Spring 2011 Update

I recently asked some informal questions to each of the beetle lab managers. These are Mark Dalusky's responses from the University of Georgia.

Q: During the recent fall and early winter, I've been hearing a lot of Hemlock Help Line callers say that their hemlocks, even ones that haven't been treated yet, are looking better this year with fewer adelgids than last year. If it's really so, do you know what it might be attributable to?

A: Regarding the hemlock recovery situation, I wonder if your constituents aren't just seeing a relative recovery in tree status compared to the miserable state these same trees were in a year ago? As you know, an attempt at recovery is part of the cycle of decline and depends on site and weather characteristics. Good site and good growing conditions equal impressive recovery growth. The timing is dependent on when new growth ceased in a particular area and when the HWA abandoned those trees.

Typically the year after new growth ceases, we see a significant decline in adelgids. Two years after this event, you can't find an adelgid anywhere! Trees will still decline even in the absence of HWA, especially if the site is poor and growing conditions are bad. This continued decline is characterized by significant reduction in needle density and an increase in dead shoots at the branch tips, leading to mortality of major branches, especially in the lower crown.

If there is an adelgid population in the area (we don't know where they go in these cases), HWA can immediately reinfest this recovery growth, which will put the tree into a steep decline. If reinfestation pressure is low, then these trees can show good recovery over several years – until the inevitable reinfestation occurs. It is hard to generalize this cycle which can be very site specific.

Q: Can you provide a recap of the progress in 2011?

A: Last year's releases were substantial in close to 90 areas from Panther Creek in the east to Lake Conasauga in the west. We also saw good recovery of at least F1 predators (both Ln and St) from about 75% of sites sampled. See next page for the Highlights of the 2010 Season.

Q: What are your plans for beetle releases in 2011?

A: We are geared up for another blitz of predator releases which will commence in early March, just around the corner!

M. Dalusky; Research Coordinator

Hemlock Woolly Adelgid Project: Activities at the Univ. of Georgia for the 2010 Release Season

Highlights for the 2010 Season

We added a fourth full-sized Percival incubator. This allowed us to ramp up our egg-twig production with the increased capacity for oviposition jars. We had 38 total jars for *Laricobius nigrinus* (Ln) and 50 total jars for *Scymnus sinuanodulus* (Ss). We placed approximately 49,000 Ln eggs on twigs and 15,741 Ss eggs on twigs in the field this year, on 61 release sites in 30 Hemlock Conservation Areas (HCA) within the Chattahoochee NF in north Georgia. We nearly doubled our Ss production thanks to our additional incubator funded by APHIS-PPQ. One thousand and 75 adult Ln from the Seattle area were released in winter 2009 on four HCA's in the Blue Ridge district. An additional 90 lab-reared Ln adults were released on GA DNR land on the Dawson Forest.

Again in the 2010 season, there was documented recovery of Ln (larvae) and *Sasajiscymnus tsugae* (St: larvae and adults) from 10 of 15 areas sampled (Sarah Osicka; NGC and State Univ.; personal comm.). Five of these recoveries were the second year for that area. We have great hope for additional recoveries on the unsampled HCA's next year. This does not represent establishment, just reproduction in the field. All recoveries were from foliage samples. Extensive beat-sheet sampling yielded zero recovery of any predators. Efforts will be confined to foliage sampling in the future. Our sampling protocol is included in APPENDIX II. For the second year, zero Ss were recovered.

Recovery 2010

This year saw the continued recovery in GA of 2 of our predator beetle species. We have released multiple predators in a low density, saturation strategy over much larger areas than standard high density, point source releases. At last count, we have released in 68 sites located on 39 Hemlock Conservation Areas (USFS designation). Due to time constraints, only 15 of our releases areas were sampled in 2010. Predators were recovered in 10 of the 15 areas. Timing of the field sampling insured that we did not sample new releases. Recoveries are at least F1 beetles. Let me emphasize that this in no way indicates establishment, but only field reproduction. Also, the best sampling technique available is still a needle-in-a-haystack venture. Any recovery under these circumstances is extremely encouraging! The following table enumerates recovery efforts for this year. Results are from Sarah Osicka with the N. GA. College and State Univ. rearing lab and MS student at UGA.

Recovery Site	Sample Date	Ln Recovered	St Recovered	Site Condition
Blackwell Creek	4/23/2010	3	0	moderate decline
Canada Creek	4/23/2010	4	4	decline severe
Cochran Crk	4/14/2010	0	2	moderate decline
Dockery Lake	4/09/2010	52	130	decline severe
Lower Panther Crk	4/17/2010	0	1	decline severe
Upper Panther Crk	4/30/2010	12	2	decline severe
Jones Creek	4/14/2010	0	1	moderate decline
Soque River	4/12/2010	2	0	decline severe
Waters Creek	4/15/2010	1	0	decline severe
Yahoola Crk	4/07/2010	1	0	moderate decline

Laricobius nigrinus Colony

Thirty-eight **oviposition** jars (ovi-jars) were set up in mid- February with 20 Ln and 12 twigs per jar. We made **egg releases** on twigs (egg-twigs) on our mid-elevation sites beginning Feb 26. HOBO data collected on-site show that weather extremes even at the low elevation sites are still common in Feb/Mar. Additionally, adequate HWA egg production does not occur until the end of Feb/early March on these low elevation sites, and even later in March for higher elevation sites. We are trying to track HWA phenology with elevation by making egg-twig releases at mid-elevation sites first and proceeding to low then high elevation sites as HWA phenology dictates. Our target stage is HWA eggs in the ovisacs. By tracking phenology with elevation, we can pretty much insure that we have suitable numbers of HWA eggs available at release sites from late Feb. through mid-June. Progrediens eggs at high elevations will actually over-lap with sisten eggs at low elevation. Then, sisten egg production continues with increasing elevation (2500' to 3200') through most of June. By early July, we are pretty much played out regarding HWA eggs in Georgia, with all HWA occurring predominantly as settled first instar nymphs. We estimate eggs per twig by hand-sorting 15 randomly selected twigs from our oviposition colony on a weekly basis. Throughout the oviposition period (late Feb. through early June) we averaged 11.0 eggs per twig from the wild colony (see Figure 1) Mid March through early June were the best months for egg production in the wild colony. All twigs from the oviposition colony were released. No larvae were held over for aestivation in soil boxes. In early June, we turned out our remaining ovipositional adults (ca. 278) on the same sites as the egg-twig releases. As noted before, we released 49,000 Ln eggs on twigs this season.

Survival of our oviposition colony was excellent again this year. Weekly mortality averaged less than 1% over the entire 16 week release season (see Figure 2).

The UGA lab will make a collecting trip to the PNW in mid-October to collect Ln for our 2011 oviposition colony. Dick McDonald will again shepherd us through this process, hopefully to good effect.

Scymnus sinuanodulus Colony

Ss Oviposition Jars

In late Feb, we set up 50 jars with 9 adults (3 males: 6 females) on 5 hemlock twigs. Egg-twig releases began in late February on our mid-elevation sites. We released egg-twigs in the same fashion and locations as reported for the Ln egg-twigs. We estimated numbers of Ss eggs on release twigs by hand-counting 15 twigs per colony, randomly selected from the ovi-jars, As with the Ln twigs, we feel that this gives a very conservative estimate of numbers of Ss eggs per twig. From late Feb. to late May, eggs per twig averaged from 4.0 to 11.0 (see Figure 3). In early June, we turned out our remaining ovipositional adults (ca. 100) on the same sites as the egg-twig releases. As mentioned earlier, 15,741 Ss eggs on twigs were placed in the field this season.

Colony survival over the 16 week release season was excellent with weekly mortality being at or frequently below 2% (see Figure 4).

Ss Larval Tents

We decided this season to limit our larval rearing to 9 tents beginning in mid-April and ending in early May. This did coincide with our highest number of eggs per twig from the oviposition colony, so ostensibly should have been an optimal time to rear larvae for our summer storage colony. We placed 25 twigs (contents from 5 ovi-jars) per tent and followed our rearing protocols. Figure 6 shows the predicted

versus actual number of Ss adults recovered from 3 tents per date for 2010. Our larval survival from egg to adult in our lab colony was dismal! We will compare this to last year's prolific reproduction and try to make some sense of it. Currently we have only 177 adults in summer storage after making a late season release of 77 adults at Emery Creek in the Cohuttas. Sarah Osicka at North Georgia College and State Univ. far exceeded her storage capacity for Ss this year, and has sent us approximately 350 adult Ss to carry over the summer. These will supplement our colony for the 2011 release season. Many thanks to Sarah for this.

Our ability to cold store beetles after an initial 4-5 week maturation period improved radically over 2009 with the addition of an additional Percival incubator. Most storage was done at 12 to13°C. After the 2010 rearing and release season, we had 255 Ss in our colony. We began summer storage in July in a Percival incubator at 12-13°C and Rh at 65%. The storage jars are large (16L), well vented and contained plentiful foliage. Development of an optimal rearing protocol for Ss is on-going! We held foliage collected at high elevation in late June, in our cold room at 6°C. This stopped HWA development, and provided us with HWA adults and eggs to feed our summer storage colonies through August into early Sept. Attempts to induce neo-sistens to break aestivation by manipulating photoperiod and temperature have been unsuccessful. Now that HWA is developing in the field (Oct. 10), quality food is again available for our storage colonies. By holding foliage in our walk-in cold room, we reduced the high mortality from 2009 to only 2% per week this year.

Heaps of praise are due our dedicated lab rats who worked their tails off producing all the egg-twigs, pumping up our Ss colony, collecting foliage to feed our predators (a feat becoming increasingly difficult in Georgia!!) and putting up with yours truly yet again. So here's to Leah, Jessica, Angee, and Jeremy for a job well done!

Finally, thanks to our sponsors for their continued support of our effort here in Georgia.

- Univ. of Georgia Dept. of Entomology, CAES
- USFS FHP Region 8; USFS-FHTET; USDA APHIS PPQ
- GA Forestry Commission; GA DNR
- Brigadoon Foundation; Turner Foundation
- Georgia Forest Watch; Georgia Wildlife Federation; Lumpkin Coalition
- Various private donors

APPENDIX I. Foliage sampling protocol for recovery efforts in release areas

Protocol for Recovery Attempts of Predators of HWA: 2010

The general idea is to intensively sample a release area that has had at least 2 consecutive years of releases of all 3 of our predators (Ln, St, Ss). We do have several areas with 3 consecutive years of work, but the trick here will be in locating healthy HWA populations to sample. Select trees in the general release area. You will know when you are in the area by presence of flagging and binder clips on the foliage of the hemlocks. Sample trees do not have to be release trees although many probably will be. Trees should be at least 3" DBH and 15' tall. Select the healthiest looking trees. Target branches with healthy, clustered HWA populations on them. We would like to collect 10 samples each from 15 different trees within each area. Do not sample foliage that can be reached b hand from the ground, as these will likely have current years' releases on them. We are after evidence of reproduction and not beetles that we have recently released. Ideally, your sample branches will come from mid-crown, BUT depending on the level of tree decline, you may have to sample higher or lower in the crown.

Clip your initial 3-4 branches (2-3 feet long) from a particular level and assess them for presence of HWA clusters large enough to accommodate predators (2-4 dozen ovisacs at least). If you don't have good adelgid at this level in the crown, then move either higher or lower and try again. Once you locate good, healthy adelgid then sample at this height. The actual sample size will be 25 cm (10 inches) long, so a 2-3 foot long branch might have multiple samples on it. We want ten of these 25 cm long samples per tree. Any particular branch can have up to 3 of these 25 cm samples on it, or they may have as few as one. The minimum number of branches you can collect per tree will be 4, and the maximum number will be 10 (only 1 sample per branch in this case). Be careful with the foliage as rough handling in the field can dislodge the larvae.

Handling of branches in field: Place a large white flat sheet under the area where you are clipping your initial 2-3 ft long branches if possible to spot any dislodged larvae/beetles as branches fall from the tree. In the field, over the sheet, cut the 10 twig samples (ca. 25 cm long) from the larger branches and put them immediately into saturated, floral foam blocks wrapped in saran wrap. Foam blocks with clipped branches are already in plastic totes (18" x 13.5" x 7" 25 qt. containers). Get the branches in the totes and into the floral foam as quickly as possible and take great care in keeping the foliage as cool as possible!! No direct sunlight on the totes or on the camper top. The 150 samples (25cm long) from the 15 trees will be set up on one or two larval rearing blocks (whatever fits your totes and your larval rearing set-up at the lab).

The larval rearing block will be brought back to the lab and reared out in a tent for a period of 6-8 weeks, adding fresh foliage as needed. After removing the larval block for placement in a tent, check the field tote boxes well for any dislodged larvae or adults. The drop jar, the tent floor and sides should be monitored daily. Any Ln larvae or lady beetle adults should be collected and recorded by date, area and tent number. Lady beetle larvae can be returned to the foliage to complete maturation or kept on foliage in petri dishes to follow maturation. Ln larvae will be kept in vials of 100 % ethanol for future genetic identification (Gina at VPI). The larval tent will be set up and maintained with fresh foliage and biweekly misting as usual. There will be only one larval tent per area sampled this year. We are not interested in which tree these predators were on, but

only if they are present or not, and a rough idea about frequency of occurrence. Suggestions to improve this protocol are welcome.

Preparation of larval rearing block for the field: Soak Oasis floral foam block in tap water until saturated. Cut foam block in half. Wrap soaked foam block 3-4 times with plastic wrap. Tape the plastic wrap at the top and bottom of larval block closed with an 'X' using masking tape. Push green metal support (used for hanging the larval block in the tent) with wire mesh on bottom through foam block. Cut 10" fresh HWA infested twigs and place two twigs in two layers of four around the bottom of the floral foam block (total=16 twigs/side). Twigs cut from the field will be layered above these fresh twigs. (This part of the protocol is highly flexible: basically it is what ever works for your rearing set-up)

Figure 1. *Laricobius nigrinus* eggs per twig in oviposition colony 2010

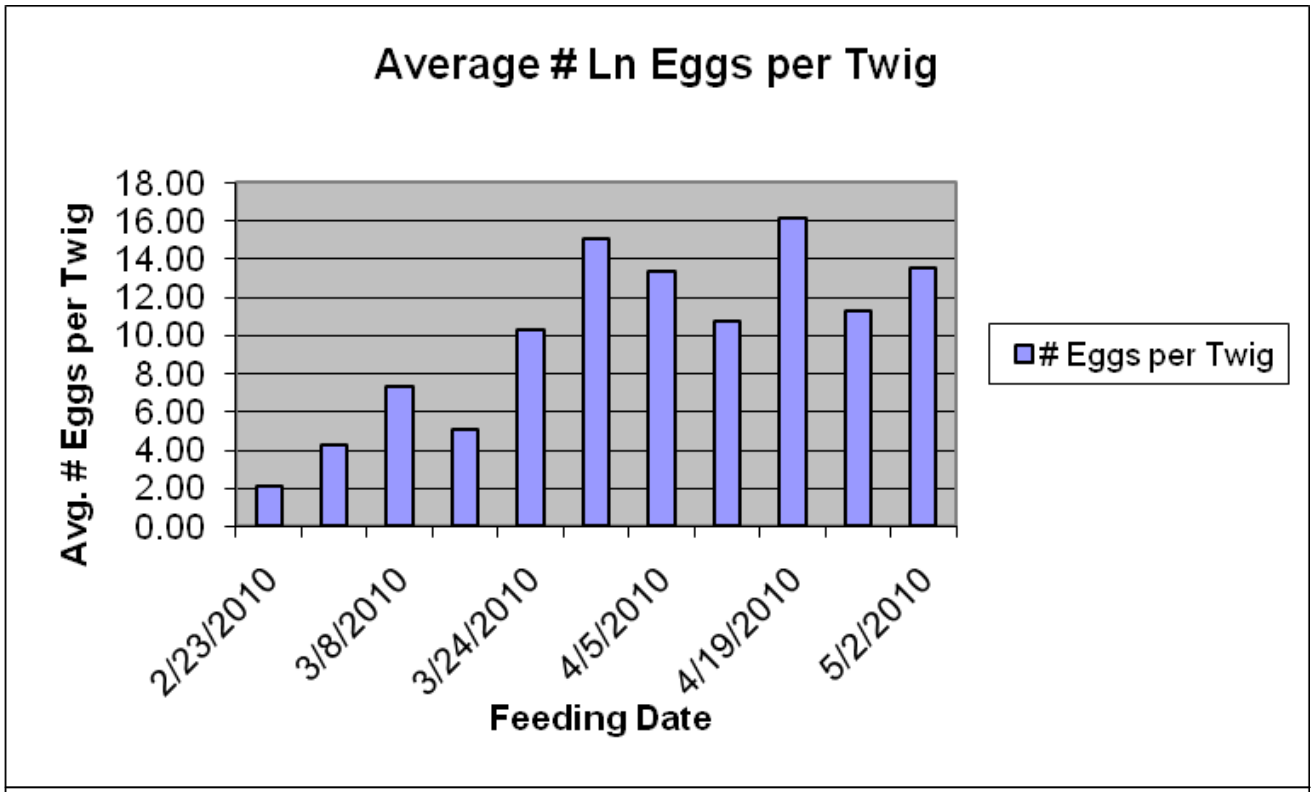


Figure 2. *Laricobius nigrinus* weekly mortality in oviposition colony 2010

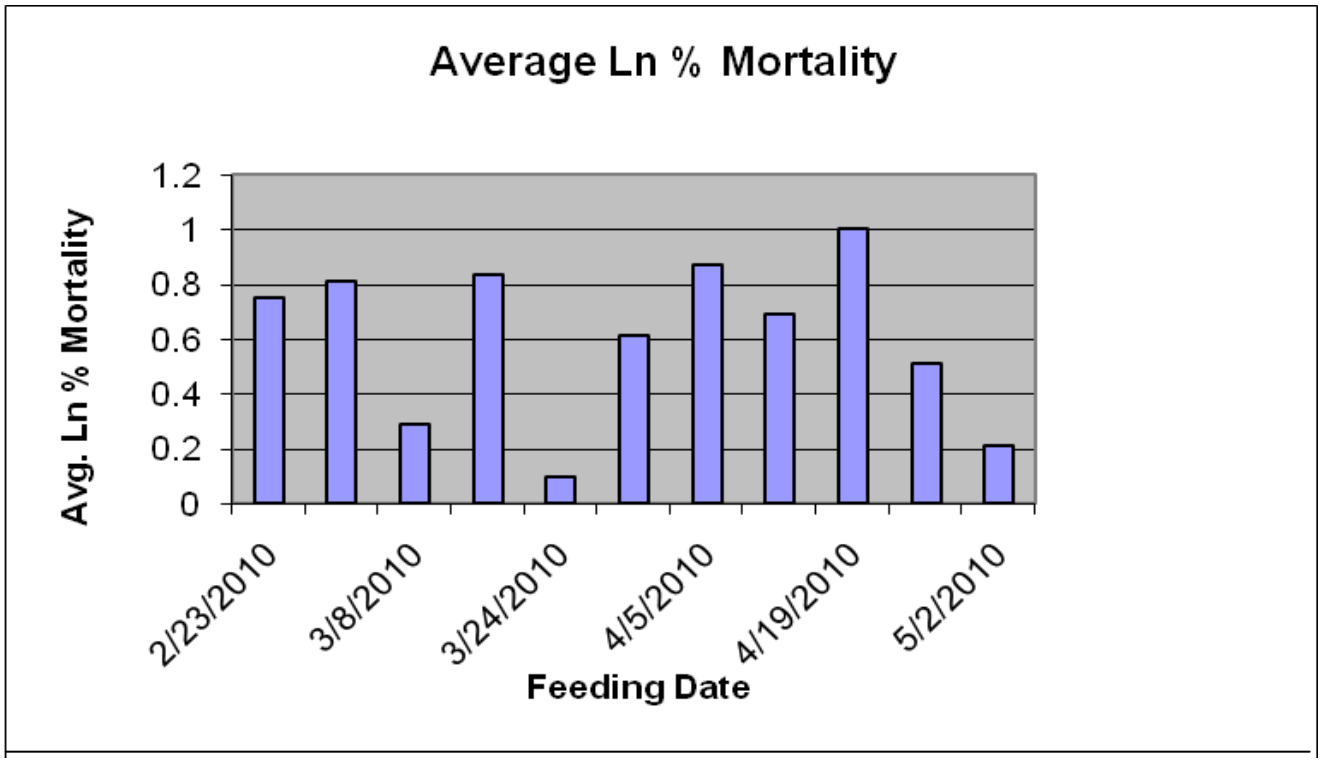


Figure 3. *Scymnus sinuanodulus* eggs per twig in oviposition colony 2010

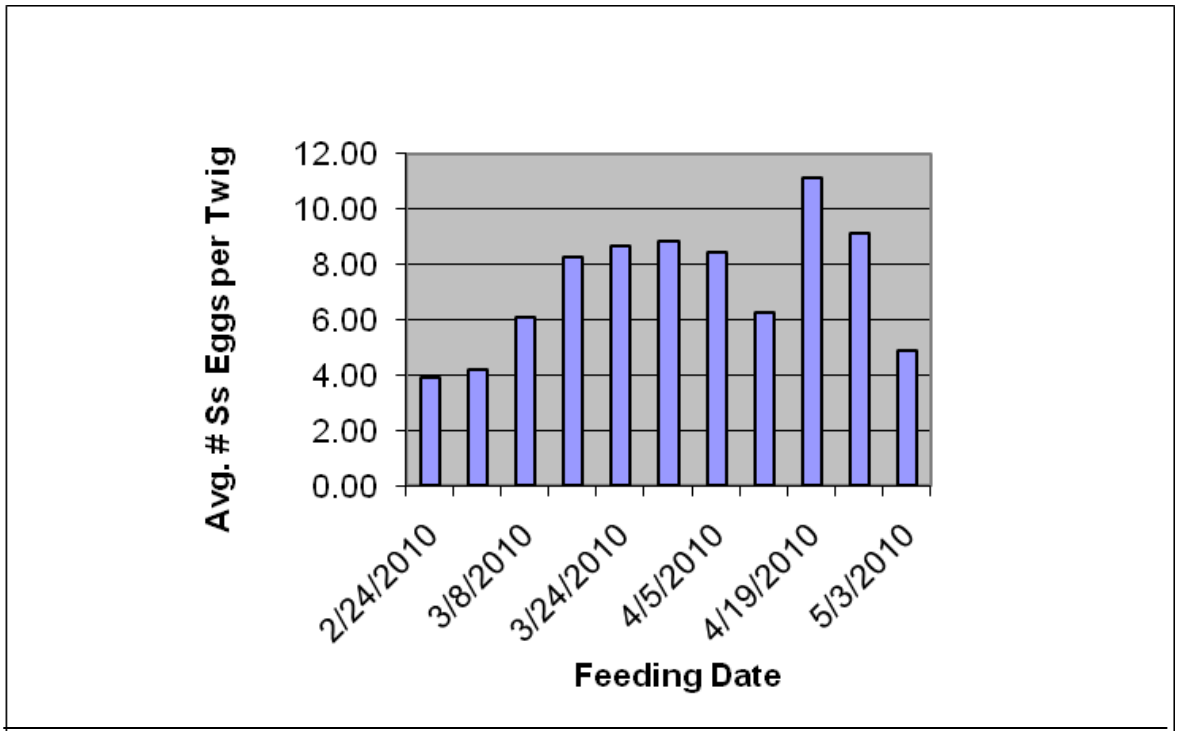
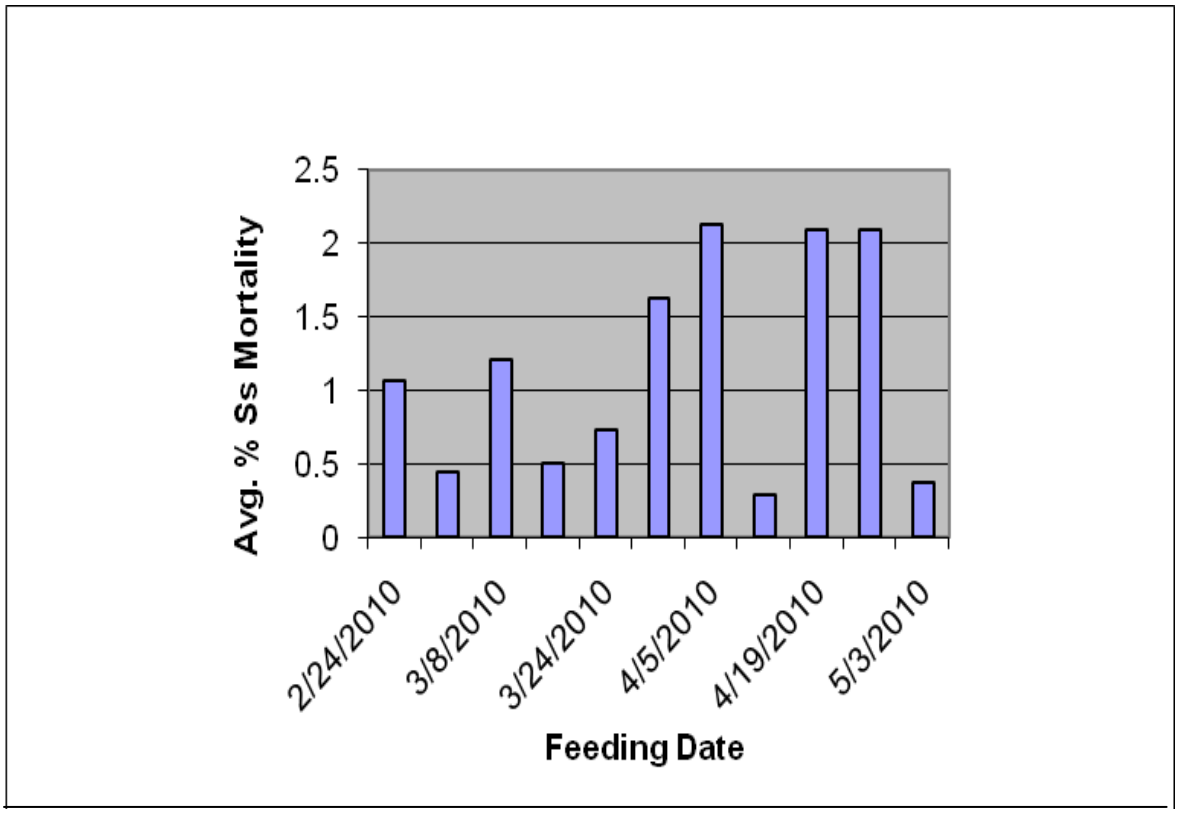


Figure 4. *Scymnus sinuanodulus* weekly mortality in oviposition colony 2010


Avg. % Ss Mortality
2.5
2
1.5
1
0.5
0
2/24/2010
3/8/2010
3/24/2010
4/5/2010
4/19/2010
5/3/2010
Feeding Date