Actigard RRV Trial Protocol

Rose Rosette Virus is spread by a mite known as *Phyllocoptes fructiphilus* which is common in Georgia but has not yet made ingress into Florida. The goal of this experiment is to test a plant resistance activator (Actigard) and its role in preventing disease progression of RRD.

# The experiment:

We will be applying Actigard at two different rates: 50 mg/L and 100 mg/L to observe the effects of Actigard on the mites. We have two controls for this experiment. The first will be a miticide (Kontos) as a positive control for the mites and the second will be water as a negative control for the spray application.

We will be recording weekly samples of mites from each rose plant during the year. This will allow us to track changes in the mite population throughout the year for each treatment. Our hypothesis is that there will be less mites on plants treated with Actigard.

# Chemical mixing

Water: 500 mL of water

Actigard 100: 417 mL of water, 31.2 mg Actigard

Actigard 50: 417 mL of water, 15.6 mg Actigard

Kontos: 417 mL of water, 110.7 µL Kontos

Mix all applications while wearing nitrile gloves under the vent hood. The Actigard should be weighed on the small scale to the right of the vent hood. The scale is calibrated for grams, please double check the decimal point to make sure you have the right number of milligrams. The Kontos is liquid which should be shaken before use. Measure out the 110.7 µL using the 1000 µl pipette to the left of the vent hood. Each chemical should be mixed directly in the tank.

# Preparing Spray Equipment

To begin spraying, first attach the CO2 regulator to the tank, then the hose to the mix tank. Attach the wand to the mixing tank. Give a firm tug on each connection to be sure that they won’t separate or leak. Then open the regulator valve slightly. The dial should read 30 PSI. If it does not, you may need to refill the tank or adjust the regulator to read 30 PSI. Once the regular valve is open, slowly open the small valve near the end of the regulator hose. This will allow the tank to pressurize. Check to make sure there is no gas leaking from the spray tank. If there is, shut off the main and the small valve, then pull the ring pin to release the pressure on the spray tank before adjusting the lid to stop a leak. **Always be sure to close all valves and release the tank pressure before opening the tank or removing hoses.**

If there are no leaks, the system is now ready to use.

# Spray Protocols

The applications should be done by block the following order: A: Water, B: Actigard 100, C: Actigard 50 then D: Kontos last.

The wand begins spraying as soon as you squeeze the handle. It sprays in a flat path and a fine mist, so you do not want to spray if wind speed is above 10 mph. You want to start a few inches away from the first plant in the row and make sure the spray is a few inches above the top of each plant. You should stop spraying a second or so before the end of the row to account for the pressure in the sprayer, which doesn’t stop spraying immediately.

You need to walk at a consistent pace to cover all the plants evenly. The first application of water should give you a good idea of how fast you need to spray to cover the entire plot. If you finish all the plots and still have liquid in the tank (you can hear it sloshing), try to make another quick pass evenly over all the plots.

Kontos has a 24-hour Restricted Entry Interval and Actigard has a 12-hour Restricted Entry Interval. Please wait the designated times before reentering the field and touching the plants.

# Recording data

We will be taking one rose bud from each plant every week, counting the number of mites and recording how many we find on a new datasheet each week. We will also be rating disease severity before we spray.

* Print off a copy of ‘actigard\_trial\_datasheet’ and write down the date on it
* Count the number of flowers and record plant size
* Calculate % disease severity according to the protocol described in ‘disease\_severity\_scale’ on the Google Drive

# Sample Collection and Processing

* Take one of the large centrifuge tubes and label it with the plot, plant position and date
* The plot map can be found on the Google Drive labeled ‘asm\_plot\_2018’
* Take a floral cutting large enough to fill the centrifuge tubes provided (about ~3 cm)
* Place the flower into the tube. Try to take flowers from the same sides of the plants when sampling and rotate the side selected each week
* Once all rose samples have been collected, return to the lab
* Follow the steps in ‘mite\_washing\_protocol’ on the Google Drive to extract the mites from the rose sample for counting
* Write the number of mites found under the column labelled ‘Count’ on the datasheet. Please double-check that you are recording for the right plant
* If you do not find any mites, record a zero on the datasheet. If there were no flowers on the rose sampled, put NA on the datasheet
* If you cannot count all the mites during the same day, it is possible to refrigerate the flowers in the mite washing solution and finish counting the following day

Notes:

* Please remember to follow all sanitation measures described in the ‘Standing Operating Procedures’ on the Google Drive
* Please read all chemical labels thoroughly and follow the safety precautions
* Please do not apply sprays when wind is greater than 10 mph or if raining
* Please try to spray on the same day every week if possible
* Please consider spray application re-entry intervals before collecting data
* Please try to collect data on a consistent weekly schedule as well
* Lastly, if you have any suggestions for changes to the protocols, questions or concerns, please call or email Austin Fife:

208-874-2283 fife@ufl.edu