Annual Report - 2012

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 2012

1. ***Brown rot blossom blight*:** In field studies, the incidence of blossom blight was significantly reduced by all treatments including the registered Quash and the experimental Topguard (both FRAC group or FG 3), Inspire Super (FG 3+9), Quadris Top and Adament (both FG 3+11), as well as the new Luna Sensation, Merivon, and Q8Y78 (FG 7+11), and the FG 7 SDHI Fontelis. In laboratory studies using detached blossoms, these fungicides were highly effective in reducing the incidence of blight when used as pre- or post-infection treatments.
2. ***Fruit brown rot***. Fungicide applications 12 days before harvest at higher gallonage (130 gal/A) in combination with a spray oil were effective for all treatments for exposed fruit that were non-wound-inoculated after harvest. For fruit inside clusters, several fungicides including Pristine, Adament, and Quash were also very effective. After wound-inoculation, Quash, Quadris Top, Adament, Topguard, Luna Experience, Inspire Super, and Q8Y78 were the most effective. These latter fungicides include a DMI compound and this fungicide class (FG 3) is known to have local systemic activity and thus, is effective in inhibiting fungal invasion of the fruit after wound-inoculation.

   Conventional and organic formulations of polyoxin-D (Ph-D and CX10440, respectively) that were used at a very low rate (e.g., Ph-D was used at 0.71 oz active ingredient/A) were very promising on non-wound-inoculated exposed fruit. Due to the recent approval of an exempt status, higher rates can be evaluated in the future. Thus, polyoxin-D has the potential to be the most effective organic treatment ever available for the fruit industries of California.
3. ***Rust***: In a late-season study, two applications of a range of selected fungicides all significantly reduced the incidence and severity of rust. No disease was detected using Luna Experience and Quash. Although Ph-D was the least effective, the result is very important because with the recent exempt status of this compound, higher rates can be evaluated in future trials.
4. ***Identification of* Aspergillus *species on dried plums.*** Of the thirteen fruit lots evaluated, *Aspergillus* spp. could only be detected at high incidence in one of the lots. Thus, current handling practices are highly effective in: preventing decay by drying fruit, sorting to remove low quality fruit after drying, and steam sanitizing fruit in preparation of processing. Using molecular methods, most isolates from the one contaminated lot detected in 2012 were assigned to *Eurotium repens* (the sexual stage of *Aspergillus reptans*). Recovery of *Aspergillus* was significantly reduced when fruit were surface-sterilized before incubation indicating that surface contamination had occurred for this fruit lot, and the fungus had not penetrated into the fruit.

   Physiological characterization of species of *Aspergillus* isolated to date from prune fruit was done to possibly obtain information as to the time (before or after drying) of fruit contamination/infection by these fungi. All species grew at temperatures of 35C, but significant growth at 45C was only found for *A. brasiliensis*. Studies on the thermal death points are ongoing. Preliminary experiments indicated that all species survived ten hours of incubation at 60C or five hours at 70C.

   The development of *Aspergillus* spp. on dried prunes is presumed to be related to the xerotolerance of these fungi on dried plums and we found that the species isolated all grew well at concentrations of 60% glucose and thus, are adapted to environments with high sugar contents.

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) 3 DMIs Tilt, Indar, Elite, and Quash; the FG 9 anilinopyrimidines Vangard and Scala; the FG 2 dicarboximide Rovral; the FG 7/11 SDHI-QoI pre-mixtures Pristine, Merivon, Luna Sensation, and Q8Y78 (the latter three are pending registration); and the FG 3/11 DMI-QoI pre-mixtures Quadris Top, Adament, Inspire Super, Quilt Excel are most effective against blossom blight. The pre- and post-infection activity of these fungicides on prune blossoms was characterized previously by us. In 2012 we evaluated several additional new fungicides (FG 3 Topguard, FG 7 Fontelis, FG unknown S-2200) and results are presented in this report. The current trend in pre-mixture fungicide registrations is done to provide high performance and 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 is known as our delayed bloom application strategy.

Bacterial blast caused by *Pseudomonas syringae* can be another serious disease of prune 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 2012, however, no disease occurred. Thus, we will continue these studies in 2013.

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 spray adjuvants (e.g., summer oils). 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. Thus, in our trials all fungicides are now applied with a summer oil and data for 2012 are presented in this report. We also provide evidence that some fungicides when applied at an increased gallonage of 130 gal/A provide good protection of fruit inside clusters. New fungicides evaluated in 2012 included Luna Sensation, Luna Experience, Merivon, Fontelis, Topguard, and Q8Y78. Additionally, we continued to evaluate the conventional (i.e., Ph-D) and organic (i.e., CX10440) formulations of polyoxin-D, and we again obtained very good disease control in some of the trials in 2012. This active ingredient recently received an exempt status, and it has been submitted to OMRI for certification for organic use on prune fruit and other crops.

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 2010 and 2011, rust started to develop late in the 2012 season at one of our trial sites. Still, data could be obtained that could be of value in high-disease seasons. Materials evaluated included Adament, Luna Sensation, Luna Experience, Inspire Super, Quadris Top, Quash, Pristine, Merivon, Ph-D, Topguard, Fontelis, and Q8Y78.

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 stored fruit of the 2011 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 started to characterize these species for their heat and high-sugar tolerance to possibly obtain information on when fruit contamination with

these fungi occurs.

OBJECTIVES

1. Evaluate the efficacy of new fungicides, pre-mixtures, and biocontrols representing different chemical classes and biological agents, as well as a new antibiotic, kasugamycin, for bacterial blast in laboratory and field trials.
   - **a.** Evaluation of fungicides for control of brown rot blossom blight and brown rot of fruit.
     - i. Pre- and post-infection activity of selected fungicides against blossom blight.
     - ii. Evaluation of preharvest fungicides in combination with selected spray adjuvants such as summer spray oil vs. Break-thru (laboratory inoculations of field-treated, harvested fruit)
   - **b.** Evaluation of fungicide efficacy against prune rust.
   - **c.** Evaluate Kasumin, Actinovate, Ph-D a new material called AgriTitan and possibly other compounds against bacterial blast in flower and stem canker inoculation studies.
2. 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.
3. Survey of *Aspergillus* species on dried plums and evaluations of the effect of water activity on fungal colonization of dried prunes.

MATERIALS AND METHODS

Evaluation of fungicides for management of brown rot blossom blight. In field studies at the UC Davis Plant Pathology field station, treatments as indicated in Fig. 1 of the results were applied to French prune trees at full bloom on March 21, 2012, using an air-blast sprayer at 100 gal/A. For evaluation of the natural incidence of blossom blight, the number of brown rot infections per tree was counted in early May for each of three single-tree replications. For post-infection laboratory studies, blossoms at popcorn stage were collected and allowed to open. They were then inoculated with a conidial suspension of *M. laxa* (20K conidia/ml), treated with a selected fungicide after 24 h using a hand sprayer, and incubated at 20C. For pre-infection studies, 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 biocontrol Actinovate as well as Kocide 3000, polyoxin-D (Ph-D), Kasumin (kasugamycin), or Mycoshield were made using a hand sprayer to run-off. After 5 h, blossoms were inoculated with *Pseudomonas syringae* (10^7 cfu/ml) by hand-spraying. Inoculated branches 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 Butte Co. to evaluate the efficacy of new fungicides. Fungicides were applied on 8-16-12 (as a preharvest application for management of fruit brown rot) and on 9-3-12 specifically for fall season rust management. Disease was evaluated on Nov. 15, 2012. For this, 10-20 leaves from each of four single-tree replications were evaluated under high magnification for the presence of sporulating rust lesions using the following scale: 0 = no disease, 1=<5 lesions/leaf; 2=<25%; 3=26-50%; and 4=>50% of leaf area affected. 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. All treatments were in combination with a spray-oil (Omni Supreme) at 1.5% v/v and were applied 12 days PHI. In the UC Davis plot, either single fruit from the tree perimeter (exposed fruit) or fruit from inside clusters (10 fruit from each tree) were collected at harvest and wound-inoculated (wounds ca. 1 mm x 2 mm x 2 mm

deep) or non-wound inoculated with conidia of *M. fructicola* (30,000 conidia/ml) on the unexposed side of the fruit. In the Yuba Co. trial, 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.

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

Identification and characterization of* Aspergillus *species on fresh and dried plums. Samples from thirteen fruit lots from Butte Co. from the 2011 harvest were collected in Jan. and Feb. of 2012 in collaboration with a processing plant. Fruit (50-100 fruit/lot) were soaked in water for 1 h and incubated in plastic tubs at 20C and >95% RH. Sub-samples were sterilized in 600 ppm sodium hypochlorite, rinsed and then soaked in water for 1 h, and incubated as the other fruit. Fruit were monitored for fungal growth and isolates were obtained from putative *Aspergillus* sp. colonies. Isolates were grouped based on colony morphology (i.e., mainly colony pigmentation on PDA and CYA) and representatives were cultured for DNA extraction. For RFLP analysis of the ITS1 region of rDNA, DNA was amplified using universal primers ITS1 and ITS2. Amplification products were digested with restriction enzymes *Alu*I, *Hin*fI, *Mbo*I, *Rsa*I, *Taq*I, *Ple*I, and *Bsm*I. DNA fragments were separated in agarose gels, fragment patterns were analyzed visually, and isolates were grouped according to their fragment patterns and compared with reference isolates. To verify species identity, a portion of the large sub-unit rDNA region was amplified using primers D1 and ITS4. 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.

Growth of representative isolates from each species of *Aspergillus* obtained to date from prune at different temperatures was determined. For this, isolates were transferred to PDA medium (three plates per isolate for each temperature) and incubated at 20, 25, 30, 35, 40, and 45C. Radial growth was measured after 5 days. Growth of the isolates was also determined at selected glucose concentrations of the PDA medium. For this, glucose was added to final concentrations of 10, 20, 30, 40, 50, and 60% (w/v) and fungal growth was measured after 5 days. Data for the temperature and glucose studies were summarized in graphical form. To determine the thermal death points of the isolates, agar plugs were transferred into microcentrifuge tubes with 0.25 ml of sterile water (water was added to prevent desiccation of the agar plugs) and were incubated for 5 to 10 h at 55, 60, or 70C. Agar plugs were then transferred to PDA plates and viability of the fungus was assessed based on growth after 3 days.

RESULTS AND DISCUSSION

Evaluation of fungicides for management of brown rot blossom blight. In field studies, the natural incidence of blossom blight was significantly reduced to very low levels ($\leq$1.7% incidence) as compared to the control (12.3% incidence) by a single, full bloom application of any of the treatments (Fig. 1). In addition to registered fungicides, these included the new FG 7+11 Luna Sensation, Merivon, and Q8Y78, as well as the FG 7 SDHI Fontelis.

In laboratory studies using detached prune blossoms, all fungicides evaluated except S2200 were highly effective as post- and pre-infection treatments (Fig. 2). S2200 did not perform well as a pre-infection treatment, which cannot be easily explained because post-infection activity was very good. 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 treatments for control of blossom blast. A study was conducted at UC Davis, however, no disease developed, possibly due to adverse environmental conditions. In 2011, 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

2013. In separate blossom studies done in the spring of 2012 on sweet cherry (cv. Coral on Mahaleb rootstock) bacterial blast developed and the results demonstrated the high efficacy of Kasumin and the moderate efficacy of Actinovate in preventing bacterial blast. Other treatments such as copper or AgriTitan were ineffective.

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). The incidence of sporulating rust lesions was reduced from 96% in the control to between 0% (i.e., Luna Experience and Quash) and 44.2% (i.e., Ph-D organic). Although Ph-D was the least effective, the result is very important because with the recent exempt status of this compound, higher rates can be evaluated in future trials. 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 fungicides applied 12 days PHI in combination with a spray-oil for control of fruit brown rot decay was evaluated in two field trials. In the UC Davis trial, the efficacy of preharvest applications was evaluated using exposed fruit collected from the outer canopy and fruit inside clusters. All fungicides evaluated reduced the incidence of decay of exposed fruit that were non-wound inoculated (Fig. 5). For fruit inside clusters, several fungicides including Pristine, Adament, and Quash also were very effective, but for the other fungicides, decay incidence decreased significantly as compared to the exposed fruit. Because efficacy for fruit inside cluster depends on the spray volume used (as we demonstrated in previous years), volumes higher than 130 gal/A that we used in our applications possibly could improve the performance of these treatments. When fruit were wound-inoculated, Quash, Quadris Top, and Adament were highly effective on the exposed fruit, and also were the most effective treatments on the fruit inside clusters. Fungicides without locally systemic activity that cannot inhibit fungal colonization of the fruit once infection has taken place, such as polyoxin-D (CX10440), Luna Sensation, Merivon, and Fontelis generally did not reduce decay levels from that of the control after wound inoculation.

In the Yuba Co. trial where fruit were randomly collected from each tree and were inoculated on the side that were exposed to the fungicide spray, all fungicides again were effective in the non-wound-inoculations (Fig. 5). Fungicides with locally systemic activity such as Quash, Topguard, Adament, Luna Experience, Inspire Super, Quadris Top, and Q8Y78 also reduced decay after wound-inoculation. Notably, Quash, Adament, Luna Experience (all containing a DMI) were the most effective.

Thus, among the fungicides used in both of these two trials, Quash, Adament, and Quadris Top were consistently highly effective in protecting fruit from brown rot decay. The highest treatment efficacy is obtained when fungicide-oil mixtures are applied at higher volumes. The spray oil is either providing better coverage or may result in better penetration of the fungicide into the fruit. Not all fungicides, however, are compatible with oils and a comparison between different spray adjuvants is warranted.

Results on the efficacy of two formulations of polyoxin-D (Ph-D and CX10440) that were used at a very low rate of 0.71 oz a.i./A (e.g., Ph-D only contains 11.2% active ingredient) were very promising for non-wound-inoculated fruit. Due to the recent approval of an exempt status, higher rates can be evaluated in the future. The fungicide has also been submitted to OMRI for organic certification. Thus, polyoxin-D has the potential to be the most effective organic treatment ever available for the fruit industries of California.

Evaluation of baseline sensitivities of* M. fructicola *isolates against SDHI fungicides. Baseline sensitivities against FG 7 SDHI fungicides were developed as part of our ongoing research on resistance monitoring and management. The evaluated fungicides belong to three subclasses of the SDHIs, fluxapyroxad and penthiopyrad being in the same sub-class, with boscalid and fluopyram in two other separate sub-groups. A range of sensitivities was found for each fungicide, with boscalid having a wider range and higher inhibitory values than the other three compounds (Fig. 6). Of interest is one isolate (isolate 2542) that showed high EC_{50} values for boscalid and fluopyram, and was also in the high-EC_{50} range for the other two fungicides. Cross-resistance between some of the sub-classes is considered low, but our data indicate that cross resistance does exist and that the risk for resistance development against SDHIs is high.

New fungicide resistance reports. We previously reported on cases of resistance in *Monilinia* spp. to AP fungicides in some prune orchards. No new detections of resistance in prune pathogens occurred in 2012. However, in our surveys of several almond orchards in 2012 where AP fungicides were applied by air and disease levels were high, we found that 20% of the isolates of *M. laxa* were resistant to this fungicide class (FG 9 which includes cyprodonil and pyrimethanil). Thus, reduced disease control was in part due to the presence of resistant isolates of the pathogen. A high potential for resistance occurs with over-usage of this mode of action (group of fungicides) and therefore, resistance management approaches should be strictly followed and include rotation, mixtures, and application that does not compromise coverage and ultimately residues.

Identification and characterization of* Aspergillus *species on fresh and dried prunes. Samples of dried prunes taken in the sorting step of fruit processing (after drying and storing for several weeks) were obtained from thirteen fruit lots. Isolates of *Aspergillus* species were obtained only from two of the lots, no fungal growth occurred on the other lots even after incubation of 3 months at 20C and >90% humidity. From the first fruit lot, two isolates of *Aspergillus* with greenish colonies and one isolate with black colonies were recovered from a total of 200 fruit incubated. From the second lot, only isolates with greenish colonies were recovered. Fungi other than *Aspergillus* species were recovered at a very low incidence in this fruit lot. Of a total of 131 fruit that were soaked in water before incubation, 96 fruit showed fungal growth of this colony type. In contrast, of the 128 fruit that were surface-sterilized in sodium hypochlorite before soaking and incubation, only 15 developed growth of *Aspergillus* with the greenish colony type. This indicates that surface contamination had occurred for this fruit lot, and the fungus had not penetrated into the fruit.

Sixteen isolates were cultured. All had identical RFLP restriction patterns using seven restriction enzymes. A comparison with reference isolates indicated that they belonged to *Eurotium repens* (the sexual stage of *Aspergillus reptans*). DNA sequencing of two isolates confirmed this identification. Thus, these results are in contrast to previous years where we identified numerous species from fresh and dried prune including *A. niger, A. brasiliensis,* and *A. carbonarius* (members of the black Aspergilli , i.e., *Aspergillus* Section *Nigri*), *A. phoenicis/tubingensis, A. flavus, A. tamarii, A. melleus,* and *Eurotium repens.* Of these, *A. flavus, A. niger,* and *A. carbonarius* are of major concern because some strains can produce aflatoxins (*A. flavus*) and ochratoxins (*A. niger* and *A. carbonarius*) that are highly toxic mycotoxins.

Based on our results from three years, colonization of prune fruit is very variable between years and fruit lots. The low recovery in this year's sampling possibly could be related to a slightly different processing, such as an increased temperature during drying or a shorter time between harvest and drying that minimizes the chance of host colonization by these fungi.

Our physiological characterization of *Aspergillus* species associated with prune fruit indicated that temperature-growth characteristics were mostly very similar for the two representatives of each species evaluated (Fig. 7). *A. carbonarius* showed a distinct temperature optimum around 30C and for *A. niger* and *A. phoenicis/tubingensis* the optimum was around 35C. The optimum of the other species was over a wider temperature range. Further characterization focused on the high-temperature range because we tried to possibly associate high-temperature tolerance with survival during the drying process of prunes. *A. melleus* (only one isolate available) and *A. carbonarius* appeared to be the most temperature-sensitive species because their growth rates declined quickly at temperatures above 30C, whereas the other species still grew well at 35C. No growth occurred at 40C for *A. melleus* and *A. tamarii*. *A. brasiliensis* was the only species that still showed significant growth at 45C. Studies on the thermal death point of these *Aspergillus* species are ongoing. Preliminary experiments indicated that all species survived ten hours of incubation at 60C or five hours at 70C (ca. 160F). Currently we are evaluating longer incubation times and higher temperatures. These data will provide an indication if contamination with *Aspergillus* species occurs before or after fruit drying and this may help to identify strategies to minimize contamination. Based on the current data it appears that *Aspergillus* species spores are contaminating prune fruit during harvest and are surviving the drying process. Still, current handling practices are highly effective in: preventing decay by drying fruit, sorting to remove low quality fruit after drying, and steam sanitizing fruit in preparation of processing.

Because the development of *Aspergillus* spp. on dried prunes is presumed to be related to the xerotolerance of these fungi on dried plums, we evaluated growth of *Aspergillus* species at different water

potentials. For this, we amended PDA media with selected glucose concentrations. As shown in Fig. 8, all species responded similarly to decreasing water potentials and all still grew at 60% glucose at approximately half their optimum growth rate. This concentration is equivalent to a water activity (A_w) of ca. 0.85 and this is considered low for most microorganisms. Most filamentous fungi require an A_w >0.95. Thus, these *Aspergillus* species can grow at high sugar concentrations and are adapted to environments with high sugar contents such as prune fruit.

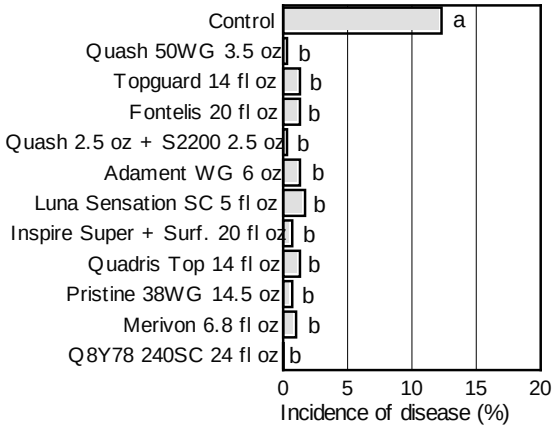
Table 1. List of available, pending registration, or research fungicides with single-site mode of action that were evaluated in our program on French prune or other stone fruit crops in California*

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		Premixture	Adament	Tebuconazole	3	Trifloxystrobin	11	Registered
6		Single	Luna Privilege	Fluopyram	7	---	---	Pending
7		Premixture	Luna Sensation	Fluopyram	7	Trifloxystobin	11	Pending
8		Premixture	Luna Experience	Fluopyram	7	Tebuconazole	3	Pending
9	Syngenta	Single	Tilt	Propiconazole**	3	---	---	Registered
10		Single	Abound	Azoxystrobin	11	---	---	Registered
11		Single	Vangard	Cyprodinil	9	---	---	Registered
12		Premixture	Quilt Excel	Propiconazole	3	Azoxystrobin	11	Registered
13		Premixture	Quadris Top	Difenoconazole	3	Azoxystrobin	11	Registered
14		Premixture	Inspire Super	Difenoconazole	3	Cyprodinil	9	Registered
15		Premixture	Inspire XT	Difenoconazole	3	Propiconazole	3	Registered
16	BASF	Single	Headline	Pyraclostrobin	11	---	---	Reg.- Cherry
17		Single	Xemium	Fluxapyroxad	7	---	---	Pending
18		Premixture	Pristine	Pyraclostrobin	11	Boscalid	7	Registered
19		Premixture	Merivon	Pyraclostrobin	11	Fluxapyroxad	7	Pending
20	DuPont	Single	Fontelis	Penthiopyrad	7	---	---	Registered
21		Single	YT 669	Picoxystrobin	11	---	---	Pending
22		Premixture	Q8Y78	Penthiopyrad	7	Picoxystrobin	11	Pending
23	Arysta	Single	Elevate	Fenhexamid	17	---	---	Registered
24		Single	Ph-D	Polyoxin-D	19	---	---	Exempt
25	Dow Agro	Single	Indar	Febuconazole	3	---	---	Registered
26	Valent	Single	Quash	Metconazole	3	---	---	Registered
27		Single	S-2200	?	?	?	?	Pending
28	Certis USA	Single	CX10440	Polyoxin-D	19	---	---	Exempt
29	Cheminova	Single	Topguard	Flutriafol	3	---	---	Registered

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

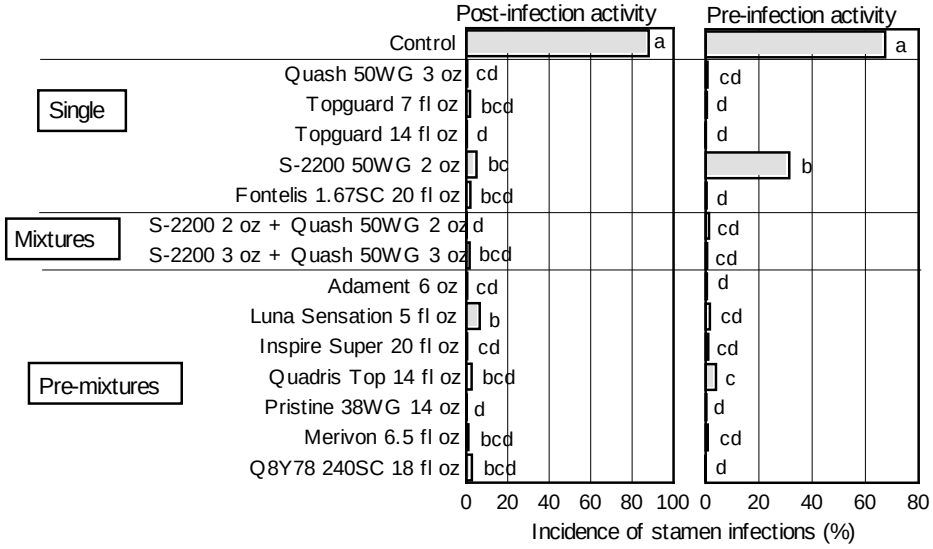
** - Generic formulations: Tebuzol, Orius = tebuconazole; Bumper = propiconazole; and Nevado, Iprodione = iprodione are also available.

Fig. 1. Efficacy of fungicide applications for management of blossom blight of French prune in Solano Co. 2011



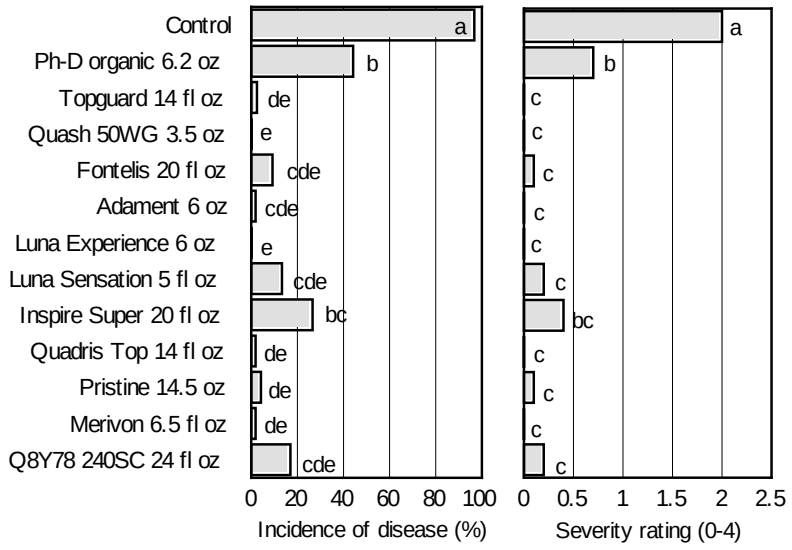
Treatments were applied in the field on 3-21-12 using an air-blast sprayer at 100 gal/A. Q8Y78 240SC is a pre-mix of picoxystrobin and penthiopyrad. For evaluation, the number of brown rot infections per tree was counted in May for each of three single-tree replications.

Fig. 2. Efficacy of post- and pre-infection treatments with fungicides in the laboratory for control of blossom blight of French prune



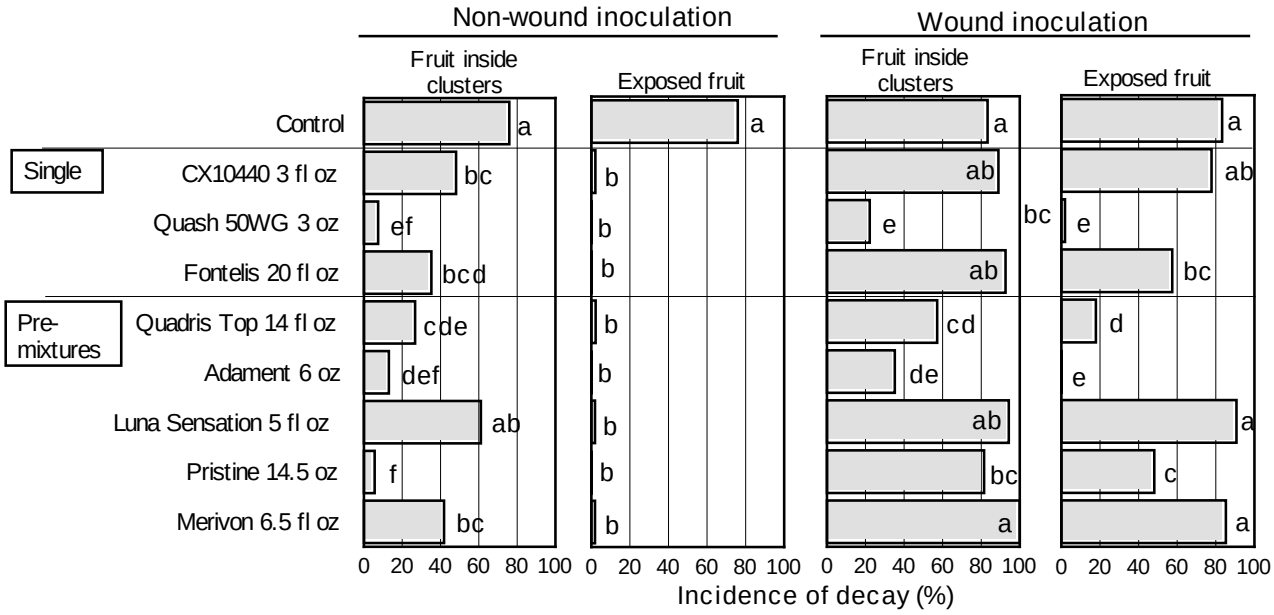
For evaluation of the pre-infection acticity, blossoms were collected in the field and treated in the laboratory using a hand sprayer. After 4 h blossoms were inoculated with a spore suspension of *M. laxa* (15K/ml). For evaluation of the post-infection activity, blossoms were collected and inoculated 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. 3. Efficacy of fungicide applications for management of rust of French prune in Yuba Co. 2012 - Late-season, after harvest evaluations -



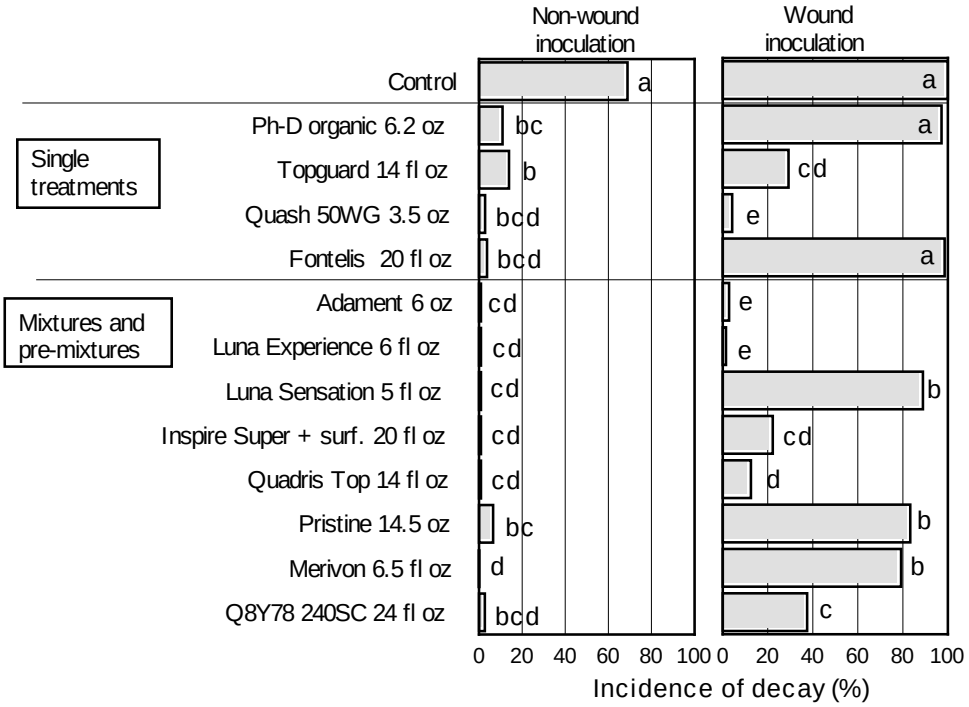
Treatments were applied in the field using an air-blast sprayer at 100 gal/A. For evaluations on 11-15-12, 10-20 leaves from each of four single-tree replications were evaluated for the presence of sporulating rust lesions using the following scale: 0 = no disease, 1=<5 lesions/leaf; 2=<25%; 3=26-50%; and 4=>50% of leaf area affected.

Fig. 4. Efficacy of 12-day PHI fungicide applications for management of postharvest brown rot of French prune at UC Davis 2012
- Comparison of exposed fruit and fruit inside clusters -



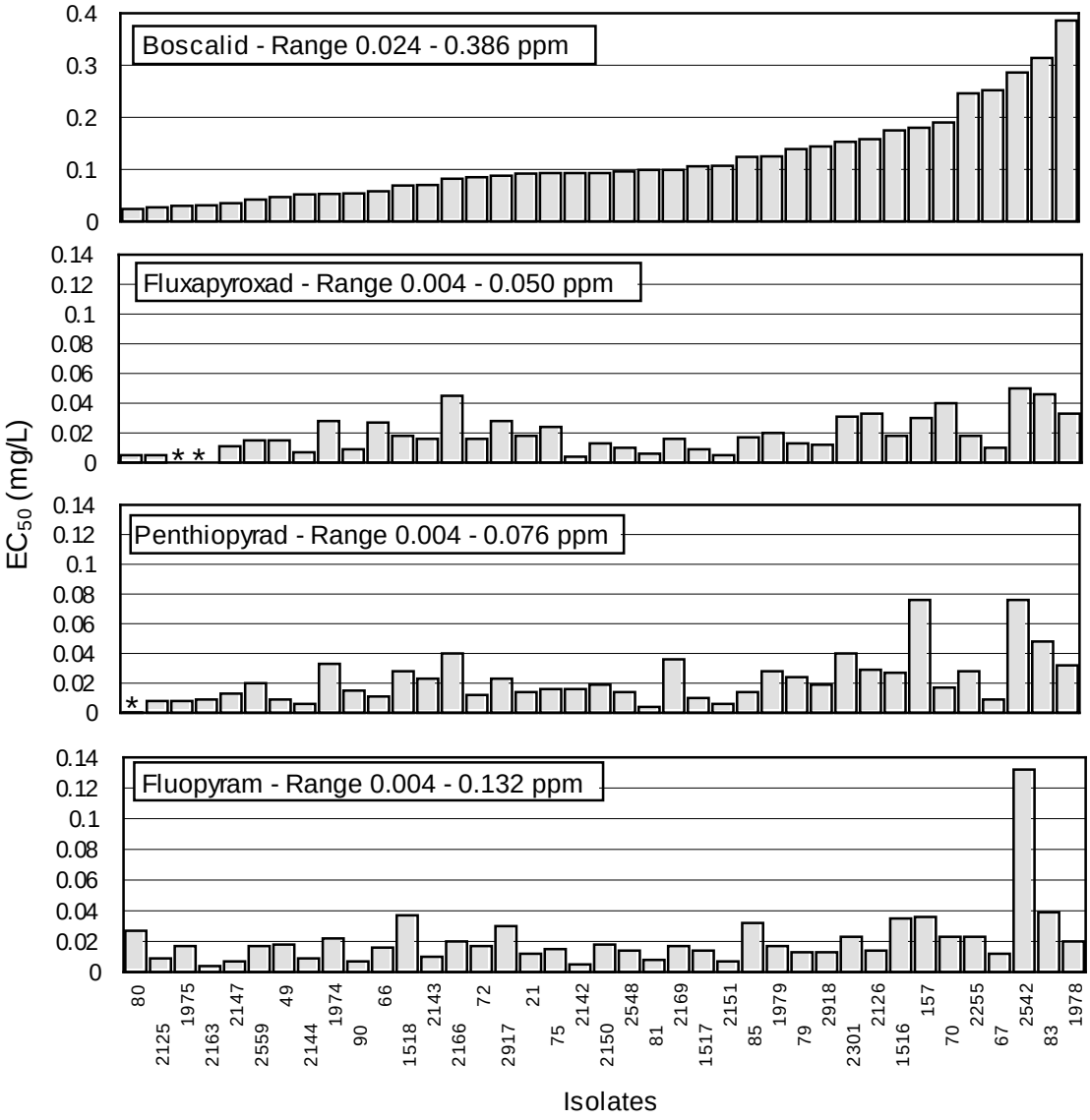
Treatments were applied in the field on 8-16-12 in combination with Omni Supreme Spray Oil (1.5%) using an air-blast sprayer at 130 gal/A. At harvest, fruit from the tree perimeter (exposed fruit) and from clusters were collected and wound- o non-wound-inoculated with conidia of *M. fructicola*(30,000 conidia/ml). Fruit were then incubated for 7 days at 20 C.

Fig. 5. Efficacy of 12-day PHI fungicide applications for management of postharvest brown rot of French prune in Yuba Co. 2012



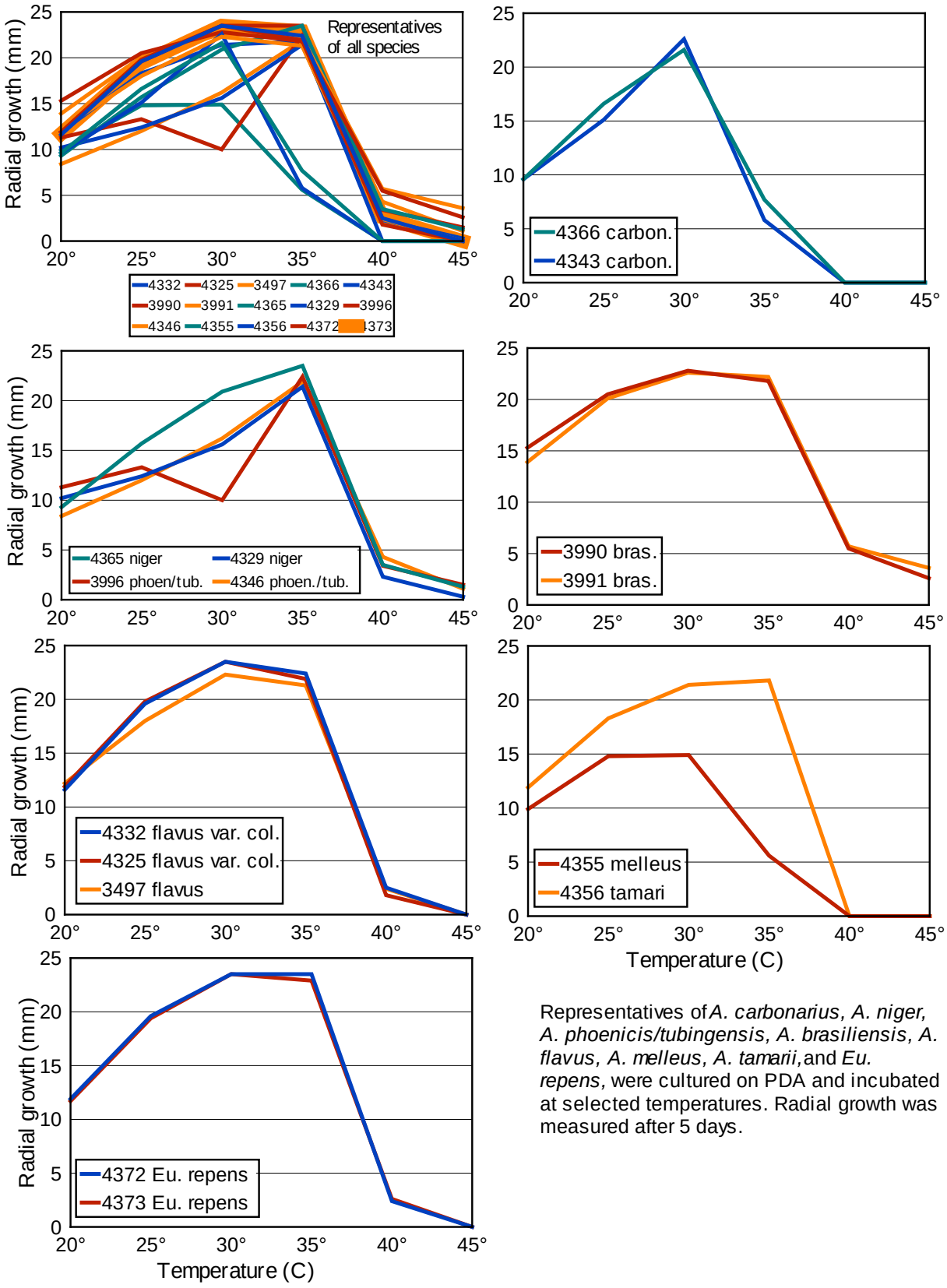
Treatments were applied in the field in combination with Omni Supreme Spray Oil (1.5%) on 8-16-12 using an air-blast sprayer at 130 gal/A. After harvest, fruit were non-wound- or wound-inoculated with conidia of *M. fructicola* (30,000 conidia/ml) and then incubated for 7 days at 20 C.

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



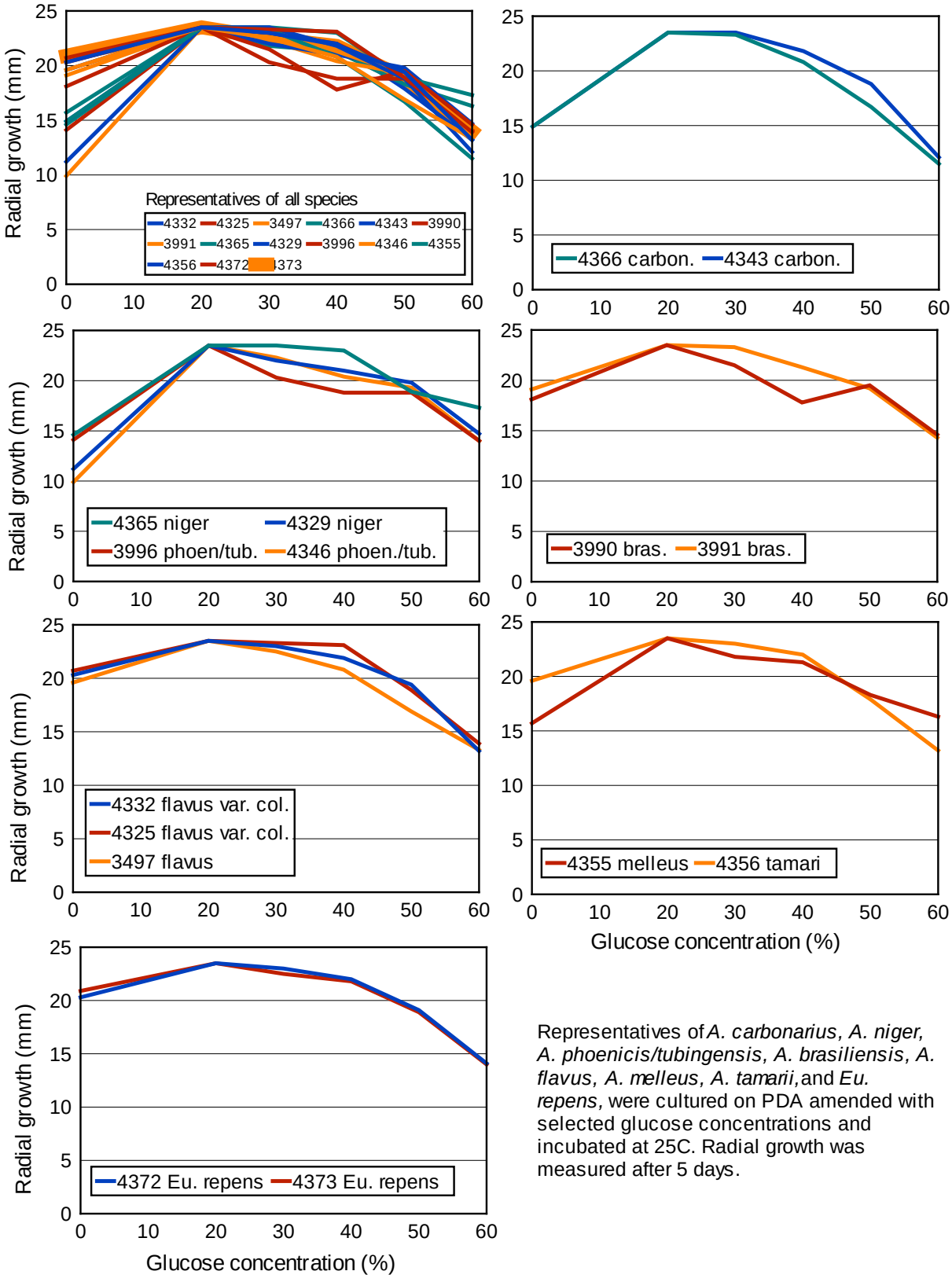
In vitro sensitivities for mycelial growth were determined using the spiral gradient dilution method. Isolates are in the same order in each of the graphs. *

Fig. 7. Growth of *Aspergillus* spp. isolated from prune at selected temperatures



Representatives of *A. carbonarius*, *A. niger*, *A. phoenicis/tubingensis*, *A. brasiliensis*, *A. flavus*, *A. melleus*, *A. tamarii*, and *Eu. repens*, were cultured on PDA and incubated at selected temperatures. Radial growth was measured after 5 days.

Fig. 8 Growth of *Aspergillus* spp. isolated from prune at selected glucose concentrations



Representatives of *A. carbonarius*, *A. niger*, *A. phoenicis/tubingensis*, *A. brasiliensis*, *A. flavus*, *A. melleus*, *A. tamarii*, and *Eu. repens*, were cultured on PDA amended with selected glucose concentrations and incubated at 25C. Radial growth was measured after 5 days.