Annual Report - 2007

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:	First-Year of Three
Principal Investigator:	J. E. Adaskaveg Department of Plant Pathology, University of California, Riverside 92521
Cooperating:	D. Thompson, H. Förster, R. Buchner (UCCE-Tehama Co.) and F. Niederholzer (UCCE-Sutter-Yuba Co.)

SUMMARY OF RESEARCH ACCOMPLISHMENTS DURING 2007

1. In laboratory studies on the pre- and post-infection activity of fungicides for managing brown rot blossom blight, all fungicides evaluated significantly reduced the incidence of disease. Rovral and mixtures of Scala or Orbit with Captan were most effective when treatments were applied before (pre-infection activity) or after (post-infection activity) inoculation with *M. laxa*. Scala by itself was also among the best treatments in post-infection studies.
2. In two field trials with preharvest applications done at 7 and 14+7 days PHI for brown rot control on naturally infected French prune, all fungicides significantly reduced the incidence of brown rot decay on harvested fruit after spray inoculations with *M. fructicola*. The addition of a spray oil to Orbit, Pristine, or Elevate in most cases significantly increased the efficacy of the fungicides. When fruit were wound-inoculated after treatment and harvest, the efficacy of most treatments was reduced as compared to the spray inoculations. Still, again when Orbit, Pristine, and Elevate were used in combination with a spray oil, in most cases a significant increase in efficacy was observed.
3. The incidence of brown rot was low in 2007 in most locations. Still, we were able to collect over 50 isolates from 12 locations. EC_{50} values for iprodione, propiconazole, and cyprodinil were compared to those of isolates collected in previous years (1999 or before). EC_{50} values for iprodione and propiconazole were in the same range or were mostly lower, respectively, for the newly collected isolates as compared to the older isolates. Thus, no resistance was detected. For cyprodinil, however, one isolate collected in 2007 had an EC_{50} value of 5.8 ppm as compared to values for all other isolates that were between 0.027 and 0.095 ppm. Thus, this is the first report of a cyprodinil-resistant field isolate of *M. fructicola*. This emphasizes the stringent use of resistance management practices to avoid the occurrence of field resistance and loss of fungicide efficacy.

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. fructicola* is the main pathogen on fruit. 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-carboxamide premixture Pristine are most effective against blossom blight. The pre- and post-infection activity of these fungicides has not been well characterized on prune blossoms, and this was one objective of our 2007 research. This information would be helpful in making decisions on treatment timing. For example, fungicides with a good post-infection activity (i.e., 'kick-back action') could be applied as a single, delayed bloom application instead of the common two-spray program. In addition, we started evaluating new fungicides (i.e., V-10135) and mixtures of the single-site mode of action materials Scala and Orbit with a multi-site mode of action material, i.e., Captan. This was done to find effective mixture treatments that could be used in resistance management to avoid the development of resistance that might easily occur against the single-site mode of action materials.

Preharvest applications with fungicides to prevent losses from fruit brown rot generally are 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 2007, we conducted field trials on preharvest treatments where fungicides were mixed with spray adjuvants that possibly could improve fungicide efficacy. Thus, the goals for our blossom and preharvest fungicide research are to develop alternative chemistries (e.g., V-10135, pre-mixtures like Pristine) to the SBI and anilinopyrimidine fungicides and mixture treatments for blossom blight and brown rot management so that preharvest rotation programs can be designed that prevent the overuse of any one class of fungicide. Without the development of new classes of fungicides, the potential of resistant populations to develop to the new single-site mode-of-action fungicides is high.

Another objective of our prune research project was the monitoring of *M. fructicola* populations for their in vitro fungicide efficacy. This was done because treatment failures after anilinopyrimidine applications have been reported by some prune growers and moreover, resistance in pathogens of other crops has been reported for this class of fungicides. Resistance against SBI fungicides has also developed in other stone fruit growing areas of the country. With these studies, we tried to find out if the reported treatment failures in prune brown rot management by fungicides was due to resistance in the brown rot fungal population or if it was due to other reasons, such as treatment timing or application method. For example, the efficacy of Vangard and Scala is generally not very high during high summer temperatures and high humidity. Monitoring of pathogen populations for their in vitro sensitivity against commonly used fungicides was also done to stay ahead of any spread of resistant populations that would eventually result in the loss of fungicide efficacy.

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 in 2007 and due to a re-prioritizing of research efforts, these studies were postponed until the next funding period.

Objectives

1. Evaluate the efficacy of new fungicides representing different chemical classes in field trials.
   - **a.** Evaluation of fungicides for brown rot blossom blight and rust control.
   - **b.** Evaluation of brown rot and rust fungicides applied during the spring based on forecasted meteorological macroclimatic conditions vs. a calendar program.
2. Epidemiological studies with prune rust.
   - **a.** Purchase and establish greenhouse potted French prune plants and selected plum rootstocks.
   - **b.** Inoculation studies of selected tissues (i.e., leaves and stems) at different stages of development from different sources of rust (i.e., peach, prune, etc.).
   - **c.** Spring surveys for sources of inoculum if 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.

MATERIALS AND METHODS

Evaluation of fungicides for management of brown rot blossom blight and preharvest fruit decay. Laboratory studies were done using French prune blossoms obtained from the UC Davis, Plant Pathology field station. For this, not fully opened blossoms were collected, forced open in the laboratory, and were either inoculated with a conidial suspension of *M. laxa* (20K conidia/ml) and treated after 24 h with each fungicide using a hand sprayer, or treated and inoculated after 24 h of incubation at 20C. Three replications of eight blossoms were used for each fungicide. Data were analyzed using analysis of variance and least significant difference (LSD) mean separation procedures of SAS 9.1.

Field plots to evaluate preharvest fungicide applications for control of fruit brown rot were established at UC Davis and in a commercial orchard in Colusa Co. Treatments of Orbit, Pristine, Elevate, Vangard, and Scala were applied at 7 and 14+7 days PHI using an air-blast sprayer calibrated to 100 gal/A. Treatments with

Orbit, Pristine and Elevate were also done in combination with a spray oil (Superior 415 Spray Oil, 2%). There were four single-tree replications for each treatment. After harvest, three replications of ten fruit each 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. Data were analyzed using analysis of variance and least significant difference (LSD) mean separation procedures of SAS 9.1.

Evaluation of the in vitro toxicity of fungicides against M. fructicola. Fungal isolations from decayed fruit that were supplied by F. Niederholzer and R. Buchner were done using standard techniques. We also collected 100 mummies from bins where fruit had been dried from the 2006 season. In vitro sensitivities for iprodione, propiconazole, and cyprodinil were determined using the spiral gradient dilution (SGD) method. With this technique, a fungicide stock solution is being diluted exponentially in a spiral, starting from the center of a Petri dish containing culture media. Conidia of the fungus were then placed on a radial line on the agar medium. For mycelial growth, the radius where 50% of growth is observed (as compared to non-amended agar media) is measured after 3 days. These radial measurements are transformed into local fungicide concentrations (ppm) using a computer program. EC_{50} values for isolates obtained in 2007 were compared to those obtained in previous years (1999 and before).

RESULTS AND DISCUSSION

Evaluation of fungicides for management of brown rot blossom blight. Because our project was initiated late in the spring, no field treatments with fungicides could be done and treatments could not be evaluated under field conditions. In a laboratory study, the pre- and post-infection activity (treatments 24 h before or after inoculation) of fungicides was compared. In both inoculation-treatment schedules, all fungicides significantly reduced the incidence of stamen infections from that of the control (Fig. 1). Rovral and mixtures of Scala or Orbit with Captan were most effective when treatments were applied before (pre-infection activity) or after (post-infection activity) inoculation with *M. laxa*. Scala by itself was also among the best treatments in post-infection studies. Thus, because selected fungicides have protective and post-infection (curative) activity and are also very persistent, single, carefully timed (e.g., late bloom) applications, can be highly effective when environmental conditions for disease are not highly conducive. This information supports a single fungicide application following our 'delayed-bloom model' for blossom blight management that is being followed for other stone fruit crops. In addition, the high efficacy of mixture treatments with Captan allows these mixtures to be used as an anti-resistance strategy. However, because there have been concerns about the toxicity of captan against bees, the efficacy of mixtures with other multi-site mode of action materials such as Bravo should be evaluated.

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 both trials, applications with selected fungicides at 7 and 14+7 days PHI all significantly reduced the naturally incidence of brown rot decay on harvested fruit after spray inoculations with *M. fructicola* as compared to the untreated control (Figs. 2A, 3A). The addition of a spray oil to Orbit, Pristine, or Elevate in most cases significantly increased the efficacy of the fungicides. Thus, overall, the most effective treatments included Orbit-Oil, Pristine-Oil, and Elevate-Oil. Scala and Vangard were less effective, but similar effective to Orbit, Pristine, and Elevate, when the latter materials were used without spray oil. With few exceptions, single treatments done at 7 days PHI were similarly effective than two treatments at 7 and 14+7 day intervals. Thus, apparently, treatments done at 14 days before harvest did not persist well. Still, it has to be considered, that fruit were inoculated after harvest in these experiments. Natural infections in the field will occur throughout fruit ripening and thus, fruit would be protected for a longer period of time when two applications are done.

When fruit were wound-inoculated after treatment and harvest, the efficacy of most treatments in both trials was reduced as compared to the spray inoculations (Figs. 2B, 3B). Most treatments performed better in the Colusa Co. trial as compared to the UC Davis trial. Comparing the efficacy in the UC Davis trial for spray- and wound-inoculated fruit, all treatments were more effective in the spray inoculations. For the Colusa Co. trial, this difference in inoculation method was not as distinct. Still, again, when Orbit, Pristine, and Elevate were used in combination with a spray oil, in most cases a significant increase in efficacy was observed. These results indicate that a significant increase in fungicide efficacy can be obtained when applications are done with a spray oil. The 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. In addition, a comparison between different spray adjuvants is warranted.

***Evaluation of the in vitro toxicity of fungicides against* M. fructicola.** The incidence of brown rot was low in 2007 in most locations and decaying fruit in orchards was hard to find. We collected mummies from bins where fruit had been dried from the 2006 season; however, no viable isolates were obtained from these mummies. With the help of farm advisors Niederholzer and Buchner, we were able to collect 51 isolates from 12 locations. EC_{50} values for iprodione, propiconazole, and cyprodinil were compared to those of isolates collected in previous years (1999 or before). EC_{50} values for iprodione and propiconazole were in the same range or were mostly lower, respectively, for the newly collected isolates as compared to the older isolates (Fig. 4A,B). Values ranged from 0.069 to 0.118 ppm for iprodione and from 0.002 to 0.009 ppm for propiconazole. Thus, no resistance was detected in these collections. For cyprodinil, the majority of isolates had EC_{50} values between 0.027 and 0.095 ppm. One isolate in the 2007 collections, however, had a value of 5.8 ppm (Fig. 4C). Thus, this is the first report of a cyprodinil-resistant field isolate of *M. fructicola*. This emphasizes the stringent use of resistance management practices to avoid the occurrence of field resistance and loss of fungicide efficacy. More extensive surveys will have to be done in 2008.