Biological Control of Oriental Fruit moth

PROJECT LEADER: Walt Bentley

COOPERATOR: Dr. Marshall Johnson and Dr. James Hagler

Objectives

1. Develop a small scale-rearing program for the *Macrocentrus ancylivorus*, the parasite of Oriental fruit moth, to be used for establishment in peach and nectarine orchards.
2. Determine the movement distance and extent of movement by the parasite from release sites into peach orchards.

Results

The initial task was to establish a small scale-rearing program for *Macrocentrus ancylivorus*, the parasitoid of Oriental fruit moth (*Grapholitha molesta*). This was accomplished in a laboratory environment at a temperature of 78-80 F. One rearing cycle requires approximately one month to complete, beginning with the adult potato tuber moth (*Phthorimaea operculella*) and concluding with emergence of the adult *Macrocentrus.* One successful cycle yields approximately 500 adult *Macrocentrus*. The process has several stages, during which the parasite and/or its host must be shifted from one venue to another, and so requires timely attention. As a necessary adjunct to the *Macrocentrus* rearing, a separate colony of potato tuber moth is maintained simultaneously at the same laboratory location. The Colorado State Department of Agriculture generously provided us with additional *Macrocentrus* pupae. This allowed us to maintain and augment our own production. See Appendix A for rearing procedures.

In fall of 2006, a single small-scale release of the above *Macrocentrus* was made in peach and nectarine orchards of two area growers. These releases were preliminary and will be followed in 2007 by regular monitored releases of the parasitoid in local orchards.

Having been established on sunflower moth since 2003, *Macrocentrus* has maintained a presence at Kearney Agricultural Center in an orchard of Crimson Lady peaches. This orchard is adjacent to a field of sunflowers, planted annually to provide an alternate, overwintering host for *Macrocentrus.* The parasitoid was established in 2003 and 2004 using augmentative releases but is now parasitizing both Oriental fruit moth (OFM) and the sunflower moth in the absence of controlled releases. The population of *Macrocentrus* declined in our samples in 2005 but has risen in 2006 and the expectation is that it will increase in 2007. OFM and parasitoid populations have been monitored using pheromone traps, shoot strikes, and larval emergence from shoot strikes. These same techniques will be used in our cooperating orchards where *Macrocentrus* releases take place.

Preliminary work was done on selection of a reliable marking technique for *Macrocentrus*, enabling us to proceed in 2007 with distance movement studies. A greenhouse study using cut sunflower heads sprayed with whole milk established that the parasitoid very successfully picked up the milk protein marker. Dr. James Hagler of the Arizona Cotton Research Lab (USDA) performed the analysis (ELISA), with excellent results showing the protein detected on all treated *Macrocentrus*. After validating the ability to detect protein markers on treated *Macrocentrus* (untreated controls were also tested) we found that parasitoids released into the field could be retrieved at 40 yards from the release site. These parasitoids had been marked with milk protein and the protein was detected on them when captured on sticky card traps.

We will focus on the actual release and capture work as well as spraying sunflowers in an attempt to detect these naturally occurring parasitoids in peach orchards in 2007.

Appendix A

Rearing Macrocentrus Parasite on Tubermoth

Adapted Process from Hilgardia Vol. 17 August 1947 No. 13 pg 437
Glenn L. Finney, Stanley E. Flanders, and Harry Smith

By Andrew Molinar 12-18-06

Requirements:

A little over 1 month per generation
Isopropyl alcohol for separating moth and parasite pupae
Household bleach for dissolving pupae cocoons of parasite and tubermoth
White pine box for holding of potatoes
Bugdorm
Fine play sand
Foil roasting trays to fit inside bugdorm
Stand to hold potatoes above tray in bugdorm for worms to drop out into sand

The process: (in lab 3 approx 78-80 degrees)

Following 1 cycle of the process is the best way to explain it. We start with tubermoth pupae. The pupae are placed in a Petri dish, (about 10-12 grams of pupae) a single layer covering the bottom of the dish. This dish is placed into a bug display case that has been modified with a window mesh screen over the top. When all or almost all of the tubermoth have emerged, the cloth is placed over the top of the screening to collect eggs (it helps to spray the cloth with water to aid in forming it to the screen). It is important that the cloth lay as flat as possible to the screen, as the tubermoth will be laying there eggs through the screen onto the cloth. Sliced potatoes can be placed on top of the sheet to aid in attraction and egg laying. Weighing the sheet with strips of wood will help for better egg collection, but may not be necessary. The cloth is left on for approximately 3 days to allow the moths to lay eggs. The number of days may change based upon number of emerged tubermoth. The goal is to have enough eggs to provide hosts but not so many to over-infest the potatoes (which will cause the moths to exit the potatoes before they are ready to pupate). Examine the egg sheet to verify presence of eggs. There should be a light sprinkling of the whitish eggs over the entire surface or large clusters of eggs where weights were placed.

Once the egg sheet is removed it can be placed in the pine box on approximately 20-25 punctured potatoes immediately, or after a few days (it may take 5 or so days for hatch so puncture the potatoes as close to the expected hatch date as possible.) The eggs will turn a yellowish color when they are about to hatch. The box is covered with the pegboard. And after approximately 5 days, when most of the tuber eggs have hatched (you can tell because they turn dark brown), the egg sheet can be removed as most of tuberworms have entered the potatoes.

The *Macrocentrus* adults can then be added to the potato cage. *Macrocentrus* pupae from the previous generation should be placed in a steel mesh box cage. They should be provided a honey water dilution and wick as they emerge. After many *Macs* have emerged and had at least a few

days to mate, they can be aspirated and blown into the potato cage. We have been putting approximately 75 adult female *Macrocentrus* into the potato cage. Everything is left in the potato cage for approx 10 days. At this time the tuberworms are nearing pupation.

The potatoes are then removed from the potato cage. The *Macrocentrus* are released. Potatoes are placed on a wooden stand with egg carton or mesh above an aluminum tray that has been lined with waxed paper and filled with sand to allow the worms to drop from the potatoes and pupate in the sand. Check the sand periodically for pupae or larvae by running your fingers thru it. You will need to separate the pupae after approximately 75 percent of the worms have pupated (1 or 2 tubermoths have emerged). This may take 5 or so days and may require test separations by soaking and stirring a sample of the pupae in a beaker of water and bleach (50/50 solution) to see if there are too many worms that have not pupated.

The separation is done by taking the sand clumps of pupae and shaking them in a metal strainer to get off as much sand as possible before putting them into the bleach solution. Just drop the pupae into the bleach and let them sink they will survive fine as long as not left in the bleach for more than a few minutes. Stir or swirl them around until most of the sand is dissolved and pour the pupae out. At this point you can see the difference in the Mac pupae and the tubermoth. Put the pupae (tuberworm and *Macrocentrus*) into a solution of (3 parts alcohol to 1 part water). The *Macrocentrus* will float and can be skimmed off the top.

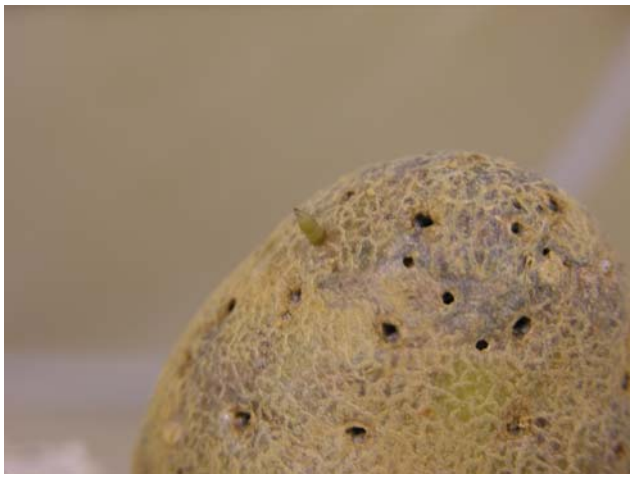
The *Macrocentrus* pupae (we've been getting approx 500) can then be placed into the metal mesh cage again. The tubermoth pupae (you will not need them all) can be placed in the screened insect display case.

Basically, the tubermoth colony takes care of itself. They lay their eggs directly on the potatoes or on a cloth with a rough surface. Puncturing the potatoes with small nail holes approx 1 cm apart and less than ¼ inch deep helps the larvae enter the potato. When the eggs hatch, they infest the potatoes through the holes.

Infested potatoes are placed on wooden rack above sand. Foil tray is lined with waxed paper to prevent pupae from sticking

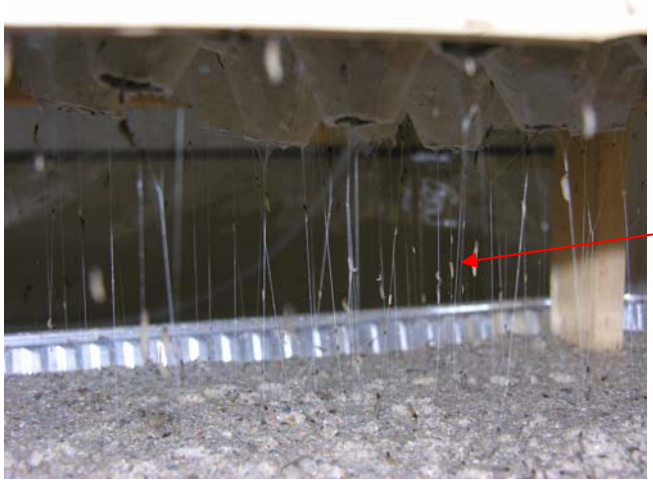
Petri dish with 1 layer of tuber pupae in modified insect display case

Place egg sheet on top of potatoes in this box (Brian's potato box). Add *Macrocentrus* as they

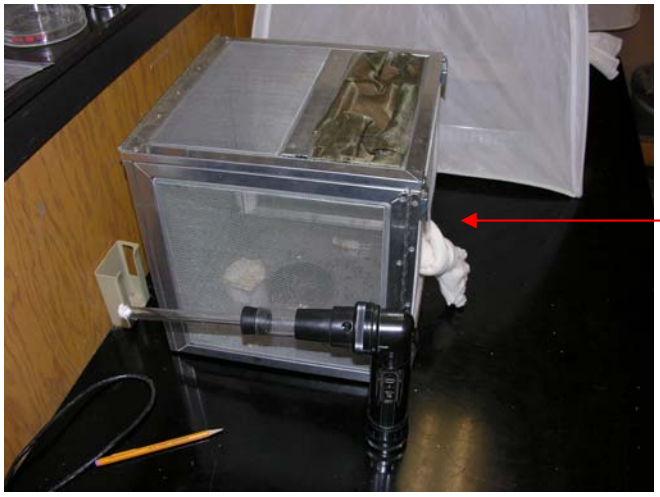


Potatoes are punctured with nail board or small nail approx 1-2cm between holes

Parasitized and non parasitized tubermoths drop to sand to pupate



Tubermoth collection cage (both parasitized and non parasitized moths drop to the sand



Wire mesh cage used for *Macrocentrus* emergence and collection.