the natural incidence of many diseases including bacterial blast and brown rot blossom blight was very low. Inoculations with the bacterial blast pathogen were also inconsistent due to warm temperatures. Blast generally is most severe during cold, wet conditions during bloom that predispose flowers to infection. Data for brown rot management could be obtained in inoculation studies. Overall, fungicide usage was low and this resulted in no new reports of fungicide failures or suspected resistance in pathogen populations from the industry.

Evaluation of fungicides for management of brown rot blossom blight. In laboratory studies using detached blossoms, five single-fungicides, three mixtures, and six pre-mixtures significantly reduced the incidence of stamen infections to very low values when applied before or after infection, and thus, were highly effective (Fig. 2). The biocontrol Botector and the natural product Fracture was also significantly reduced the incidence of stamen infections from that of the control, but were not as effective as the fungicides. Fracture is registered in California on stone fruit and other crops. Botector is currently registered on grapes and berry crops in California and registration on stone fruit crops is pending.

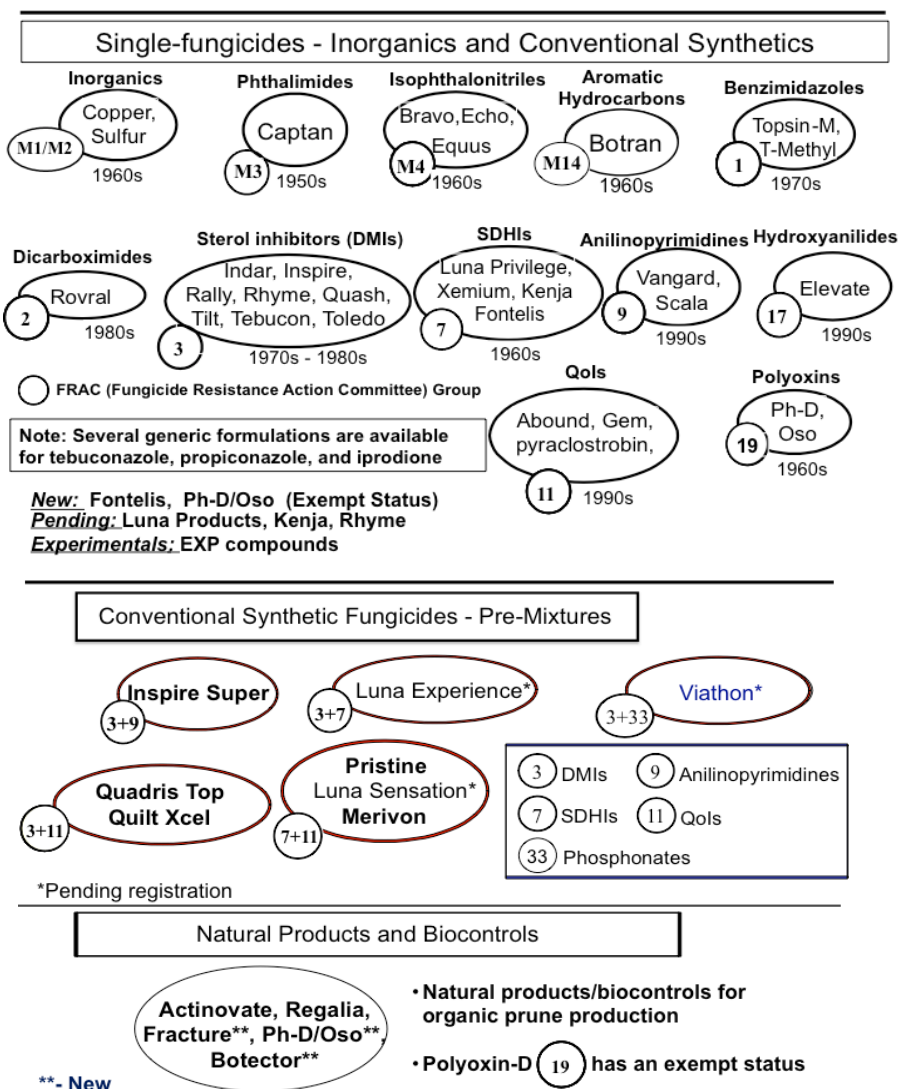
The post-infection activity was evaluated in these experiments to assess the potential efficacy of the treatments as a single application in a delayed bloom application when recent blossom infections need to be controlled. This strategy has been successfully used on other tree crops in spring seasons when precipitation is low to moderate.

Currently registered fungicides with high pre- and post-infection activity include single active ingredients such as the FG 2 dicarboximide Rovral (-oil) and generics; the FG 3 DMIs Tilt (and generics), Indar, Tebucon (and other generics), and Quash; the FG 7 SDHI Fontelis; the FG 9 anilinopyrimidines (APs) Vanguard and Scala; and the FG 17 hydroxylanilide Elevate. Pre-mixtures include the FG 7/11 Pristine and Merivon, the FG 3/11 Quilt Xcel and Quadris Top, and the FG 3/9 Inspire Super. These pre-mixtures provide consistent, broad-spectrum high efficacy with built-in resistance management.

Evaluation of fungicides for management of fruit brown rot. We previously demonstrated that the efficacy of preharvest fungicides applications to prevent losses from fruit brown rot is considerably improved when used in combination with agricultural, spray oil (e.g., 415, Gavicide). We also demonstrated that some fungicides when applied at an increased gallonage of 130 gal/A provide better protection of fruit inside clusters.

Therefore, all treatments were evaluated using these methods. Preharvest fungicides were applied 14 days PHI in a commercial orchard and fruit harvested from the outside of the tree canopy or from inside fruit clusters were non-wound- or wound-inoculated with the brown rot pathogen. In the first trial where fruit were harvested from

Fig. 1. Fungicides registered and in development for managing prune diseases



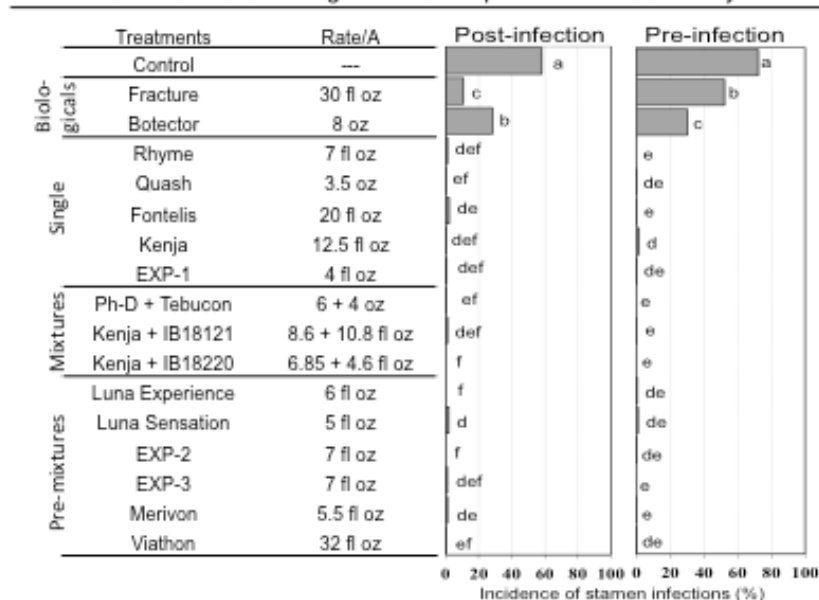
the outside tree canopy, all fungicides evaluated significantly reduced the incidence of brown rot as compared to the control after non-wound inoculation (Fig. 3). Most fungicides were highly effective, but Ph-D, Protexio, and Fontelis were less effective. After wound-inoculation, only fungicides containing a FG 3 DMI and the new experimentals EXP-1, -2, and -3 were effective; efficacy of Fontelis, Luna Sensation, and Merivon was much reduced as compared to the non-wound inoculation studies. Therefore, DMIs have local systemic activity, whereas other fungicides are contact materials.

In the second trial where fruit were either harvested from the outside of the tree canopy or from within clusters and were then non-wound inoculated, Quash and the Quash-Protexio and Ph-D-Bumper mixtures were highly effective in reducing brown rot on both types of fruit (Fig. 4). Merivon was highly effective on the exposed fruit, and moderately effective on the clustered fruit indicating that fruit clusters are often so tight that even at

higher gallonage, applications sometimes cannot provide adequate coverage. Protexio and Ph-D showed intermediate efficacy on both types of fruit.

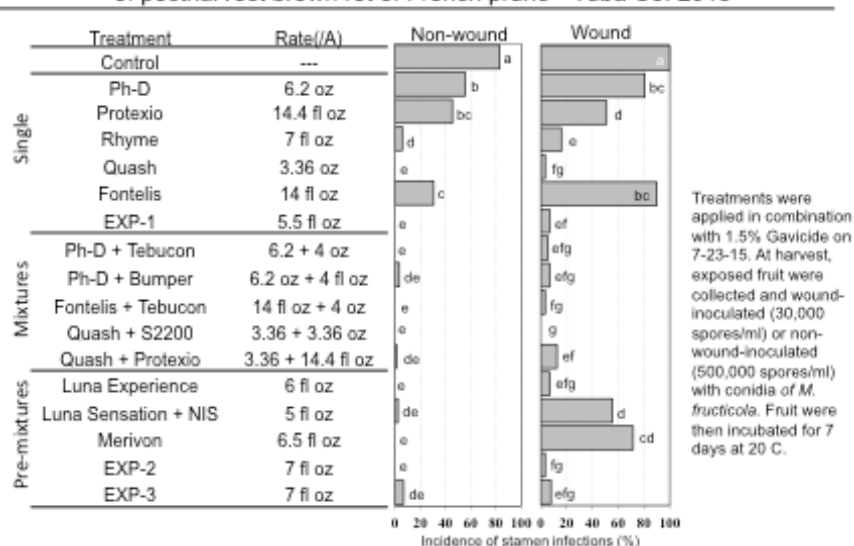
Thus, several fungicides with high efficacy are available to the industry to protect fruit from brown rot decay even when applied 14 days before harvest. The highest treatment efficacy is obtained when fungicide-oil mixtures are applied at higher volumes. Spray oil provides improved coverage of fruit (acting as a spreader on waxy fruit surfaces) and likely also improves penetration of some fungicides into the fruit. Not all fungicides, however, may be compatible with oils. It is important to prevent fruit injuries during and after harvest. To reduce brown rot of mechanically harvested fruit in bins, fruit should be processed for drying within 48 h of harvest.

Fig. 2. Efficacy of pre- and post-infection treatments for management of brown rot blossom blight of French prune in the laboratory 2015



For evaluation of the pre-infection activity, closed blossoms were collected in the field, allowed to open, and treated in the laboratory using a hand sprayer. After 4 h blossoms were inoculated with a spore suspension of *M. fructicola* (20K/ml). For post-infection activity, blossoms were inoculated, incubated at 22 C, and treated after 19 h. Blossoms were evaluated for stamen infections after 4-5 days of incubation at 20 C.

Fig. 3. Efficacy of 14-day preharvest fungicide treatments for management of postharvest brown rot of French prune - Yuba Co. 2015



Evaluation of fungicides for management of prune rust. The severity of rust was low in the 2015 growing season. In a late-season study, two applications of a range of fungicides (the first application was part of the pre-harvest brown rot fruit decay study and the second one was applied after harvest) all significantly reduced the incidence and severity of rust developing in the upper tree canopy as compared to the non-sprayed control trees (Fig. 5). Protexio did not significantly reduce disease severity from the control, and Ph-D showed intermediate effectiveness in reducing rust. All other fungicides were highly effective. These data indicate that effective treatments against prune rust are available. Over the years, treatments that include FGs 3, 7, or 11 have

been the most effective. Prune rust occurs sporadically and protective treatments are generally not warranted. These fungicides, however, should still be very effective if applied when the very first rust lesions are detected in an orchard during regular scouting and monitoring of orchards.

Fig. 4. 14-day PHI treatments for management of brown rot decay of French prune – Yuba Co. 2015
- Efficacy on exposed fruit and fruit inside clusters -

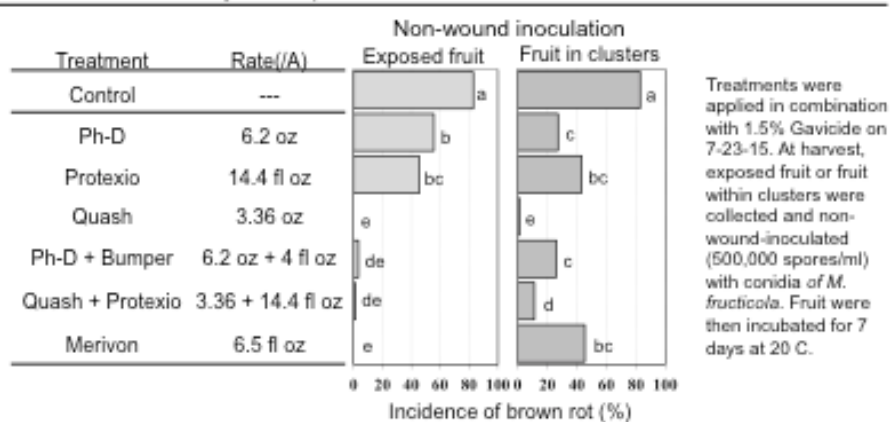
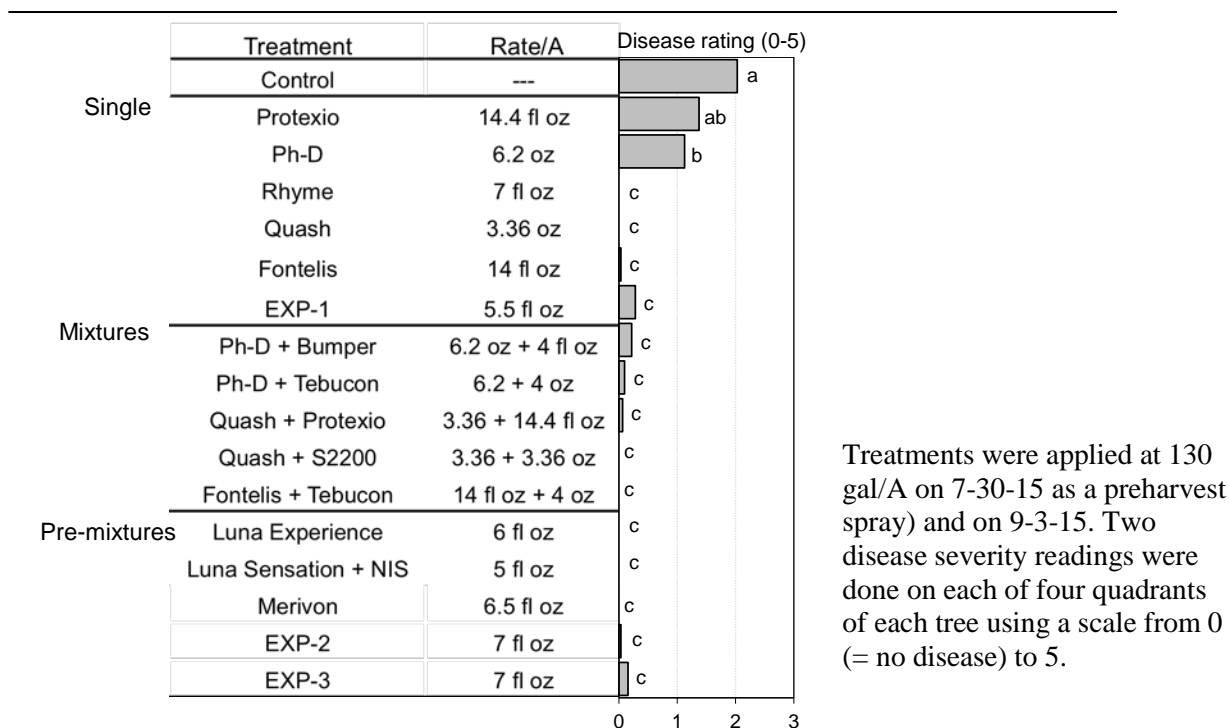


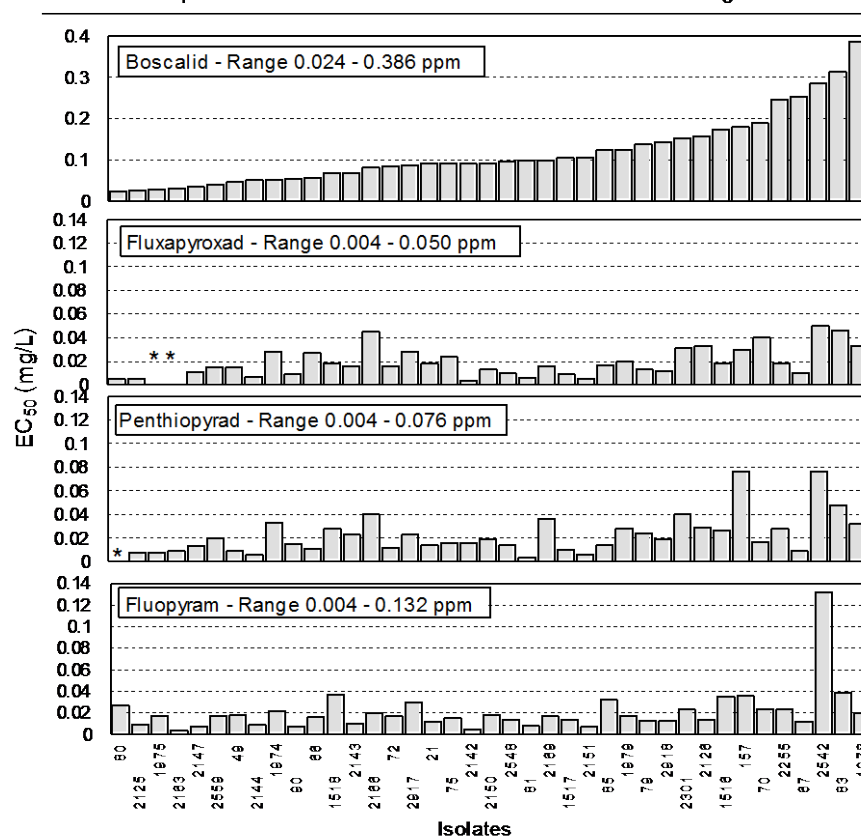
Fig. 5. Efficacy of fungicide applications for management of rust of French prune - Yuba Co. 2015



Evaluation of baseline sensitivities of *M. fructicola* isolates against polyoxin-D and SDHI fungicides.

Baseline sensitivities were developed as part of our ongoing research on resistance monitoring and management. The sensitivity range for polyoxin-D among 32 isolates was determined to be 0.08 to 3.6 ppm (mean 1.34 ppm). The evaluated FG 7 SDHI fungicides belong to three subgroups, fluxapyroxad and penthiopyrad are in the same sub-group, and boscalid and fluopyram are in two other sub-groups. Boscalid had a wider range and higher inhibitory values than the other three compounds (Fig. 6). Of interest is one isolate (isolate 2542) that showed high EC_{50} values for boscalid and fluopyram, and was also in the high- EC_{50} range for the other two fungicides. Cross-resistance between some of the sub-classes is considered low, but our data indicate that cross resistance does exist and that the risk for resistance development against SDHIs is high. Isolates used were collected between 1992 and 2005, a time before the widespread use of this fungicide class. The occurrence of isolates with reduced sensitivity in a relatively small sample indicates that isolates with lower natural sensitivity are present at relatively high incidence. There is a risk that such isolates may adapt to higher fungicide concentrations, emphasizing the strict rotation between fungicide classes as a critical component of fungicide resistance management.

Fig. 6. *In vitro* sensitivity of isolates of *Monilinia fructicola* collected from stone fruit crops in California between 1992 and 2005 to four SDHI fungicides



In vitro sensitivities for mycelial growth were determined using the spiral gradient dilution method. Isolates are in the same order in each of the graphs. *

Contamination of dried plums in storage with *Aspergillus* species. Samples of plums were taken after drying from several 2015 harvest lots. After re-hydrating fruit and incubation for 4 weeks at high relative humidity, growth of *Aspergillus* spp. occurred at high incidence (80 to 95%) on three of three lot samples that have been evaluated currently. Based on morphological characteristics, four species of *Aspergillus* were observed, including yellow-green (potentially *A. flavus* or *E. repens*) and black *Aspergilli* (probably *A. niger*, *A. carbonarius*, or *A. brasiliensis*), and species identification is ongoing. There was a low incidence (<10%) of a xerotolerant, toxin-producing Basidiomycota fungus, *Wallemia* sp., that was also found in 2014, and of fungi belonging to other genera including *Alternaria* and *Monilinia* spp. Thus, as in previous years, the incidence of contamination was high, but the diversity of fungal organisms was low.

Fungal growth on fruit that were surface-sterilized with 100 ppm chlorine for 1 min after re-hydration was low, and the incidence of *Aspergillus* spp. growth was very low (<5%). These results are similar to previous years' data and they indicate that fungal contamination is superficial, because little fungal growth developed on surface-sterilized fruit.

Aspergillus spp. are known to be xerotolerant and they can occur on dried plums that have a high sugar content, whereas most other fungi cannot survive due to low water availability. In our studies on thermal death points of hydrated mycelial agar plugs, some species still survived incubation at 75C (167F) for 18 h, although at low incidence. Conidia were more heat-sensitive than mycelium, and conidia of all species examined were completely inactivated after 14 h at 70C (158F). At 75C (167F), conidia of all species were inactivated after 5 h except for *A. brasiliensis* where 10 h were needed to kill all conidia. Thus, all species of *Aspergillus* identified from prune fruit to date were killed at temperatures (71 to 85C or 160 to 185F) and drying durations used in commercial fruit drying. This would imply that the fruit were not sanitized and dried properly or that contamination occurs after drying in storage. It is possible, however, that our laboratory assays on heat sensitivity

are not reflecting the exact conditions on the fruit surface. Fungal structures on the fruit surface may be in a dehydrated state and more heat tolerant than when they are in a hydrated state in an aqueous environment. Fruit contamination before the drying process is supported by the fact that last and this year's samples were taken shortly after drying. Thus, xerotolerant fungi may survive commercial drying as surface contaminants, especially if drying conditions are not carefully monitored.

The origin of *Aspergillus* spp. contamination of dried plum fruit is still unclear, but improper sanitation, drying, and storage of fruit and improper re-hydration during storage may have key roles. Because *Aspergillus* spp. contamination was determined to be mostly superficial on the fruit, one of the most important strategies to prevent fungal growth from developing before processing is to ensure that storage facilities are dry and well ventilated, as well as maintain fruit moisture content between 18-22%. In order to prevent any contamination, surface sterilization procedures may need to be developed and implemented immediately after harvest and before drying. A standard method for surface disinfestation is the use of sodium hypochlorite washes. Concentrations of 50 to 100 ppm of the active ingredient hypochlorous acid are commonly used in the fruit industry.