FHP TECHNOLOGY DEVELOPMENT PROJECT PROGRESS REPORT

PROJECT STATUS: Continuing (Funds are being requested for FY 1999 to continue the project)

PROJECT NUMBER: R8-98-04

PROJECT TITLE: Evaluating the potential of native or established predators as biological control agents for hemlock woolly adelgid in eastern hemlock forests.

SUBJECT: Biological Control, Hemlock Woolly Adelgid, Adelges tsugae (Homoptera: Adelgidae), Predators in the family Derodontidae and Coccinellidae, Diapause, Rearing

PROJECT OBJECTIVES:

1. Evaluate effectiveness, feeding preferences, feeding rates, and fecundity of Laricobius nigrinus, L. rubidius, L. erichsonii and Aphidecta obliterata on hemlock woolly adelgid (HWA).

2. Determine synchrony of Laricobius and Aphidecta life stages with HWA

3. Determine temperature and photoperiod conditions necessary to break aestival diapause of HWA that will allow for continuous rearing of the adelgids and their predators.

PROJECT DESCRIPTION: The project has two major facets:

Evaluate native or established beetle species for potential as biological control agents of HWA. Laricobius spp. (Coleoptera: Derodontidae) are predators specific to Adelges spp. Three species are currently established in North America, two are native and one introduced for the balsam woolly adelgid, Adelges piceae. Additionally, Aphidecta obliterata (Coleoptera: Coccinellidae), introduced for control of balsam woolly adelgid is also established in North America. These candidate predators will be evaluated for their potential as biological control agents for the hemlock woolly adelgid.

Develop a continuous rearing procedure for HWA that is not constrained by the 4-month long aestival diapause of 1st instar sistens nymphs. We have identified a temperature and photoperiod that allows 25% of the adelgids to continue development without going into diapause. This will be further investigated to obtain the highest percentage possible.

FHP PERSON WHO WILL LEAD THE PROJECT: James R. Rhea, FHP, Asheville, NC.

CO-PRINCIPAL INVESTIGATORS: Scott M. Salom, Assistant Professor and Loke T. Kok, Professor (Department of Entomology, Virginia Tech)

COOPERATORS: Lee Humble, Research Entomologist (Forestry Canada), Philip Eggborn, Program Manager (Virginia Dept. of Agric. and Consumer Services), Tim Tigner, State forest Entomologist (Virginia Dept. of Forestry), John Neal, Retired Research Entomologist (USDA, ARS, Beltsville, MD).

ACCOMPLISHMENTS AND RESULTS:

Evaluate native or established beetle species for potential as biological control agents of HWA:

A. Laricobius spp. (Coleoptera: Derodontidae) are known to feed specifically on adelgids. L. nigrinus has been observed in the field to be closely associated with HWA in western North America. It appears the L. nigrinus is synchronized with its host (HWA) but this needs to be quantified. The known distribution of L. nigrinus is British Columbia, western Washington, Oregon and northern Idaho. This predator has been poorly studied and hence very little is known on its biology.

1. Collection and rearing of L. nigrinus:

Overwintered L. nigrinus adults were collected from HWA infested western hemlock (Tsuga heterophylla) at the Lost Lake Conifer Seed Orchard owned and operated by Western Forest Products Ltd. near Victoria B.C., Canada in March 1998 and brought back to the quarantine facility at the Prices Fork Research Center of Virginia Tech.

L. nigrinus were reared on HWA infested T. canadensis (eastern hemlock) in environmental chambers between 13 and 15C, 85-95% RH and 12:12 L:D photoperiod. Approximately 10 to 15 adults (males and females) were placed in a 500 ml volume translucent plastic rearing container with aeration provided from a 6 cm hole cut at the bottom and covered with #45 mesh hardware cloth. In the lid of the container, an approximate 0.25 cm hole was drilled to which a hemlock twig stem could be inserted. The stem of the twig was wrapped with cotton gauze before it was inserted into the hole in the lid to keep it in place. The container was inverted and the end of the twig was placed in a vial with water. Twigs were replaced every three to four days and twigs with eggs were placed in a separate container as described above. This rearing method appeared to work well for ovipositing adults but was not suited for developing larvae, as when larvae searched out food, they often migrated to the base of the twig and were drowned from the water saturated gauze. The rearing method for larvae was subsequently adjusted, whereby the end of the twig was placed in moistened floral foam and the base of the container was covered by 1 cm thickness of sterilized peat (Premier Horticulture Inc., Red Hill, PA 18076) that was presifted using 3 X 3 mm mesh size hardware cloth. The sterilized peat served as a pupation site. Twigs were checked every three to four days and replaced as necessary. Dead larvae were collected weekly and placed in alcohol whereby head capsule widths were later measured. In addition, dead adults were collected and placed in water to be later sexed and body size measured. After 4 weeks, the peat was sifted weekly to collect pupae. Pupae were placed in 100 X 15 mm

Falcon`` containing sterilized peat lightly moistened with methyl paraben and checked weekly for adult emergence. Emergent adults were placed under two conditions. In one environmental chamber they were kept at 15C 13:11 photoperiod constant. In another environmental chamber photoperiod and temperature was adjusted to follow natural conditions. Increasing in L:D at 0.5 h intervals weekly until 14:10 L:D, which was maintained until mid -September with temperatures following natural conditions, followed by a decrease of 0.5 h biweekly.

L. nigrinus successfully mated, oviposited and developed on a diet of HWA indicating it is a suitable host. Mating was observed readily between March and April at the rearing temperatures. Males lie lateral to females when copulating. Copulation lasted at least 10 minutes. Eggs were predominately laid singly in the woolly ovisacs of HWA. Females were also found to occasionally lay eggs on the stem and needles of hemlock. The eggs appeared to be deposited with a sticky secretion on the surface to aid in adhering to the wool. Eggs are bright yellow when first laid, oblong in shape and smooth surfaced. Mean egg size was 0.28 mm in width and 0.46 mm in length (Table 1). The egg undergoes a color change as it develops. Changing from bright yellow to pale greenish-yellow. A few days before hatch, black compound eyes can be seen through the chorion. The ovipositional period lasted from March to May. Four instars were determined from head capsule measurements (Fig.1, Table 1). All life stages of L. nigrinus were observed to feed on HWA. First and second instar larvae fed only on eggs while later instars fed on eggs and developing HWA. Adults fed on all life stages of HWA. Mean adult body length was 2.69 mm (Table 1). Early instars fed within the woolly sacs and could only be found by picking open the wool. Later instars fed within the wool but were also active on the stem in search of prey. Larvae appear to have well developed mandibles, which they use to grasp prey and suck out body fluids. They also camouflage themselves by adhering wool to their bodies. Mature fourth instars lose this camouflage prior to migrating to the soil to pupate. Time from egg deposition to adult emergence ranged from 56 to 63 d between 13 and 15C. The first F1 adults were found on 27 May. Emergent adults stayed in the peat and exhibited negative phototaxis. From this behavior we believe they were summer aestivating. Approximately 80 F1 individuals were reared to adulthood. On October 6, the first activity of L. nigrinus adults was observed at 15C, 13:11 (L:D). The adults exhibited positive phototaxis and were found at the top of the rearing containers, closest to the light source.

2. L. nigrinus development study:

A development study was conducted at three temperatures; 13, 15, 18C at L:D 12:12 photoperiod and RH 85-95% between 30 April and 7 July 1998. Because males and females are difficult to distinguish morphologically, four to six adults were placed in each of ten disposable polystyrene sterile 100 X 15 mm petri dishes (Falcon``, Becton Dickinson & Co, Franklin Lakes, NJ) with 2 layers of filter paper (Whatman #1) at base moistened with distilled water and a 5-10 cm twig of eastern hemlock infested with HWA. Adults were kept at 15C. Twigs were replaced every 24 h. Eggs were removed from the twigs and placed individually in 50 X 9 mm disposable polystyrene sterile airtight petri dishes (Falcon``) with 2 layers of filter paper at the base moistened with

methyl paraben (0.42 g/250 ml distilled water) as a fungal inhibitor, and randomly assigned to one of the three temperatures until approximately 50 eggs were assigned to each temperature.

Mean egg development (± S.D.) was 6.6 ± 0.14, 9.1 ± 0.18, and 10.7 ± 0.20 d at 18, 15 and 13C, respectively.

3. Discussion and Future Work:

L. nigrinus at all development stages was observed to feed, mate and develop on a diet of HWA indicating it is a suitable host. The association of L. nigrinus with HWA has not been reported previously in the literature.

Biology of L. nigrinus described from laboratory observations is similar to biology described by Franz (1958) for L. erichsonii, a European species. However, Franz (1958) reports that emergent adults feed prior to undergoing aestivation from August to October. This was not observed for L. nigrinus under laboratory conditions. Aestivation for L. nigrinus occurred from May to October in the laboratory. First activity of L. nigrinus adults was observed early October in the laboratory. However, at present there are still adults that have not broken aestivation. Biology of L. nigrinus has not been previously reported in the literature.

Determination of thermal development requirements not only will provide baseline information on biology and distribution of L. nigrinus, but relating temperature requirements with that of HWA will help determine whether it is synchronized with HWA. In addition, optimal rearing temperatures will be determined which is important for laboratory rearing. Because of high early instar mortality, which could have been exacerbated because of fungal problems in the tight petri dishes where individual larvae were reared, development time of larvae to adulthood could not be determined. This study will be repeated at the same temperatures and three additional temperatures (9, 21 and 27C) for a total of six temperatures in order to determine the relationship between mean development rate and temperature at each development stage.

No-choice and choice host preference and suitability studies will be conducted in the Spring of 1999 when L. nigrinus adults begin reproducing in the laboratory. A minimum of six species of adelgid and non adelgid homopterans will be tested against HWA. The expected results from these studies will indicate host range of L. nigrinus which is key in removal of it from quarantined status.

B. Harmonia aryxidis, (Coleoptera: Coccinellidae) the multicolored ladybeetle, has been found preying on HWA, and is known as generalist predator of homopteran insects. It is present throughout the range of HWA in eastern North America and has been found sporadically feeding on the adelgids during the spring. Voracity, feeding preference, and field survey efforts were undertaken to evaluate H. aryxidis as a potential predator of HWA.

1. Harmonia aryxidis voracity of HWA:

Adult H. axyridis beetles were placed individually in moistened 100 mm petri dishes at 20o C, 12:12 photoperiod. Each petri dish had a grid marked on the underside to simplify counting. The dishes were numbered sequentially. Three prey types were used: HWA eggs, developing HWA nymphs, and HWA adults. Thirty trials were run per prey type, with different individual beetles. The eggs were collected locally, and removed from ovisacs on hemlock twigs with a fine brush. The nymphs and adults were also collected locally, and presented to the predator on a small cutting from a hemlock twig. The remaining prey were counted after 24 hours, and subtracted from the original total to find the number consumed. This was done for each dish in the order numbered on the previous day.

Adult H. axyridis consumed ca. 3 eggs/h over a 24 h period (Fig. 2). The number of nymphs and adults consumed averaged ca. 1/h over a 24 h period.

2. Harmonia aryxidis feeding preference:

An arena study was conducted to determine diet preference of H. axyridis when offered both HWA and one of its known food sources, the green pea aphid, Acyrthosiphon pisum. Adult HWA and pea aphids were offered at the same time. The predator was an adult beetle, starved for 24 hours in a bare petri dish and then released at the marked center of a 100 mm petri dish arena. It was observed until it attacked its prey. Prey were presented on 20 mm clips of foliage (from hemlock twigs for adelgids and fava bean stems for aphids). This experiment was carried out in a controlled laboratory setting at room temperature under steady overhead fluorescent lighting. Aphids were presented as a mixture of various life stages. 100 trials were run, with 5 blocks of 20 trials using different individual beetles in each block.

In 100 trials, aphids were chosen 71 times, and adult adelgids were chosen the remaining 29 times. Thus the preference ratio for adelgids as prey was .29, (i.e. they were chosen 29% of the time).

3. Harmonia aryxidis survey on hemlock:

Weekly samples were made in both urban and forested settings to monitor the levels of H. axyridis larvae and adults feeding on HWA in the spring. A total of four sites were used, two where ornamental hemlocks were infested in an urban setting, and two rural sites where stands were notably infested. At each site, five samples were taken in a random order from the lower branches in the stand. Samples were taken by grasping a large infested branch and shaking it vigorously into an empty five gallon bucket. The bucket's contents were then carefully sorted, and any predators found were counted and collected.

All H. axyridis collected were larvae. At all four sites, the number the number of H. aryxidis captured tended to decrease during the month-long survey (Fig. 3), with

signinficant reductions observed at Olean (F value = 4.89; DF = 4; P = 0.0065) and Blue Ridge Parkway (BPR) (F value = 5.13; DF = 4; P = 0.0052).

C. Aphidecta obliterata (Coleoptera: Coccinellidae), a native to Europe, was imported into North America in an effort to help control the balsam woolly adelgid. They have not been found in the eastern U.S. for over 30 years. Therefore, we imported them from British Columbia with goals to both rear and evaluate them as potential natural enemies of HWA.

1. Aphidecta obliterata voracity of HWA:

Adult A. obliterata beetles were placed individually in moistened 100 mm petri dishes at 18°C, 12:12 photoperiod. Each petri dish had a grid marked on the underside to simplify counting. The dishes are numbered sequentially. One trial was run with 9 different individual beetles, each offered 50 Adelges tsugae eggs. The eggs were collected locally, and removed from ovisacs on hemlock twigs with a fine brush. The remaining eggs were counted after 24 hours, and subtracted from the original total to find the number consumed. This is done for each dish in the order numbered on the previous day.

Egg consumption ranged from 11 to 35, with an average of 21.1 eggs consumed in 24 hours.

2. Aphidecta obliterata voracity of eastern spruce gall adelgid

Eastern spruce gall adelgids were collected locally. Galls were collected on the Virginia Tech campus from infested Norway spruce trees. Galls were then taken to the lab, where they were carefully broken open; causing adelgids to fall out. Galls collected a day in advance have higher numbers of adelgids that are unattached when the galls are opened. The free adelgids fall into a dish and the process is continued for many galls until several hundred adelgids are collected. For each experimental block, 70 adelgids were counted into each of ten clear 100 mm plastic petri dishes. Each dish had a grid marked on the underside to simplify counting. The time was noted, and one adult A. obliterata is placed in each dish. The dishes are numbered sequentially. The dishes are placed into a chamber with controlled temperature and photoperiod (20°C, 12:12 photoperiod). After 24 h the dishes are removed and the number of remaining adelgids are counted. This is done for each dish in the order numbered on the previous day. The number remaining is subtracted from the original 70, thus calculating the number consumed. The number of adelgids consumed is recorded, and the beetle is returned to its cage. The experiment was run four times, with each run counted as a block.

Consumption of A. abietis ranged from 6 to 51 nymphs. Overall, an average of 31.4 nymphs were consumed by A. obliterata in a 24 hour period.

Since the voracity tests for A. tsugae and A. abietis were run at different temperatures, the data are not directly comparable. While the data are encouraging, more individual

insects are required for completion of these experiments. Rearing methods must still be perfected. From three adults collected in mid-spring, mating was observed and viable eggs were laid at 18°C at a 12:12 (L:D) photoperiod. Later experimentation showed the beetles to be slightly more voracious at 20°C. No viable eggs were produced by colonies collected and shipped to us in the summer, possibly indicating that eggs are normally laid only in the spring. In late summer, during the aestival diapause of HWA, eastern spruce gall adelgids were successfully substituted as a food source for A. obliterata.

Develop a continuous rearing procedure for HWA that is not constrained by the 4-month long aestival diapause of 1st instar sistens nymphs.

We initiated studies that investigated conditions responsible for inducing aestival diapause in HWA. If we could identify these conditions and determine when HWA is sensitive to them, we may be able to develop a protocol that allows us to continuously rear HWA without too much impediment.

Diapause 1997: On March 21, 1997, hemlock twigs infested with HWA sisten adults and progredien eggs in ovisacs were attached to 2-yr old hemlock seedlings. On March 31, twelve of these infested seedlings were placed in each of six environmental chambers. The temperature and photoperiod settings as well as location for each chamber are presented in the Table 2.

Development of progrediens was monitored weekly at 12°C and twice a week at 17°C and 22°C as part of a development rate study for progrediens. After progrediens developed to adult stage and oviposited, plants from the different chambers were rearranged to determine:

1. Which temperature and photoperiod combination would prevent diapause from occurring.

2. If progrediens preconditioned at cooler temperatures would go into diapause at warmer temperatures.

3. If progrediens preconditioned in warmer temperatures would go into diapause at cooler temperatures.

Rearrangement of plants took place when approximately 50% of sistens crawlers settled onto the seedlings. For both photoperiods, of the twelve plants held at 12°C, three were placed in the 17°C chamber, three were placed in the 22°C chamber, and the remaining six were left at 12°C. Of the twelve plants held at 17°C, three were placed in the 12°C chamber and the remaining nine were kept at 17°C. Of the twelve plants held at 22°C, three were placed in the 12°C chamber and the remaining nine were kept at 22°C.

Plants were sampled twice per week. A different seedling was chosen randomly for each sample. In each sample the lifestage of the first 100 live sistens observed was recorded,

including diapausing first instars. No assessment was made whether a nymph was in diapause, other than it did not molt into a 2 nd instar.

All sistens exposed to a photoperiod of 16:8, at all temperatures, went into aestival diapause. At the 12:12 photoperiod, only adelgids preconditioned at 12 and 17°C, and kept at those temperatures through the sistens lifestages, developed with a reduced occurrence of diapause. At 12°C, over 40% molted at least once within 24 days (Fig. 4). At 17°C, duration of diapause lasted 3 months as opposed to 4 months documented in field studies (McClure 1996, Gray and Salom 1996). These data were a first indication that aestival diapause is not obligative, but rather induced by environmental cues.

Diapause 1998 - Experiment 1: Seedlings infested in 1997 but not used in the experiment described above were held in a greenhouse where daily and nightly temperature means (± S.D.) were 18.4± 1.5°C and 17.3±1.3°C, respectively. Thus, sistens that typically develop from October to March in the field (McClure 1996, Gray and Salom 1996), developed more quickly, with ovipositing adults appearing by January, 1998. On January 21, when presence of progredien crawlers appeared to be at its peak, five plants were placed in each of three chambers, all held at 12°C. The differences among the chambers was in (L:D) photoperiodic settings (12:12, 11:13, and 10:14). Progredien development was monitored on a weekly basis. In April, when sisten eggs began eclosing and crawlers began to settle, sampling of the seedlings was intitiated. Each of the five hemlocks in each chamber was sampled weekly. The lifestages of the first 30 adelgids encountered from clipped twigs was recorded. This provided us with a sample size of 150 adelgids per chamber per sampling period. Sampling was done on a weekly basis.

Because we could not follow individual adelgids through their lifecycle, lifestage frequency distributions were determined for each sample date. Weighted mean lifestage was then calculated for each treatment. Analysis of variance was run for each sample date to determine if photoperiod influenced sistens diapause and development. If significant for either dependent variable, the Tukey-Kramer test (a = 0.05) was used for comparing among treatments.

For all three 12°C treatments, no sistens entered aestival diapause. 100% of all 1st instar sistens molted within 49, 63, and 63 days at 12:12, 11:13, and 10:14 (L:D) photoperiods, respectively (Fig. 5). Overall development progressed more quickly at 12:12 than at the other two photoperiods (Fig. 3), taking only 63 days to go from settled 1st instar nymph to adult. Time of development was lengthened with a decrease in photoperiod.

Diapause 1998 - Experiment 2: In this experiment our objective is to see if diapause can be avoided at higher temperatures. Two- and 3-yr old hemlock seedlings were infested with HWA on April 10th, 1998. Nine plants were then placed in five separate environmental chambers each held at different constant temperatures. Two of the temperatures used, 12 and 14.5°C, were included in this experiment. Once half the

sampled adelgids we determined to be settled sistens, the seedlings were placed in the treatments as shown in Table 2 except where noted:

Plants with progrediens held at 12°C, were divided into three photoperiod treatments for evaluation of sistens development (Table 3). The same was done for plants with progrediens held at 14.5°C. In addition, hemlock twigs collected from infested trees were used to infest hemlock seedlings on June 19th, 1998. Five plants were immediately placed in each of two chambers, one at 12°C and the other at 14.5°C, both at a photoperiod of 12:12 (L:D) (Table 2). Because of the limited number of plants, we could not evaluate ``wild sistens' development in the other photoperiod treatments. The lifestages of the first 30 adelgids encountered from clipped twigs was recorded for each sample. This provided us with a sample size of 90 adelgids for each sample of pre-conditioned sistens and 150 adelgids for each sample of wild sistens. Sampling was done on a weekly basis.

This experiment is still ongoing. Results will be reported in the next year's progress report.

DOCUMENTATION: Only presentations made so far.

Salom, S.M., J.W. Neal, and W.T. Mays. 1998. Duration of hemlock woolly adelgid lifestages and aestival diapause under different constant temperatures and photoperiods. Joint meeting of working parties S7.03.03 (Insects Affecting Reforestation), S7.03.05 (Integrated control of scolytid bark beetles), and S7.03.07 (Population dynamics of forest insects) of IUFRO. San Juan, Puerto Rico.

Salom, S.M. 1998. Removing aestival diapause in hemlock woolly adelgid: implications on rearing its predators. 43rd Southern Forest Insect Work Conference. Asheville, N.C.

Zilahi-Balogh, G., S.M. Salom, and L.T. Kok. 1998. Development of Laricobius nigrinus (Coleoptera: Derodontidae) on hemlock woolly adelgid in the laboratory. 43rd Southern Forest Insect Work Conference. Asheville, N.C.

Cunningham, A., S.M. Salom, and L.T. Kok. 1998. Evaluating the potential of two arboreal coccinellids for biological control of hemlock woolly adelgid. 43rd Southern Forest Insect Work Conference. Asheville, N.C.

FIRST YEAR FUNDED: FY 1998

YEAR SCHEDULED TO END: FY 2000

ACTUAL YEAR TO END: FY 2001

PRODUCTS AND DUE DATES IDENTIFIED IN ORIGINAL PROPOSAL:
Predator(s) may be available for limited releases by the year 2000, and perhaps full scale

releases one or two years later. Protocol A continuous rearing procedure could be ready by the end of 1999.

STATUS OF PRODUCTS: HWA appears to be a suitable host for feeding and completing development for L. nigrinus. Biology of the insect needs to be investigated further, more research on other potential suitable hosts are needed, and development of an efficient continuous rearing protocol is necessary. Harmonia and Aphidecta feed on HWA, but it is not clear yet whether they can complete a generation on HWA as the sole food source. Aspects of critical photoperiod and temperature plus determination of sensitive stages required for initiation of diapause in HWA are being worked out. Recommendations on protocol for avoiding diapause may be available after FY 1999.

FUNDS OBLIGATED FROM BEGINNING OF PROJECT THROUGH END OF FY 1998: $35,000

FUNDS CARRIED OVER FROM FY 1998 TO FY 1999: All FY 1998 funds were obligated and used.

Table 1. Egg size, head capsule width and body length measurements of various stages of L. nigrinus.

Stage	N	Mean ± S.D. (mm)
Egg width	7	0.28 ± 0.02
Egg length	7	0.46 ± 0.03
1st instar head capsule width	16	0.20 ± 0.01
2nd instar head capsule width	25	0.27 ± 0.01
3rd instar head capsule width	74	0.35 ± 0.02
4th instar head capsule width	110	0.46 ± 0.03
Adult body length	155	

2.69 ± 0.02

Table 2. Location of treatments in 1997 sistens diapause experiment.

Photoperiod (L:D)
Temperature °C

12
17
22
12:12
VT
VT
VT
16:8
ARS
ARS
ARS

Table 3. Number of plants exposed to the following temperature and photoperiodic settings in 1998 sistens diapause experiment 2.

Treatment (Temperature; Photoperiod)

Progrediens
Pre-conditioned
Sistens
Wild Sistens
12°C; 12:12 (L:D)
9
3
5
12°C; 13:11 (L:D)

3

12°C; 14:10 (L:D)

3

14.5°C; 12:12 (L:D)
9

3
5
14.5°C; 13:11 (L:D)

3

14.5°C; 14:10 (L:D)

3