Annual Report - 2016

Prepared for the Dried Plum Board of California

Title:	Epidemiology and management of blossom, leaf, and fruit diseases of prune
Status:	2nd Year
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SUMMARY OF RESEARCH ACCOMPLISHMENTS DURING 2016

1. ***Brown rot blossom blight.*** Natural incidence of blossom blight in 2016 was moderate, and data on fungicide efficacy was obtained in laboratory studies using detached blossoms and in field studies. Among conventional fungicides, single-active-ingredients (Rhyme, Quash, UC-1, EXP-A, and R-106506), the tank mixture of Quash and Intuity, and the pre-mixtures (Luna Experience, Luna Sensation, Merivon, Quadris Top, and experimentals UC-1 and UC-2B) demonstrated excellent activity.

   The biocontrol Serenade Opti was moderately effective in laboratory assays; whereas Botector (*Aureobasidium pullulans*) and the natural product Fracture (*Lupinus alba*) were very effective in field trials and significantly reduced blossom blight from that of the control, similar to conventional fungicides.

2. ***Bacterial blossom blast.*** Due to unfavorable environmental conditions (strong winds) and failed experimental methods (bagging of branches with flowers) during bloom in the spring of 2016, no disease developed.

3. ***Fruit brown rot***. In applications done at 130 gal/A in combination with 1.0% oil, all fungicides evaluated significantly reduced the incidence of brown rot when harvested fruit were non-wound-inoculated. Treatments containing FRAC group 3 (i.e., a DMI that has locally systemic activity) as well as EXP-A, -AD, and -AF resulted in very low levels of brown rot. The contact fungicides Luna Sensation and Merivon, as well as FRAC Group 9, the experimentals R-106506 and UC-1 were slightly less effective.

4. ***Rust***. In a late-season study on the management of rust, most fungicides were highly effective. Rhyme, Luna Sensation, Luna Experience Merivon, Quadris Top, and the experimentals UC-2B and IL-54111 were highly effective; whereas FRAC Group 9 fungicides and the experimentals R-106506 and EXP-A were the least effective.

5. ***Contamination of dried plums with* Aspergillus *species.*** When dried fruit from 14 lots from the 2015 harvest were re-hydrated and incubated at high relative humidity, the incidence of fruit contaminated with *Aspergillus* spp. ranged from 20 to 100%, but was mostly >75%. Several colony types were observed. Molecular studies indicated that the majority of isolates belonged to *Eurotium repens*, the sexual state of *A. reptans*. Other species included *A. niger, A. carbonarius*, *A. ochraceus*, and *A. tamarii*. All 22 samples including fruit with known *A. flavus* contamination that were submitted to DFA for aflatoxin testing were negative for aflatoxin.

   When conidia of eight species of *Aspergillus* were incubated on dried plums at an average temperature of 71.5C for 18 h, >95% of conidia were inactivated as compared to incubation at 25C. Thus, these species were all inactivated at temperatures (71-85C or 160-185F) and drying durations used in commercial fruit drying. The origin of *Aspergillus* spp. contamination of dried plum fruit is still unclear. As a general strategy to minimize fungal contamination, fruit storage facilities should be dry and well ventilated, and we still recommend fruit surface sterilization immediately after harvest and before drying.

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 have been identified over the years: the currently registered FRAC group (FG) 2 Rovral/Nevado/Iprodione; FG

3 Tebucon/ Toledo, Indar, Tilt/Bumper, Quash, and recently registered Rhyme; the FG 7 Fontelis; FG 9 Scala and Vangard; FG 11 Abound and Gem; FG 17 Elevate, and FG 19 Ph-D or Oso (Table 1). Pre- mixtures also provide excellent control, and products evaluated include: FG 3/9 (Inspire Super); FG 3/11 (Quadris Top, Quilt Excel); FG 7/11 (Pristine, Merivon, and the recently registered Luna Sensation), and FG 3/7 (Luna Experience). A pending registration on dried plum includes FG 7 Kenja. Several experimental pre-mixtures such as UC-2B, EXP-AD, -AF, IL-54111, and R-106506 are also planned for registration. 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 biocontrols Serenade Opti, and Botector (*Aureobasidium pullulans*). Results obtained in 2013-2016 demonstrated good to intermediate brown rot blossom blight control. Polyoxin-D in mixture with Scala was also very effective against fruit brown rot. Products such as Ph-D and Oso containing the active ingredient polyoxin-D have exempt status in the United States. Potentially, the National Organic Standards Board and the 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).

Laboratory inoculation and field studies provide information on the protective and local systemic action of compounds and should help growers and PCAs in the selection of materials and treatment timing to optimize individual management programs. Fungicides that have post-infection activity (i.e., 'kick-back action') could be applied as a single, delayed bloom application when environmental conditions are not favorable for disease. Under high disease pressure, a two-spray bloom program should be followed using protective or locally systemic fungicides. 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. Having several highly effective fungicides belonging to different FRAC Groups for managing diseases of prune allows for rotations and reduces the risk of selecting for resistance. 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 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. 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.

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-mixture FGs 3/11, 3/7, 3/19, and 7/11. 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.

At the request of farm advisors, another objective of our research in the last several years was the occurrence and identification of molds on dried plums with an emphasis on *Aspergillus* species. Prune fruit were obtained from 14 lots of the 2015 crop after drying, and the presence of fungal contamination was determined after incubation of fruit at high humidity. We previously determined that conidia of all *Aspergillus* species that we have identified from prune fruit to date were killed when incubated as aqueous suspensions at temperatures of 71-85C (160-185F) and exposure durations used in commercial fruit drying. This indicated that contamination with *Aspergillus* species likely originated after fruit drying and during storage in the processing facility. In 2016, we evaluated the heat sensitivity of these species by incubating conidia directly on the surface of dried plum fruit. This was done to rule out that the fruit micro-environment affects the heat sensitivity of conidia. Additionally, fruit samples with known A. *flavus* contamination were submitted to DFA for aflatoxin testing.

OBJECTIVES

1. Evaluate the efficacy of new fungicides (e.g., polyoxin-D, EXP-A, UC-1), pre-mixtures (Viathon, EXP-AD-, -AF, UC-2B), and biocontrols (Botector, Fracture) 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, *Bacillus*-containing products, Blossom Protect – *Aureobasidium* sp.).
   b. Antibiotics – Kasugamycin and other antibiotics.
3. Continue to develop baseline sensitivity data for SDHI and new fungicides (e.g., EXP-A, UC-1).
4. Survey of *Aspergillus* species on dried plum, identify the species using molecular methods, and test for aflatoxin on fruit samples in cooperation with DFA.
   a. Evaluate heat tolerance of dry conidia on dried plum fruit surfaces
   b. Evaluate surface sterilization with on harvested fruit prior to drying plums
   c. Develop a UC-extension bulletin on proper drying and storage of dried 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 mean separation procedures of SAS 9.4.

Evaluation of bactericides and biocontrols for management of bacterial blast. Flowers at full bloom on the trees were treated with selected treatments (the antibiotic Kasumin, the biologicals Actinovate, Double Nickel 55, and Blossom Protect,) 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. Data were analyzed using analysis of variance and 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 7 days before harvest using an air-blast sprayer calibrated at 130 gal/A. All fungicides were applied in combination with 1.0% of a spray oil (i.e., Gavicide). Single fruit (24 fruit from each of four paired replications, 8 trees in total) were collected at harvest and non-wound inoculated with conidia of *M. fructicola* (5

x 10^5 conidia/ml). After inoculation, fruit were incubated for 7-10 days at 20 C. Data were analyzed using analysis of variance and 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 8-9-16 (as a preharvest application for management of fruit brown rot) and on 9-7-16 specifically for fall season rust management. Disease was evaluated on 10-28-2016. Disease severity was determined for four quadrants of each tree using a scale from 0 (= no disease) to 5. Data were analyzed using analysis of variance and mean separation procedures of SAS 9.4.

Contamination of dried plums in storage with* Aspergillus *species. Dried prune fruit from 14 lots of the 2015 harvest were obtained between November 2015 and January 2016. Fruit were surface-sterilized in 100 ppm sodium hypochlorite for 1 min or not surface-sterilized and were then re-hydrated by immersing in sterile water for 3 to 6 h. Fruit were then placed into fruit trays (1-2 fruit/tray cavity) in plastic boxes. There were between 40 and 160 fruit for each sanitized and non-sanitized sample. Fruit were incubated at 20C, >90% RH for 3-5 weeks, misted periodically with water to maintain high humidity, and evaluated for the presence of *Aspergillus* and other fungal species. For evaluation, the number of *Aspergillus* spp. were enumerated for each colony pigmentation (e.g., black, green, olive-green, yellow, yellow-green, orange). Representative isolates were obtained from different colony types by culturing on potato dextrose agar. The level of *Aspergillus* contamination was determined by calculating the average number of *Aspergillus* spp. colonies per fruit.

For the molecular grouping of isolates, an RFLP analysis of the ITS1 region of rDNA was performed as described previously (*see* Annual Report 2012). DNA was amplified using universal primers ITS1 and ITS2 and amplification products were digested with restriction enzymes *Alu*I, *Hin*fI, *Mbo*I, *Rsa*I, *Taq*I, and *Bse*I. DNA fragments were separated in agarose gels, and isolates were grouped according to their fragment patterns. For species identification of representative isolates, a portion of the large sub-unit rDNA region was amplified using primers ITS5 and D2R, and primers D1 and ITS4 were used in sequencing reactions. Sequences were aligned using ClustalW and compared to those of isolates of *Aspergillus* spp. deposited in Genbank and with our own sequences that were obtained previously. A total of 22 samples including 12 fruit with known *A. flavus* contamination, as well as 10 cultures of the fungus were submitted to DFA for aflatoxin testing.

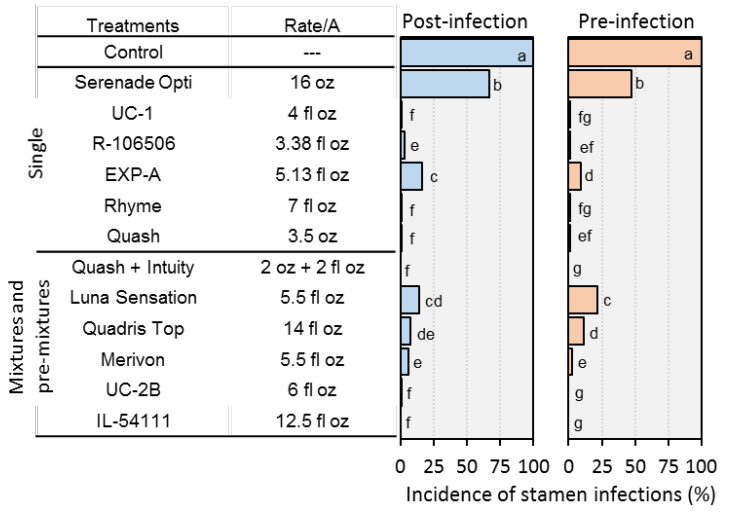
The heat sensitivity of conidia of *Aspergillus* spp. and *Eurotium repens* was previously evaluated using aqueous suspensions. In 2016, we determined the heat sensitivity of conidia on dried plum fruit. For this, conidial suspensions (10^7 conidia/ml) were applied to surface-sterilized dried plum fruit, and fruit were incubated for 18 h at 25C or at an average temperature of 71.5C. Conidia were re-suspended in sterile water, plated onto agar media, and conidial germination was evaluated after 18 h. As a control, conidia were also evaluated for their germination on agar medium following incubation at 25C.

RESULTS AND DISCUSSION

Overview. Rainfall occurred in the spring of 2016, and the natural incidence of brown rot blossom blight was moderate. Data on fungicide efficacy for managing brown rot blossom blight were obtained in laboratory inoculation studies and in field studies where natural incidence of disease was recorded. Later in the summer, natural incidence of rust was also higher than in previous years and results were obtained in field trials. Inoculations with the bacterial blast pathogen, however, were inconsistent due to windy conditions that injured and removed flowers inside bagged branches. Blast generally is most severe during cold, wet conditions during bloom that predispose flowers to infection. Overall, fungicide usage was higher than in previous years, but still there were no new reports of fungicide failures or suspected resistance in pathogen populations from the industry.

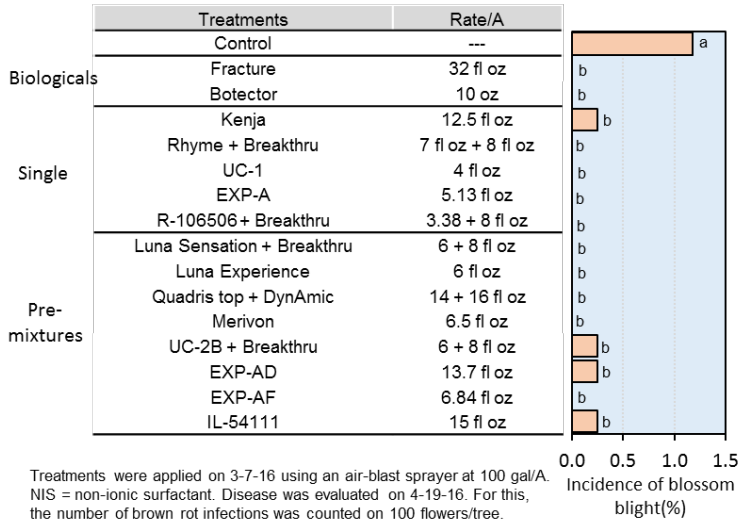
Evaluation of fungicides for management of brown rot blossom blight. In laboratory studies using detached blossoms, five single-fungicides, one mixture, and five pre-mixtures significantly reduced the incidence of stamen infections to very low values when applied before or after infection, and thus, were highly effective (Fig. 1). The new DMI fungicide Rhyme, the experimentals UC-1, R-106506, the experimental tank mixture of Quash and Intuity, and most of the premixtures such as Quadris Top, Merivon, UC-2B, and IL-54111 were highly effective in these trials. The biocontrol Serenade Opti (*Bacillus subtilis*) significantly reduced the incidence of stamen infections from that of the control, but was less effective than conventional fungicides. Serenade Opti is registered in California on stone fruit crops including prune.

Fig. 1. Efficacy of pre- and post-infection treatments for management of brown rot blossom blight of French prune in the laboratory 2016



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. 2. Efficacy of fungicide applications in the field for management of brown rot blossom blight of French prune – Yuba Co. 2016



Treatments were applied on 3-7-16 using an air-blast sprayer at 100 gal/A. NIS = non-ionic surfactant. Disease was evaluated on 4-19-16. For this, the number of brown rot infections was counted on 100 flowers/tree.

In a field trial, Fracture and Botector along with most conventional fungicides including single-site modes of action Rhyme, UC-1, EXP-A, and R106506, as well as the premixtures Luna Sensation, Luna Experience, Quadris Top, Merivon, and EXP-AF showed excellent performance. IL-54111, Kenja, UC-2B, and EXP-AD had a small amount of disease (<0.5%) that was still significantly less than in the untreated control (Fig. 2). The biological controls Serenade Opti, Botector (*Aureobasidium pullulans*), and Fracture (extract of *Lupinus albus*) are currently registered on stone fruit crops in California.

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

Table 1. Efficacy of single mode-of-action fungicides and pre-mixtures against major diseases of prunes (dried plum)

				Brown rot		Jacket rot/	
Fungicide product	**Active ingredients**	**FRAC Group**	**Resistance risk**	**Blossom**	**Fruit rot**	**Green fruit rot**	**Rust**
Topsin-M/T-Methyl/ Incognito*	thiophanate methyl	1	high	++++	+++	++++	----
Rovral + oil	iprodione-oil	2	low	++++	NL^	++++	++
Rovral, Iprodione, Nevado	iprodione	2	low	+++	NL	+++	+
Bumper/Tilt/Propiconazole	propiconazole	3	high	++++	++++	----	++++
Tebucon/Toledo	tebuconazole	3	high	++++	++++	++	+++
Indar	fenbuconazole	3	high	++++	+++	----	+++
Quash	metconazole	3	high	++++	++++	++	+++
Rally	myclobutanil	3	high	+++	+++	----	---
Rhyme	flutriafol	3	high	+++	+++	ND	++++
Fontelis	penthiopyrad	7	high	++++	+++	++++	+++
Vangard	cyprodonil	9	high	++++	+++	+++	---
Scala	pyrimethanil	9	high	++++	+++	+++	---
Abound	azoxystrobin	11	high	+++	+	----	+++
Gem	trifloxystrobin	11	high	+++	++	----	+++
Elevate	fenhexamid	17	high	+++	+++	++++	---
Ph-D, Oso	polyoxin-D	19	high	++	++	+++	++
Luna Experience	tebuconazole/fluopyram	3/7	medium	++++	++++	+++	++++
Inspire Super	difenoconazole/cyprodinil	3/9	medium	++++	++++	+++	+++
Quadris Top	difenoconazole/azoxystrobin	3/11	medium	++++	++++	++	++++
Quilt Xcel	propiconazole/azoxystrobin	3/11	medium	++++	++++	++	++++
Viathon	tebuconazole/KPO_3	3/33	medium	++++	++++	+++	+++
Luna Sensation	fluopyram/trifloxystrobin	7/11	medium	++++	++++	+++	+++
Pristine	boscalid/pyraclostrobin	7/11	medium	++++	++++	+++	+++
Merivon	fluxapyroxad/pyraclostrobin	7/11	medium	++++	++++	+++	+++

^ -Rating: ++++ = excellent and consistent, +++ = good and reliable, ++ = moderate and variable, + = limited and/or erratic, +/- = minimal and often ineffective, ---- = ineffective, ND = no data, NL = not on label,

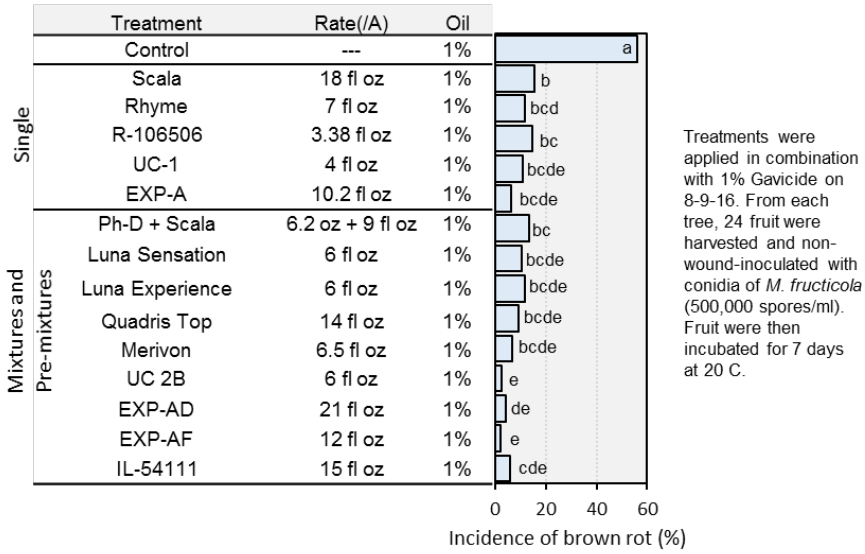
* - Resistant sub-populations have been detected in some pathogens.

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, Rhyme, Tebucon (and other generics), and Quash; the FG 7 SDHI Fontelis; the FG 9 anilinopyrimidines (APs) Vangard and Scala; and the FG 17 hydroxyanilide Elevate. Pre-mixtures include the FG 7/11 Pristine and Merivon, the FG 3/11 Quilt Xcel and Quadris Top, the FG 3/9 Inspire Super, and the FG 3/7 Luna Experience (Table 1). 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 1-1.5% agricultural spray oil (e.g., Gavicide). We also demonstrated that some fungicides when applied at an increased volume of 130 gal/A provide better protection of fruit inside clusters. Therefore, all treatments were evaluated using these methods. Preharvest fungicides were applied 7 days PHI in a commercial orchard and harvested fruit from each tree and fruit were non-wound-inoculated with the brown rot pathogen.

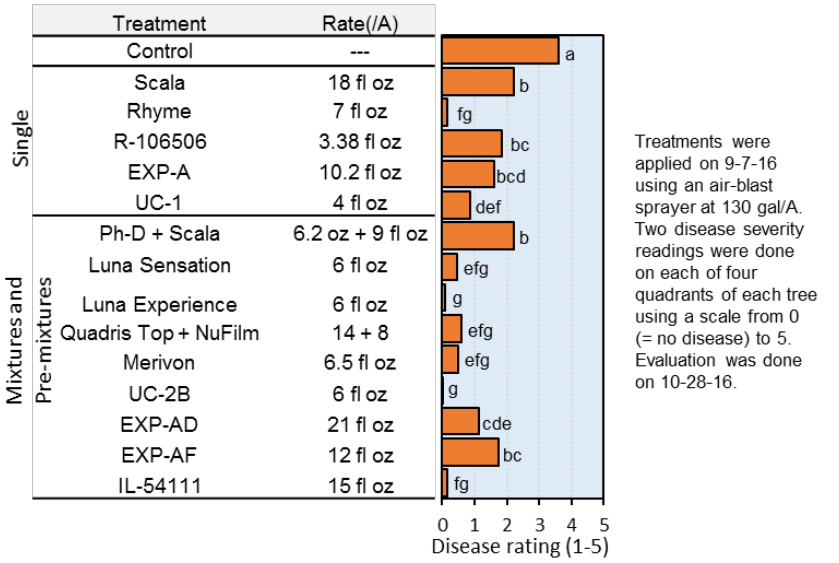
All fungicides evaluated significantly reduced the incidence of brown rot as compared to the control after non-wound inoculation (Fig. 3). Most of the fungicides evaluated were highly effective. The most effective materials with the lowest diseases levels were EXP-A, UC-2B, EXP-AD, EXP-AF, and IL-54111. Fungicides containing DMIs have locally systemic activity, whereas other fungicides function as contact materials.

Fig. 3. Efficacy of 7-day preharvest fungicide treatments for management of postharvest brown rot of French prune - Yuba Co. 2016



Treatments were applied in combination with 1% Gavicide on 8-9-16. From each tree, 24 fruit were harvested and non-wound-inoculated with conidia of *M. fructicola* (500,000 spores/ml). Fruit were then incubated for 7 days at 20 C.

Fig. 4. Efficacy of fungicide applications for management of rust of French prune - Yuba Co. 2016

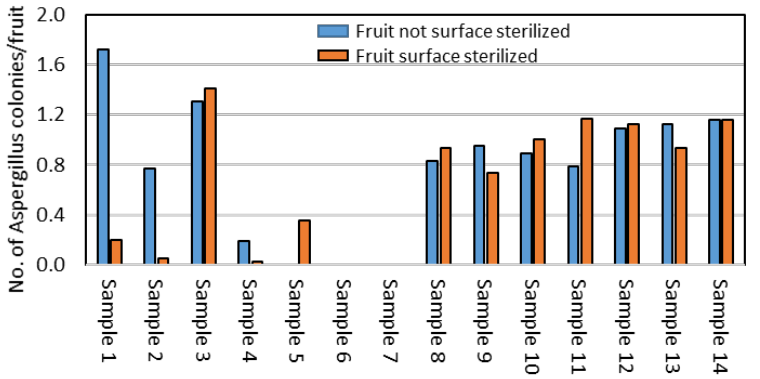


Treatments were applied on 9-7-16 using an air-blast sprayer at 130 gal/A. Two disease severity readings were done on each of four quadrants of each tree using a scale from 0 (= no disease) to 5. Evaluation was done on 10-28-16.

Thus, several fungicides with high efficacy are available to the industry to protect fruit from brown rot decay even when applied 14- to 7-days before harvest (PHI). 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.

Evaluation of fungicides for management of prune rust. The severity of rust was moderate in the 2016 growing season. In a late-season study, two applications of several 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 tree canopy as compared to the non-sprayed control trees (Fig. 4). Scala, R-106506, EXP-A, Ph-D-Scala, and EXP-AF were significantly less effective than the other fungicides reduce disease severity from the control. The highest efficacy was obtained using Rhyme, Luna Sensation, Luna Experience, UC-2B, and IL-54111. These data indicate that effective treatments against prune rust are available.

Fig. 5. Effect of surface-sterilization with sodium hypochlorite on the presence of viable *Aspergillus* spp. on stored, dried plums



Samples of dried fruit from 14 lots were received between Nov. 2015 and Jan. 2016. Fruit were surface-sterilized in 100 ppm sodium hypochlorite for 1 min or not surface-sterilized and then re-hydrated in sterile water for 6 h. Fruit were then placed into fruit trays (1-2 fruit/cavity), incubated at 20C, >90% RH for 3-5 weeks and evaluated for the presence of *Aspergillus* and other fungal species.

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 during April through June.

Contamination of dried plums in storage with* Aspergillus *species. Samples of dried plums were obtained from 14 fruit lots of the 2015 harvest. After re-hydrating fruit and incubation for 3 to 5 weeks at high relative humidity, growth of *Aspergillus* spp. occurred on all but two samples. The incidence of contaminated fruit ranged widely from 20 to 100%, but was mostly >75%. Several *Aspergillus* spp. colony types were often present on individual fruit, and at least ten colony types were differentiated based on pigmentation. Thus, in contrast to fruit from the 2014 harvest season, a wider range of species was observed, but contamination levels in both years were mostly high. Contamination of fruit in the laboratory can be ruled out because fruit were incubated in bagged-up plastic boxes and were never exposed to open-air circulation. Additionally, cross contamination during re-hydration can be ruled out because for some lots, fruit were re-hydrated individually. *Wallemia* sp. was present in six lots, and few other fungal genera were observed. Colony types on plum fruit often did not match those on agar media after fungal isolation. Therefore, for grouping of isolates they were placed into molecular groups by RFLP analysis of a short rDNA region. Sequencing of representative isolates indicated that a majority of cultures growing on the dried plums belonged to *Eurotium repens*, the sexual state of *Aspergillus reptans*. Other species identified were *A. niger, A. carbonarius* (both in the *Aspergillus* Section *Nigri*), *A. ochraceus,* and *A. tamarii*. These species, except *A. ochraceus*, as well as *A. brasiliensis*, *A. flavus*, *A. melleus*, *A. phoenicis,* and *A. tubingensis* were identified in our lab previously from dried plum fruit.

When fruit were surface-sterilized before re-hydration and incubation, a high level of contamination was still present in most lots, and contamination was significantly reduced in only three of the lots (Fig. 5). Therefore, the majority of fungal contaminants apparently had penetrated the fruit. In contrast, our previous studies on surface-disinfestation of fruit had indicated that fungal contamination was superficial because little fungal growth developed on sterilized fruit. As a precautionary procedure, samples were submitted to DFA for aflatoxin testing. All samples including known samples with *A. flavus* contamination and cultures of the fungus were negative for the presence of aflatoxin.

Because the origin of *Aspergillus* spp. contamination of dried plum fruit is still unclear, we continued to evaluate the heat sensitivity of eight selected species of *Aspergillus*. Previously, when conidia in aqueous suspensions were exposed to heat (wet heat), they were completely inactivated after 14 h at 70C (158F). In this year's studies, conidia were placed onto dried plum fruit, and fruit were incubated for 18 h at 25C or at an average

of 71.5C (dry heat). When conidia where incubated on potato dextrose agar and incubated at 25C (a control treatment), germination rates were all >95%. Average germination rates of conidia on fruit at 25C, however, were variable and ranged from 26 to 90% in the two studies conducted (Table 2). When conidia on fruit were incubated at an average temperature of 71.5C, germination was reduced by >95% as compared to 25C. No germination was observed for *A. phoenicis/tubingensis, A. niger/brasiliensis* (these two pairs of species could not be separated by the methods we used), *A. carbonarius, A. melleus*, and *Eu. repens*. Thus, these species of *Aspergillus* identified from prune fruit were all inactivated at temperatures (71-85C or 160-185F dry heat) and drying durations used in commercial fruit drying. This indicates, as in some previous years, that contamination occurs after drying in storage. Still, last year's data of 2014 fruit favored the hypothesis that fruit become contaminated before the drying process because samples were taken shortly after drying, and not after several months of storage. As a general strategy to minimize fungal contamination, fruit storage facilities should be dry and well ventilated. Any rehydration of dried fruit in storage is a non-sanctioned practice that risks mold contamination. Thus, we still recommend surface sterilization procedures immediately after harvest and before drying. A standard method for surface disinfestation prior to drying fruit 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. Subsequent steam sanitation before processing of stored, dried fruit further minimizes the risks associated with fungal contamination of fruit.

Table 2. Heat sensitivity of conidia of *Aspergillus* spp. and *Eurotium repens* on dried plum fruit

		% germination	
Isolate No.	Species	18 h 25C	18 h 71.5C
3991	*A. brasiliensis*	27	0.2
4325	*A. flavus*	30	1.2
3996	*A. phoenicis/tubingensis*	50	0.0
4329	*A. niger/brasiliensis*	90	0.0
4366	*A. carbonarius*	32	0.0
4356	*A. tamarii*	34	1.5
4355	*A. melleus*	26	0
4373	*E. repens*	72.5	0

Conidial suspensions (10^7 conidia/ml) were applied to surface-sterilized dried plum fruit and fruit were incubated for 18 h at 25C or an average of 71.5C. Conidia were re-suspended in sterile water, plated onto agar media, and conidial germination was evaluated after 18 h. Data presented are the average of two experiments.