Annual Report - 2009

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 of Three
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SUMMARY OF RESEARCH ACCOMPLISHMENTS DURING 2009

1. In laboratory studies, the post-infection activity of a biocontrol (i.e., Actinovate), natural products (i.e., Cerebrocide, Regalia, and two additional formulations of the plant extract in Regalia), and of fungicides (single-active ingredients – Scala, Vangard, Quash, Luna Privilege, pre-mixtures – Adament, Inspire Super, Inspire XT, Luna Sensation, Quadris Top, Quilt Xcel) was evaluated for managing brown rot blossom blight. When treatments were applied 24 h after inoculation with *Monilinia laxa*, all fungicides evaluated were highly effective and the incidence of stamen infections was reduced from 74.8% in the control to between <1% (i.e., Quash, Luna Privilege, Adament, Inspire Super, Inspire XT, Quilt Xcel) and 12.7% (i.e., Scala) among the treatments. The biocontrol and the natural products also significantly reduced the incidence of stamen infections and their efficacy was intermediate between the control and fungicide treatments. Although the natural products were less effective than any of the fungicides, they still have a potential to be useful in organic production. Pre-infection activity of biologicals and natural products was low; whereas synthetic fungicides demonstrated very good pre- and post-infection activity.
2. In two field trials, the efficacy of preharvest fungicide applications in combination with a spray oil against brown rot decay of French prune fruit inoculated with *M. fructicola* was evaluated. No highly effective single-active-ingredient alternatives to the SBIs (Indar, Orbit, Quash) were identified that provided a high reduction of decay incidence of treated fruit in wound-inoculation studies. Still, Elevate and Abound also significantly reduced the incidence of decay. On non-wound inoculated fruit, however, the SDHI Luna Privilege (USF2015) was very effective. Furthermore, among the pre-mixtures, Quilt Xcel (SBI/QoI premixture) proved to be highly efficacious using both inoculation methods. Biologicals and natural products were ineffective as protective treatments of fruit.
3. In another field trial, the efficacy of preharvest applications with five fungicides was evaluated using different application volumes (80 and 160 gal/A). For wound-inoculation, exposed fruit collected from the outer canopy and fruit inside clusters were used. More decay developed on fruit collected inside clusters as compared to exposed fruit. Application at higher gallonage was beneficial for two of the fungicides applied to the exposed fruit whereas it was beneficial for four of the fungicides applied to the fruit clusters.
4. More evidence was found for a major shift in sensitivity of *Monilinia* spp. populations to AP fungicides is occurring in some locations in Butte Co. Isolates of *M. laxa* collected from fruit in a commercial orchard had several isolates with reduced sensitivity. EC_{50} values increased by 10 to 30 times as compared to baseline sensitive wild-type isolates.
5. Rust trials were initiated in early June to evaluate the efficacy of new fungicides. Unfortunately, rust did not develop in this trial.
6. There were no new reports on fungal growth on dried plums and thus, no additional isolates of *Aspergillus* sp. were obtained. We initiated the development of molecular identification methods for *Aspergillus* spp. A DNA sequence search and comparison indicated that there is sufficient sequence variability to achieve our goal to allow rapid identification of suspect fungi that may produce mycotoxins..

INTRODUCTION

Brown rot, caused by *Monilinia* species is the most important blossom and preharvest disease of prune in California. In the main growing areas of the state, *M. laxa* is the primary pathogen on blossoms, whereas *M. fructico*la is the main pathogen on fruit. Still, both species can be found causing blossom blight and fruit rot in California. Currently, fungicide treatments that are properly timed are the most effective method to control this disease. Among the registered fungicides, the SBI fungicide Orbit, the anilinopyrimidines Vangard and Scala, the dicarboximide Rovral, and the strobilurin-SDHI (succinate dehydrogenase inhibitor) pre-mixture Pristine are most effective against blossom blight. The pre- and post-infection activity of these fungicides on prune blossoms was characterized previously by us. In 2009 we continued to evaluate several new fungicides and pre-mixtures and results are presented in this report. In 2008, the natural products MOI-104, MOI-107, (different formulations of Regalia) and Cerebrocide were not very effective. Regalia and two other formulations of this plant extract, Cerebrocide, and the biocontrol Actinovate were evaluated in 2009. The information provided will help to identify new effective materials and will help in making decisions on treatment timing. For example, fungicides with a good post-infection activity (i.e., 'kick-back action') in addition to pre-infection activity could be applied as a single, delayed bloom application instead of a standard two- or -three spray program described for preventing infections of sepal (green tip), petals (white tip), and stamen/pistils (full bloom) of prune blossoms.

Preharvest applications with fungicides to prevent losses from fruit brown rot are generally not as highly effective on prune as on other stone fruit crops such as peaches and nectarines. This is because the waxy bloom on the prune fruit prevents a sufficient coverage of the fruit surface by most formulated fungicide products. In our field trials in 2007 and 2008 on preharvest treatments, we demonstrated that when fungicides were mixed with spray adjuvants (e.g., summer oils) the efficacy of most fungicide treatments was significantly improved. Fungicide-spray oil mixtures were again evaluated in 2009 and the new materials Gem (trifloxystrobin), Inspire (difenoconazole), Luna Privilege (fluopyram), and Quash (metconazole), as well as the pre-mixtures Luna Sensation (fluopyram + trifloxystrobin), Quadris Top (difenoconazole + azoxystrobin), and Quilt Xcel (propiconazole + azoxystrobin) were included in the studies. These materials represent new groups and new pre-mixtures representing FRAC groups 3, 7, 9, and 11 and the combinations 3/11 and 7/11. Additionally, because prune fruit developing in clusters are often not well protected and more likely develop brown rot, different application volumes were used in one of the studies to potentially increase the efficacy of the treatments.

Rotations and mixtures of fungicides with different modes of action are critical to prevent the overuse of any one class of fungicide that may lead to the selection of resistant pathogen populations. In 2009 we again confirmed the presence of anilinopyrimidine (FRAC group 9)-resistant isolates of *M. laxa* in one orchard location where treatments with this fungicide class resulted in unsatisfactory decay control. Thus, without the development of new classes of fungicides or new combinations, the potential of resistant populations to develop against new single-site mode-of-action fungicides is high. Thus, in order to prevent the loss of the AP fungicides (FRAC group 9) we have recommended the development of 3/9 and 9/11 pre-mixtures to registrants.

Studies had also been planned on the epidemiology and management of prune rust. These studies were to focus on the host specificity of isolates of stone fruit rust, sources of spring inoculum in orchards, and on disease management. Because, the incidence of rust was very low again in 2009 (as in 2007 and 2008), these studies could not be conducted. Isolation and identification of molds on dried plums was pursued at the request of farm advisors. Although no new isolates could be obtained, we initiated the molecular characterization of previously collected isolates of *Aspergillus* species. The goal is to have a simple, rapid method available to differentiate between harmless saprobes and potentially harmful mycotoxin-producing species.

Objectives

1. Evaluate the efficacy of new fungicides and pre-mixtures representing different chemical classes 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 (laboratory inoculations of field-treated, harvested fruit)

b. Evaluation of fungicide efficacy against rust (If rust does not develop in spring and summer, fall trials will be initiated in selected areas prone to rust disease).

2. Epidemiological studies with prune rust.
   a. Inoculation studies in the greenhouse using potted plants (peach, prune, and selected roostocks) and inoculation of different tissues (i.e., leaves and stems) at different stages of development. For inoculation, different sources of rust (i.e., peach, prune, etc.) will be used.
   b. Spring surveys for sources of inoculum in orchards that had outbreaks in the previous growing season.
3. Monitoring of *Monilinia* spp. populations obtained from decaying fruit for their in vitro sensitivities against commonly used fungicides.
4. Molecular identification of *Aspergillus* species on dried plums using rDNA sequence data.

MATERIALS AND METHODS

Evaluation of fungicides for management of brown rot blossom blight. Laboratory studies were done using French prune blossoms obtained from the UC Davis, Plant Pathology field station. For post-infection studies, blossoms at popcorn stage were collected and allowed to open in the laboratory. They were then inoculated with a conidial suspension of M. laxa (20K conidia/ml), treated with natural products or fungicides after 24 h using a hand sprayer, and incubated at 20C. for pre-infection studies, blossoms were first treated and then inoculated. Three replications of eight blossoms were used for each fungicide. Treatments were applied using rates suggested by the treatment manufacturers. 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 plots to evaluate preharvest fungicide applications for control of fruit brown rot were established at UC Davis and in a commercial orchard in Sutter-Yuba Co. All treatments were in combination with a spray oil (Omni Supreme) used at 1.5% or 2%. In the Sutter-Yuba plot, treatments were applied on 8-4-09 and fruit were harvested randomly from each of the four single-tree replications 14 days after treatment. Ten fruit from each tree were either spray- or wound-inoculated with conidia of *M. fructicola* (30,000 conidia/ml). Spray-inoculations were done on non-wounded fruit, whereas for the wound-inoculations, drops of inoculum were placed on wounds of fruit (ca. 2 mm x 2 mm x 2 mm deep). Fruit were then incubated at 20C for 7 days. In the UC Davis plot, 14 and 0 day PHI treatments were applied in the field on 8-14 and 8-28-09 using an air-blast sprayer at 80 or 160 gal/A. At harvest, either single fruit from the tree perimeter (exposed fruit) or fruit from clusters were collected and wound-inoculated with conidia of *M. fructicola* (30,000 conidia/ml) on the unexposed side of the fruit. Fruit from inside clusters were inoculated on the inside facing side. Fruit were then 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.

A rust trial was established in a commercial orchard to evaluate the efficacy of new fungicides on prune. Fungicides were applied on July 7 and July 21 at the onset of rust symptoms in the test portion of the orchard.

In vitro fungicide sensitivity tests. The sensitivity of isolates of *M. laxa* obtained from prune fruit in an orchard in Butte Co. against cyprodinil and propiconazole was assessed using the spiral gradient dilution method. Conidia were used to inoculate amended PDA agar plates along the fungicide concentration gradient and plates were evaluated for fungal growth after 3 days of incubation at 20C. EC_{50} values of isolates were compared to isolates from the baseline population.

Identification of molds on dried plums. A sequence comparison of rDNA internal transcribed spacer regions among species of *Aspergillus* including *A. nidulans* (1 isolate), *A. niger* (2), *A. flavus* (3), *A. chevalieri* (3), and *A. parasiticus* (4) was conducted to gain information about sequence diversity within this fungal genus. For this, sequences of the ITS region were obtained from GenBank (NCBI) and from studies found in the literature.

RESULTS AND DISCUSSION

Evaluation of fungicides for management of brown rot blossom blight. In laboratory studies using detached prune blossoms, the post-infection activity of a biocontrol (i.e., Actinovate), natural products (i.e.,

Cerebrocide, Regalia, and two additional formulations of the plant extract in Regalia), and of fungicides (single-active ingredients – Scala, Vangard, Quash, Luna Privilege, pre-mixtures – Adament, Inspire Super, Inspire XT, Luna Sensation, Quadris Top, Quilt Xcel) was evaluated for managing brown rot blossom blight. The post-infection activity was evaluated 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.

When treatments were applied 24 h after inoculation with *M. laxa*, all fungicides evaluated were highly effective and the incidence of stamen infections was reduced from 74.8% in the control to between <1% (i.e., Quash, Luna Privilege, Adament, Inspire Super, Inspire XT, Quilt Xcel) and 12.7% (i.e., Scala) among the treatments (Fig. 1A). The biocontrol Actinovate and the natural products also significantly reduced the incidence of stamen infections and their efficacy was intermediate between the control and the fungicide treatments (Fig. 1B). There was no statistical difference in efficacy between Regalia and the two other formulations of this product. The addition of an adjuvant (i.e., Breakthru) to Cerebrocide decreased the activity of this natural product. Although the natural products were less effective than any of the fungicides, they still have a potential to be useful in organic production.

Evaluation of fungicides for management of fruit brown rot. The efficacy of preharvest fungicides for control of fruit brown rot decay was evaluated in two field trials. In the Sutter-Yuba trial, most of the fungicides were more effective on non-wound inoculated fruit (Fig. 2). Only the fungicides of the SBI class (i.e., Indar, Orbit, Quash – Inspire, however, was less effective) or the pre-mixture Quilt Xcel than contains the SBI propiconazole were also very effective when fruit were wound-inoculated after treatment and harvest. Thus, no highly effective single-active-ingredient alternatives to the SBIs were identified that provided a high reduction of decay incidence of treated fruit in wound-inoculation studies. Still, the registered Elevate and Abound, as well as Gem and Luna Sensation also significantly reduced the incidence of decay.

In the UC Davis trial, the efficacy of preharvest applications with five fungicides was evaluated using different application volumes (80 and 160 gal/A). For wound-inoculation, exposed fruit collected from the outer canopy and fruit inside clusters were used. More decay developed on fruit collected inside clusters as compared to exposed fruit indicating that fungicide residues on these fruit were lower than on the exposed fruit (Fig. 3). Applications at higher gallonage were beneficial for two of the fungicides (Orbit, Quash) applied to the exposed fruit whereas this strategy was beneficial for four of the fungicides (Orbit, Quash, Pristine, Luna Sensation) applied to the fruit clusters. Overall the incidence of decay was still relatively high for all treatments in the fruit cluster evaluation because fruit were inoculated on the side facing away from the spray direction. Thus, in this assay, the efficacy of the fungicides was highly challenged.

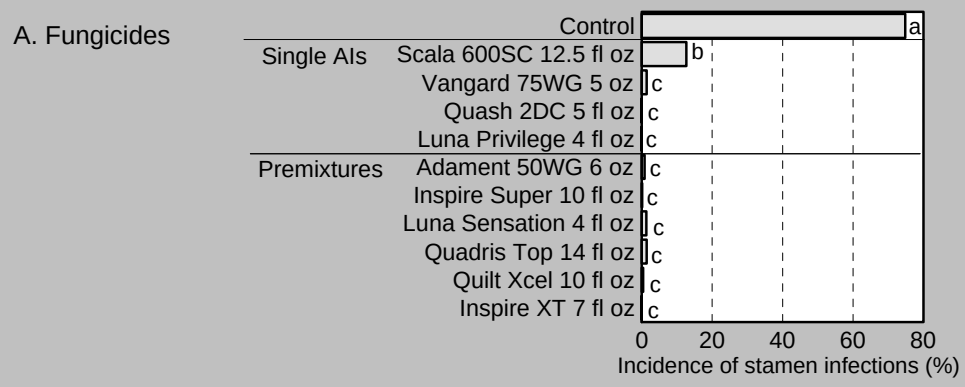
In summary, the highest efficacy of pre-harvest treatments is obtained when fungicide-oil mixtures are applied at higher volumes. The spray oil is either providing better coverage or may result in better penetration of the fungicide into the fruit. Not all fungicides, however, are compatible with oils and a comparison between different spray adjuvants is warranted. Additionally, no highly effective single-active-ingredient alternatives to the SBIs were identified that provided a high reduction of decay incidence of treated fruit in wound- and non-wound-inoculation studies. Still, Luna Sensation (a pre-mixture of the SDHI fluopyram and the QoI trifloxystrobin) was also quite effective.

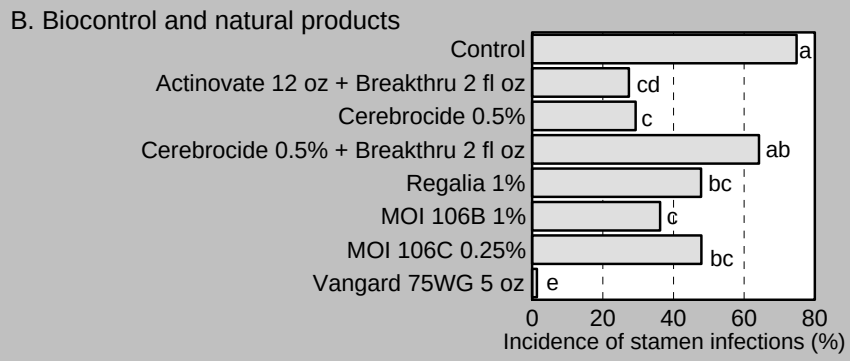
Another objective of our prune research project was to evaluate pathogen isolates for their in vitro fungicide efficacy against important fungicide classes (i.e., the SBIs and anilinopyrimidines). We are focusing our samplings on locations where fungicide treatments do not provide satisfactory decay control. We obtained isolates of *M. laxa* from an orchard that was treated with Vangard and two applications of Orbit between July and August. Our assay indicated that 8 of the 9 isolates evaluated had a reduced sensitivity to cyprodinil, whereas all isolates were highly sensitive to propiconazole (Fig. 4). EC_{50} values increased by 10 to 30 times as compared to baseline sensitive wild-type isolates. We previously had found cyprodinil-insensitive isolates of *M. fructicola* in an orchard in 2007. Thus, without the development of new classes of fungicides, new pre-mixtures, or the use of rotations, the potential of resistant populations to develop against new single-site mode-of-action fungicides and replace the sensitive population is high.

Identification of* Aspergillus *on dried plums. There were no new reports on fungal growth on dried plums and thus, no additional isolates of *Aspergillus* sp. could be obtained. We initiated the development of molecular identification methods for *Aspergillus* spp. and we started evaluating the isolates that were obtained in 2008 together with reference isolates. A sequence search in the GenBank DNA database and in the literature indicated

that there is sufficient sequence variability in the ribosomal DNA spacer regions to differentiate species of *Aspergillus* (Fig. 5). Thus, we are currently preparing DNA extracts of the isolates obtained and we will proceed with the sequence analysis and species identification. The goal is to have a simple method available to differentiate between harmless saprobes and potentially harmful mycotoxin-producing species. Several species of *Aspergillus* are known to produce toxins. These in addition to *A. flavus* (toxin = aflatoxins) include *A. ochraceus* (toxin = ochratoxin A) and *A. terreus* (toxin = citrinin).

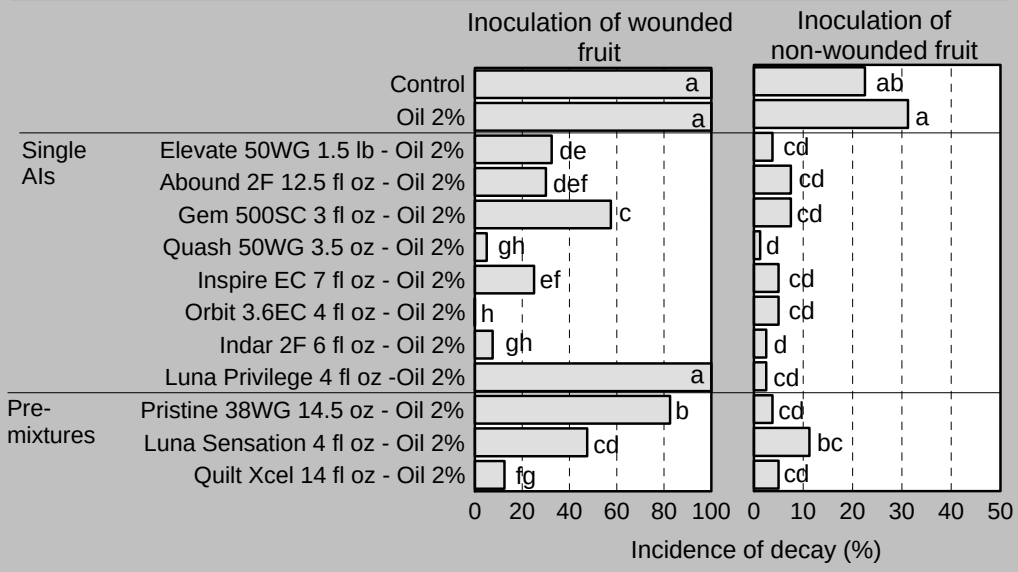
Fig. 1. Efficacy of post-infection treatments with a bioncontrol, natural products, or fungicides for management of blossom blight of French prune





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

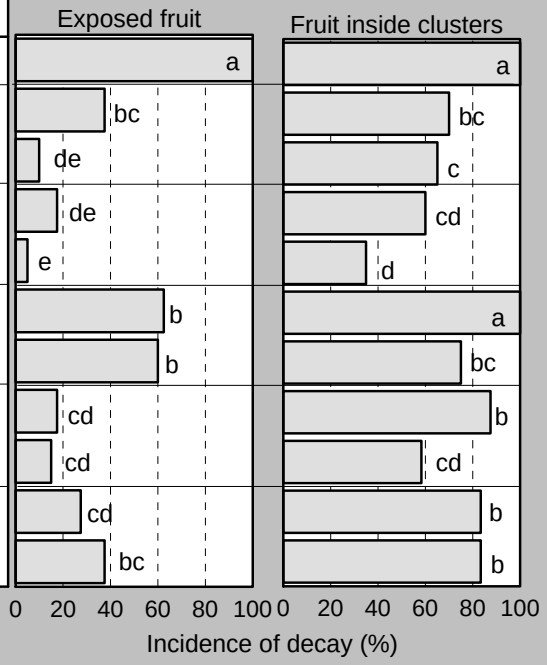
Fig. 2. Efficacy of 14-day PHI fungicide applications for management of postharvest brown rot in a field trial in Sutter-Yuba Co. 2009.



Treatments were applied in the field on 8-4-09 using an air-blast sprayer (100 gal/A). Omni Supreme Spray oil was used. After harvest, fruit were either spray- or wound-inoculated with conidia of *M. fructicola* (30,000 conidia/ml). Fruit were then incubated for 7 days at 20 C.

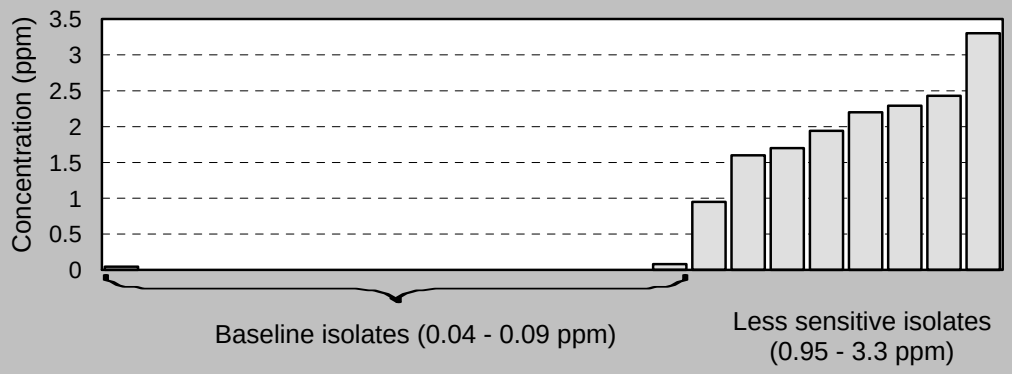
Fig. 3. Efficacy of 14+0-day PHI fungicide applications for management of postharvest brown rot of French prune at UC Davis 2009.
- Evaluation of application volumes in preventing decay of exposed fruit and fruit inside clusters -

Treatment	Application volume/A	Exposed fruit	Fruit inside clusters
Control	---	a	a
Orbit 3.6EC 4 fl oz - Oil 1.5%	80 gal	bc	bc
Orbit 3.6EC 4 fl oz - Oil 1.5%	160 gal	de	c
Quash 50WG 3.5 oz - Oil 1.5%	80 gal	de	cd
Quash 50WG 3.5 oz - Oil 1.5%	160 gal	e	d
Pristine 38WG 14.5 oz - Oil 1.5%	80 gal	b	a
Pristine 38WG 14.5 oz - Oil 1.5%	160 gal	b	bc
Luna Sensation 7 fl oz - Oil 1.5%	80 gal	cd	b
Luna Sensation 7 fl oz - Oil 1.5%	160 gal	cd	cd
Quadris Top 14 fl oz - Oil 1.5%	80 gal	cd	b
Quadris Top 14 fl oz - Oil 1.5%	160 gal	bc	b



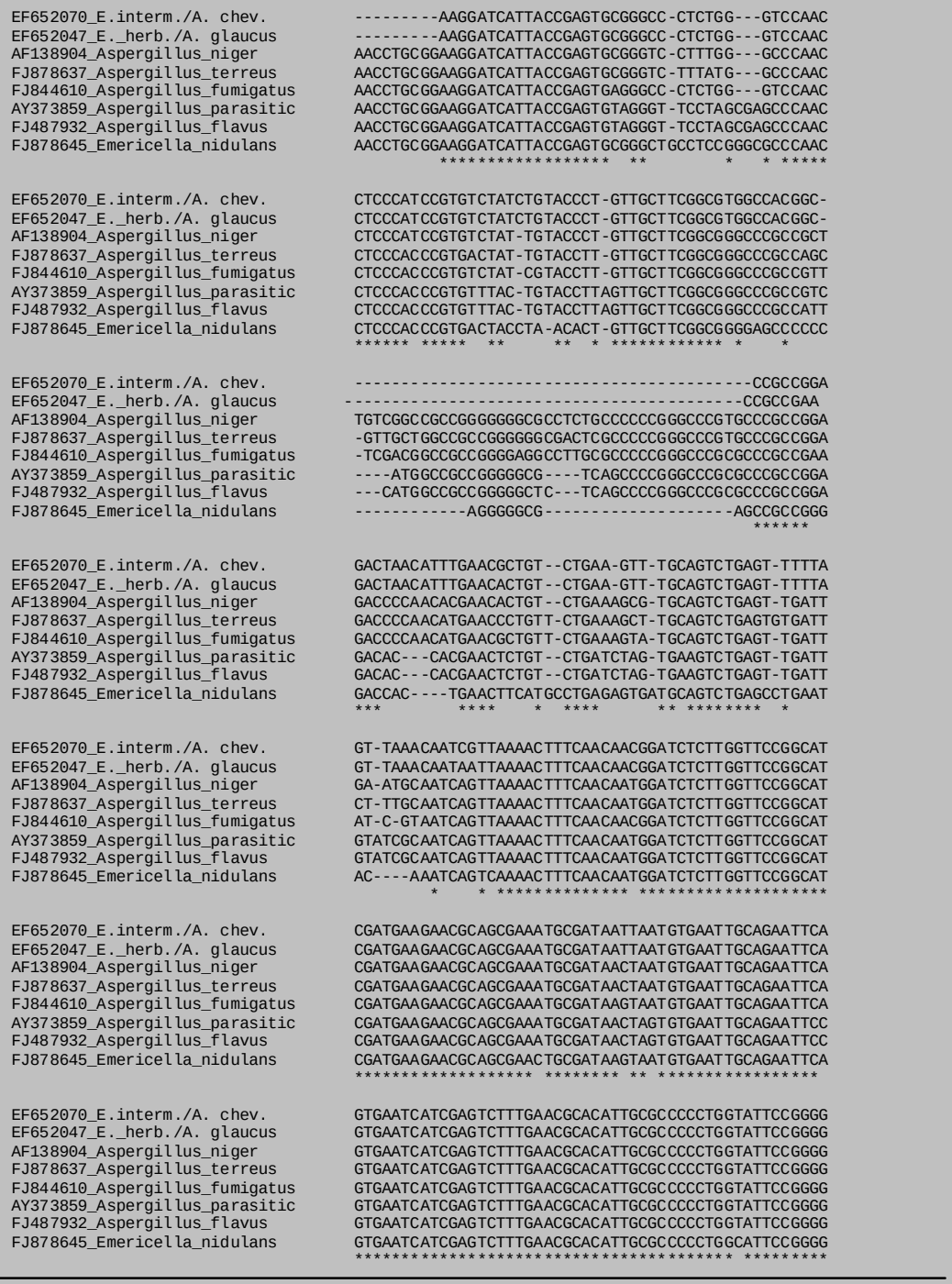
Treatments were applied in the field on 8-14 and 8-28-09 using an air-blast sprayer at 80 or 160 gal/A. Omni Supreme Spray oil was used. At harvest, either single fruit from the tree perimeter (exposed fruit) or fruit from clusters were collected and wound-inoculated with conidia of *M. fructicola* (30,000 conidia/ml) on the unexposed side of the fruit. Fruit from inside clusters were inoculated on the inside facing side. Fruit were then incubated for 7 days at 20 C.

Fig. 4. *In vitro* sensitivity of isolates of *Monilinia laxa* to cyprodinil



Inhibitory concentrations were determined on PDA using the SGD method.

Fig. 4. Alignment of the rDNA ITS 1 region of selected species of *Aspergillus*



Sequences were obtained from Genbank and the alignment was done using Clustal W. Alignments are based on 3 isolates of *Aspergillus chevalieri* (teleomorph *Eurotium intermedium*), 3 isolates of *A. glaucus* (teleomorph *E. herbariorum*), 2 isolates of *A. niger*, 3 isolates of *A. terreus*, 3 isolates of *A. fumigatus*, 4 isolates of *A. parasiticus*, 3 isolates of *A. flavus* and 1 isolate of *A. nidulans*.