EVALUATION OF THE JAPANESE *LARICOBIUS* SP. N. AND OTHER NATURAL ENEMIES OF HEMLOCK WOOLLY ADELGID IN JAPAN

Ashley Lamb[1], Shigehiko Shiyake[2], Scott Salom[1], Michael Montgomery[3], and Loke Kok[1]

[1]Department of Entomology, Virginia Tech, Blacksburg, VA, USA

[2]Osaka Museum of Natural History, Nagai Park, Osaka, Japan

[3]USDA, Forest Service, Northern Research Station
51 Mill Pond Road, Hamden, CT 06514 USA

ABSTRACT

Since the initial importation of the Japanese *Laricobius* (Coleoptera: Derodontidae) to Virginia in 2006, we have studied the development of the predator at two different temperatures, measured feeding and oviposition rates, conducted preliminary host-range testing, and reared two generations. During this time, we have learned this predator differs from *Laricobius nigrinus* (Fender) in biology and life cycle. Host range tests have been relatively inconclusive, and laboratory survival has been low. These results prompted further investigation of this *Laricobius* beetle and hemlock woolly adelgid in Japan.

KEYWORDS

Laricobius sp. n., Japan, HWA predators, phenology, mortality factors

INTRODUCTION

As part of coordinated foreign exploration effort for hemlock woolly adelgid (HWA) predators in Asia, a new *Laricobius* species was discovered in Japan (Montgomery and Shiyake, unpublished report). The purpose of this project is to evaluate the newly imported biological control agent, *Laricobius* sp. n., in quarantine and in its native range in Japan. Preliminary basic biology and host-range studies have been conducted in the U.S. Results are summarized below. Phenology studies of *Laricobius* and HWA life cycles are underway in Japan. The presence of each life stage of HWA and its predators will be recorded over an entire year. The overall impact of natural enemies on the sistentes and progredientes generations will be estimated by examination of foliage under the microscope and by use of exclusion cages. Results from studies in Japan will support the effort to receive permission for removal of this species from quarantine in the U.S.

BIOLOGY AND LIFE CYCLE OF *LARICOBIUS* SP. N.

Field-collected adults had a high ovipositon rate (up to 11 eggs/day) at 9°C. Egg development rate is similar to *L. nigrinus*, but larval development rate is much faster at 9°C and 12°C (Table 1).

Table 1. The mean development rate (days±S.D.) of Japanese *Laricobius* eggs and larvae compared to the development rate of *L. nigrinus*.

TEMPERATURE	STAGE	JAPANESE *LARICOBIUS*	*L. NIGRINUS*
9° C	Egg	17.31±0.63	16.3±0.86
12° C	Egg	12.2±0.92	11.5±1.05
9° C	Larva	24.14±5.33	32.8±2.92
12° C	Larva	15.45±3.55	25.1±2.77

Pupae have similar survival when maintained at 12°C (65%) and 15°C (64%) after 30 days. Most individuals at 15°C had eclosed. If left undisturbed throughout the summer, adult survival is good at 15°C or at 18°C, if the temperature is higher than at pupation.

In 2006, over 9,000 larvae were reared to prepupae. Rearing conditions were suboptimal at ~18°C compared to the desired 12°C. Nevertheless, 1,530 adults emerged between August and December 2006 and held at 3°/5°C (night/day) (Figure 1b). A greater proportion of males emerged first, whereas ~80% of adults emerging in November and December were female. F_1 adults began laying eggs soon after emergence, first observed in mid-November, two weeks following emergence. Adults layed eggs at 0°C and were then cooled to as low as -7°C to get them to stop. Adults survived this temperature and still showed signs of feeding. In December, eggs began to hatch, and it was discovered that the larvae were feeding on HWA sistentes nymphs, something rarely observed in *L. nigrinus*. The Japanese predator larvae were surviving on this food source; however, they appeared dark in color and emergence from aestivation was much lower than the previous year (Figure 1c). In addition, many individuals experienced extreme temperatures as eggs and larvae (-7°C, and later, 4°C), which may also explain the poor summer survival.

A total of 9,000 larvae were reared, as in the previous year, but larval drop was slightly later; furthermore, adult emergence from aestivation was delayed compared to the previous year (Figure 1d). This may be due to the slow development at low temperatures, which caused larval drop to occur later in the spring, or it may be normal for individuals to emerge much later in the year and immediately begin laying eggs. These F_2 adults did begin to lay eggs in December; thus, the period between emergence and oviposition is very short (two to three weeks).

Between October and January 2007, 550 adults emerged and were held at 4°/6°C (night/day). Survival through aestivation was lower than the previous year and most adults to emerge were females. These F_2 adults did begin to lay eggs in December; thus, oviposition and funnel production is well underway as of January, and it is assumed individuals will develop under more normal conditions this year. The understanding of *Laricobius* life-cycle gained from field research in Japan will support this effort.

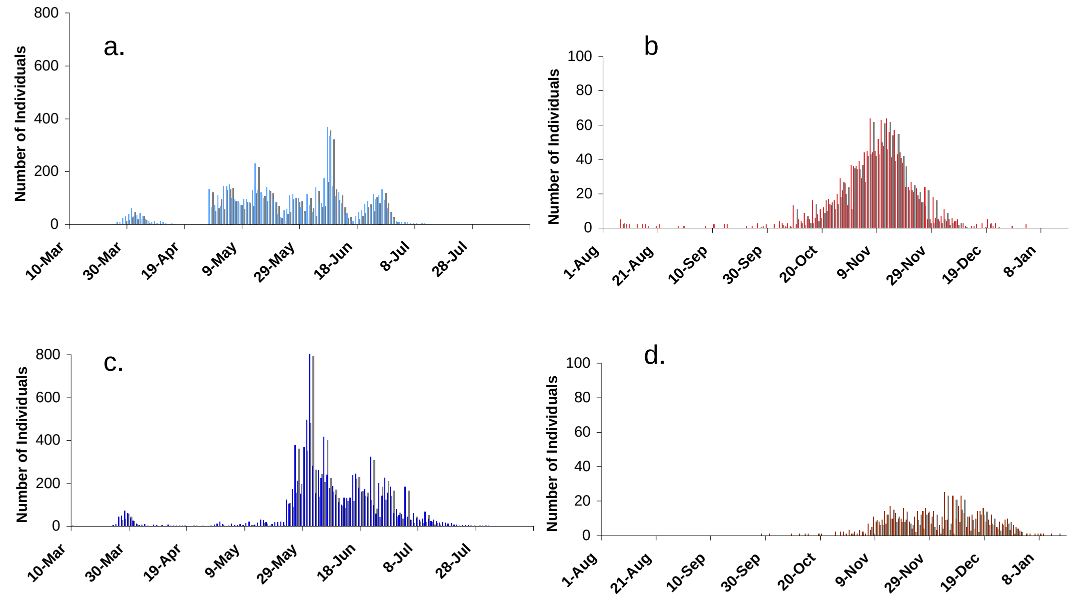

Figure 1. The number of *Laricobius* larvae to mature each day in spring 2006 (a) and 2007 (c) and the number of adults to emerge each day in fall 2006 (b) and 2007 (d).

HOST-RANGE TESTING

Host-range testing studies will follow the format and procedures used by Zialhi-Balogh et al. (2002) for *L. nigrinus*. The following non-target prey species are used in choice and no-choice feeding and oviposition bioassays: balsam woolly adelgid (BWA), pine bark adelgid (PBA), eastern spruce gall adelgid (ESGA). larch adelgid, woolly alder aphid, pine needle scale, elongate hemlock scale, and mealybugs. Development studies with some of these species have been conducted.

No-choice feeding and oviposition tests indicate adults prefer HWA over BWA and PBA but will feed and lay eggs on BWA and PBA when given no choice (Table 2).

Table 2. The mean number of adelgids eaten and eggs laid (±SE) when Japanese *Laricobius* is offered only one prey item: hemlock woolly adelgid (HWA), balsam woolly adelgid (BWA), and pine bark adelgid (PBA).

Host	n	Feeding Rate (eggs/day)	Oviposition Rate (eggs/day)
HWA	8	17.7±5.9	3.7±1.9
BWA	10	2.0±26	0.1±0.1
PBA	11	7.1±3.5	0.5±0.8

Development tests were inconclusive: fewer individuals developed to pre-pupae on BWA and PBA than on HWA, but no individuals successfully pupated. In a separate test, larch adelgid and woolly alder aphid do not appear to be suitable hosts (Table 3). The results of a third test were similarly inconclusive as no adults emerged from the soil. However, more individuals developed to pre-pupae on PBA than on HWA or ESGA (Table 4). BWA was not available for testing. It is possible that individuals are not being provided with a sufficient amount of food during these tests. When more food is provided, mold rapidly appears and larvae die. A different bioassay will be used in 2008.

Table 3. Number and percentage of *Laricobius* individuals surviving each life stage when offered only one prey item.

Stage	Hemlock Woolly Adelgid		Balsam Woolly Adelgid		Pine Bark Adelgid		Hemlock Woolly Adelgid		Larch Adelgid		Woolly Alder Aphid	
	n	%	n	%	n	%	n	%	n	%	n	%
Egg	26		22		20		10		20		20	
L1	11	100	14	100	18	100	9	100	0	0	0	0
L2	10	91	2	14	12	67	8	88	0	0	0	0
L3	9	82	1	7	12	67	7	78	0	0	0	0
L4	9	82	1	7	10	56	7	78	0	0	0	0
Pre-pupa	6	55	1	7	3	17	5	56	0	0	0	0
Pupa	0	0	0	0	0	0	?	?	0	0	0	0
Adults	0	0	0	0	0	0	4	44	0	0	0	0

Table 4. Number and percentage of individuals surviving each life stage when offered only one prey item.

Stage	Hemlock Woolly Adelgid		Pine Bark Adelgid		Eastern Spruce Gall Adelgid	
	n	%	n	%	n	%
Egg	30		30		14	
L1	28	93	29	97	14	100
L2	24	86	26	90	14	100
L3	11	46	21	80	12	86
L4	7	64	18	86	8	67
Pre-pupa	5	71	13	72	6	75
Pupa	0	0	0	0	0	0
Adults	0	0	0	0	0	0

Both behavioral activity and rearing experiences with this species have been different than with *L. nigrinus* and difficult to understand. We also had issues with understanding behavior and rearing of Chinese *Laricobius* spp. (Gatton 2005). As we began working with *L. nigrinus* back in 1997, we had access to field plots to study the insect's seasonal activity in its native habitat. We have not had that luxury with both the Chinese and the Japanese

Laricobius species. Without knowing the normal behavior and life cycle of *Laricobius* in the field, it is difficult to make appropriate adjustments to obtain optimal rearing conditions. A phenology study to determine the life history of this predator and its host in its native range will enhance our ability to research and rear this species in the U.S. The results of the host suitability tests are currently inconclusive as *Laricobius* sp. n. has been able to develop on other adelgids, sometimes with better survival than those on HWA.

Mausel (2007) determined that *L. nigrinus* has a significant impact on the populations of HWA in the Pacific Northwest. Since HWA is native to Japan (Havill et al. 2006), assessing the impact of natural enemies on HWA populations in its native range would help us predict what impact the biological control agents can potentially have in the U.S.

RESEARCH IN JAPAN

Phenology Study

The phenology of HWA and *Laricobius* sp. n. will be studied using an approach similar to that used by Zilahi-Balogh et al. (2003). The trees at Kobe Arboretum, Takatsuki, and Nara were ranked according to HWA infestation levels. Twelve trees with sufficient HWA were identified at three locations. There are nine trees at the Kobe Municipal Arboretum, one tree at the Myo-on-ji Temple in Takatsuki, and two trees on a mountaintop, Wakakusayama, east of Nara City. These locations are west, north, and east of Osaka, respectively. Beginning in early December 2007, each tree is being sampled for adult and immature predators and the number and life stage of HWA. Adult predators are sampled by beating three branches per tree; all predators are returned to the tree after counting. Immature predators and HWA are sampled by removing four 10-cm branch tips from each tree for examination under the microscope. The stage and number of adelgids are recorded, as well as dead adelgids and the cause of death (chewing insect, sucking insect, fungus, or mortality during aestivation). Immature numbers and stages are also recorded, and if unknown predators are found, they are reared out for identification or preserved in alcohol. These data will reveal which life stages of *Laricobius* and other natural enemies are present with respect to the stage of their host.

Predator Exclusion Study

The impact of natural enemies on HWA populations is being measured by comparing the number of adelgids on caged branches with no predators to branches open to predators. The four treatments include: caged branches from December to March, caged branches from January to May, open branches from October to March, and open branches from March to May (caged January to March). Two trees were selected at Wakakusayama, east of Nara City. HWA infestation levels were estimated on 40 branches and assigned 20 pairs. Each pair was randomly assigned a cage or open treatment. Branches assigned to cages were caged on December 26, 2007, after permission to use the trees was granted. Four 10-cm branch tips will be sampled from each of these 40 branches in March and April. The number of surviving sistentes will be counted, and treatment means will be compared using a standard t-test to determine the impact of predators from December to March and April.

PRELIMINARY RESULTS

HWA Phenology

Most adelgids were fourth instar sistentes on the first sample date (December 6), and by mid-January, most were adults, but very few eggs have been observed (Table 5). Interestingly, in the Takatsuki samples, very young adelgids (first and second instars) have been observed under the bud scales. In samples from December 19, two eggs were observed under the bud scales and hatched into crawlers a few days later. These eggs are likely the source of the young adelgids found there; however, the insects that laid these eggs are unknown. Samples have been sent to Nathan Havill for genetic analysis. They have been confirmed to be *Adelges tsugae*; however, their morphology and DNA differ from all other individuals collected in the past except one. They group with Japanese HWA but do not cluster with the two clades that were found in the mtDNA paper. It is out by itself with another sample collected at Unpenji Temple in Tukishima.

Table 5. Percentage of HWA at each life stage at three different sites during each sample period.

Sample Period	Site	Percentage of Individuals at each Life Stage						
		1st Instar	2nd Instar	3rd Instar	4th Instar	Adult	Adults with Eggs	Eggs/ Ovisac
Dec 6 -11	Kobe	0.9	0	7.6	88.7	1.4	0	
	Nara	2.9	0	2.9	88.1	6.0	0	
	Takatsuki	10.3	5.1	3.8	80.8	0	0	
Dec 14 - 19	Kobe	0	0	0.6	89.1	10.2	0	
	Nara	0	0.9	0.9	68.8	29.2	0	
	Takatsuki	18.4	0	5.2	73.7	2.6	0	
Dec 22 - 27	Kobe	0	0.5	3.2	73.3	18.4	0	
	Nara	0	1.3	0	56.3	42.5	2.9	1
	Takatsuki	26.2	5.6	4.4	59.8	3.8	0	
Dec 31 - Jan 3	Kobe	0.7	0	2.8	62.8	31.7	0	
	Nara	0	0	0	68.3	31.7	15.4	2
	Takatsuki	19.3	5.3	14.4	56.1	5.3	33.3	2
Jan 8 - 12	Kobe	0	0.5	2.5	42.8	52.7	9.4	2
	Nara	0	0	0	37.5	62.5	6.7	3
	Takatsuki	43.1	7.7	0	46.2	3.1	0	
Jan 14 - 18	Kobe	0	0	0	36.6	63.1	11.9	3.4
	Nara	0	0	0	11.8	88.2	20.0	5.3
	Takatsuki	12.6	14.3	19.8	46.2	13.1	0	
Jan 22 - 26	Kobe	0	0.5	3.6	30.1	65.8	8.5	2.55
	Nara	0	0	4.5	0	95.5	26.2	
	Takatsuki	5.8	2.5	15.0	75.8	0.8	0	3.45

Natural Enemies

The HWA at each site seem to experience different mortality factors. By early December, significant mortality could be observed, and the fall and winter predation are likely important contributors to HWA population regulation (Figure 2). There appears to be a chewing predator, particularly in Nara, that is responsible for some mortality (shown in grey); however, it has not been collected in the beatsheet samples yet. This predator could have been feeding throughout the fall and is now dormant.

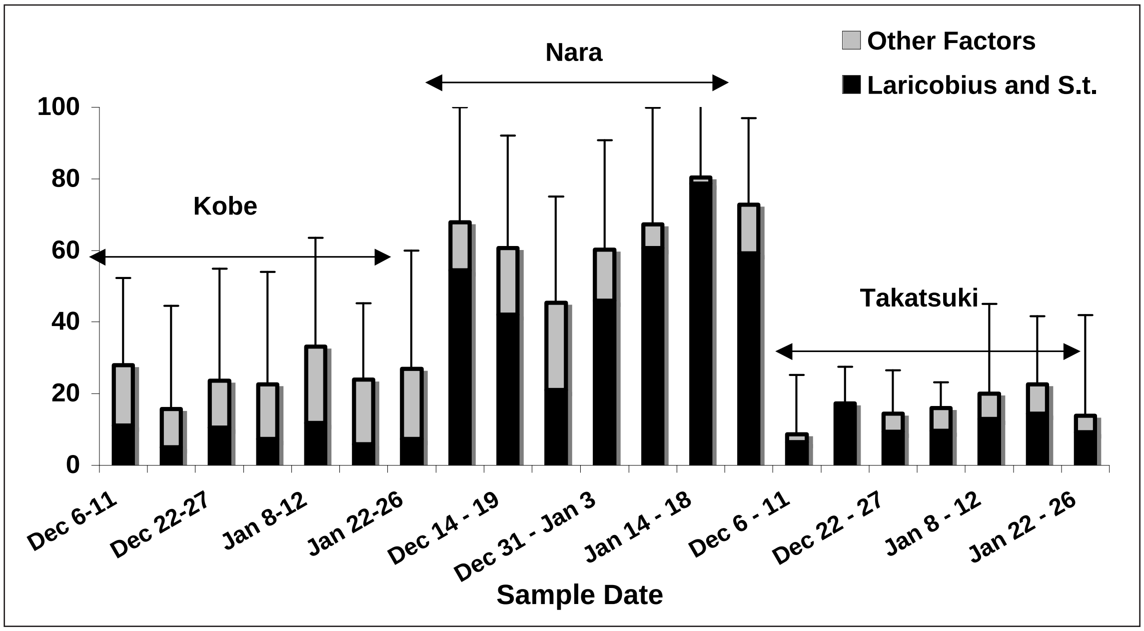

Figure 2. The average mortality observed in foliage samples during each sample period at each site. Proportions shown in black indicate the contribution of *Laricobius* and *St* adults to HWA mortality, and proportions shown in gray represent the impact of other natural enemies.

Laricobius is rather abundant at all three sites, particularly Nara, where nearly every beatsheet sample yields adult *Laricobius*, sometimes more than 25 individuals. At the other two sites, adults are less abundant but consistently present. Mortality caused by *Laricobius* "type" feeding (includes *St*) is indicated by black in Figure 2. It is very apparent that they are making an impact on adelgid populations at the Nara site but less so at the other two sites. *Laricobius* eggs were first observed in mid-December, when most adelgids were still fourth instars. Eggs seem to be laid in pairs on a single adelgid (often the adelgid has already been eaten); therefore, they appear to be less selective about their site of oviposition than *L. nigrinus*. Larvae were first observed in mid-January, and surprisingly, there are often no HWA eggs present. In effort to determine exactly what they are feeding on is currently underway.

Adult *Sasajiscymnus tsugae* (Sasaji and McClure) (Coleoptera: Coccinellidae) have been observed in the beatsheet samples at the Nara and Takatsuki sites. It is the first record of *St* in Nara Prefecture. They are much less abundant than *Laricobius*; however, they have been observed during four of seven sample periods. (It is difficult to distinguish an adelgid consumed by *St* from one that has been consumed, for example, by *Laricobius*.)

Other predators consistently found at each site include several Dipteran larvae. Specimens have been difficult to collect as they are currently dormant within the budscales and are often damaged when the budscales are removed. They do not become active when placed on HWA, so further investigation of this predator will have to be done later in the year, when they become active in the field.

Perhaps the most exciting and unexpected find is one or more entomopathogenic fungi. Fortunately, there are two scientists in Japan (Drs. Degawa and Sakuma) that have taken an interest in them and are currently working on identification. Thus far, they have determined that it is within Entomophthoraceae, Entomophthorales. Dr. Degawa has been unable as yet to match the asexual and sexual stages. It has been found on four of the nine sample trees at the Kobe site, and where it is present, HWA mortality is high.

CONCLUSION

Based on field samples from December and January, it seems the behavior of *Laricobius* observed in the lab may be normal. Adults are active and ovipositing on fourth instar HWA in Japan in December. With increased knowledge of *Laricobius* and HWA in Japan, rearing and research should improve in the following years. Providing that all the alternative hosts can be attained for testing, host range studies will continue and may be completed in the near future.

The mortality agents operating at each of the three sites in Japan vary by site and by tree. Although *Laricobius* is making a measurable impact on HWA populations, it seems there are other factors contributing to the overall mortality. Field sampling at more sites will be required to understand the diversity of natural enemies and the roles they play in regulating HWA.

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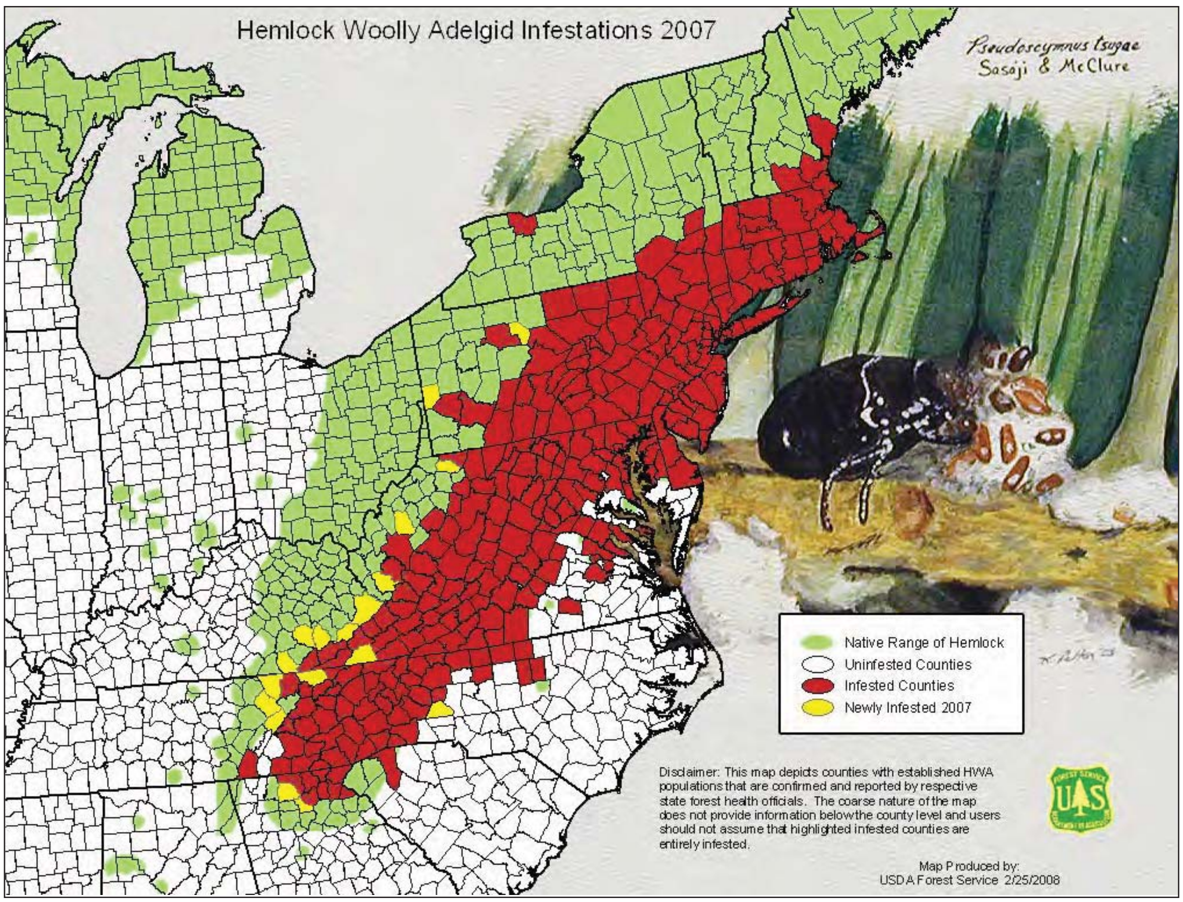

Brad Onken and Richard Reardon, Compilers

Forest Health Technology Enterprise Team—Morgantown, West Virginia

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