Chapter 7: *Laricobius osakensis*, a Hemlock Woolly Adelgid Predator from Japan

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INTRODUCTION

The approach for the biological control of hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, has been to release multiple species of host-specific predators in order to reduce HWA populations below damaging thresholds. Beetles in the genus *Laricobius* prey excusively on adelgids and have life histories matched closely to that of their prey (Lawrence and Hlavac 1979). Thus, this group has high potential for biological control of adelgids.

The genus has 21 recognized species—1 native to Europe, 3 native to North America, and 17 native to Asia (Leschen 2011). The European species, *L. erichsonii* Rosenhauer was released widely in North America for biological control of the balsam woolly adelgid, *A. piceae* (Ratzenburg), and although it initially established, it did not thrive and disappeared (Schooley et al. 1984). The only *Laricobius* species native to eastern United States, the target area for biological control of HWA, is *L. rubidus* Leconte, a predator of pine bark adelgid, *Pineus strobi* (Hartig). A western North American species and known predator of HWA, *L. nigrinus* Fender, has recently been introduced in the eastern United States (Mausel et al. 2010). Because of the presence of two congeners in the eastern United States, it is important that distinctions between the species and how they would interact is known prior to the introduction of another *Laricobius* species.

Recently, *Laricobius osakensis* Shiyake and Montgomery was discovered in Japan (Shiyake et al. 2008, Montgomery et al. 2011). It is very promising for biological control because it comes from the same location as the source of the population of HWA in the eastern United States (Havill et al. 2006). Therefore, this species has evolved with the HWA strain affecting *T. canadensis*. This chapter will review information on its (1) identification, (2) the phenology and distribution of the predator in its native habitat and (3) experiments conducted in a quarantine laboratory to assess its host-specificity and compatibility with a native and introduced congeneric species.

IDENTIFICATION

Laricobius osakensis, like other members of the family Derodontidae, is a small beetle between 2-3 mm in length, elongate, dorsally convex and ventrally flattened, with a vestiture of fine hair, a head with large pores or canals and 11-segmented antennae with a 3 segment club, a narrow prothorax with esplanate sides, elytra seriate or striate, and an abdomen with five visible sternites. Like other members of the genus, it has strongly lobed tarsi, open procoxal cavities, and elyta with a scutellary striole and 10 rows of punctures. Distinguishing *L. osakensis* from the other two

Laricobius species now in the eastern United States can be difficult in the field with live specimens, but fairly easy with dead, mounted specimens in the laboratory. A detailed description of *L. osakensis* can be found in Montgomery et al. (2011).

Live adult *L. osakensis* have two color morphs—one has reddish elytrae with darker maculation on the lateral edge, similar to *L. rubidus* (Fig. 1A), whereas the other color form has nearly black (not piceous) elytrae, similar to *L. nigrinus* (Fig. 1B). When a group of beetles are sorted according to color and then dissected to confirm the sex by examination of their genitalia, about 90% of the reddish specimens were female, while about 80% of the dark-brown specimens were male. Following death, the reddish form becomes various shades of brown. The eggs are light yellow and about 50% larger than an HWA egg (Fig. 1C). The larvae are covered by white wax and usually feed inside the HWA woolly ovisac (Fig.1D).

There are several characteristics, visible under a good stereomicroscope at high power, that can be used to distinguish *L. osakensis* from the other *Laricobius* species already present in the eastern United States. Most Derodontidae have two small ocelli on the head, each mesal of the compound eyes. *Laricobius osakensis*, *L. taiwanensis* Yu and Montgomery, and *L. kangdingensis* (Zilahi-Balogh and Jelenik 2003) are exceptions to this rule and have no ocelli (Montgomery et al. 2011). *Laricobius rubidus* and *L. nigrinus* have small rudimentary ocelli. Careful

Figure 1. *Laricobius osakensis* life stages. (A) Adult female, (B) adult male, (C) two eggs beside dead adult HWA, and (D) fourth instar lava feeding on HWA eggs.

examination of the frons area of the head is the most reliable way to distinguish *L. osakensis* from *L. rubidus* and *L. nigrinus* (Fig. 2). The patterns of the deep pits and punctures on the head are variable and are not a sure way to separate these three species. There are small differences in the dimensions and shape of the pronotum of the three species, but these are difficult to discern (see Montgomery et al. 2011). If the genitalia are dissected and the specimen is a male, *L. osakensis* can readily be distinguished from the North American species by its narrow, acutely pointed parameres and slender median lobe (Fig. 3).

Based on PCR-based molecular diagnostics, *L. nigrinus* and *L. rubidus* are closely related sister species but *L. osakensis* is in a separate clad with other Asian species (Montgomery et al. 2011). Molecular information is not only a way to identify adults, but can be also used to identify larvae.

DISTRIBUTION

Laricobius osakensis has been collected in 14 prefectures on the Japanese islands of Honshu, Kyushu and Shikoku (Fig. 4) at elevations from 80-1850 m on both hemlock species native to Japan. The southern Japanese hemlock, *Tsuga sieboldii* (Carrière), grows naturally at elevations between 200-1500 m on the southern half of Honshu and the more southern islands. The northern Japanese hemlock *T. diversifolia* (Maxim.) Masters grows at elevations from 700-2100 m, mostly in the more northern areas of Honshu Island. Both hemlocks are grown as ornamental or landscape trees, and HWA and the beetle are more common in landscape plantings or semi-natural settings compared to forest sites.

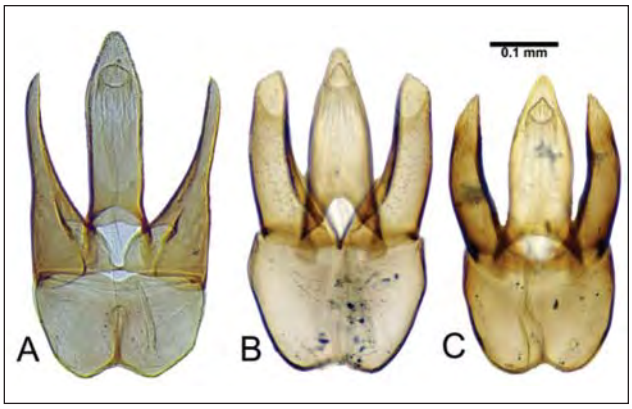

Figure 3. Sexual organs of *Laricobius* males: (A) *Laricobius osakensis*, (B) *L. rubidus*, and (C) *L. nigrinus*.

PHENOLOGY

The seasonal history of *L. osakensis* and HWA was monitored in the Kansai area of Japan to predict its ability to thrive in the eastern United States. HWA-infested *T. sieboldii* trees in 3 prefectures were sampled weekly for 2 years. At each sample period, beat sheet samples were conducted at each

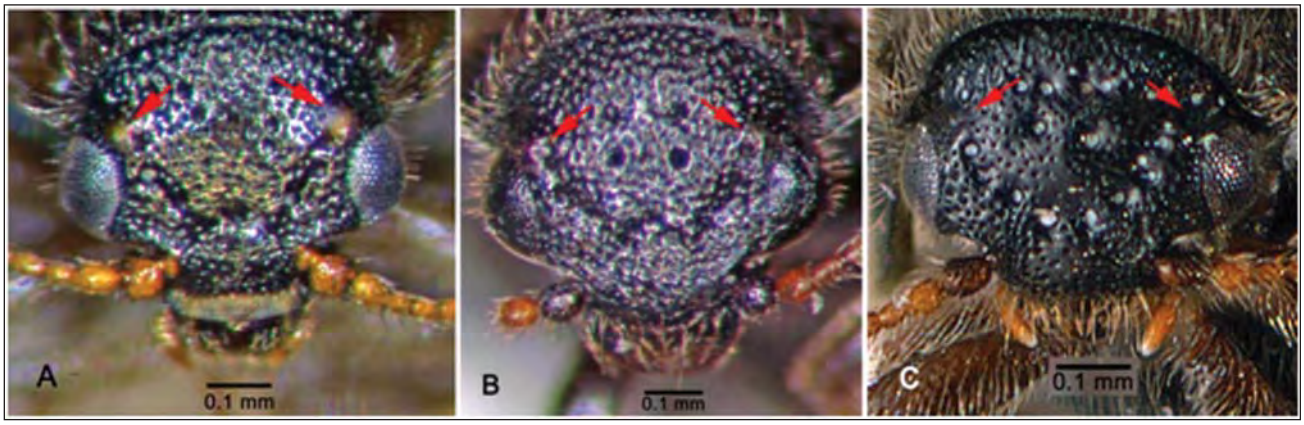

Figure 2. (A) *Laricobius rubidus* and (B) *L. nigrinus* with arrows pointing to ocelli; (C) *L. osakensis* with arrows showing where ocelli are absent.

Figure 4. Map of Japan indicating the prefectures where *Adelges tsugae* has been collected (yellow shading) and locations where *Laricobius osakensis* has been collected (red dots).

tree to determine the presence of adult predators. In addition, branch samples were removed from the trees and examined microscopically for immature stages of the predator and to determine the stages of HWA. Both species were present and active on the hemlock foliage during all months except for June through October (Fig. 5). *L. osakensis* adults first appeared on the trees in the Kansai region in mid-November, approximately one week after HWA had resumed feeding. These adults remained on the trees throughout the winter and early spring. In late December, adult *L. osakensis* began laying eggs in the woolly masses, in synchrony with HWA oviposition. *L. osakensis* continued to lay eggs throughout the winter and early spring. *L. osakensis* eggs hatched from January to April and larvae fed on HWA eggs. After developing through four instars, the larvae dropped from the hemlock branches in the early spring and pupated in the soil. *L. osakensis* aestivate as adults from May through October in the Kansai region. Phenology of *L. osakensis* is synchronized with the winter generation of HWA on *T. sieboldii* in Japan. Both species are present and active from

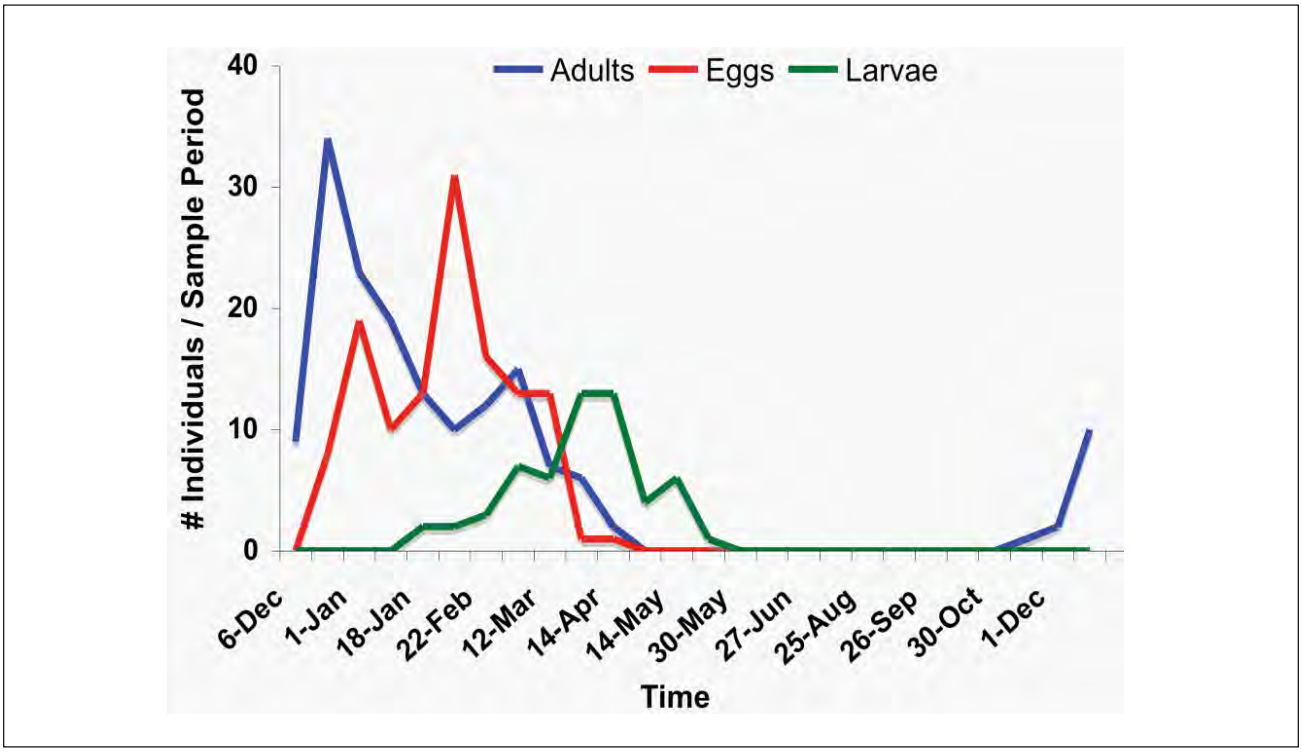

Figure 5. The number of *Laricobius osakensis* adults (blue), eggs (red), and larvae (green) found at each sample period on *Tsuga sieboldii* in the Kansai region of Japan.

November through May, feeding and ovipositing at the same time. In addition, both species undergo a dormant period throughout the summer months.

The genotype of HWA in this area is identical to the genotype of HWA in the eastern United States (Havill et al. 2006). The seasonal temperatures of the study area in Japan are comparable to the temperatures of potential release areas in North America (Fig. 6). In the Kansai region of Japan, *L. osakensis* adults became active in mid-November, a little later than expected based on emergence in British Columbia (Zilahi-Balogh et al. 2003) and emergence of *L. nigrinus* and *L. rubidus* in Virginia (Mausel et al. 2008). The delay in emergence of *L. osakensis* in Japan is likely because HWA on *T. sieboldii* in Japan breaks dormancy slightly later, probably due to the extended summer at low latitudes in Japan.

Phenology of *L. osakensis* on *T. diversifolia* at higher altitudes has not been studied in detail. Adults have been found on *T. diversifolia* in late October and early May. Trees were extensively sampled in early April, but adults were not collected. Since temperatures are very low and a large amount of snow falls in these high mountain areas, *L. osakensis* may not be active throughout the winter. Although more sampling is needed to determine the phenology of *L. osakensis* in colder regions, it's clear that it can survive in areas with average winter minimum temperatures below -20° C.

IMPACT ON HWA IN JAPAN

Since *Tsuga* species in Japan do not appear to be injured by HWA, the impact of predatory species on HWA populations were of interest. A predator exclusion study was conducted at 2 sites in the Kansai area of Japan. At one site, 48 HWA-infested branches were chosen and adult predators were removed by beat-sheeting. Half of the branches were caged from January to April. In April, 2 branch samples (10 cm in length) were removed from each branch and the density of

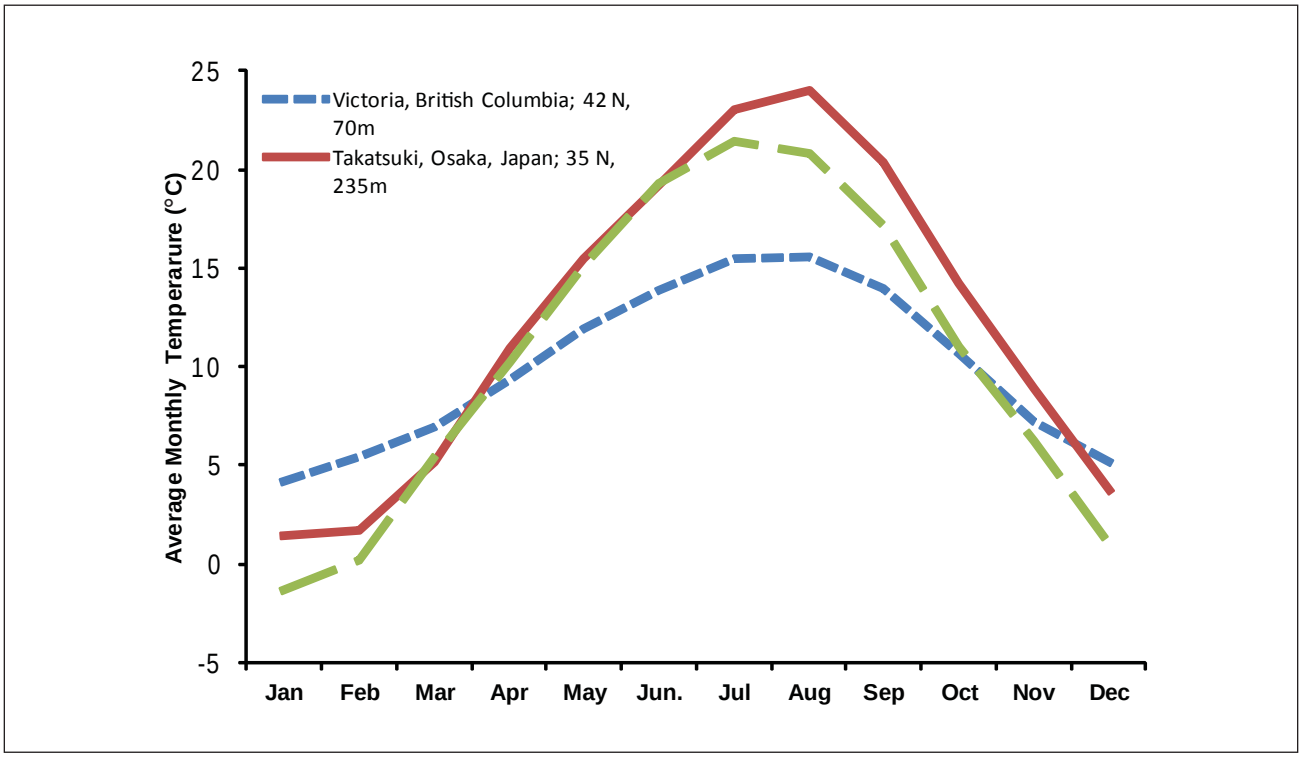

Figure 6. Average monthly temperatures (°C) of seasonal phenology study areas of *Laricobius nigrinus* in British Columbia and *L. osakensis* in Japan compared to a representative, prospective release area for both species in the eastern United States.

HWA was determined. On the caged branches (not exposed to predators), approximately half the HWA were alive and produced ovisacs, whereas only 5% of the adelgids were alive on the open branches. Thirty branches were selected at the second site. Half of these branches were caged after predators were removed by beat-sheeting. The cages were left on the branches from May until the following June. The branches that were caged for the 14-month period were heavily infested with adelgids (>3/cm), whereas branches left exposed had almost no adelgids (<0.1/cm).

Additionally, uncaged branches were sampled for predators throughout the year. *Laricobius osakensis* was the primary predator during the winter months at both sites. During the spring and summer, many generalist predators were present on the trees (Coccinellidae, Cantharidae, Elateridae, Syrphidae, Reduviidae, and others) (Shiyake et al. 2008). One Coccinellid species of particular interest found in these samples was *Sasajiscymnus tsugae* (Sasaji and McClure), a predator that has been released extensively in eastern North America since 1997 (Cheah et al. 2004). *S. tsugae* was collected fairly consistently on several, but not all, of the study trees during May and June. This beetle was not consistently present throughout the other months of the year.

The exclosure experiment suggests that the predators in Japan contribute to population control of HWA in the Kansai region. The sampling for predators indicates that *L. osakensis* is the primary predator during the winter and early spring. In late spring and early summer, generalist predators can be abundant, especially on hemlock with dense HWA infestations.

EVALUATION OF HOST RANGE AND DEVELOPMENT IN THE LABORATORY

Development tests were conducted by placing *L. osakensis* eggs on hemlock with HWA or on an alternate host. Larvae were observed for survival and maximum developmental stage reached. Choice and no-choice tests were set up in petri dishes and the amount of host consumed and number of eggs laid on each host were determined. The alternate hosts used were: balsam woolly adelid (BWA), pine bark adelgid (PBA), eastern spruce gall adelgid (ESGA), *Adelges abietis* (Linneaus), woolly alder aphid (WAA), *Paraprociphilus tessellates* (Fitch), elongate hemlock scale (EHS), *Fiorina externa* Farris, and pine needle scale (PNS), *Chionaspis pinifoliae* (Fitch). In choice tests, single adults were put in petri dishes containing a known number of HWA and an alternate host. The amount of prey consumed and number of eggs laid on each were counted after 7 days. In the no-choice tests, single adults were put in petri dishes with a known number of HWA or alternate host and the number of host consumed and eggs laid were determined after 5 days.

Laricobius osakensis consumed more HWA and laid more eggs on HWA than on any other host in both the choice and no-choice tests. In addition, these predators were only able to complete development to adults on HWA. They were only able develop up to 4th instar larvae on the adelgid alternate hosts and no development occurred on the non-adelgid alternate hosts. The host range tests indicate that this predator is host-specific on HWA and does not develop on other species tested (Vieira et al. 2011).

CURRENT STATUS IN UNITED STATES

These data suggest this predator poses no risk to native fauna within the eastern United States. This information was provided in the petition to release *L. osakensis* from quarantine. In May 2010, this predator was issued a FONSI (Finding Of No Significant Impact) and is no longer required to be in quarantine. In October 2010, a large field collection of *L. osakensis* was brought to Virginia Tech for mass rearing. The University of Tennessee is also rearing a small colony. Further research will be conducted at Virginia Tech and based on the results, *L. osakensis* will be tested in the field. If field tests are favorable and large numbers can be reared, field releases of this beetle will begin. Given what we know about its life history, *L. osakensis* has the potential to contribute to biological control of HWA in the future.

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LITERATURE CITED

Cheah, C.A.S.-J.; Montgomery, M.E.; Salom, S.M.; Parker, B.L.; Costa, S.; Skinner, M. 2004. *Biological Control of Hemlock Woolly Adelgid*. Reardon, R.; Onken, B., tech. coords. FHTET-2004-04. Morgantown, WV: U.S. Department of Agriculture, Forest Service: 22 p.

Havill, N.P.; Montgomery, M.E.; Yu, G.Y.; Shiyake, S.; Caccone, A. 2006. Mitochondrial DNA from hemlock woolly adelgid (Hemiptera: Adelgidae) suggests cryptic speciation and pinpoints the source of the introduction to eastern North America. *Annals of the Entomological Society of America* 99(2): 195-203.

Lawrence, J.F.; Hlavac, T.F. 1979. Review of the Derodontidae (Coleoptera: Polyphaga) with new species from North America and Chile. *The Coleopterists Bulletin* 33: 369-413.

Leschen, R.A.B. 2011. World review of *Laricobius* (Coleoptera: Derodontidae). *Zootaxa* 2908: 1-44.

Mausel, D.L.; Salom, S.M.; Kok, LT.; Davis, G. 2010. Establishment of the hemlock woolly adelgid predator, *Laricobius nigrinus* (Coleoptera: Derodontidae), in the eastern United States. *Environmental Entomology* 39(2): 440-448.

Mausel, D.L.; Salom, S.M.; Kok, L.T.; Fidgen, J.G. 2008. Propagation, synchrony, and impact of introduced and native *Laricobius* spp. (Coleoptera: Derodontidae) on hemlock woolly adelgid in Virginia. *Environmental Entomology* 37: 1498-1507.

Montgomery, M.E.; Shiyake, S.; Havill, N.P.; Leschen, R.A.B. 2011. A new species of *Laricobius* (Coleoptera: Derodontidae) from Japan with phylogeny and a key for native and introduced congeners in North America. *Annuals of the Entomological Society of America* 104: 389-401.

Schooley, H.O.; Harris, J.W.E.; Pendrel, B. 1984. *Adelges piceae* (Ratz.), balsam woolly adelgid (Homoptera: Adelgidae). In: Kelleher, J.S.; Hulme, M.A., eds. *Biological Control Programmes against Insects and Weeds in Canada 1969-1980*. Farnham Royal, Slough, England: Commonwealth Agricultural Bureax: 229-234.

Shiyake, S.; Miyatake, Y.; Montgomery, M.E.; Lamb, A.B. 2008. Hemlock woolly adelgid phenology and predacious beetle community on Japanese hemlocks. In: Onken, B.; Reardon, R., comps. *Fourth Symposium on Hemlock Woolly Adelgid in the Eastern United States*; 2008 February 12-14; Hartford, CT. FHTET-2008-01. U.S. Department of Agriculture, Forest Service, Forest Health Enterprise Team: 261-266.

Vieira, L.C.; McAvoy, T.; Chantos, J.; Lamb, A.B.; Salom, S.M.; Kok, L.T. 2011. Host Range of *Laricobius osakensis* (Coleoptera: Derodontidae), a new biological control agent of hemlock woolly adelgid (Hemiptera: Adelgidae). *Environmental Entomology* 40: 324-332.

Zilahi-Balogh, G.M.G.; Humble, L.M.; Lamb, A.B.; Salom, S.M.; Kok, L.T. 2003. Seasonal abundance and synchrony between *Laricobius nigrinus* (Coleoptera: Derodontidae) and its prey, the hemlock woolly adelgid (Hemiptera: Adelgidae). *The Canadian Entomologist* 135: 103-115.

Implementation and Status of Biological Control of the Hemlock Woolly Adelgid

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