**Manduca sexta and Cotesia congregata temperature variation experiment (25, 25+/-10, 30, 30+/-5, 30+/-10)**

**25C and 25+/-10 Protocol (colony diet):**

* Set up bins of eggs from the colony in the constant temperature (25), and check every day for hatching. Record the date that the eggs were set up on the bin.
* Once eggs have hatched, transfer 1st instar larvae into the big thin petri dishes by either cutting out the diet with the larvae on it, or transferring larvae with a brush onto new diet. Record the date the eggs were set up, and the date that they hatched, on the petri dish.
* Place newly hatched 1st instar larvae into one of the pair of temperature treatments (25 and 25+/-10). Try to divide hatchlings evenly into the treatments each day.
* Check caterpillars in each treatment, replacing food as needed, until they reach the 3rd instar. On the first day of the 3rd instar, put each caterpillar into its own small petri dish, give it a unique ID number, and weigh it. Record the rearing temperature and temperature variation, the date hatched, the date of 3rd instar, and the mass at 3rd instar into the data sheet. **NOTE:** Don’t use 3rds that are >100 mg, as they probably are not newly molted.
* Roughly half of the new 3rds each day will be parasitized. After weighing and recording their information, take those destined for parasitization into the wasp colony room, and follow the protocol for parasitizing 3rds. Record the date and time of oviposition, as well as the number of ovipositions (we’re aiming for only 1) in the data sheet. Write “para” in the petri dish, as well as how many ovipositions. Record “para” in the treatment column of the data sheet for these caterpillars. For the others, write “control”.
* Check all caterpillars in all treatments every day. Record the date, time and mass when they molt into the 4th and 5th instars. Replace food as need.
* For the control caterpillars (unparasitized), record the date, time, and mass at wandering. To make it easier to weigh them, put them in the fridge for 10 minutes to “chill out”. Just don’t forget them in there! After being weighed, they can be frozen for disposal later. If you have several, combine them into one dish to save freezer space.
* For the parasitized caterpillars, check them every day for wasp emergence. Be vigilant, as this can happen as early as the 4th instar! The ones in the fluctuating treatments might have very low emergence numbers, so inspect each caterpillar and its petri dish carefully. Once the wasps have started emerging, transfer the caterpillar and all wasp larvae carefully into a new petri dish, or condiment cup. DON’T give the caterpillar any food. Be sure to copy all the information for that caterpillar into the new dish. On the lid, write “count cocoons \_\_\_\_” and the date 2 days later. EX: If the wasps emerge on 6/2, the cocoons will be counted on 6/4. Record the date of emergence in the data sheet. Sometimes the caterpillars will bleed when the wasps emerge. If they do, record a 1 in the bled.em column. If they don’t, record a 0.

* Two days after wasp emergence, scrape cocoons off of the caterpillar, being careful not to squish any. Count the number of cocoons, and the number of wasp larvae that failed to spin, and enter both into the data sheet. They will automatically sum in the number emerged column. Put the cocoons into a clean condiment cup, and write the ID number, the date of emergence, the number of cocoons, and the temperature treatment on the lid. Put the condiment cup in a new tub, and put back into the temperature treatment. Weigh the caterpillar and record the mass in the mass48.em column. Freeze the caterpillar in the petri dish (taped shut) or condiment cup for later dissection.
* Check the condiment cups full of cocoons every day for adult wasp eclosion. Once the wasps eclose, record the date in the data sheet and on the lid of the cup. Then write “Freeze \_\_\_\_\_” and put the next day’s date. EX: Wasps eclose on 6/4, are frozen on 6/5. Put the condiment cup back in the temperature treatment.
* Check the chambers every day for wasps that need to be frozen. Make sure all pertinent information is on the lid of the cup, and then stick them in the freezer.
* When you have time, work on dissecting the caterpillars to find the number of unemerged wasp larvae, and count the number of wasps from the cups of cocoons. Try to at least count the wasps regularly, as they don’t keep as well in the freezer as the caterpillars. Record the number of adults in the num.ecl column. When dissecting the caterpillars, record the number of unemerged wasp larvae in the num.unem column.

**NOTE:**

If you ever find a caterpillar that has not fully shed its cuticle, please gently remove it with forceps and small scissors (if necessary). The closer this is done to the molt, the better (but be sure they are done molting first!). The unshed cuticle will restrict their growth and movement, and may alter our data. Make a note in the comment section about when (date and molt #) you removed the cuticle from that caterpillar. If you feel uncomfortable doing this, ask Elizabeth or Christina for help!

**30C, 30+/-5 and 30+/-10 Protocol (tobacco diet):**

* Set up bins of eggs from the colony in the constant temperature (30), and check every day for hatching. Record the date that the eggs were set up on the bin. For field eggs, place 20 eggs in a petri dish, record the collection date and location and the number of eggs. Replace food every 2 days.
* Once eggs have hatched, transfer 1st instar larvae into the big thin petri dishes by or transferring larvae with a brush onto new diet. Record the date the eggs were set up, and the date that they hatched, on the petri dish. For field larvae, only set up 10-15 per dish--record the number on the dish. Replace food for field larvae every 2 days.
* Place newly hatched 1st instar larvae in the 30C chamber until molt to 3rd--this helps prevent early larval mortality at stressful fluctuating temperatures.
* Check 1st and 2nd instar dishes every day for new 3rds. On the first day of the 3rd instar, put each caterpillar into its own small petri dish, give it a unique ID number, and weigh it. Record the rearing temperature and temperature variation, the date hatched, the date of 3rd instar, and the mass at 3rd instar into the data sheet. Also record whether it is from the lab or the field, what diet it is fed (colony or tobacco), the date of collection and the location of collection (for field bugs). **NOTE:** Don’t use lab 3rds that are >100 mg, as they probably are not newly molted. Since field bugs are limited, use all 3rds for the experiment.
* Roughly half of the new 3rds each day will be parasitized. Check the counts sheet to see what treatments need more caterpillars. After weighing and recording their information, take those destined for parasitization into the wasp colony room, and follow the protocol for parasitizing 3rds. Record the date of oviposition, as well as the number of ovipositions (we’re aiming for only 1) in the data sheet. Write “para” in the petri dish, as well as how many ovipositions. Record “para” in the treatment column of the data sheet for these caterpillars. For the others, write “control”.
* Check all caterpillars in all treatments every day. Record the date, time and mass when they molt into the 4th and 5th instars. Replace food as need.
* For the control caterpillars (unparasitized), record the date, time, and mass at wandering. To make it easier to weigh them, put them in the fridge for 10 minutes to “chill out”. Just don’t forget them in there! After being weighed, they can be frozen for disposal later. If you have several, combine them into one dish to save freezer space. DO NOT FREEZE FIELD WANDERERS--follow the protocol for field pupation and eclosion.
* For the parasitized caterpillars, check them every day for wasp emergence. Be vigilant, as this can happen as early as the 4th instar! The ones in the fluctuating treatments might have very low emergence numbers, so inspect each caterpillar and its petri dish carefully. Once the wasps have started emerging, transfer the caterpillar and all wasp larvae carefully into a new petri dish, or condiment cup. DON’T give the caterpillar any food. Be sure to copy all the information for that caterpillar into the new dish. On the lid, write “count cocoons \_\_\_\_” and the date 2 days later. EX: If the wasps emerge on 6/2, the cocoons will be counted on 6/4. Record the date of emergence in the data sheet. Sometimes the caterpillars will bleed when the wasps emerge. If they do, record a 1 in the bled.em column. If they don’t, record a 0.

* Two days after wasp emergence, scrape cocoons off of the caterpillar, being careful not to squish any. Count the number of cocoons, and the number of wasp larvae that failed to spin, and enter both into the data sheet. They will automatically sum in the number emerged column. Put the cocoons into a clean condiment cup, and write the ID number, the date of emergence, the number of cocoons, and the temperature treatment on the lid. Put the condiment cup in a new tub, and put back into the temperature treatment. Weigh the caterpillar and record the mass in the mass48.em column. Freeze the caterpillar in the petri dish (taped shut) or condiment cup for later dissection.
* Check the condiment cups full of cocoons every day for adult wasp eclosion. Once the wasps eclose, record the date in the data sheet and on the lid of the cup. Then write “Freeze \_\_\_\_\_” and put the next day’s date. EX: Wasps eclose on 6/4, are frozen on 6/5. Put the condiment cup back in the temperature treatment.
* Check the chambers every day for wasps that need to be frozen. Make sure all pertinent information is on the lid of the cup, and then stick them in the freezer.
* When you have time, work on dissecting the caterpillars to find the number of unemerged wasp larvae, and count the number of wasps from the cups of cocoons. Try to at least count the wasps regularly, as they don’t keep as well in the freezer as the caterpillars. Record the number of adults in the num.ecl column. When dissecting the caterpillars, record the number of unemerged wasp larvae in the num.unem column.

**30+/-10 5ths and mongos:**

This treatment is having very low wasp emergence, and a very high occurrence of “mongos”--very large, very long lived caterpillars. There are also very old caterpillars that are not mongos. To try and track what they do, I have been weighing them sporadically. After at least 10 days have passed since they molted to 5th, I have been putting half at 25C (less stressful) and leaving the rest at 30+/-10. There is a separate sheet for this data.

On death, most have been frozen for future dissection (unless they are very gross, and it is unlikely that we could see melanized wasp eggs upon dissection).

One so far has wandered (as well as a not very old para treatment), and was put in a pupae box. It died as a larval/pupal intermediate, and was frozen for future dissection (as was the wandered para--it pupated, but did not fully shed larval cuticle).