Standing Operating Procedures

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. These protocols have been designed to avoid accidentally introducing Rose Rosette Disease or *P. fructiphilus* into Florida while working on our projects.

## Transportation of Plant Materials

The transfer of infected materials from Griffin, GA and Gainesville, FL represents a possibility of contamination and introduction of *Phyllocoptes fructiphilus* and Rose Rosette Virus, so we need to follow some strict rules regarding the transportation of plant materials.

We need three infested roses to form our quarantine colony. The initial mite-infested plants will be transported in a sealed large cooler which will not be opened again until arrival in Gainesville. The exterior of the cooler will be sprayed over the entire surface with absolute alcohol before transport. When transporting this cooler, the roses will be taken directly from Griffin to Gainesville without visiting any other agricultural areas. These roses will be immediately placed in the quarantine facility upon arrival and not allowed to leave until the termination of the experiment. The roses will need to be kept away from other roses, and other plants if possible. There should not be fans which are blowing on the infested plants to avoid spreading the mites. The roses are to be kept in moats with soap to break the surface tension and kill any mites which fall off the plants. All predatory experiments will be done enclosed in ‘Munger cells’: an enclosed acrylic cage attached to a leaf designed to contain mites and prevent them from escaping. The researcher will wear gloves and a lab coat when handling mites and dispose of gloves in a biohazard bag which will be autoclaved before disposal. All metal instruments will also be autoclaved, or flame sterilized before and after use. After handling contaminated plants or mites the researcher will wash their arms and hands with soap and water. It is strongly recommended that the researcher shower and change clothes after experiments. The researcher should avoid entering areas with uncontaminated roses after working with the infested colonies. Beyond the initial three roses, no more roses are to be transferred from Griffin to Gainesville for the duration of the project. No roses will be transferred from Gainesville to Quincy during this project. Plant materials required for PCR may only be transported if sealed in plastic baggies in a chilled cooler which has been sprayed with absolute alcohol to sanitize all surfaces. Infested material will be disposed of only after sanitizing with bleach, absolute alcohol, and/or autoclaving contaminated materials.

## Transportation of Mites

No living mites are to be transported to or from Gainesville apart from the initial establishment of the quarantine colony. All mites to be transferred to the Quincy facility must have been killed either by freezing overnight before transport or by other chemical means, i.e. acaricides, ethanol, bleach, etc. There is no exception to this rule.

There is also a risk of cross contamination of mites. Researchers who handle predatory mites should not work with *P. fructiphilus* for the remainder of the day or enter plots where we are conducting research which do not involve predatory mites. Researchers who have worked with acaricides should also avoid entering areas where mites are being cultured until they have showered and changed clothes.

## Sanitation measures:

Shears for cuttings should only be used for their respective projects. The shears should be routinely sanitized with 70% ethanol and/or bleach after use to avoid cross-contamination of infected plant material. All plant materials destined for disposal should be maintained in a separate designated container and sanitized by autoclaving.