RRV Volatiles Protocol

Rose Rosette Virus (RRV) is spread by a mite known as *Phyllocoptes fructiphilus* which is common in Georgia but has not yet made ingress into Florida. The purpose of this experiment is to observe any differences in headspace volatiles among RRV-infected and healthy roses treated with the systemic acquired resistance inducer, Acibenzolar-S-methyl, (ASM, Actigard).

# The experiment:

We will initially collect and analyze baseline headspace volatiles from 20 pink double knockout roses. We will then apply Actigard at 100 mg/L weekly to observe the effects of Actigard on rose volatiles. We will then graft-inoculate 10 pink double knock-out roses with RRV. We will use water as a negative control for the spray application.

We will be taking headspace volatiles consistently throughout this experiment for a year. This will allow us to track changes in headspace volatiles for each treatment. Our hypotheses are that RRV reduces the amount of Methyl Salicylate (MeSA) and other defensive compounds produced by roses, while Actigard increases these defensive compounds.

# Chemical mixing

Water: 500 mL of water

Actigard 100: 417 mL of water, 31.2 mg Actigard

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. 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

Water applications should be done first, followed by Actigard.

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.

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 daily recordings of. We will then sample from one row per week, rotating through the rows until each row has been sampled three times. We will also collect entire roses at the end of the trial.

We will 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’ in the shared drive
* Record data on the ‘actigard\_trial\_datasheet’ on tab ‘RRD Trial 2019’

# Sample Collection and Processing

* Take one of the large centrifuge tubes and label it with the plot, plant label (letter and number) and date
* The plot map can be found in the shared folder labeled ‘asm\_plot\_2019’
* Take a floral cutting large enough to fill the centrifuge tubes provided (about ~3 cm)
* Place the flower into the tube
* Refrigerate the rose samples until there are enough to ship to Quincy.

**Notes:** 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 questions or concerns, please call or email Austin Fife:

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