Annual Report - 2014

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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SUMMARY OF RESEARCH ACCOMPLISHMENTS DURING 2014

1. ***Brown rot blossom blight.*** Natural incidence of blossom blight in 2014 was very low, and data on fungicide efficacy could only be obtained from laboratory studies using detached blossoms. Among conventional fungicides, Luna Experience showed the best pre-infection activity, whereas Rhyme, Quash, Fontelis, Luna Experience, Luna Sensation, and Merivon demonstrated excellent post-infection activity. The biocontrol Botector and the exempt-from-tolerance polyoxin-D (Ph-D, Tavano) were similarly effective as some of the fungicides, whereas the natural plant extract Fracture was less effective in this year's evaluation.
2. ***Bacterial blossom blast.*** Due to unfavorable environmental conditions in the spring of 2014, no disease developed.
3. ***Fruit brown rot***. In applications done at 130 gal/A in combination with 1.5% oil, all fungicides evaluated, including the exempt-from-tolerance polyoxin-D (Tavano, Ph-D), were highly effective when harvested fruit were non-wound-inoculated. After wound-inoculation, most of the field treatments with products containing FRAC group 3 (i.e., a DMI that has locally systemic activity) including Quash, Ph-D+Tebucon, Ph-D+Bumper, Quash+S2200, Quash+Protexio, and Luna Experience 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 not effective.
4. ***Rust***. In a late-season study, most effective were Rhyme, Quash, Luna Experience, Luna Sensation, Quadris Top, and Inspire Super. Ph-D was also effective and highly efficacious when mixed with a FRAC Group 3 fungicide.
5. ***Contamination of dried plums with* Aspergillus *species.*** When dried fruit from 15 lots from the 2014 harvest were re-hydrated and incubated at high relative humidity, growth of several xerotolerant fungi including *Aspergillus* spp. occurred on all but one sample. The incidence of *Aspergillus*-contaminated fruit ranged widely from 3.7 to 96.6%. Only two species of *Aspergillus* were observed among all lots, both distinct from the black Aspergilli. No fungi grew under ambient (low humidity) conditions.

   The origin of *Aspergillus* spp. contamination of dried plum fruit is still unclear. We previously concluded that contamination most likely occurs during long-term storage of dried fruit before processing. Because this year's samples were obtained shortly after drying, it appears that contamination originates from before the drying process. Presumably, heat does not completely inactivate these xerotolerant organisms adhering to the high-sugar fruit surface. Because *Aspergillus* spp. contamination was determined to be superficial on the fruit and only developed after several weeks of incubation at high relative humidity, one of the most important strategies to prevent fungal growth developing on the fruit before processing is to ensure that storage facilities are dry and ventilated. Sanitation of harvested fruit with oxidizing treatments is suggested as a future research direction.

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. Among the fungicides registered, the FRAC (Fungicide Resistance Action Committee) Group (FG) 1 benzimidazole carbamate Topsin; the FG 2 dicarboximide Rovral; the FG 3 DMIs Tilt (or generic formulations of propiconazole), Indar, Tebucan (or generic formulations of tebuconazole), and Quash; the FG 7 Fontelis; the FG 9 anilinopyrimidines Vangard and Scala; the FG 3/11 DMI-QoI pre-mixtures Quadris Top and Quilt Excel; the FG 3/7 DMI-SDHI Luna Experience (pending); the FG 3/9 DMI-AP Inspire Super, and the FG 7/11 SDHI-QoI pre-mixtures Pristine, Merivon, and Luna Sensation (pending) are most effective against blossom blight. The pre- and post-infection activity of numerous fungicides on prune blossoms was again characterized by us in 2014. We also included new fungicides (FG 3 Rhyme - flutriafol; FG 17 Protexio - fenpyrazamine; the experimentals EXP-1 and EXP-2); as well as the biofungicide polyoxin-D (FG 19), the plant extract Fracture (*Lupinus albus*) and the biocontrol Botector (*Aureobasidium pullulans*) in these studies and results are presented in this report. The current trend is to register fungicides as pre-mixtures. This is done to provide consistent activity and high performance with a broader range of activity against several diseases. Registrants also want to protect proprietary rights of older products and a mixture of old and new fungicides facilitates this goal. Additionally, pre-mixtures reduce the risk of resistance development to any single class of fungicides. Thus, pre-mixtures as part of rotation programs are more likely to provide a sustainable use of these active ingredients in California where the total number of applications per season is limited.

The information we are providing with our research is defining the comparative efficacy and spectrum of activity of new materials. We are also optimizing treatment strategies and providing information on the best usage of these fungicides. For example, fungicides that have post-infection activity (i.e., ‘kick-back action’) in addition to pre-infection activity can be applied as a single, delayed bloom application instead of a standard two- or three-spray program. This prevents infections of sepals (green tip), petals (white tip), and stamens/pistils (full bloom) of prune blossoms under conditions that are less favorable for disease. This delayed bloom application strategy helps to reduce costs and reduces unnecessary contamination of the environment while providing highly effective management of the disease.

Bacterial blast caused by *Pseudomonas syringae* can be another serious disease of prune in the springtime and we have been evaluating new management strategies in the last few years. Kasumin (kasugamycin) and the biocontrol Actinovate (*Streptomyces lydicus*) were the most effective compounds evaluated. Actinovate is registered and Kasumin is pending registration on cherry and other crops. Due to unfavorable environmental conditions in the spring of 2014, however, no disease was observed on prune.

We previously demonstrated that the efficacy of preharvest fungicides applications to prevent losses from fruit brown rot is generally considerably improved when used in combination with agricultural spray oil (e.g., 415). The waxy bloom of prune fruit prevents sufficient coverage when using aqueous applications. We also demonstrated that in general, fungicides when applied at an increased gallonage of 130 gal/A provide better protection of fruit inside clusters. This strategy was used in the evaluation of treatments in 2014. New fungicides evaluated in 2013 included Luna Sensation, Luna Experience, Merivon, Fontelis, Rhyme (Topguard), Protexio in mixture with Quash, and Ph-D.

Because of the sporadic occurrence of prune rust among growing seasons, fungicide efficacy data for this disease are difficult to obtain. The disease has to be re-initiated each year due to the lack of overwintering twig infections and the absence of known alternate hosts adjacent to prune production areas in California. As in previous seasons, rust started to develop late in 2014 at one of our trial sites and we were able to obtain results that will be of value in high-disease seasons. New materials evaluated included the same fungicides that we used in our preharvest brown rot studies.

Another objective of our research in the past years was the occurrence and identification of molds on dried plums with an emphasis on *Aspergillus* species. This was pursued at the request of farm advisors. Prune fruit were obtained from 15 lots of the 2014 crop after drying. We previously determined that all species of *Aspergillus* that we identified from prune fruit to date are killed at high temperatures (71-85C or 160-185F) and exposure durations used in commercial fruit drying. This indicated that *Aspergillus* species contamination likely originated after fruit drying and during storage in the processing facility.

Objectives

1. Evaluate the efficacy of new fungicides, pre-mixtures, polyoxin-D, BLAD (Fracture), and other biocontrols representing different modes of action for management of brown rot blossom blight and fruit rot in laboratory and field trials, as well as rust in field trials.
   a. Pre- and post-infection activity of selected fungicides against blossom blight.
   b. Evaluation of preharvest fungicides in combination with selected spray adjuvants such as summer spray oil vs. adjuvants (laboratory inoculations of field-treated, harvested fruit)
   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, Double Nickel 55, 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 (polyoxin-D and Fracture) and evaluate in vitro fungicide sensitivity of *Monilinia* spp. where failures have been reported.
4. Survey of *Aspergillus* species on dried plum, evaluate heat tolerance of these fungi, and air-sample dried plum storage facilities.

Table 1. List of available, pending registration, or research fungicides with single-site mode of action that were evaluated in our program on French prune in 2014*

No.	Registrant	Type of Formulation	Trade Name	Active Ingredient-1	FRAC Group	Active Ingredient-2	FRAC Group	Registration Status
1	Bayer	Single	Elite	Tebuconazole**	3	---	---	Registered
2		Single	Gem	Trifloxystrobin	11	---	---	Registered
3		Single	Luna Privilege	Fluopyram	7	---	---	Pending
4		Premixture	Luna Sensation	Fluopyram	7	Trifloxystrobin	11	Pending
5		Premixture	Luna Experience	Fluopyram	7	Tebuconazole	3	Pending
6	Syngenta	Single	Tilt	Propiconazole**	3	---	---	Registered
7		Single	Abound	Azoxystrobin	11	---	---	Registered
8		Single	Vangard	Cyprodinil	9	---	---	Registered
9		Premixture	Quadris Top	Difenoconazole	3	Azoxystrobin	11	Registered
10		Premixture	Inspire Super	Difenoconazole	3	Cyprodinil	9	Registered
11	BASF	Single	Headline	Pyraclostrobin	11	---	---	Reg.- Cherry
12		Single	Xemium	Fluxapyroxad	7	---	---	Pending
13		Premixture	Merivon	Pyraclostrobin	11	Fluxapyroxad	7	Pending
14	DuPont	Single	Fontelis	Penthiopyrad	7	---	---	Registered
15	Arysta	Single	Ph-D	Polyoxin-D	19	---	---	Exempt
16	Valent	Single	Quash	Metconazole	3	---	---	Registered
17		Single	Protexio	Fenpyrazamine	17	---	---	Pending
18		Single	S-2200	?	?	---	---	Pending
19	Cheminova	Single	Topguard, Rhyme	Flutriafol	3	---	---	Registered
20	Experimentals	Single	EXP-1	?	?	---	---	Pending
21		Single	EXP-2	?	?	---	---	Pending
22	Biologicals	?	Fracture	*Lupinus albus*	?	---	---	Pending
23		?	Botector	*Aureobasidium pullulans*	?	---	---	Pending

* - Some of the fungicides were only evaluated in pre-mixtures. Multi-site fungicides such as copper, chlorothalonil (Bravo, Echo, Equus), and captan are also registered.

** - Generic formulations: Tebuzol, Orius, Tebucon, Toledo = tebuconazole; Bumper = propiconazole; and Nevado, Iprodione = iprodione are also available.

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 24 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. 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). Perimeter fruit were inoculated on the exposed, outside side of the fruit, whereas fruit from inside clusters were inoculated at random contact points. 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 7-30-14 (as a preharvest application for management of fruit brown rot) and on 9-18-14 specifically for fall season rust management. Disease was evaluated on 10-16-14. For this, 40 random leaves (10 per quadrant) were sampled from each of four single-tree replications and rated for severity of sporulating rust lesions. A rating scale was used from 0 to 4 (1 = 1-5; 2 = 6-15; 3 = 16-25; and 4 = >25 sporulating lesions/leaf). Data were analyzed using analysis of variance and least significant difference (LSD) mean separation procedures of SAS 9.4.

Contamination of dried plums in storage with* Aspergillus *species. Dried prune fruit from 15 lots of the 2014 harvest were obtained in September from a processing plant. Samples of 20-30 fruit were re-hydrated in sterile distilled water for 1 h, placed into plastic trays in plastic boxes, bagged, and incubated at 20 C, >95% RH for up to 8 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 2014, 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. Still, data for brown rot 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.

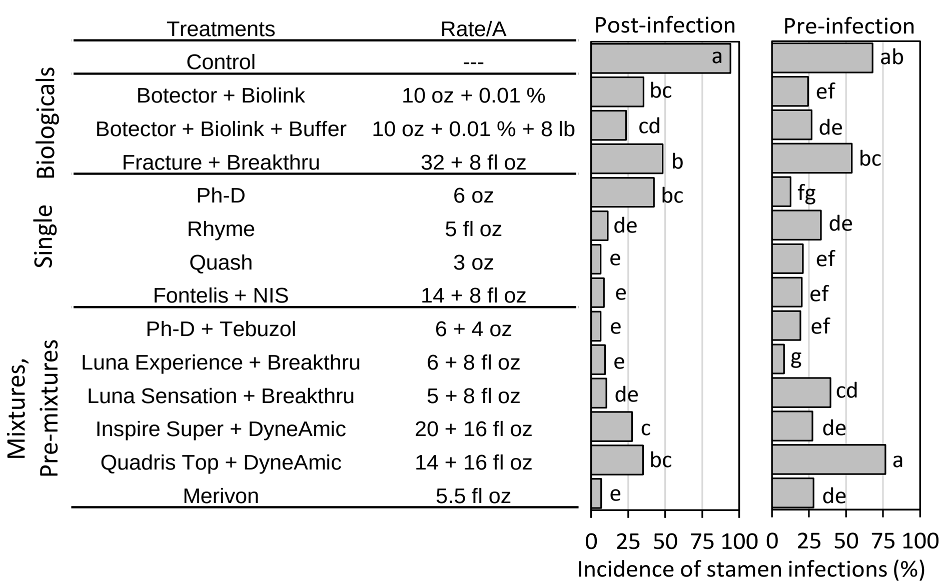
Evaluation of fungicides for management of brown rot blossom blight. In laboratory studies using detached blossoms, most treatments, including two biologicals, four single-fungicides, and six mixtures/pre-mixtures, significantly reduced the incidence of stamen infections when applied before or after infection (Fig. 1).

The biocontrol Botector was equally effective as some of the fungicides, whereas the natural plant extract Fracture was less effective (Fig. 1). Botector is planned for registration, whereas the registrant of Fracture is postponing registration. With good efficacy in this laboratory test under highly conducive disease conditions, Botector should be included again in 2015 studies, possibly also in field applications. The exempt-from-tolerance Ph-D was similarly effective as Botector and showed especially a high pre-infection activity. This treatment could potentially be another brown rot material for the organic industry.

Among conventional fungicides, Luna Experience showed the best pre-infection activity; whereas Rhyme, Quash, Fontelis, Luna Experience, Luna Sensation, and Merivon demonstrated excellent post-infection activity. 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.

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 generally considerably improved when used in combination with agricultural, spray oil (e.g., 415). 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 randomly harvested fruit were non-wound- or wound-inoculated with the brown rot pathogen. All fungicides evaluated, including the exempt-from-tolerance Ph-D, were highly effective in the non-wound-inoculation study (Fig. 2A). After wound-inoculation, most of the field treatments with products

Fig. 1. Efficacy of pre- and post-infection treatments for management of brown rot blossom blight of French prune in the laboratory 2014



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 24 h. Blossoms were evaluated for stamen infections after 4-5 days of incubation at 20 C.

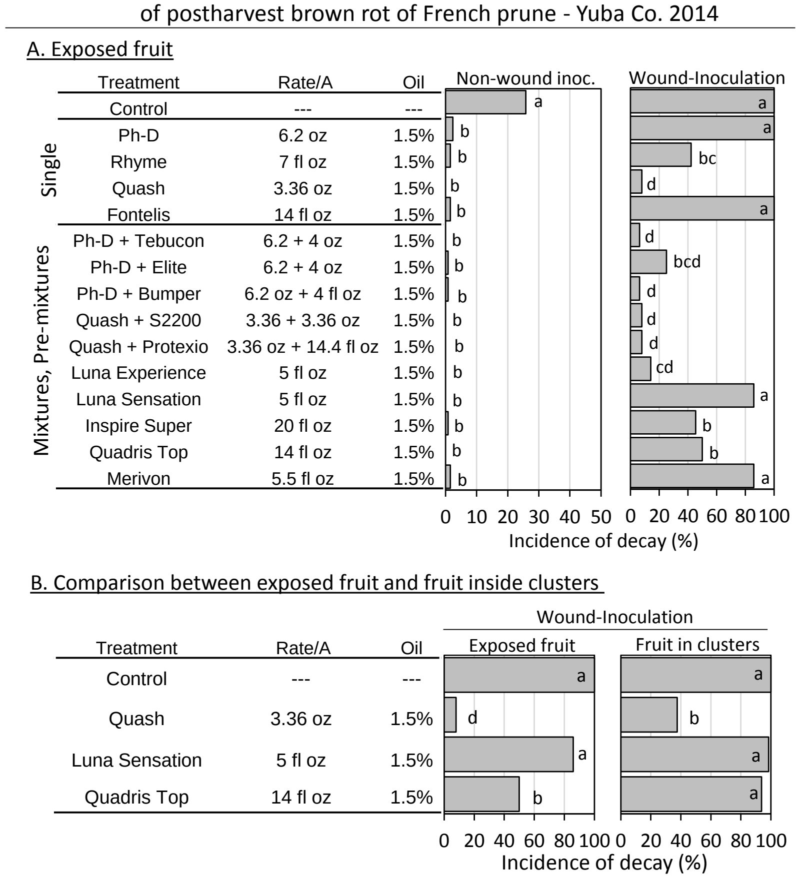
containing FG 3 (i.e., a DMI that has locally systemic activity) including Quash, Ph-D+Tebucon, Ph-D+Bumper, Quash+S2200, Quash+Protexio, and Luna Experience resulted in very low levels of brown rot, whereas the contact fungicides Fontelis, Luna Sensation, and Merivon, as well as the fermentation product Ph-D were not effective.

In another study, comparisons were done of selected fungicides for their efficacy in treating exposed fruit (i.e., fruit from the tree periphery) or fruit in clusters. Quash and Quadris Top showed higher performance on exposed fruit when harvested fruit were wound-inoculated (Fig. 2B), indicating that fruit clusters are often so tight that even at higher gallonage, applications cannot provide adequate coverage. The contact fungicide Luna Sensation was not effective on both types of fruit in these wound-inoculation studies. Because all fungicides were highly effective on randomly picked fruit in non-wound-inoculation studies (see above), treatments will still be beneficial in protecting fruit. This emphasizes the importance in preventing injuries to fruit 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.

Thus, several fungicides with consistent 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, are compatible with oils. Considering that polyoxin-D potentially could be approved as an organic treatment, its high efficacy on non-wound inoculated fruit is quite exiting.

Evaluation of fungicides for management of prune rust. 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. 3). As in last year's studies, most effective treatment were Rhyme, Quash, Luna Experience, Luna Sensation, Quadris Top, and Inspire Super, reducing severity from a rating of 3.6 to less than 0.5 on a scale from 0 to 4. Ph-D was also effective by itself or highly

Fig. 2. Efficacy of 14-day preharvest fungicide treatments for management of postharvest brown rot of French prune - Yuba Co. 2014

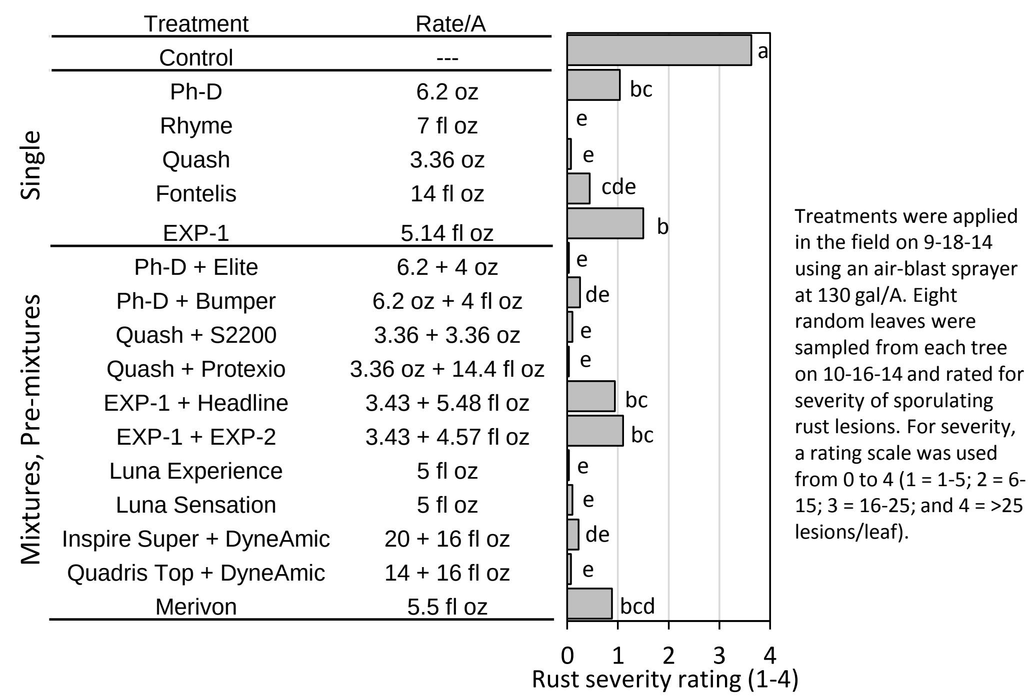


Treatments were applied in the field on 7-30-14 using an air-blast sprayer at 130 gal/A. At harvest, exposed fruit or fruit within clusters were collected and wound-inoculated (30,000 spores/ml) or non-wound-inoculated (500,000 spores/ml) with conidia *of M. fructicola*. Fruit were then incubated for 7 days at 20 C.

effective in a mixture with a FG 3 fungicide. These data indicate that effective treatments against prune rust are available. The disease 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.

Contamination of dried plums in storage with* Aspergillus *species. Samples of plums taken after drying were obtained from 15 fruit lots. After re-hydrating fruit and incubation for 4 to 7 weeks at high relative humidity, growth of *Aspergillus* spp. occurred on all but one sample and the incidence ranged widely from 3.7 to 96.6% (Table 2). Only two species of *Aspergillus* were observed among all lots, both distinct from the black Aspergilli. The only other fungal contaminant found was tentatively identified as *Wallemia* spp. Thus, the incidence of contamination was very high, but the diversity of fungal organisms was very low.

Fig. 3. Efficacy of fungicide applications for management of rust of French prune - Yuba Co. 2014



Aspergillus spp. are known to be xerotolerant and can occur on dried plums because of their high sugar content where most other fungi cannot survive due to low water availability. The low diversity of *Aspergillus* spp. found in 2014 is in contrast to other years when a wider range of *Aspergillus* species was observed. Additionally, we had observed that the range of species found varied among years. For example, in some years, mostly black Aspergilli occurred, whereas in other years, mostly other *Aspergillus* spp. were present in the samples.

The origin of *Aspergillus* spp. contamination of dried plum fruit is still unclear. The same species of fungi were contaminating all 15 fruit lots in 2014, indicating a common source. Still, the degree of contamination varied widely among lots. Contamination 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, *Wallemia* sp. is not a common air-borne fungus and is found in soil.

Previous studies with surface-disinfested fruit indicated that fungal contamination is superficial, because little fungal growth developed on sterilized fruit. We also previously established thermal death points of mycelial agar plugs (hydrated state) and of aqueous conidial suspensions (a hydrated state). Some species still survived incubation at 75C (167F) for 18 h, although at low levels. These species include *A. niger*, *A. flavus* var. *columnaris*, and *A. tamari*. Other species such as *A. brasiliensis*, *A. carbonarius*, *A. phoenicis/A. tubingensis*, *A. flavus*, *A. melleus*, and *E. repens*, however, did not survive. 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 (71-85C or 160-185F) and drying durations used in commercial fruit drying. This would imply 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 may be somewhat dehydrated of the fruit surface and more heat tolerant than when in an aqueous environment or hydrated state. Fruit contamination before the drying process is supported by the fact that this year's samples were taken shortly after drying, and not after several months of storage as in previous years. Thus, this year's results indicate that spores of xerotolerant fungi may survive commercial drying as surface contaminates.

Because *Aspergillus* spp. contamination was determined to be superficial on the fruit and only developed after several weeks of incubation at high relative humidity, one of the most important strategies to prevent fungal growth from developing on the fruit before processing is to ensure that storage facilities are dry and well ventilated. In order to prevent any contamination, surface sterilization procedures may need to be developed 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.

Table 2. Fungal contamination of prune fruit collected shortly after commercial drying

Lot No.*	Incidence of *Aspergillus* spp. contamination**	Incidence of *Wallemia* sp. Contamination
1	0.0	100.0
2	3.4	100.0
3	3.7	11.1
4	7.7	7.7
5	9.7	0.0
6	9.7	16.1
7	13.8	58.6
8	16.1	100.0
9	22.2	44.4
10	27.6	58.6
11	33.3	100.0
12	42.9	100.0
13	53.6	0.0
14	71.9	78.1
15	96.6	0.0

* Dried prune fruit samples were obtained from 15 lots from the current harvest in September 2014. Fruit were re-hydrated, incubated at >95 RH, 20C, and were periodically evaluated. Fungal growth was first observed after 4 weeks. A final evaluation of fruit was done after 7 weeks.

** Two *Aspergillus* species were present among all lots.