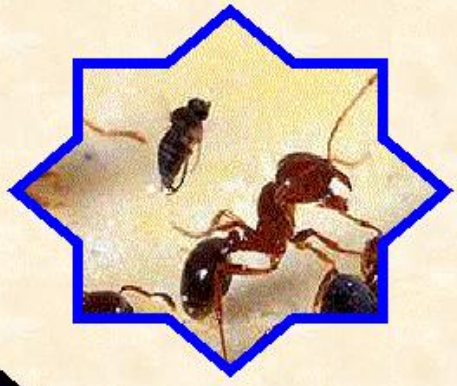
Beneficial Insects Laboratory

2005 Annual Report of Activities



North Carolina Dept. of Agriculture
and Consumer Services

2005 REPORT OF ACTIVITIES

Beneficial Insects Laboratory
Plant Protection Section
North Carolina Department of Agriculture and Consumer Services
1060 Mail Service Center
Raleigh, NC 27699-1060

http://www.ncagr.com/plantind/index.htm

Introduction

This report is a summary of the projects undertaken by the Beneficial Insects Laboratory (BIL) of the Plant Industry Division of the North Carolina Department of Agriculture and Consumer Services during 2005. The BIL addresses two programs, biological control and apiary inspection. The Biocontrol program implements classical biological control projects, in which the natural enemies of pest insects and weeds are released in the environment with the goal of stabilizing pest populations below their economic threshold. The Apiary Inspection program is designed to maintain a viable bee and honey industry in North Carolina through inspection for mites, diseases, and other hive pests.

The insects featured on our cover this year are representative of the diversity of our biocontrol and apiary inspection programs, geographically and biologically. From the top left, pictured are: larva of *Sasajiscymnus tsugae*, a predator of the hemlock woolly adelgid; top right - phorid fly attacking red imported fire ants, (USDA photo), center - honey bee; bottom left - cereal leaf beetle; bottom right - *Harmonia axyridis*.

USDA-APHIS, ARS, and Forest Service, as well as the Cooperative Extension Service, faculty, and staff of North Carolina State University all played roles in the implementation of our programs during 2005. We are grateful for the cooperation of other members of the NCDA Plant Protection Staff, including Support Services, and the statewide field staff under the supervision of Dan Wall.

Implementation of our 2005 programs included release of approximately 54,000 beneficial insects; the lab personnel reared some, others originated from out-of-state. Cooperative work with USDA-APHIS for cereal leaf beetle continued, as well as studies on the biology of the adventive predator *Harmonia axyridis*. A study of germination of oriental bittersweet seed was conducted in conjunction with David Patterson, Weed Specialist, NCDA&CS, and is included in this report.

The Quarantine Facility housed at the laboratory has been used by our personnel, entomologists from NCSU, and by the Museum of Natural Sciences. Rebecca Norris currently serves as the Quarantine Officer, and welcomes inquiries about the facility.

One paper was published by BIL personnel during 2005:

Nalepa, C.A., G.G. Kennedy, and C. Brownie. 2005. Role of visual contrast in the alighting behavior of *Harmonia axyridis* (Coleoptera: Coccinellidae) at overwintering sites. Environ. Entomol. 34(2): 425-431.

The personnel of the BIL during 2003-2004 were:

Dr. Kathleen Kidd, Biological Control Administrator
Dr. Christine Nalepa, Laboratory Research Specialist
Mrs. Rebecca Norris, Ag. Res. Tech II & Quarantine Officer
Mrs. Phyllis Straughn, Office Assistant
Ms. Janet Griffiths, Ag. Res. Technician
Mr. Arne Anderson, Ag. Res. Technician
Mrs. Anitha Boniface, Ag. Res. Technician
Ms. Jessica Bridges, Ag. Res. Technician

Mr. Mike Gusefski, Ag. Res. Technician
Mrs. Karin Hess, Ag. Res. Technician
Mrs. Betsy Megalos, Ag. Res. Technician

Personnel of the Apiary Inspection Program were:
Mr. Donald Hopkins, State Apiarist and Apiary Inspection Supervisor
Mr. Glenn Hackney, Agricultural Research Technician
Mr. Will Hicks, North Central Piedmont Area Apiary Inspector
Mr. Adolphus Leonard, Eastern Area Apiary Inspector
Mr. William Sheppard, Sandhills Area Apiary Inspector
Mr. Richard Lippard, Western Piedmont Area Apiary Inspector
Mr. Jack Hanel, Mountain Area Apiary Inspector

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K.A. Kidd, C.A. Nalepa, and R. S. Norris
Editors
21–III-06

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Records of Beneficials Released during 2005.

DATE	HOST	BENEFICIAL	#	SOURCE	RELEASE LOCATION
27 April - 10 May 05	RIFA	*Pseudacteon curvatus*	13,708	USDA-ARS, Gainesville, FL	Beneficial Insect Lab Wake County, NC
2004/2005	HWA	*Sasajiscymnus tsugae*	44,000	NCDA & CS-BIL Lab	Western section, NC*
May 05	CLB	*Diaparsis temporalis*	113	Piedmont Research Station	Cherry Farm Wayne County, NC
May 05	CLB	*Tetrastichus julis*	575	Piedmont Research Station	Cherry Farm Wayne County, NC

RIFA = Red Imported Fire Ant, *Solenopsis invicta*; CLB = Cereal leaf beetle, *Oulema melanopus*, HWA = Hemlock wooly adelgid, *Adelges tsugae*

* All beetles were released by USDA-Forest Service personnel.

Approximately 58,396 beneficial insects were released in North Carolina during 2005.

NCDA & CS Beneficial Insects Laboratory

Summary of Quarantine Activities 2005

The NCDA &CS Insect Quarantine Facility received one shipment of foreign material during 2005, and four shipments from previous years remain in the facility.

ID #	SPECIES	FAMILY	STAGE	#	ORIGIN	STATUS
Q02-1	***Lymantria dispar***	Lymantriidae	Larvae	126	NC	Insects dissected with some held in refrigerator.
Q03-1	***Aethina tumida***	Nitiduliade	Adults		NC	Colony being maintained in quarantine for research.
Q04-1	***Calidiellum rufipenne***	Cerambycidae	Pupae/ larvae/ adults	Unknown 6 emerged to date	NC	Cedar logs being held in quarantine for maturation and emergence of adult beetles.
Q04-2	***Celastrus orbiculatus***	Celastraceae	Seeds	2000	NC	Treatments currently being investigated to allow the sale of oriental bittersweet wreaths and preclude movement of viable seeds include the use of heat, ethylene oxide fumigation, and spray paint.
Q05-1	***Lymantria dispar***	Lymantriidae	Larvae	51	NC/VA	Insects dissected and autoclaved.

Cereal Leaf Beetle Insectary Program 2005

Kathleen Kidd and Rebecca Norris

The cereal leaf beetle (*Oulema melanopus* (L.)) (CLB) (Coleoptera: Chrysomelidae) is native to Europe and Asia, and is a pest of small grains. This species was first detected in North Carolina in 1977 in 19 counties, mostly along the Virginia border, and is now found in all grain-growing regions of the state. CLB can cause severe damage to the leaves of wheat, oats, barley and other cereal crops, and when heavy feeding occurs, grain yields may be reduced.

Fig. 1. Cereal leaf beetle larvae damage.

Cereal leaf beetle was discovered in Michigan in the early 1960s, and a biological control program was initiated in 1963 (Haynes and Gage 1981). Parasitoids were collected in Europe, and parasitoid nurseries (or field insectaries) were established in Michigan and other midwestern states by the late 1960s. Field days were held to distribute parasitoids to regional extension personnel and farmers. USDA originally imported one species of egg parasitoid and three species of larval parasitoids from Europe. These became established in North America, and all have been released in North Carolina. *Anaphes flavipes* (Foerster) (Hymenoptera: Mymaridae) (the egg parasitoid) was released as early as 1978. This species disperses well and has spread across NC. Three larval parasitoids, *Tetrastichus julis* (Walker) (Hymenoptera: Eulophidae), *Diaparsis temporalis* Horstmann (Hymenoptera: Ichneumonidae) and *Lemophagus curtus* Townes (Hymenoptera: Ichneumonidae) have also been released.

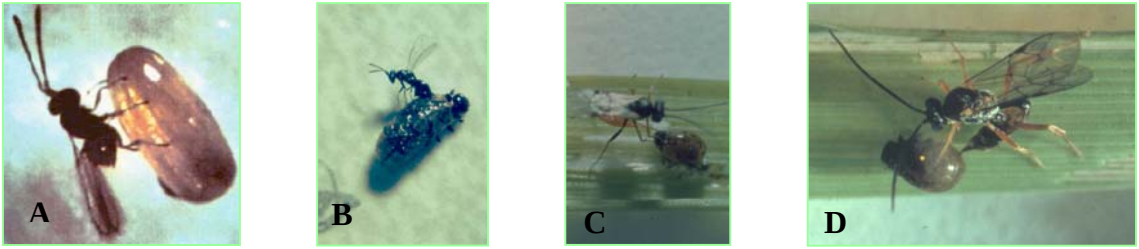


Fig. 2. Parasitoids of cereal leaf beetle. A. *Anaphes flavipes,* B. *Tetrastichus julis,* **C.** *Diaparsis temporalis,* D. *Lemophagus curtus.* (Photos courtesy of USDA).

The first parasitoid releases were made in North Carolina in 1978, and a field insectary program, similar to the program in Michigan, was started in the fall of 1987; insectaries were seeded with parasitized larvae from an insectary in Virginia. An insectary is currently located at the Piedmont Research Station near Salisbury. *Tetrastichus julis* has persisted there since the insectary was established. Adults of *Diaparsis temporalis* and *Lemophagus curtus*, emerged from material collected in Europe, were released at the Piedmont insectary in 1996 and 1998. Larval ichneumons were found parasitizing CLB larvae at Salisbury in 2000 and subsequent years. The egg parasitoid is prevalent at the insectary, but does not appear until late in the season.

Materials and Methods

The insectary program for cereal leaf beetle *Oulema melanopus* (CLB) and its parasitoids continued in 2005, with one insectary planted at the Piedmont Research Station near Salisbury, NC. The Piedmont insectary consists of two plots, each divided into four or more subplots. Wheat is planted in one subplot of each plot, followed by three or more sequential plantings of spring oats in adjacent subplots. No-till planting methods are used in all plots.

Plots were monitored beginning 9 April and continued on a weekly basis through 31 May. Larvae and adults were counted on one sq ft of grain (20.5" on 7" centers) at three locations within a subplot. Up to 25 eggs and larvae were collected in each subplot as populations allowed. Larvae were placed in vials containing 20% ETOH and returned to the laboratory for dissection. Larvae were dissected with a stereo microscope and numbers and life stages of parasitoids were recorded. Eggs were collected on the leaf blade, placed in zipper bags and returned to the lab. Eggs were placed on a cover slip in a small petri dish and held at room temperature until CLB hatched or *A. flavipes* emerged. The number of adult wasps emerged was divided by 1.7, the mean number of adults found in previous years. To calculate percent parasitism, the estimated number of parasitized eggs was divided by the total number of eggs.

Fig. 3. Cereal leaf beetle eggs parasitized by *Anaphes flavipes.*

Results and Discussion

CLB populations were low in the field, but parasitoids were abundant (Figs. 4 and 5). Parasitism by *Tetrastichus julis*, a multivoltine species peaked in late April and late May (Fig. 5). Ichnuemons were most prevalent in mid-May. Larvae were collected from the insectary on 24 May 2005 for release at Cherry Farm, near Goldsboro NC. A total of 217 larvae were collected, and based on dissections, we estimated that 113 *D. temporalis/L. curtus* and 575 *T. julis*

immatures were released. Populations will be monitored to determine if the parasitoids become established. *Anaphes flavipes* was found beginning in early May, attacking late eggs.

We gratefully acknowledge the staff at the Piedmont Research Station for their contributions to this project.

Literature Cited

Haynes, D.L. and S.H. Gage. 1981. The cereal leaf beetle in North America. Ann. Rev. Entomol. 26: 259-287.

Fig. 4. Cereal leaf beetle populations, Piedmont Research Station, Salisbury, NC, 2005.

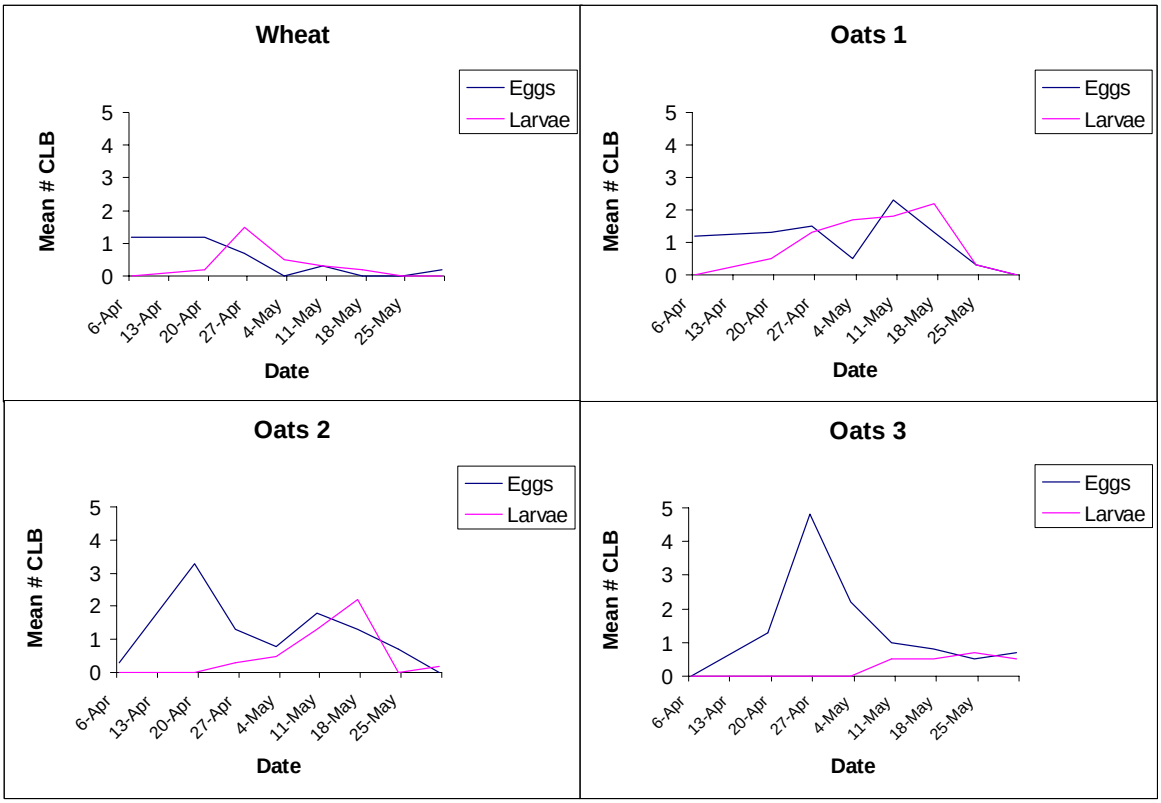
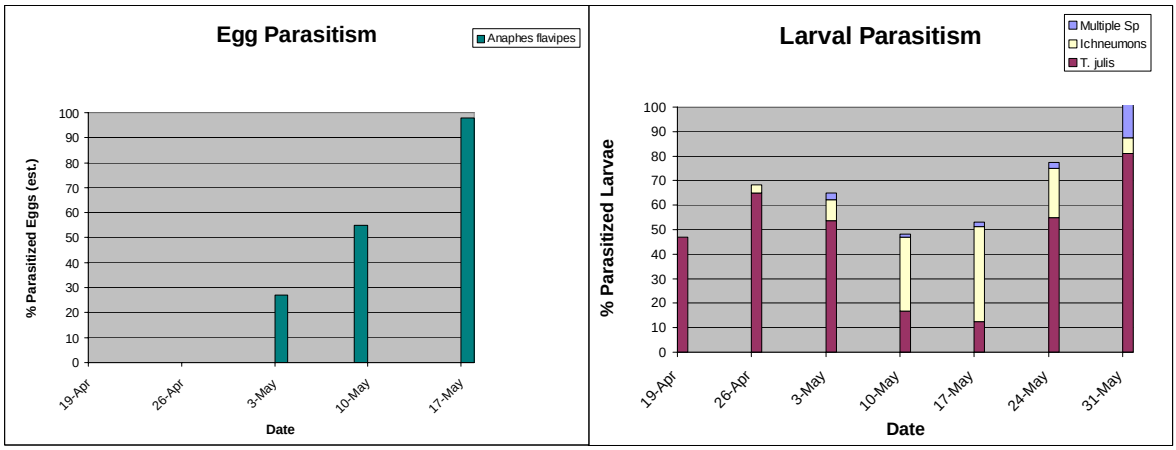


Fig. 5. Cereal leaf beetle parasitism in all plots, Piedmont Research Station, Salisbury, NC, 2005.



Rearing *Sasajiscymnus tsugae,* a Natural Enemy of Hemlock Woolly Adelgid

Kathleen Kidd

The hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, a pest of hemlocks (*Tsuga* spp.), is native to Asia (Fig.1). The insect was first found in the eastern US in the 1950s near Richmond, VA, but its spread was slow until it reached areas where hemlock is endemic. Upon reaching large, natural stands of hemlock, the insect population increased rapidly and was spread by birds and the movement of nursery stock, in addition to natural dispersal. Infestations are now found from Georgia to New Hampshire. The first infestations in NC were recorded in 1995 in two counties, and currently at least 25 counties are infested. After foreign exploration in Japan, part of the native range of HWA, several natural enemies were identified, and a small coccinellid, *Sasajiscymnus tsugae* (Sasaji and McClure) has now been reared and released from Georgia to New England as a biological control agent. As HWA infestations became more widespread, the need to rear additional beetles became acute, so cooperative agreements between NCDA&CS Beneficial Insect Laboratory (BIL), the USDA-Forest Service, and USDA-APHIS were initiated in 2002 to establish a rearing lab for beetles in NC.

Fig. 1. Hemlock woolly adelgid on hemlock**.**

The rearing facility was established in space on the second floor of the BIL located in Cary, NC, with a large room for rearing and smaller cool room for storage of beetles. A walk-in cooler is used for storage of adelgid-infested hemlock boughs. Procedures for rearing were developed by the NJDA, and followed with minor modification. Groups of beetles in a ratio of two females to one male are placed in 4 liter jars for oviposition (Fig 2). Whenever possible each jar contains 15 beetles, 10 females and 5 males. Each jar contains a bouquet of hemlock twigs and three pieces of gauze (5cm X 5cm) placed on the twigs. Females lay their eggs on the gauze as well as the twigs in a 50:50 ratio (D. Palmer, NJDA, personal comm.). Gauze and twigs are removed weekly, and eggs on the gauze squares counted.

Fig. 2. *Sasajiscymnus tsugae* oviposition jars and rearing cages.

Gauze and twigs are placed in acrylic rearing cages (61 X 61 X 48 cm) with additional fresh twigs and provided with honey and twigs until they completed their life cycle on average, 5-6 weeks.

Adults are transferred to a storage cage and held until released. Various life stages of *S. tsugae* are shown in Figs 3 and 4.

Fig. 3. *S. tsugae* larva (blue circle).

Fig. 4. *S. tsugae* pupa (red circle) and adult (yellow circle).

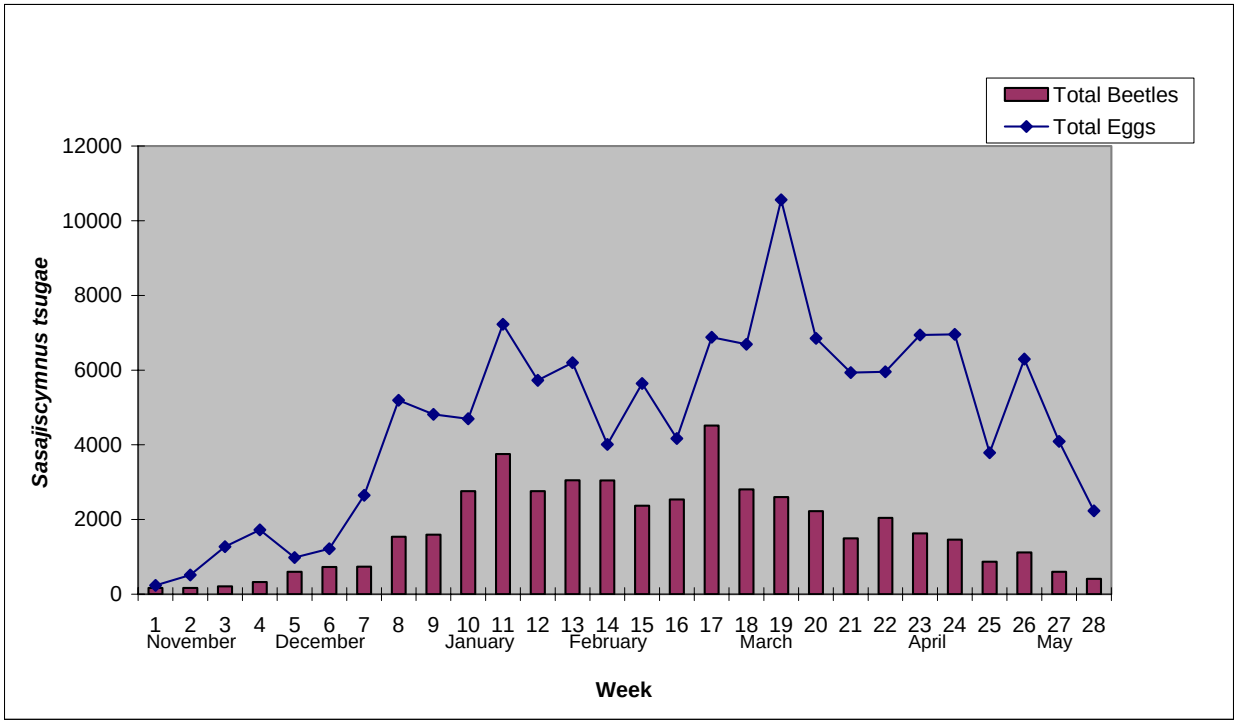
Current Results and Benefits

The season begins in mid-November, and oviposition occurs through mid- May. Eggs that were laid in May complete their development by the end of July. Production started with 40 oviposition units and increased to 60 by mid-season. Peak egg-laying occurred January through March (Table 1, Fig 5). A total of 48,075 beetles were produced, and approximately 4200 beetles were maintained at the lab to initiate fall rearing efforts once the adelgid broke its aestivation in the fall. Forty four thousand beetles were turned over to Forest Service personnel to release in NC State Forests and State Parks (Stone Mountain, South Mountains, and Hanging Rock).

Table 1. *Sasajiscymnus tsugae* production by month.

Month	# Weeks	Eggs	Adults	% Return
November	3	2016	542	26.9%
December	5	11754	3929	33.4
January	4	22464	10861	48.3
February	4	20020	10988	54.9
March	5	36912	13614	36.9
April	4	23640	5991	25.3
May	3	12618	2123	16.8

Fig. 5. Beetle production 2004-05, NCDA&CS.



Harmonia axyridis Program 2005

Christine Nalepa

Three studies were conducted on the introduced multicolored Asian lady beetle *Harmonia axyridis* during 2005.

1) Between 3 March and 21 July 2005, a survey of the coccinellids captured by pitcher plants was completed on the grounds of the Beneficial Insects Laboratory in Cary. The collected data will be pooled with the data of previous years and analyzed. The goal of the study was to compare the species composition of coccinellids attracted to the plants to historic data collected by Dr. D.L. Wray of the NCDA in the early 1940's.

2) A mark-recapture study was initiated on 18 October 2005 on the grounds of the BIL with the goal of determining the distance traveled between field sites and aggregation sites. A separate report of the study has been written and follows this one.

3) Two light traps were run on the grounds of the BIL between 4 April and 31 October 2005 and all coccinellids collected. Starting on 30 August 2005, *Harmonia* was also collected from dog fennel (*Eupatorium capillifolium*) in a nearby field. All insects were preserved, packaged and shipped to cooperators at the State University of New York; they are studying infection patterns of the fungus *Hesperomyces virescens* on the beetle.

One paper was published in a professional journal during 2005:

Nalepa, C.A., G.G. Kennedy, and C. Brownie. 2005. Role of visual contrast in the alighting behavior of *Harmonia axyridis* (Coleoptera: Coccinellidae) at overwintering sites. Environ. Entomol. 34(2): 425-431.

Two posters were e presented at the Annual Meeting of the Entomological Society of America in Fort Lauderdale, Florida 15-18 December 2005:

"Infection of *Harmonia axyridis* by *Hesperomyces virescens*: Role of Mating Status and Mating Behavior" by C.A. Nalepa and A. Weir

"Infection of *Harmonia axyridis* (Coleoptera: Coccinellidae) by *Hesperomyces virescens*: Pattern of Infection during the Mating/Feeding Season" by Fang Zhou, Christine Nalepa and Alex Weir.

How Far Does *Harmonia axyridis* Fly to Aggregation Sites? A Preliminary Experiment and Notes on Autumn Field Activity

Christine Nalepa

On 29 August 2005, substantial populations of *Harmonia axyridis* were observed in a field dominated by dog fennel (*Eupatorium capillifolium* (Lam.)) on which two species of aphid in the genus *Aphis* (identification by Dave Stephan, NCSU) served as food. The field measured approx. 540 m^2, and was located about 55 m northwest of a barn on the grounds of the Beneficial Insects Laboratory (Fig. 1); this barn typically serves as a winter aggregation site for the lady beetles.

Because little is known regarding the distance flown between feeding/ breeding sites of the beetles and their winter aggregation site, it was decided to take advantage of this opportunity to study the phenomenon. The goal was to mark the adults present in the field, then to survey the winter aggregation in the barn to determine if it contained any of the locally marked beetles. Although the study was compromised because the beetles did not have a strong flight in the piedmont during the autumn of 2005, some useful observations on the beetles were obtained.

Fig. 1. Southeast view of barn aggregation site (building on left). During fall flight, beetles typically land on this face of the barn, then form an aggregation behind a loose board framing the hay loft door. Photo was taken standing in a field of dog fennel infested with *Aphis* on which *Harmonia* were feeding.

Methods

The study was initiated on 18 October, and continued until a hard freeze on 18 November 2005; additional sporadic observations were made until 28 November. Each morning at dawn or shortly after, a prescribed route was walked through the field and the dog fennel plants examined for *Harmonia*. Newly encountered beetles were collected for marking; previously marked beetles were counted, noted, and left in place. Unmarked beetles were brought into the laboratory and the pronotum dotted with Testors brand enamel paint. The paint was allowed to dry, and the beetles were re-released later the same morning at the base of fennel plants in the same field. The paint color used for marking the insects was changed every eight days (Fig. 2). A total of 2,454 beetles were marked.

On afternoons appropriate for autumn flight of beetles to aggregation sites (clear, calm days > 21^{o}C – Nalepa et al., 2005), the barn pictured in Fig. 1 was monitored for alighting *Harmonia* for two hours, between 13:00 and 15:00 hours. Beetles landing on the barn within reach were collected, brought into the laboratory and examined for markings. The aggregation

site within the barn was examined for beetles the morning following flight days and for several days subsequent.

Fig. 2. Marked beetles copulating on dog fennel 6 November 2005. Beetles marked in blue (female, bottom) were marked 18-25 October; those marked in green (male, top) were marked 3 - 10 November.

Results

The mean temperatures at RDU Airport for both October and November 2006 were above normal; many days were warm enough to allow for beetle flight. The majority of days above 21^{o}C, however, were also windy, keeping the beetles grounded. Beetles flew on just 6 days in the Cary area in the autumn of 2005. On none of these days could the flight be described as a strong one when compared to flights of previous years. A total of 587 beetles were collected; just 3 (~0.5%) of these beetles were marked (Table 1).

Two beetles were seen in the usual aggregation location within the barn on 2 November, but these were gone by 5 November. Three beetles were observed on 7 November but these had disappeared by the time the barn was checked on 16 November. No further *Harmonia* were noted in that location, and none overwintered there.

Observations made while surveying the fennel field indicate remarkably extended feeding, mating and reproductive activity of the beetles during the warm autumn of 2005. Mating pairs were observed in the field throughout the period of observation. Freshly laid eggs were seen as late as 10 November 2005; larvae, pupae and aphids were noted in the field on the following day. A freshly eclosed adult was seen on 17 November. On the last day of systematic observations, 28 November, large larvae were still present. The fennel was nearly completely dead by that time.

Minimum temperatures were at or near freezing (29 – 32^{o}F) on the last five days of October. Harder freezes did not occur until 18 and 19 November, when temperatures sank to 26 and 23^{o}, respectively. Two interesting behaviors were observed during early morning field surveys after a cold night. First, if a pair is mating when the temperature begins dropping in the evening, they may remain coupled all night. There were several early observations on cold mornings where an immobile female was clinging to a vertical stem, with an immobile male hanging from her by his genitalia. Second, there were many cases of small clusters (n = 2 to 4) of beetles on plant stems. In one case a *Harmonia* was huddled together with a *Coccinella septempunctata.* The clustering behavior typical of winter aggregations, then, occurs in response to cold temperatures and does not necessarily require a period of flight as a prerequisite.

Discussion

The collection of just three marked beetles on the barn does not allow us to reach any conclusions regarding the distance traveled to overwintering sites, particularly since no beetles settled into the aggregation site within the barn. The continued development of larvae into late autumn when temperatures allow it explains the high proportion of virgins that fly to aggregation sites in some years (Nalepa *et al*. 1996).

The lack of a strong flight in the Cary area during the autumn of 2005 does emphasize that in years where fall days are largely inappropriate for flying (strong winds on warm days, in this case), the beetles have an alternate strategy. Instead of flying to buildings or mountains, they move to shelters more proximate to their feeding/breeding grounds. These include the interior of hollow logs, beneath the loose bark of standing or prostrate dead trees, or under leaf litter (R.M. Lippard and C.A. Nalepa, unpublished observations).

References

Nalepa, C.A. K.A. Kidd and K.R. Ahlstrom. 1996. Biology of *Harmonia axyridis* in winter aggregations. Ann. Entomol. Soc. Amer. 89(5): 681-685.

Nalepa, C.A., G.G. Kennedy and C. Brownie. 2005. Role of visual contrast in the alighting behavior of *Harmonia axyridis* (Coleoptera: Coccinellidae) at overwintering sites. Environ. Entomol. 34(2): 425-431.

Table 1. Autumn flight to overwintering quarters: beetles collected as they were alighting on the barn on the grounds of the Beneficial Insects Laboratory.

Date	Number of Beetles Collected	Number of Marked beetles Collected
31 Oct 06	3 seen on barn; none collected	-
4 Nov 06	64	0
7 Nov 06	175	1
8 Nov 06	133	2
9 Nov 06	77	0
14 Nov 06	138	0

Field Release and Monitoring Of Phorid Flies (Diptera: Phoridae: *Pseudacteon*) For Red Imported Fire Ant Control

Rebecca Norris and Kathleen Kidd

The release of phorid flies in North Carolina for biocontrol of Red Imported Fire Ant (RIFA) was initiated in 2000 as a cooperative effort between NCDA & CS and USDA ARS, FL. Since then two phorid fly species *Pseudacteon tricuspis* and *P. curvatus* have been released in this state.

The species of phorid flies released at each locality are based on the predominant social form of ant colonies present: monogyne colonies with one functional queen or polygyne colonies with multiple functional queens. *P. tricuspis* flies typically attack larger workers found in the monogyne colonies whereas *P. curvatus* prefer smaller ants in polygyne colonies (Glancey et al, 1973).

Pseudacteon tricuspis Release and Field Monitoring

Pseudacteon tricuspis were released in the following North Carolina counties: Beaufort 2000, Duplin 2002, Robeson 2003, and Wayne 2004 (Fig 3). Release sites are usually monitored annually for the presence of phorid flies for at least four years. Monitoring ceases after four consecutive years of no phorid flies sightings. Monitoring for phorid flies in 2005 commenced in September for all sites except Beaufort. One phorid fly was sighted but not collected at the Wayne county site on 31 October 2005. This is the second account of a phorid fly overwintering in NC. The first account was in Robeson County on 1 November 2004. No flies were found at the Robeson county site this year. The lack of flies may be attributed to the extremely dry weather experienced this past summer. Thus far no flies are known to overwinter at the Duplin or Beaufort sites.

Pseudacteon curvatus- Release and Field Monitoring

The first field release of *P. curvatus* was made in Wake county between April 27 and May 10, 2005. Field release of *P. curvatus* involved collection of ants from 6 mounds weekly for three weeks for a total of 18 mounds. Approximately 3 grams of ants were retrieved from each mound. This was achieved by placement of plastic stakes into the mounds (Fig 1.). As the ants crawled up the stakes they were removed from the mound and the ants knocked off into a gallon plastic bucket. Fluon (polytetrafluoroethylene) applied on the stakes prevented the ants from crawling to the top. Collected ants were placed into specially designed containers for shipment to

Fig 1. Collecting RIFA from mounds.

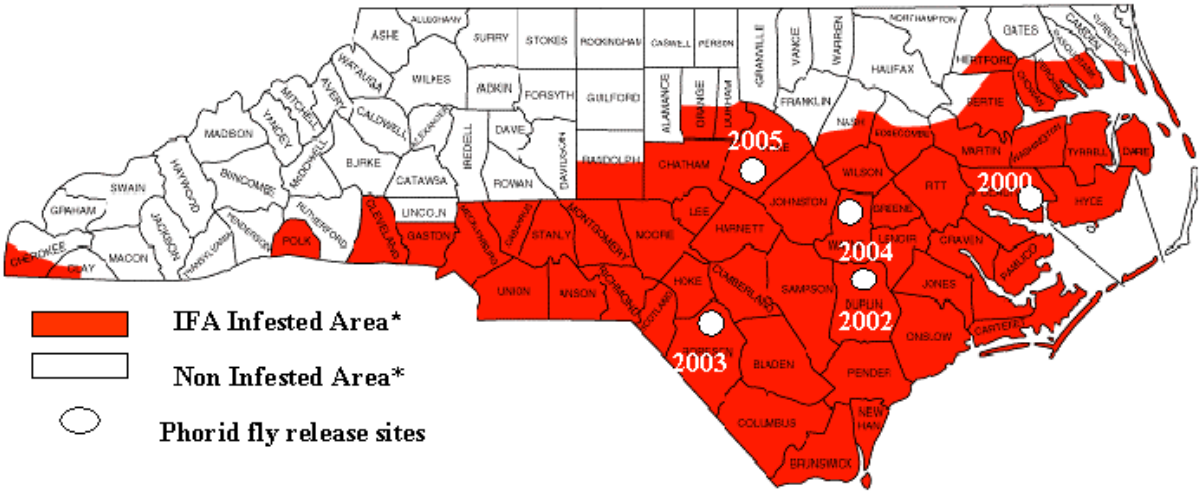
USDA ARS in Florida, where they were exposure to the phorid flies for seven days. Approximately 70.3 grams of parasitized ants were shipped back for placement in the same mounds from where they were collected. An estimated 13,708 emerged based on 650 ants per gram and a 30% parasitization rate (Debbie Roberts' personal communication). Materials and release protocol for *P. curvatus* were provided by Debbie Roberts and Shannon James, USDA, CPHST, ANPCL, SIPS, (www.cphst.org/projects/Phorid_rearing).

Monitoring for flies in Wake County (Fig. 2) began about 32 days after the initial release of parasitized ants. The first generation of flies was found on 5 June 2005. Seven generations of flies were found before temperatures fell too low (< 70° F) for flies to be observed. On 11 November 2005 (the last monitoring date), flies were observed at all of the 9 mounds checked. Numbers ranged from 1 to 10 flies with an average of 4.6 flies per mound. A delimiting survey was made on 11 November, to determine range expansion. Phorid flies were found to have spread at least a 1/4-mile beyond the release area. Eighty three percent of the approximately 30 mounds checked outside the release area had flies present.

Fig. 2 Wake Co. phorid fly release site**.**

Fig. 3 Phorid Fly Release Sites In North Carolina

2000 - 2005



* Effective Date March 3, 2004

Map: NCDA Plant Industry

Glancey, B.M., C. H. Craig, C.E. Stringer and P.M. Bishop. 1973. Multiple fertile queens in colonies of the imported fire ant. *Solenopsis invicta*. J. Entomol. Sci. 8: 237-238.

Roberts, D. 2005. USDA, CPHST, ANPCL, SIPS, Personal Communication.

2005 Apiary Inspection Program

Don Hopkins

Relative to the 2004 spring, the bees overwintered fairly well. Spring buildup was good and weather appeared to be favorable for a good honey flow. Unfortunately, this was realized only early in the season and later on turned out not to be the case. Those beekeepers who depend upon the sourwood honey crop were for the most part disappointed. The bees of the coastal plain seemed to fair better throughout the season than those of the piedmont and the mountains.

As had been mentioned in the previous year, there appeared to be a shortage of colonies for pollination. NCDA & CS and NCSU have provided a website to assist in this problem of matching beekeepers to growers. This program is in its infancy, however, and still needs to be further developed through more promotional and educational efforts to the growers.

The Varroa mites continue to be a major threat to the beekeeping industry in North Carolina. There have been several new products registered for use in controlling this pest. However, the mites continue to develop resistance in short order to some of these materials and make it difficult to maintain the efficacy of these products. Another perplexing factor is the growing use of unregistered materials that may or may not be effective. This and, in some cases, the improper use of antibiotics can further complicate useful treatment regimes.

We continue to enjoy a good working relationship with our friends at NCSU. We have had the opportunity to assist them in some of their projects and would like to express our gratitude for their assistance in many of our projects.

The Inspectors continue to help the beekeepers through field inspections, educational meetings, and field days, and to make every attempt to be available to assist the beekeepers in any way necessary. We look forward to a successful 2006 season. Our goal is to continue to improve our overall inspections and in particular our documentation of them. Our ultimate goal is to reduce the rate of honeybee disease and pest problems. The numbers of colonies documented for inspection for 2005 are 8,983 inspected, and of these, 40 hives were documented to have American Foulbrood.

Investigation Of Methods To Preclude Movement Of Viable Oriental Bittersweet (*Celastrus orbiculatus* Thunb.) Plant Propagules Through The Sale Of Wreaths

Rebecca Norris

Oriental bittersweet (*Celastrus orbiculatus,* Thunb.) was designated a State Noxious Weed in 2003 by the North Carolina Department of Agriculture & Consumer Services (NCDA & CS Plant Industry Division, Noxious Weed Regulations, Section 1700). Bittersweet is an exotic invasive woody vine. Invasive characteristics include high reproductive rate, aggressive growth and the ability to root sucker (USDA Forest Service, 2003). Because of its aggressive growth it has the ability to displace native vegetation by forming dense thickets. Since its introduction into the US as an ornamental vine in the late 1800's, it has escaped ornamental planting and invaded natural and managed areas throughout the eastern United States (Patterson, 1974). In North Carolina bittersweet has been found predominately in the more western counties.

Due to its attractive and persistent fruit the vines of bittersweet are often used in ornamental wreaths and dried flower arrangements. The fruits are globose, loculicidal capsules, approximately 1 cm in diameter. The capsules are three valved with each valve containing one or two seeds completely enclosed in a fleshy red aril. Upon ripening, the outer yellow covering splits open to reveal the red aril (Radford et al., 1968).

Fig 1. Oriental bittersweet (photo: James H. Miller, USDA Forest Service, www.foresstryimages.org)

Fig 2. Oriental bittersweet seeds, (photo: Leslie J. Mehrhoff, Univ. of Conn. www.forestryimages.org)

The designation of bittersweet as a state noxious weed prohibits the sale or distribution of the plant or viable plant propagules in NC outside the known infested counties without a permit. Counties where bittersweet is now known to occur include Alleghany, Ashe, Avery, Buncombe, Cherokee, Clay, Graham, Haywood, Henderson, Jackson, Macon, Madison, Mitchell, Orange, Swain, Transylvania, Watauga, Wilkes, and Yancey. Methods investigated to preclude movement of viable oriental bittersweet seeds through the sale of wreaths and not adversely affect appearance included dry heat, clear spray paint and ethylene oxide fumigation. Crafters often use spray paints to coat the wreaths to prevent the yellow ovary walls from falling from the vine.

Methods and Procedures

Bittersweet vines were collected from a site in Chapel Hill, NC on 8 November 2004. Since bittersweet is a regulated State Noxious weed, its storage and research was conducted in the NCDA & CS Beneficial Insects Laboratory Quarantine Facility located in Cary, NC. The fruits were stored at room temperature at temperatures that fluctuated between 18 - 26^{o} C. Fruits were removed from the vine for all treatments. For the heat treatments fruits were placed in the bottom of a glass 100 x 15-mm petri dish and exposed to dry heat in a Precision Econotherm oven at temperatures ranging from 50 to 80^{o} C ± 2^{o}C for 24 hours. To determine the effects of spray paint on seed viability, fruits were sprayed with clear paint. For combination treatments using spray paint and heat, fruits were sprayed prior to the heat treatment. Fruits were fumigated in a chamber using an 8.5% ethylene oxide and carbon dioxide gas mixture for a minimum of 16 hours at 38^{o} C ± 10^{o} C. Chamber pressure was 26 psig. Fruits were secured in nylon cloth bags for treatment. Treatments were replicated 3x with 20 fruits per replicate.

A preliminary test was conducted to determine if the stratification of fruits was necessary for seed germination. Patterson (1974) indicated that stratification of fruits in moist sand at 5^{o} C for over 30 days enhanced seed germination. Greenberg et *al.* (2002) however found that defleshed seeds germinated as well as stratified seeds. In this test stratification of fruits did not significantly improve seed germination (Table 1), therefore seeds in future tests were not stratified. To determine seed viability pulp and skin were removed from seeds 5 days after treatment. Thirty seeds were placed in 60 x 15mm dishes. Moistened Whatman No.3 filter paper served as the medium for germination. Seeds were held in a germination chamber at 30/20^{o}C day/night temperatures for 40 days. Seeds were checked for germination every 3 to 4 days.

Results

The development of a regulatory treatment for bittersweet fruits proved difficult. The condition of the fruit at time of treatment, moisture content and seed maturation influenced treatment efficacy. As expected, freshly harvested seeds were easier to kill than dry mature seeds with the dry heat treatments (Table 2). Treatments resulting in total seed kill were the dry heat treatment - 80^{o}C for 24 hours (Table 2) and ethylene oxide fumigation (Table 1). Coating the fruits with paint had no effect on seed viability but acted as a protective barrier against heat penetration (Table 3).

Summary

The most promising regulatory treatment for bittersweet wreaths that did not adversely affect the seeds appearance was the use of ethylene oxide fumigation. More tests are needed to ascertain its effectiveness on fruits and to establish treatment parameters; these include dosage, exposure time, and chamber temperature, humidity level and load configuration.

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Table 1. Comparison of bittersweet seed germination: Stratified verses non-stratified

Treatment	% Germination	
	Stratified	Non-stratified
Painted	66	60
50°C for 24 hrs.	10	11
Painted + 50C - 24hrs	65	51
Ethylene oxide	0	0
Nontreated	65	64

Ave. of 3 reps.
Seeds stratified - 5°C in moist sand for 35 days.

Table 2. The Effects of Heat on Oriental bittersweet seed germination

Treatment	DAC	% Germ	DAC	% Germ.	DAC	% Germ.	DAC	% Germ.
50°C	8	5	30	17	60	40	90	65
60°C	38	28	83	69				
70°C	60	30	86	40				
80°C	65	0	92	0				
Non-treated	8	64	30	61	60	50	90	61

Duration of treatment - 24 hours
DAC = Days After Collection
Seeds Collected - 11-8-04
Seeds were held in a germination chamber at 30/20°C day/night temperatures for 40 days after treatment.

Table 3. The Effects of Paint + Heat on Oriental bittersweet Seed Germination

Treatment	% Germination
Painted	69
Painted + 50°C - 24 hrs.	56
Painted + 60°C - 24 hrs.	68
Nontreated check	66