***Trissolcus japonicus* Colony Care**
Welty Lab at OSU
by Kristina Fox Vik, 17 December 2020

The *Trissolcus japonicus* colony is kept in 50mL plastic vials with a fine meshed fabric held on by a rubber band at the top of the container closing the opening. A small strip of paper towel is placed inside the container for the wasps to rest on. About 100 individuals, or wasps from four to five egg masses, are in a vial. Overcrowding the container has caused mass die off or aggressive fighting within the vial during feeding.

Colony is kept in a rearing chamber at a 50-55 degrees F (= 10-13 degrees C) temperatures year round and a 8:16 day:night cycle. (The Oregon colony keeps the rearing chamber dark unless the door is open). The cooler temperature slows down their metabolism and keeps them from being aggressive.

The colony is removed from the rearing chamber once per week for at least one hour but no longer then 3 hours to allow wasps to feed. Colony is fed by brushing a thin layer of honey water mix (1:1) onto the mesh. Dip the paint brush in the honey water then wipe the brush on the side of the container to remove any excess honey water on the brush. Placing too much honey at the top of the mesh will cause the honey to seep and drip into the vial creating mold and the wasps to get stuck in the honey. The objective is to just have a thin layer of honey with care not to brush the honey water on the wasps that are resting on the top of the mesh. Once the colony has had time to feed (1-2 hours), the colony will go back into the rearing chamber or individual vials will be randomly selected to parasitize egg masses.

Host eggs are attached to card stock using permanent double sided sticky tape. Corn starch is sprinkled onto the exposed parts of the double sided sticky tape so the wasps do not get stuck to the tape. One egg mass is placed on one end of a 1 inch by 0.5 inch strip of card stock. For each host egg mass, randomly select one colony vial. Tap the vial on the table before taking the mesh off this will bring the wasps to the bottom of the vial. Then in one quick motion, remove the mesh, and tip the vial upside down on top of the egg mass. The dead wasps will come to the bottom but the live ones will scramble up towards the bottom of the vial. If there are a lot of dead, wait until the wasps go up to brush the dead wasps off the egg mass. If the wasps do not go to the egg mass and all travel up, give the vial a tap to get some of the wasps to fall onto the egg mass. At this point you can leave this set up until 2 wasps start parasitizing the eggs. If there are 3 or more, remove the vial from the top of the egg mass (interested females will rarely try to fly away from the host eggs and will aggressively defend it), brush or aspirate excess females off of the eggs (host eggs with too many female wasps parasitizing it will have a reduced emergence of wasps) place these females in their original vial. If no wasps in the vial are interested in the host eggs, select another wasp vial to parasitize the host egg. No interest is considered with attempts at knocking the wasps onto the host eggs a few times and all wasps go back up in the vial and the wasps have been given 10-15 minutes with the host eggs. This often occurs in unmated females or older females (9months – 1 year). Older females will tend to produce fewer parasitized egg masses and will show very little interest in the egg mass. Once

2 females are interested in the host eggs and are actively ovipositing, carefully fold the card stock in half towards the host eggs. Place the host eggs in a new 50mL vial with mesh on top. Place 4 – 5 host eggs in one vial careful that the host eggs are not touching card stock (this is the reason for the fold, as it helps prevent anything from touching the host eggs). Write the date on the vial to keep track on the age of the vial. This is now a host vial. Place the colony vials mesh back on, and put it with the rest of the colony vials in the 50-55 degree chamber with the rest of the colony. If the host vial goes into the 50-55 chamber, the host eggs will have no wasp emergence.

The host egg vial is stored at a 68-70 degrees F (= 20-21 degrees C) with 16:8 day:night cycle (ambient humidity; the Oregon lab keeps a 40% RH). Brush honey water mix on top of the mesh of the host egg vial and place a lid on it to keep the honey from drying out. Feed the wasps every 2-3 days or until the females die. The vial remains in these conditions until all eggs have emerged or 4 to 5 days since the first emergence (17-20 days from exposure). Place the host vial in the freezer for 2 minutes to calm the wasps so the host egg card stock can be removed. If egg card stock are left in the container, females will attempt to oviposit on already emerged egg masses. Place the vial in with all the other colony vials. Males will emerge one to two days before females and will mate with the females immediately after they emerge. Host eggs will typically have 3-5 males on an egg mass of 28 eggs. Older females or eggs that have remain frozen for more than a month will typically have a higher number of emerged males. Males also seem to have a shorter life expectancy, so older colony vials will tend to have very few or no males.

Cleaning and maintaining the colony. Mesh is replaced every month or earlier if mold is developing. The vials and paper towel strips are replaced once every 4 months or sooner if mold is developing. Replacing the vials by removing the mesh from the old vial and placing a new vial on top of it to allow the wasps to “move in” and the dead to remain at the bottom of the old vial. Place new mesh on top of the new vial and transfer the date from the old vial to the new so the age of the wasps are known. When new mesh is placed on top of the vial, feed the wasps honey water. Combining vials have resulted in mass die off within the vial.