Annual Report - 2013

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:	Second Year
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SUMMARY OF RESEARCH ACCOMPLISHMENTS DURING 2013

1. ***Brown rot blossom blight.*** Natural incidence of blossom blight in field studies in 2013 was very low, and data on fungicide efficacy could not be obtained. In laboratory studies using detached blossoms, all fungicides, including one single-treatment, seven pre-mixtures, and the exempt from residue tolerance compound Oso (polyoxin-D), were highly effective as pre- and post-infection treatments. The organic and natural product Fracture had very good post-infection activity and also significantly reduced the amount of disease as a pre-infection treatment.
2. ***Bacterial blossom blast.*** A field trial using the biocontrols Actinovate and Botector, the antibiotics Kasumin (kasugamycin) and Mycoshield, Kocide 3000, as well as selected mixtures was conducted, but no disease developed.
3. ***Fruit brown rot***. Applications at higher gallonage (130 gal/A) with Bumper, Luna Sensation, or Quadris Top in combination with an agricultural spray oil or the surfactant Dyne-Amic 14 days before harvest were highly effective when harvested fruit were non-wound inoculated. In wound-inoculations, treatments were significantly more effective on fruit from the outside tree canopy when used with oil as compared to using the surfactant. Among the three fungicides, Bumper reduced decay to the lowest level and was still very effective when fruit from inside clusters were wound-inoculated.

   In a second trial, six single and seven mixture or pre-mixture treatments were applied 14 or 21 days before harvest. All treatments (including polyoxin-D) reduced the amount of decay to very low levels after non-wound inoculation. Treatments that included FRAC group 3 (i.e., a DMI) with local systemic activity were generally more effective, and some of them such as Bumper, Luna Experience, Luna Sensation, Quash, Quadris Top, and Inspire Super resulted in very little decay even when applied 21 days before harvest. On wound-inoculated fruit, again most treatments containing a DMI were effective. Quash, Bumper, Ph-D + Tebucon, and Luna Experience were still effective after the 21-day PHI application.
4. ***Rust***. In a late-season study, one application of a range of selected fungicides all significantly reduced the severity of rust. Most effective were Topguard, Quash, Luna Experience, Luna Sensation, Quadris Top, and Inspire Super. Ph-D was among the least effective treatments, but disease was still reduced significantly from the control.
5. ***Contamination of stored dried plums with* Aspergillus *species.*** A high incidence (26.3 to 79.2%) of growth of *Aspergillus* spp. was observed when dried plums from five fruit lots were re-hydrated and incubated. In contrast, fruit that were surface-sterilized before incubation developed little growth of *Aspergillus* spp. indicating that these fungi were surface-contaminants that had not penetrated into the fruit. In addition to black Aspergilli, all non-surface-sterilized fruit developed a high incidence of Aspergillus species with different pigmentation.

   Previously, we determined that all species of *Aspergillus* from dried plum grew at temperatures of 35C, but significant growth at 45C was only found for *A. brasiliensis*. Studies on thermal death points in 2013 indicated that some species still survive incubation at 75C (167F) for 18 h, although at low levels. Conidia were more heat-sensitive than mycelium and were completely inactivated after 14 h at 70-75C (158-167F). Thus, *Aspergillus* spp. on dried plums are mostly surface contaminants that can be killed at temperatures used for commercial fruit drying and, furthermore, are effectively removed during sanitation in processing. We conclude that *Aspergillus* contamination likely originates during storage in the processing facility.

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. A list of the materials that are registered, pending registration, or used in evaluations on French prune is shown in Table 1. Among the fungicides registered, the FRAC (Fungicide Resistance Action Committee) Group (FG) 1 benzimidazole carbamates Topsin; the FG 2 dicarboximide Rovral; the FG 3 DMIs Tilt or Bumper, Indar, Tebuzol (Elite), 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 (pending), and Luna Sensation (pending) are most effective against blossom blight. The pre- and post-infection activity of most of these fungicides on prune blossoms was again characterized by us in 2013. We also included new fungicides (FG 3 Topguard; FG 3/11 Custodia), as well as polyoxin-D (FG 19) and the plant extract Fracture (*Lupinus albus*) in these studies and results are presented in this report. The current trend in pre-mixture fungicide registrations is done to provide consistent high performance, a broader range of activity against diseases, to reduce the risk of resistance development to any single class of fungicides, and to protect proprietary rights of products. 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 is limited. The information we are providing with our research is helping to identify new effective materials and treatment strategies. 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 for preventing 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. Trials were also conducted in the spring of 2013, however, as in 2012 no disease occurred. Thus, we will continue these studies in 2014 in studies using the same inoculation method on detached shoots incubated at 6 C (43F).

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). Due to the waxy bloom of prune fruit, aqueous applications do not provide sufficient coverage as they do on other stone fruit crops such as peaches and nectarines. In 2013, we compared the addition of oil to that of a surfactant (i.e., Dyne-Amic). We also demonstrated that some fungicides when applied at an increased gallonage of 130 gal/A provide better protection of fruit inside clusters. New fungicides evaluated in 2013 included Luna Sensation, Luna Experience, Merivon, Fontelis, Topguard, and polyoxin-D (Ph-D).

Due to the sporadic occurrence of prune rust among growing seasons, fungicide efficacy data are difficult to obtain. The disease has to be re-initiated each year due to the lack of overwintering twig infections and the lack of known alternate hosts adjacent to prune production areas in California. As in previous seasons, rust started to develop late in 2013 at one of our trial sites and we were able to obtain results that will be of value in high-disease seasons. Materials evaluated included Ph-D, Indar, Propiconazole, Topguard, Quash, Fontelis, and six pre-mixtures mentioned above.

Another objective of our research was the isolation and identification of molds on dried plums with an emphasis on *Aspergillus* species. This was pursued at the request of farm advisors. Isolates were collected from five lots of stored fruit of the 2012 crop. We previously developed a molecular characterization method for species of *Aspergillus* found on prune fruit. The goal is to have a method available to differentiate between

harmless saprobes and potentially harmful mycotoxin-producing species. Furthermore, we continued to characterize these species for their heat tolerance to deduce the origin of fungal fruit contamination and how it can be avoided.

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 or other stone fruit crops in California*

No.	Registrant	Type of Formulation	Trade Name	Active Ingredient-1	FRAC Group	Active Ingredient-2	FRAC Group	Registration Status
1	Bayer	Single	Rovral	Iprodione**	2	---	---	Registered
2		Single	Elite	Tebuconazole**	3	---	---	Registered
3		Single	Gem	Trifloxystrobin	11	---	---	Registered
4		Single	Scala	Pyrimethanil	9	---	---	Registered
5		Single	Luna Privilege	Fluopyram	7	---	---	Pending
6		Premixture	Luna Sensation	Fluopyram	7	Trifloxystobin	11	Pending
7		Premixture	Luna Experience	Fluopyram	7	Tebuconazole	3	Pending
8	Syngenta	Single	Tilt	Propiconazole**	3	---	---	Registered
9		Single	Abound	Azoxystrobin	11	---	---	Registered
10		Single	Vangard	Cyprodinil	9	---	---	Registered
11		Premixture	Quilt Excel	Propiconazole	3	Azoxystrobin	11	Registered
12		Premixture	Quadris Top	Difenoconazole	3	Azoxystrobin	11	Registered
13		Premixture	Inspire Super	Difenoconazole	3	Cyprodinil	9	Registered
14	BASF	Single	Headline	Pyraclostrobin	11	---	---	Reg.- Cherry
15		Single	Xemium	Fluxapyroxad	7	---	---	Pending
16		Premixture	Pristine	Pyraclostrobin	11	Boscalid	7	Registered
17		Premixture	Merivon	Pyraclostrobin	11	Fluxapyroxad	7	Pending
18	DuPont	Single	Fontelis	Penthiopyrad	7	---	---	Registered
19		Single	YT 669	Picoxystrobin	11	---	---	Pending
20	Arysta	Single	Elevate	Fenhexamid	17	---	---	Registered
21		Single	Ph-D	Polyoxin-D	19	---	---	Exempt
22	Dow Agro	Single	Indar	Febuconazole	3	---	---	Registered
23	Valent	Single	Quash	Metconazole	3	---	---	Registered
24	MANA	Single	Custodia	Azoxystrobin	11	Tebuconazole	3	Pending
25	Certis USA	Single	Oso	Polyoxin-D	19	---	---	Exempt
26	Cheminova	Single	Topguard	Flutriafol	3	---	---	Registered
27	FMC	Single	Fracture	*Lupinus albus*	Biocontrol	---	---	Exempt

* - Multi-site fungicides such as copper, chlorothalonil (Bravo, Echo, Equus), and captan are also registered.

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

OBJECTIVES

1. Evaluate the efficacy of new fungicides, pre-mixtures, polyoxin-D, and biocontrols representing different chemical classes for brown rot blossom blight, brown rot fruit rot, and rust in laboratory and 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. a spreader surfactant (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 – large-scale trials once federally registered.
   c. Sanitizers - AgriTitan and Citrox

d. Systemic acquired resistance (SAR) compounds – Actigard, PM-1, and possibly others.

3. Continue to develop baseline sensitivity data for SDHI fungicides and monitor populations of *Monilinia* spp. where failures have been reported for their in vitro sensitivities against utilized fungicides.
4. Survey of *Aspergillus* species on dried plums and evaluate heat tolerance of these fungi.

MATERIALS AND METHODS

Evaluation of fungicides for management of brown rot blossom blight. Field applications with selected fungicides were done at our experimental orchard at the UC Davis Plant Pathology field station, however, no natural incidence of blossom blight developed. Fungicides were also 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. laxa* (2×10^4 conidia/ml), treated with a selected fungicide 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 9.1.

Evaluation of antibacterial treatments for protection of inoculated blossoms of French prune against bacterial blast in the field. A trial on bacterial blossom blast was done in a French prune orchard at UC Davis. Blossoms were wounded by cutting off pistils, stamens, and part of the petals. Treatments with the biocontrols Actinovate and Botector, the antibiotics Kasumin (kasugamycin) and Mycoshield, Kocide 3000, as well as selected mixtures were made using a hand sprayer to run-off. After 5 h, blossoms were inoculated with *Pseudomonas syringae* (10^8 cfu/ml) by hand-spraying. Inoculated branches (four single-tree replications) were covered with white bags overnight and were evaluated for disease after 1 to 3 weeks. Data were analyzed using analysis of variance and least significant difference (LSD) mean separation procedures of SAS 9.1.

Evaluation of fungicides for management of prune rust. A field trial was established in a commercial orchard in Sutter Co. to evaluate the efficacy of new fungicides. Fungicides were applied on 8-2-13 (as a preharvest application for management of fruit brown rot) and on 9-19-13 specifically for fall season rust management. Disease was evaluated on 10-15-13. For this, ten random leaves 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 9.1.

***Evaluation of fungicides for management of preharvest fruit decay*.** Field trials to evaluate preharvest fungicide applications for control of fruit brown rot were done at UC Davis and in a commercial orchard in Yuba Co. In the UC Davis plot, treatments were applied 14 days before harvest using an air-blast sprayer calibrated at 130 gal/A. Quadris Top, Luna Sensation, or Bumper were applied in combination with 1.5% of a spray oil or with the surfactant Dyne-Amic (16 fl oz/A). 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 (2.5×10^5 conidia/ml). Exposed fruit were inoculated on the exposed, outside side of the fruit, whereas fruit from inside clusters were inoculated at random sites. In the Yuba Co. trial, all treatments were done 14 or 21 days before harvest in combination with a spray-oil (Omni Supreme) at 1.5% v/v. Fruit were collected randomly from each tree and were inoculated on the side that was exposed to the fungicide spray. After inoculation, fruit were incubated for 7 days at 20 C. Data were analyzed using analysis of variance and least significant difference (LSD) mean separation procedures of SAS 9.1.

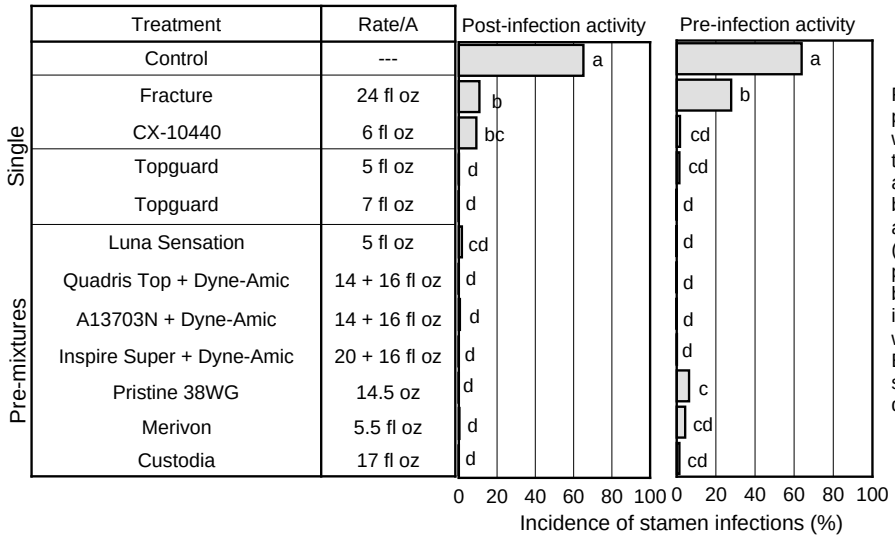
Identification and characterization of* Aspergillus *species on fresh and dried plums. Cull fruit from five fruit lots from Butte Co. from the 2012 harvest were obtained in April of 2013 from a processing plant. Fruit were split into two sub-samples (71-94 fruit/sub-sample) and were surface-sterilized or not sterilized for 1 min in 100 ppm sodium hypochlorite. All fruit were re-hydrated in water for 1 h, placed into plastic trays, and incubated at 20 C, >95% RH. Fruit were evaluated for the presence of *Aspergillus* spp. growing on the fruit after 14 days. *Aspergillus* spp. were grouped into those with black or light-colored sporulation. Representative colonies were also cultured for later species identification based on RFLP analysis of the ITS1 region of rDNA.

Representative isolates from each species of *Aspergillus* obtained from prune in previous years were evaluated for their thermal death points. For this, four PDA agar plugs (with fungal mycelium and sporulation) were incubated with 1 ml sterile water (water was added to prevent desiccation of the agar plugs) in microcentrifuge tubes at 70C or 75C for 5, 10, 14, or 18 h and then plated onto PDA to determine survival (mycelial growth). Viability of the fungus was assessed based on growth after 3 days. For determining thermal death points of conidia, conidial suspensions prepared from CMA cultures (10^5 conidia/ml) were incubated in microcentrifuge tubes at 70C or 75 C for selected times and then plated onto PDA. Survival of conidia was based on microscopic evaluation of conidial germination after 18 h.

RESULTS AND DISCUSSION

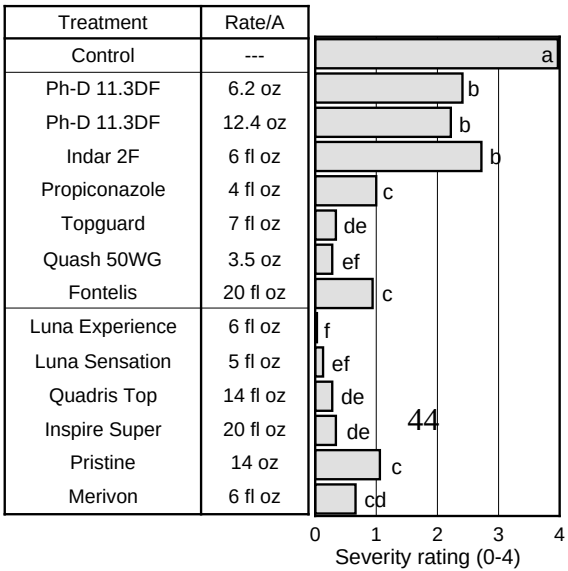
Evaluation of fungicides for management of brown rot blossom blight. Natural incidence of blossom blight in field studies in 2013 was very low, and data on fungicide efficacy could not be obtained. In laboratory studies using detached blossoms, all fungicides, including one single-treatment, seven pre-mixtures, and the exempt from residue tolerance compound Oso (polyoxin-D), were highly effective as pre- and post-infection treatments (Fig. 1). The natural product Fracture had very good post-infection activity and also significantly reduced the amount of disease as a pre-infection treatment. Thus, it will be important to evaluate this product in field studies in 2014. 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

Fig. 1. Efficacy of post- and pre-infection treatments with fungicides in the laboratory for control of blossom blight of French prune



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

Fig. 2. Efficacy of fungicide applications for management of rust of French prune in Yuba Co. 2013
- Late-season, after harvest evaluations -



Treatments were applied in the field using an air-blast sprayer at 100 gal/A. Ten random leaves were sampled from each tree on 10-15-13 and rated for severity of sporulating rust lesions. For severity, a rating scale was used from 0 to 4 (1 = 1-5; 2 = 6-15; 3 = 16-25; and 4 = >25 lesions/leaf).

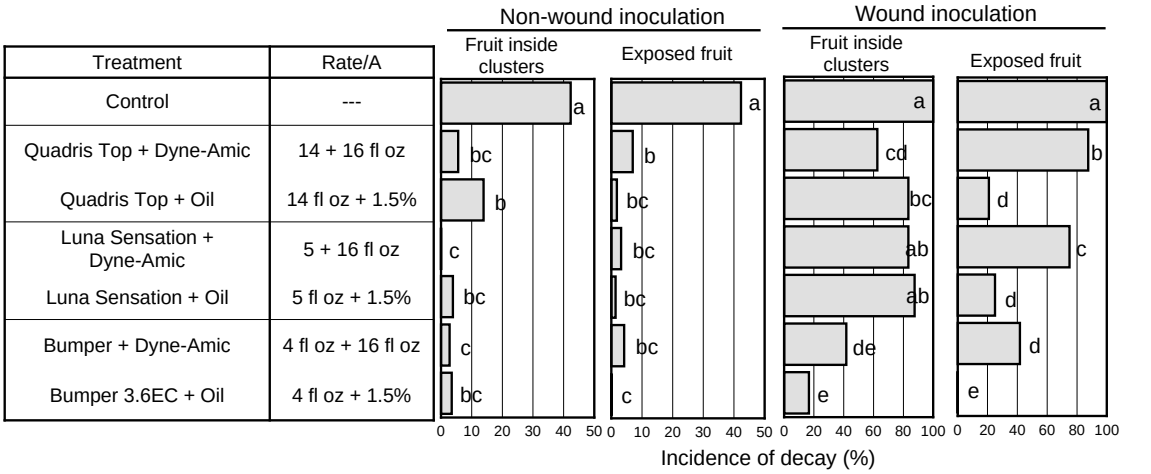
controlled. This strategy has been successfully used on other tree crops in spring seasons when precipitation is low to moderate.

Evaluation of treatments for control of blossom blast. An inoculation study was conducted at UC Davis, however, as in 2012 no disease developed, possibly due to adverse environmental conditions. Previously, polyoxin-D (Ph-D), kasugamycin (Kasumin), Mycoshield, and the biocontrol Actinovate significantly reduced the disease from that of the control. Thus, effective treatments for blossom blast control were identified that potentially could be registered for this use and be used in combination with a brown rot application. Studies need to be repeated in 2014 using the same inoculation method but using detached shoots incubated at 6 C (43 F).

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. 2). Most effective were Topguard, Quash, Luna Experience, Luna Sensation, Quadris Top, and Inspire Super, reducing severity from a rating of 4 on a scale from 0 to 4 to ratings between 0.03 to 0.34. Ph-D was among the least effective treatments, but disease was still reduced significantly from the control. 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.

Evaluation of fungicides for management of fruit brown rot. The efficacy of preharvest fungicides was evaluated in two field trials. At the UC Davis trial, applications at higher gallonage (130 gal/A) with Bumper, Luna Sensation, or Quadris Top in combination with a spray oil or the surfactant Dyne-Amic 14 days before harvest were highly effective when harvested fruit were non-wound inoculated (Fig. 3). Because efficacy for fruit inside cluster depends on the spray volume used (as we demonstrated in previous years), spray volumes of 130 gal/A were used to improve the performance of these treatments. In wound-inoculations, treatments were significantly more effective on fruit from the outside tree canopy when used with spray oil as compared to using the surfactant (Fig. 3). Among the three fungicides, Bumper reduced decay to the lowest level and was still very effective when fruit from inside clusters were wound-inoculated.

Fig. 3. Efficacy of 14-day PHI fungicide applications for management of postharvest brown rot of French prune at UC Davis 2013
- Comparison of exposed fruit and fruit inside clusters -

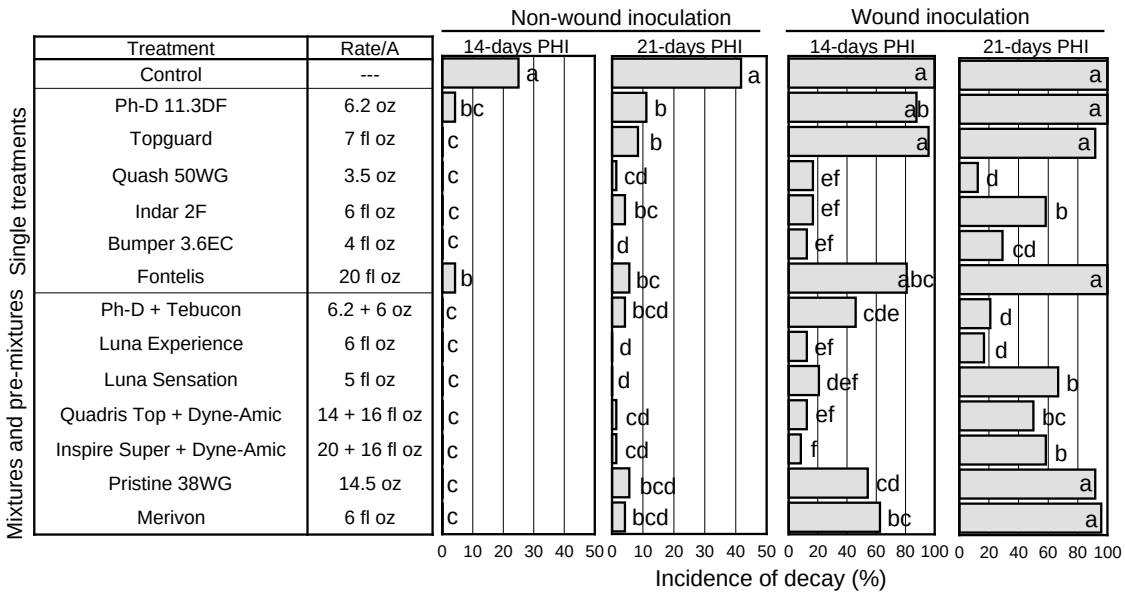


Treatments were applied in the field on 8-8-13 in combination with Omni Supreme Spray Oil or Dyne-Amic using an air-blast sprayer at 130 gal/A. At harvest, fruit from the tree perimeter (exposed fruit) and from clusters were collected and wound- (30,000 conidia/ml) or non-wound-inoculated (250,000 conidia/ml) with *M. fructicola*. Fruit were then incubated for 7 days at 20 C.

In the second trial, six single and seven mixture or pre-mixture treatments were applied 14 or 21 days before harvest. All treatments (including polyoxin-D) reduced the amount of decay to very low levels after non-

wound inoculation (Fig. 4). Treatments that included FRAC group 3 (i.e., a DMI) with local systemic activity were generally more effective, and some of them such as Bumper, Luna Experience, Luna Sensation, Quash, Quadris Top, and Inspire Super resulted in very little decay even when applied 21 days before harvest. On wound-inoculated fruit, again treatments containing a DMI (with the exception of Topguard) were effective. Quash, Bumper, Ph-D + Tebucon, and Luna Experience were still effective after the 21-day PHI application. Fungicides without locally systemic activity that cannot inhibit fungal colonization of the fruit once infection has taken place, such as polyoxin-D (Ph-D, Oso), Fontelis, Pristine, and Merivon, generally did not reduce decay levels from that of the control after wound inoculation.

Fig. 4. Efficacy of 14- and 21--day PHI fungicide applications for management of postharvest brown rot of French prune in Yuba Co. 2013



Treatments were applied in the field in combination with Omni Supreme Spray Oil (1.5%) on 8-2-13 using an air-blast sprayer at 130 gal/A. After harvest, fruit were wound- (30,000 conidia/ml) or non-wound-inoculated (250,000 conidia/ml) with *M. fructicola*. Fruit were then incubated for 7 days at 20 C.

Thus, several fungicides with consistent high efficacy are available to the industry to protect fruit from brown rot decay. The highest treatment efficacy is obtained when fungicide-oil mixtures are applied at higher volumes. The spray oil is providing better coverage and likely also results in better penetration of the fungicide into the fruit because the surfactant Dyne-Amic as an adjuvant was less effective. 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.

Table 2. Contamination of stored prune fruit with species of *Aspergillus*

Fruit lot	Incidence of black Aspergilli (%)	Incidence of other Aspergilli (%)	Incidence of Aspergillus contamination total (%)
A	9	20.2	29.2
A chlorine	0	3.6	3.6
B	15.4	46.2	61.5
B chlorine	0	0	0
C	39	40.3	79.2
C chlorine	1.1	1.1	2.3
D	52.1	25.4	77.5
D chlorine	0	1.1	1.1
E	3.9	22.4	26.3
E chlorine	1.1	1.1	2.3

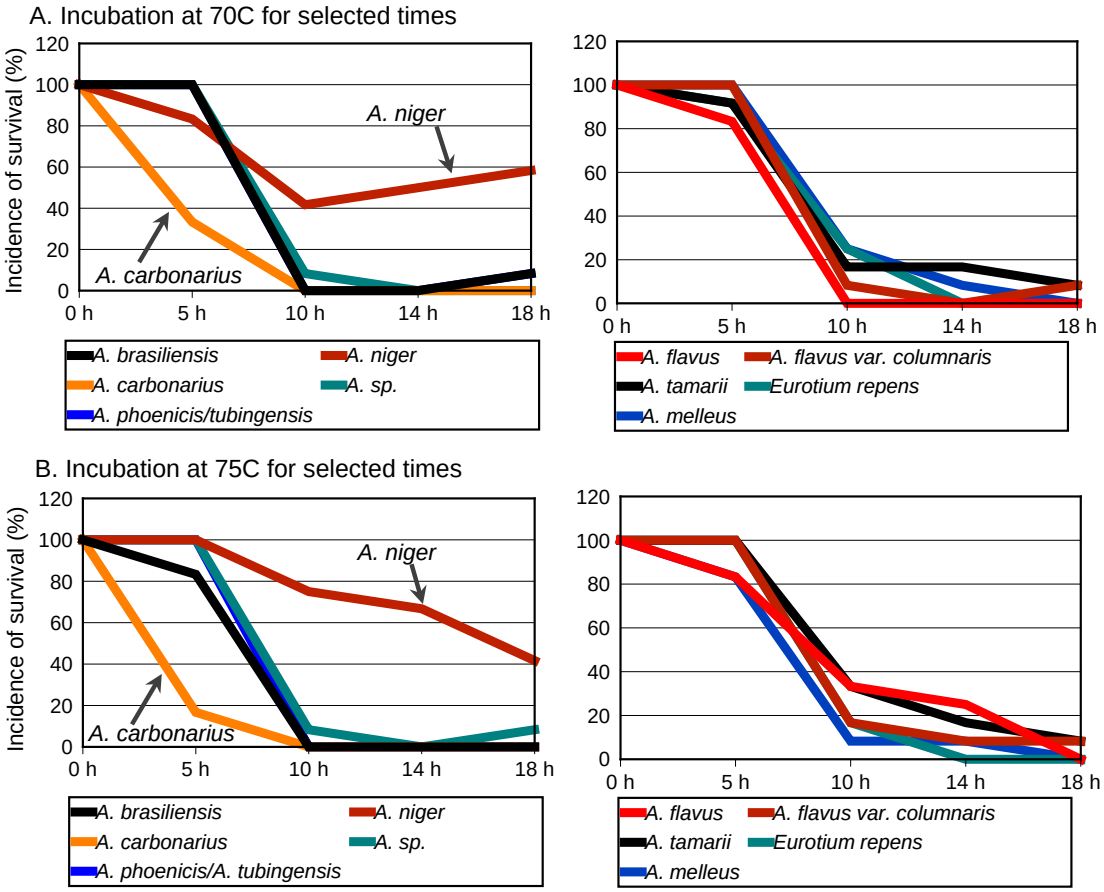
Culled prune fruit from a processing plant (received mid-April 2013) were surface-sterilized or not sterilized for 1 min in 100 ppm sodium hypochlorite. All fruit were re-hydrated in water for 1 h, placed into plastic trays, and incubated at 20 C. There were between 70 and 94 fruit for each sterilized and non-sterilized fruit lot. Fruit were evaluated for the presence of *Aspergillus* spp. growing on the fruit after 14

days. *Aspergillus* spp. were grouped into black and other Aspergilli. Species identification for these isolates is pending. After longer incubation, additional fruit showed growth of *Aspergillus* spp.

Contamination of stored dried plums with* Aspergillus *species. Samples of dried plums taken from cull piles in the sorting step of fruit processing (after drying and storing for several months) were obtained from five fruit lots. A high incidence (26.3 to 79.2%) of growth of *Aspergillus* spp. was observed when fruit were re-hydrated and incubated (Table 2). In contrast, fruit that were surface-sterilized before incubation developed little growth of *Aspergillus* spp. indicating that these fungi were surface-contaminants that had not penetrated into the fruit. In addition to black Aspergilli, all non-surface-sterilized fruit developed a high incidence of *Aspergillus* species with different pigmentation. In comparison among years, prune fruit contamination with *Aspergillus* spp. varies in incidence from very high as in 2013 to very low as in most lots in 2012 and also varies regarding species found. For example, in some years mostly black Aspergilli occur, whereas in other years like in 2013 mostly other Aspergilli were present in our samples.

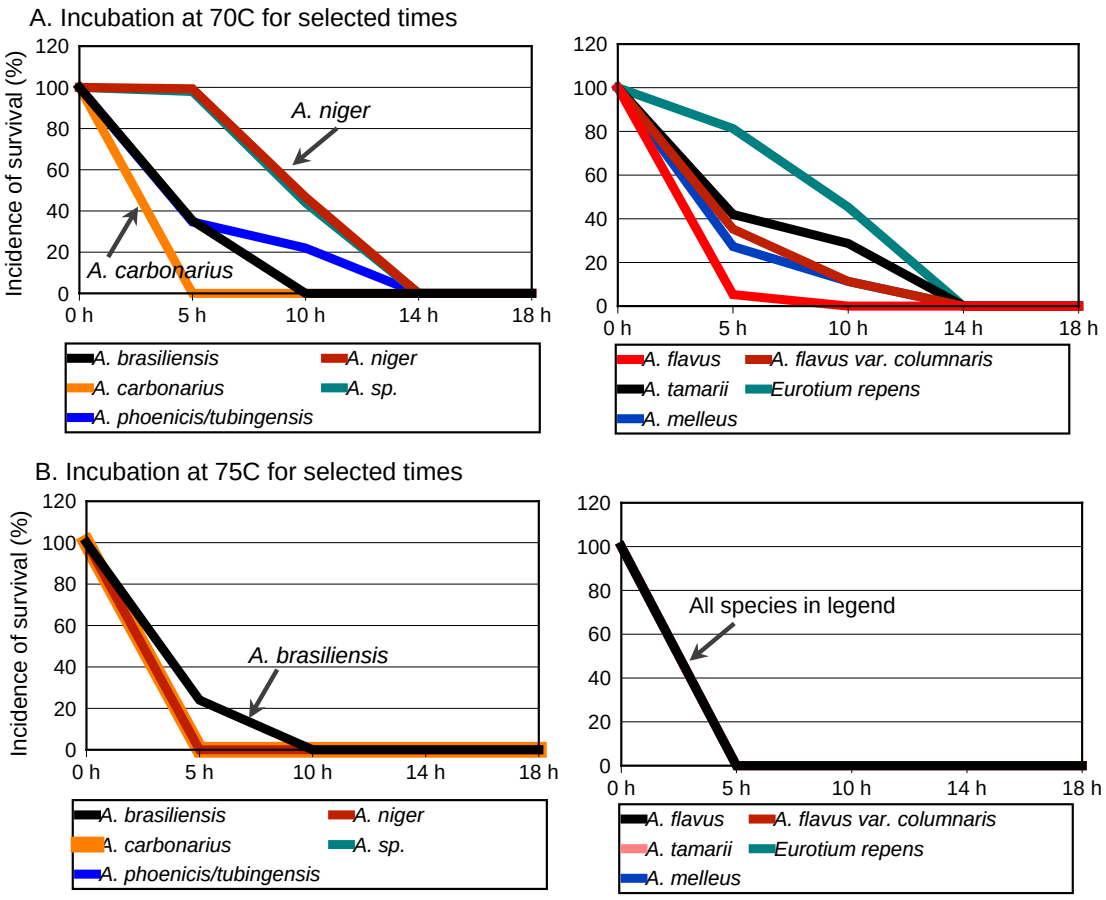
Previously, we determined that all species of *Aspergillus* from dried plum grew at temperatures of 35C, but significant growth at 45C was only found for *A. brasiliensis*. Studies on thermal death points of mycelial agar plugs containing conidia and of conidial suspensions in 2013 indicated that some species still survive incubation at 75C (167F) for 18 h, although at low levels (Fig. 5). These species include *A. niger*, *A. flavus* var. *columnaris*, and *A. tamari*. Other species such as *A. brasiliensis*, *A. carbonarius*, *A. phoenicis/A. tubingensis* (our sequence analysis of a representative of this RFLP group could not distinguish these two species), *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) (Fig. 6). 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.

Fig. 5. Heat sensitivity of mycelial cultures (mycelium and conidia) of *Aspergillus* spp. recovered from prunes



Four PDA agar plugs (with fungal mycelium and sporulation) were incubated with 1 ml sterile water in microcentrifuge tubes at 70C or 75C for selected times and then plated out onto PDA to determine survival.

Fig. 6. Heat sensitivity of conidia of *Aspergillus* spp. recovered from prunes.



Conidial suspensions prepared from CMA cultures (1 x 10^5 conidia/ml) were incubated in microcentrifuge tubes at 70C or 75 C for selected times and then plated out onto PDA to evaluate survival (conidial germination).

In summary, the xerotolerant *Aspergillus* spp. occur on dried plums because the high sugar content lowers water availability and excludes most other fungi from growing. The *Aspergillus* species found in this study are mostly surface contaminants that can be effectively removed during sanitation in the processing chain. Furthermore, all species of *Aspergillus* identified from prune fruit to date are killed at temperatures (71-85C or 160-185F) and drying durations used in commercial fruit drying. This indicates that *Aspergillus* species contamination likely originates after fruit drying during storage in the processing facility. A common source of contamination is supported by the fact that species composition among fruit lots has been relatively uniform for each year with black Aspergilli dominating in some years and a high incidence of species with other pigmentation occurring in other years like in this year's 2012 harvested lots. If contamination was originating from the field, more variability would be expected among fruit lots. Thus, additional air-sampling studies should be done in storage room ventilation systems to determine how and when dried fruit are contaminated.