

## **Rearing *Pseudacteon cultellatus*: Notes and Photos**

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### ***Changes to Minimize Chewed-out Heads***

1. Collect dead workers 6 days a week.
2. Use larger trays (#3 Rubbermaid TakeAlongs).
3. Use a stainless steel cup (2.5 oz., ~\$0.50 each) over plaster block with foil patch on tray bottom (Beaty Restaurant Supply, 352 376-5939).

### ***Changes to Minimize *P. curvatus* Contamination***

1. Hold workers from field for 10-21 days in lab at 21-24°C to allow as many *P. curvatus* as possible to pupate before use. Don't hold workers longer than 3 weeks to avoid possibility that they become infected with SINV-3 virus.
2. Sieve workers to remove larger ants which *P. curvatus* prefers.
  - Collect colonies with a predominance of small workers.
  - Rapid pre-sieve using #12 U.S. Standard sieve (1.7 mm diameter opening) and heavily etherized workers, especially if sex brood and sexuals are common (optional).
  - Remove brood with sorting sheets.
  - Slow sieve the remaining workers using a #25 sieve (0.71 mm opening). Use workers passing through this sieve. We used 1 g workers & 1.5 g brood. (The brood was needed to keep workers running when flies attacked.)
3. Discard all dead in boxes with parasitized workers at 12 days (oldest date) when holding parasitized workers at 27.5±0.5°C. We tag all boxes with the date at which the oldest parasitized ants turn 12 days (or 11 days if 12 is on a Sunday). Collect and plate dead workers and pupating maggots beginning at 13 days. **Note:** This works because most *P. curvatus* pupate between 10 and 12 days at 27°C.
4. Hold pupal trays in reach-in boxes at 23-25°C until day before *P. cultellatus* begin emerging. Usually this is 21-24 days depending on temperature. Best to use two boxes: one for oldest date and another for the next 4-5 dates for flies that emerge early. **Note:** This works because most *P. curvatus* flies emerge from the pupae 1-3 days before the *P. cultellatus* pupae do. The exact time depends on temperature.
5. Monitor species of flies emerging in reach-in boxes to adjust timing and abundance of *curvatus*.
6. Monitor random samples of 30-40 pupae 1-2 times a week to warn of buildup of *curvatus*.
7. If all else fails, be prepared to check all emerging flies to remove *curvatus* females. This is best done under a microscope using CO<sub>2</sub> to knock them down and a cold table (9-12°C) to keep them immobile while they are inspected. If caught early enough, this can be done to half of the colony at a time.
8. Keep *P. curvatus* and *P. cultellatus* colonies in different rooms.

### ***Changes to Minimize Mites and Trash Phorids***

1. Use tight seals on all holding boxes.
2. Move to new holding box after about 5 days so adult mites or adult flies do not have time to emerge and re-infest new trays.
3. Put holding boxes in rooms where they will be separated from trash phorid flies (especially, with new trays).

### ***For further information about *Pseudacteon* fly rearing see:***

Vogt, J. T., S. D. Porter, D. A. Nordlund, and R. Smith. 2003. A modified rearing system for production of *Pseudacteon curvatus* (Diptera: Phoridae), a parasitoid of imported fire ants. Biol. Control 28: 346-353.



1. Parasitized ants. They are kept for 1 month or 28 days at 27-28°C.



2. Vented 1 gal RubberMaid TakeAlong box with parasitized ants. Ants like to place dead workers in the corner depressions.





3. Test tube for water. Castone (dental plaster) cap keeps ants from digging into cotton and cotton fibers getting mixed with dead ants which makes it hard to count the pupae. Two Castone moisture blocks were used to maintain the high humidity required by the pupating fly larvae. Metal cup with 2 small notches cut in lip inverted over a third Castone moisture block. Sugar wick on plastic lid. Foil tape is affixed under bottom of tray to blackout inside of metal cup and help keep negatively phototactic ants inside.



4. Castone block under cup.

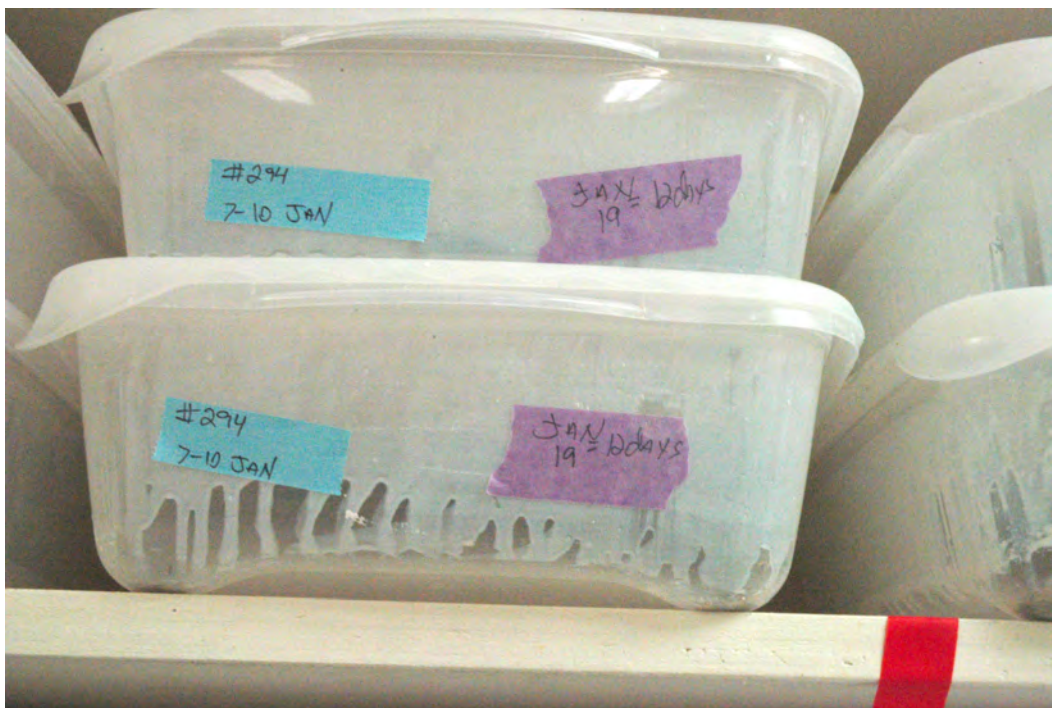


5. Large Kimwipes used to make sugar wicks with 2 M sugar solution.



6. Moisture blocks are made of a very soft mixture of Castone so they absorb a lot of water quickly.





7. At 12 days old, we throw away all dead workers and then begin collecting the dead on day 13. This helps to reduce numbers of *P. curvatus* getting through. If 12 days falls on a Sunday, we pull on day 11 instead. Timing may need adjusting if temperature varies.



8. Orphan box: used for live workers accidentally collected with dead workers. It is replaced monthly.



9. Plaster tray for holding dead workers. This tray is partly moistened before putting dead workers on and then again after they are evenly spread across the tray. The 11 lines creating 12 strips are used for conducting 1/2- or 1/4-count estimates of pupal production.



10. Trays with dead parasitized ants in Holding Box. Workers are spread evenly over trays. Several bank trays are added and moistened to make sure humidity is in the 85-95% range. High humidity is critical for Box 1 and good for box 2. Holding boxes may need smaller vents or watering more often if room is drier.





11. *Pupae Holding Box 1. Pupae are placed here immediately after collecting dead. Blank trays are watered twice a week with regular water and pupae trays are watered on day four or five. After collection, it is best not to water pupae again until they have extended their respiratory horns at 3-4 days. All water must soak in so plaster in trays is not shiny. We leave the trays in Holding Box 1 for 4-5 days and then move them to Holding Box 2.*



12. *Pupae Holding Box 2. Transferring trays to box 2 at five days keeps mites and trash phorids from completing development inside the box and contaminating new trays. Trays in Holding Box 2 are watered with 1% bleach solution twice a week to keep humidity high. Trays are transferred from this holding box to Emergence Box B and then to Emergence Box A.*



13. 4-5 days of trays are held in Emergence Box B along with several additional trays for moisture. This box is backup in case our aim with Box A is too long.



14. Emergence Box A with Emergence Box B behind. We hold pupae trays in Box A until just before the *P. cultellatus* emerge. The trays are moved out into the attack box at about 18 days but when the room is cooler it can be as much as day 20 or 21. This is done to make sure the *P. curvatus* have a chance to emerge first. If more than a dozen or so *P. cultellatus* flies begin emerging, the waiting period is too long (unless we are still getting a lot of *P. curvatus*).





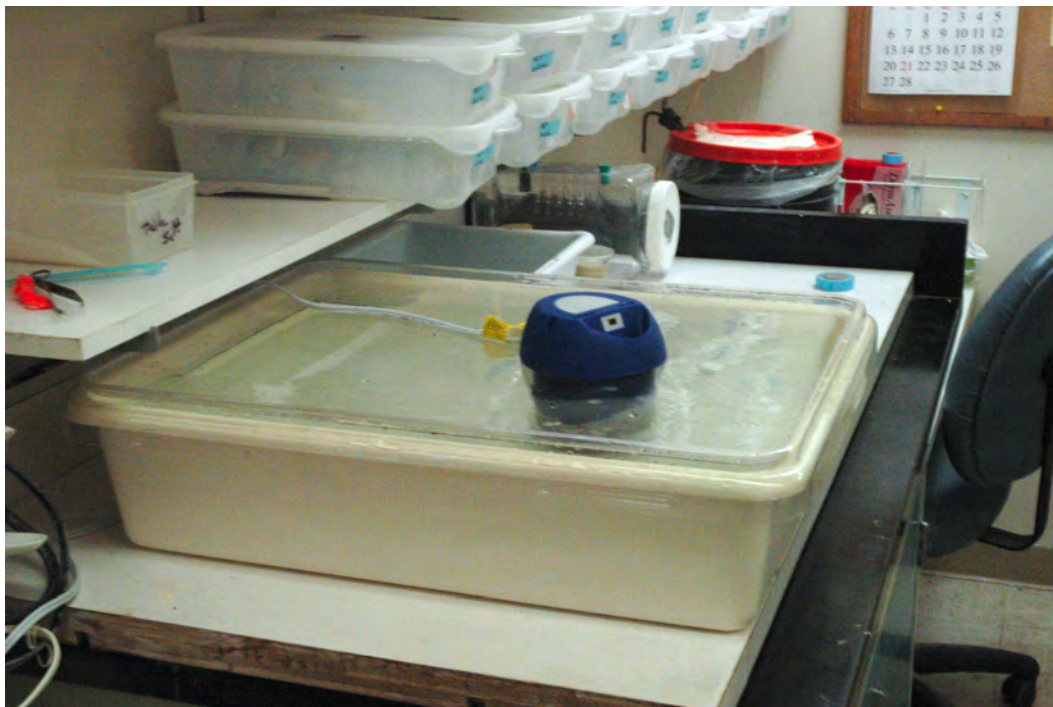
15. Only one date is held in Box A. Other trays are to ensure moisture remains high. Water is added daily to trays except on Sunday.



16. Copper tubing port in center of photo used to attach Allen aspirator to a tube inside the box which is used with hands through the two side access ports to extract flies in the box.



17. Black strip in corner of Box A. Flies are attracted to strip so they are easier to collect and the strip may aid in mating.



18. Vaporizer attached to a humidistat used in winter to keep room from getting too dry ( $<35\%$ ).





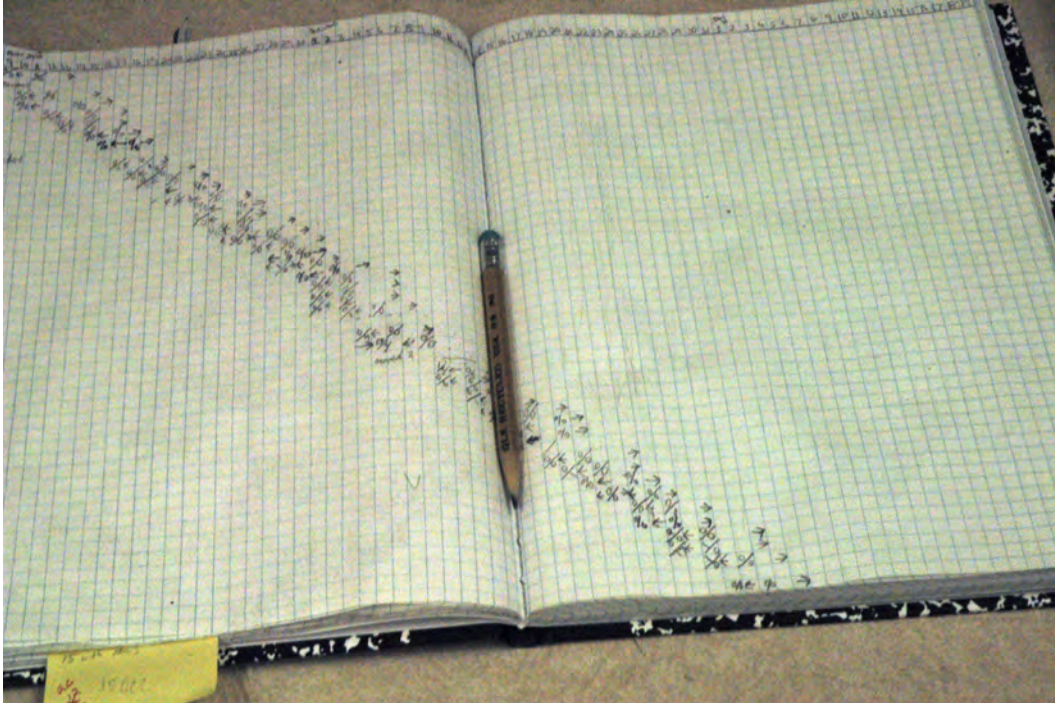
19. Room humidistat.



20. Preferred room temp and humidity.

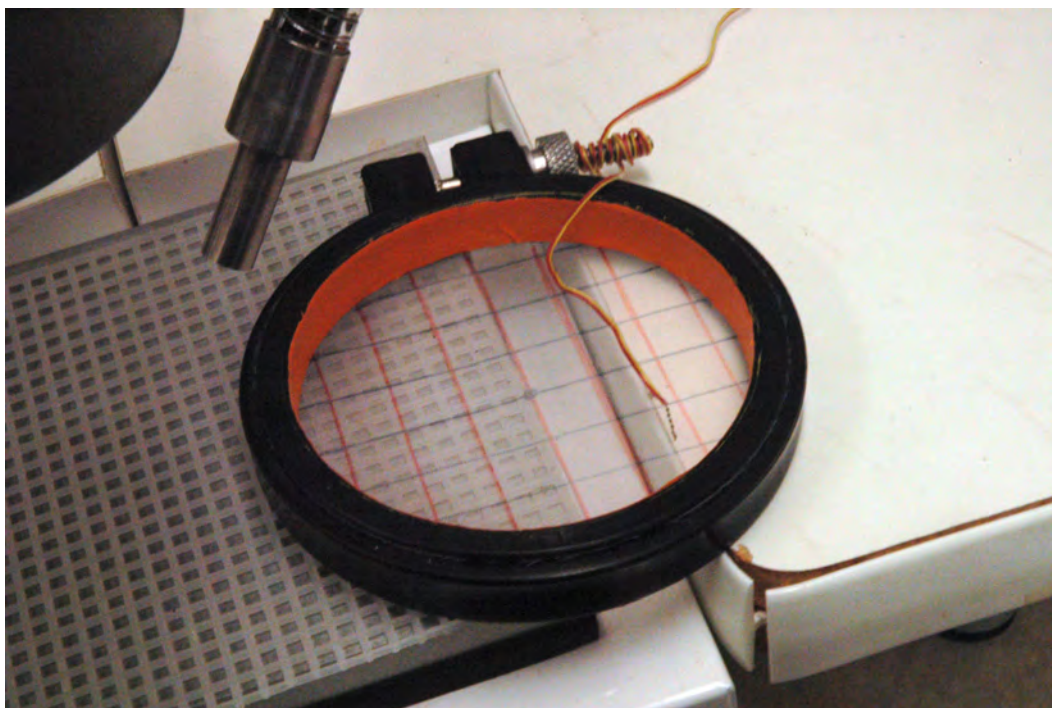


21. Cold table. Note embroidery hoop with sheer cloth for holding flies. We used several squares of plastic needlework canvas from a craft store below the hoop to maintain temperature at 10-12°C. More of the plastic squares are added if the hoop gets too cold or removed if it gets too warm.

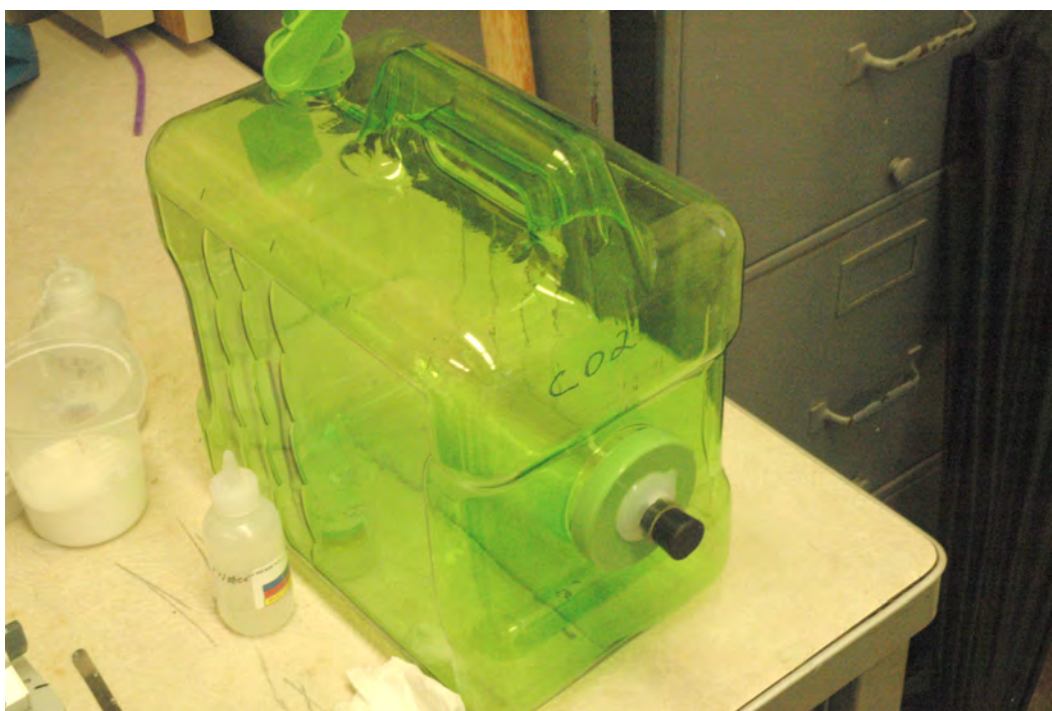


22. Data book for recording numbers of female *P. cultellatus* and *P. curvatus* emerging in Box A and B. Might want to keep track of total males too.

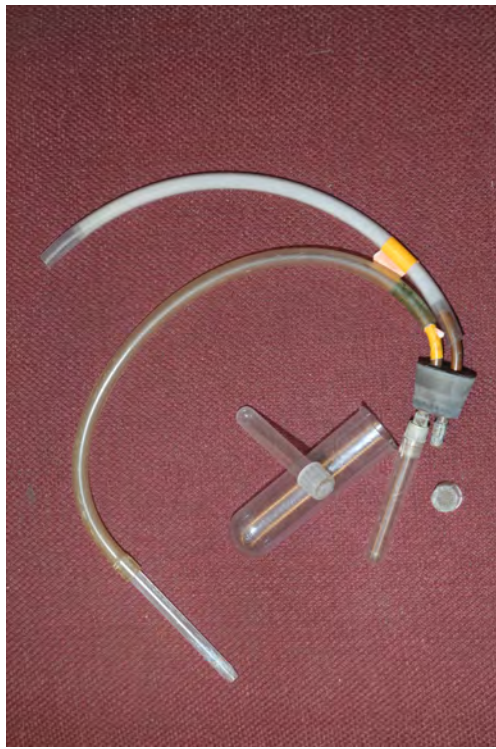




23. Embroidery hoop with guidelines drawn on sheer material with thermocouple to monitor temperature.



24. CO<sub>2</sub> box for knocking flies down prior to putting on cold table. Screened vials are inserted in place of the black stopper so the CO<sub>2</sub> can diffuse into vial.



25. Double-chambered Allen-type aspirator fitted to handle vials with 60-mesh screen caps that can be fitted to the CO<sub>2</sub> container to anesthetize the flies prior to sorting on cold table.



26. Attack box. The temperature in the box is controlled by the halogen lights during the day and the room temperature during the night. Humidity is controlled by a vaporizer placed in a box under the attack box. A fan continually circulates moist air up to and back from the attack chamber. A bank of fluorescent lights illuminate the attack tray during the day. The emergence box on the right with the big "C" is where pupae trays are put just prior to the flies emerging.





27. Attack trays and "lifter lids". The ants run back and forth to between the two lids in each tray as the lids are raised or lowered by the PVC rod above. Note that each tray has a vial of sugar water and a vial of tap water.



28. "Lifter Lids" used in large attack box. Workers use chains workers to escape when lid is raised. The brass clip glued to bottom lip of the black spray paint can lid shims it up thus avoiding crushing ants when it is lowered. A wooden dowel is screwed to the top of the black cap and a fluted PVC pipe is slipped over the dowel. Two inverted cups are attached to the top of the dowel. The inside of the outer cup and both sides of the inner cup are fluted to ensure that ants do not escape up the lifter screen. The black lid is filled to within 7-10 mm of the lip with plaster and the plaster is coated with paraffin wax to discourage ants from simply riding up and down under the lid.



29. Pupae trays in attack box with pupae ready to emerge. Trays are transferred to the attack box from Emergence Box A. Seven trays is usually enough to ensure all flies emerge before removal. About 85% emergence rates indicate good conditions. Trays are watered each afternoon.

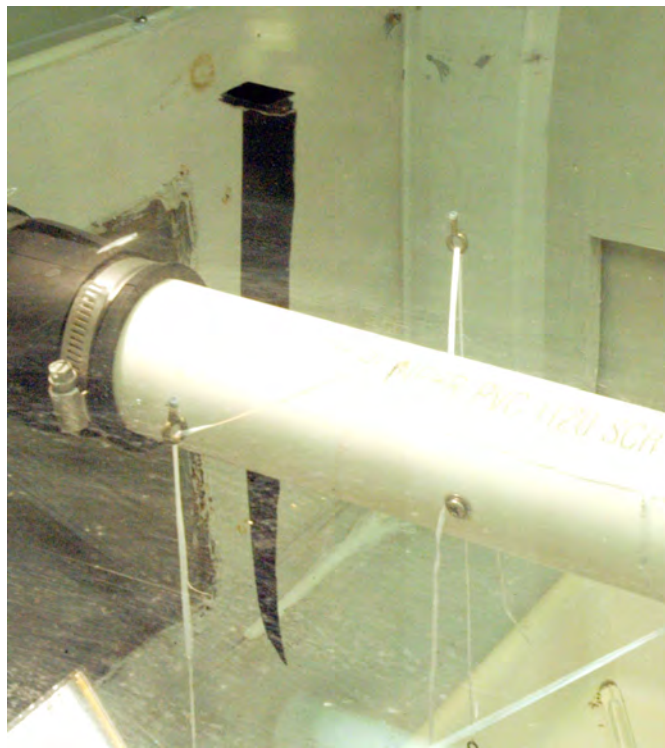


30. Preferred temp in attack box is 27°C during the day and about 23-24°C at night with and 80-85% humidity maintained 24 hours a day.





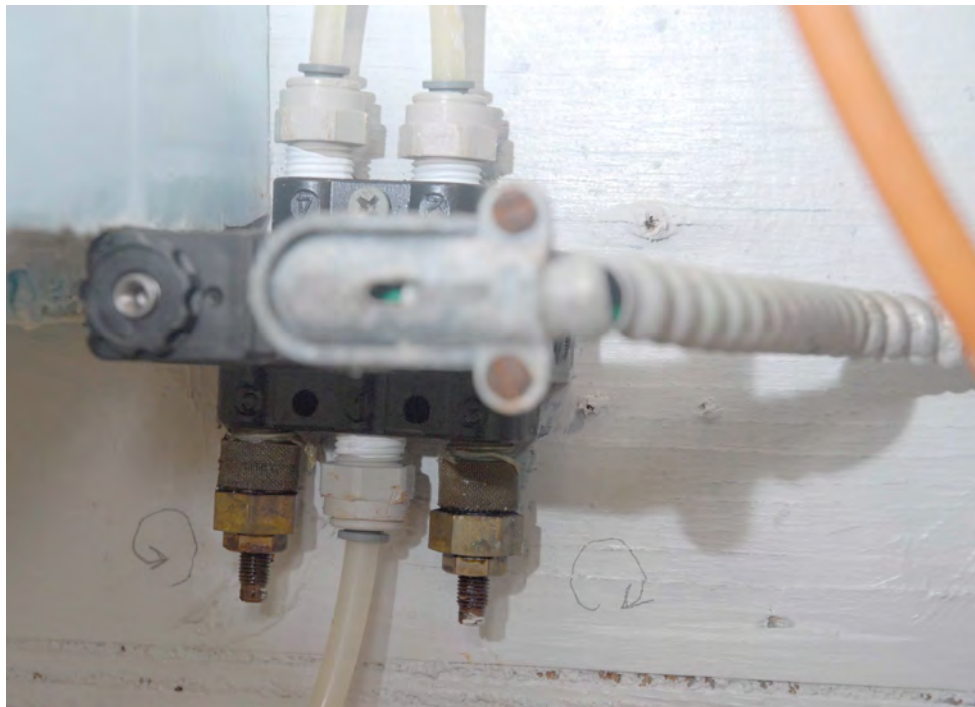
31. Black strips in attack box held on with Velcro. Mating appears to occur around the black strips. *P. cultellatus* males do not mate with female whiles they oviposit on the ants so they are not collected in the field like *P. tricuspis* or *P. obtusus* males.



32. Black strips in attack box held on with Velcro.



33. Pneumatic motor used to drive the large central PVC pipe which raises and lowers the lifter lids.



34. Solenoid which controls the direction the pneumatic motor turns. The bleeder valves on the bottom adjust the rate of turn so that the cups rise and fall slowly.





35. Timers for halogens (on at 10 am and off at 6 pm), fluorescent lights (on at 9 am and off at 6:30 pm) and lifter motor (on at 12 noon and off at 6 pm).



36. Room light timer (on at 7:00 am and off at 7:00 pm).



*37. Dusk light on for 30 min before and after room light (6:30am-8:00pm). 40 watts.*

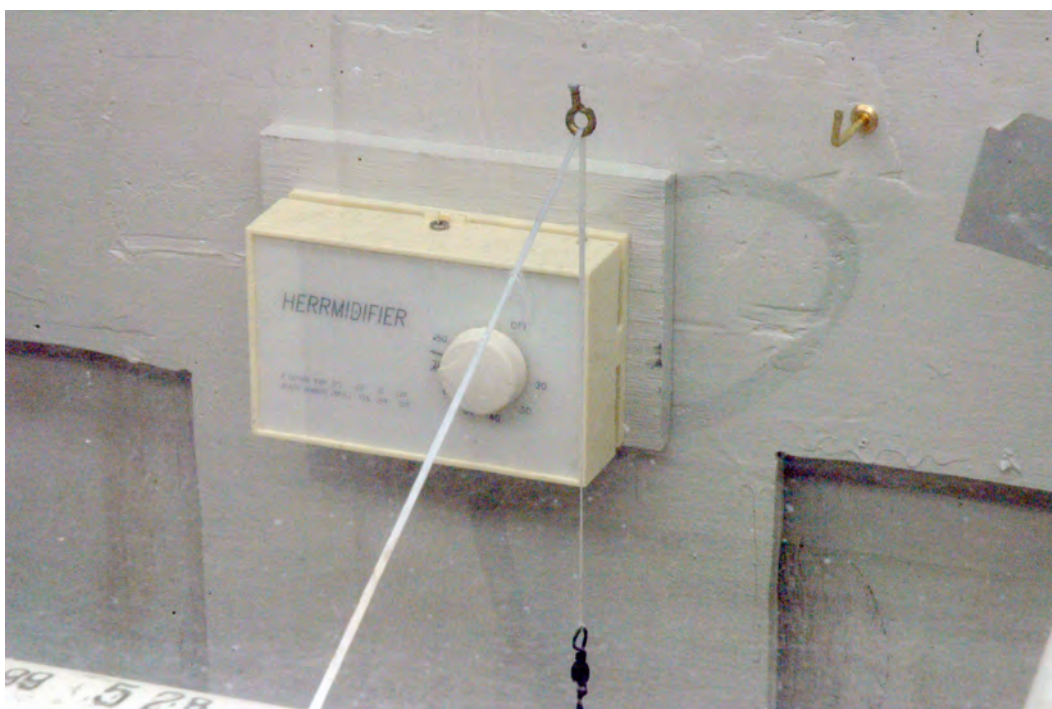


*38. Second dusk timer on for another 30 min in morning and evening (6:00am-8:30pm). 7.5 watts.*





39. Custom made lifter timer: about 11 minutes one way and 13 the other. Controls pneumatic motor from noon to 6 pm.



40. Attack box humidistat. Dental tape used to raise and lower cups. Swivel fishing clip used to attach and remove cups for cleaning and repair.



41. Safety off thermostat. Senses when water in vaporizer gets too hot because humidistat is stuck on. Turns vaporizer off. Set to about 90°F. Without this safety, the vaporizer would occasionally keep pumping vapor into the air in the box until it condensed on the plexiglas top and rained into the trays filling them with water and drowning many of the ants. Many of the flies would also die when they were trapped in the condensation.



42. Vaporizer for controlling humidity in attack box. A vaporizer is much better than a ultrasonic humidifier because the vaporizer does not leave calcium dust everywhere. Water was added to tray daily during the week. Water was drained and replaced monthly to avoid accumulations of salts which may have caused the vaporizer to run too hot so that the relay in the humidistat would be stuck on. Holes in the vaporizer reservoir allowed water in from the larger tray.





43. Using an aspirator to collect pupae for shipping or study. Ant heads with pupae are sucked into double-chambered Allen-type aspirator (BioQuip) so that they are not wind-tunneled as would happen in a single-chamber aspirator. We connected the aspirator to a vacuum line and used a small flexible tube with a trimmed pipette tip to suck up the pupae.