Approval of *Laricobius naganoensis* (Coleoptera: Derodontidae), a Predatory Beetle for Biological Control of Hemlock Woolly Adelgid (*Adelges tsugae*), in the Continental United States

Environmental Assessment 2017

Agency Contact:

Robert Tichenor, Jr.
Sr. Entomologist, Biological Control
Pests, Pathogens and Biocontrol Permitting
Plant Health Programs, USDA APHIS PPQ
4700 River Road, Unit 133, Riverdale, MD 20737

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I. Background and Introduction

The hemlock woolly adelgid (HWA) (*Adelges tsugae* Annand) is an introduced pest destructive to forest and ornamental hemlock trees (*Tsuga* spp.) in the eastern United States. The adelgid feeds at the bases of needles, causing them to desiccate and resulting is needle loss. This prevents trees from producing new apical buds, and leads to branch dieback and often eventual death of the tree. Heavy infestations have killed trees in as little as 4 years, but some survive longer with only a sparse amount of foliage at the very top of the crown (McClure et al. 2001). The hemlock woolly adelgid (HWA) has become a very important pest of eastern hemlock, *Tsuga canadensis,* and Carolina hemlock, *T. caroliniana* in the eastern United States because of the damage and mortality to trees ranging from landscape shrubs to old large forest trees. This pest has impacted National Parks, Recreation Areas, and Forests; State Forests; commercial and private landowners; and urban and suburban communities.

The first report of HWA in eastern US came from the Virginia Department of Agriculture and Consumer Services (VDACS) in 1951 on planted eastern hemlock in Richmond, VA (Stoetzel 2002). HWA infestation is fatal to eastern hemlocks of all ages, regardless of health prior to infestation (McClure 1990). Depending on environmental conditions, death may occur within four years or prolonged over ten or more years (Orwig and Foster 1998, McClure et al. 2001). Mode of dispersal includes any combination of birds, deer, humans (via movement of nursery stock), or wind (McClure 1990). HWA now infests 20 states and it is estimated that approximately 60% of the *T. canadensis* range within the U.S. and 100% of the *T. caroliniana* range is infested (USDA Forest Service 2015). Because HWA is adapted to high elevations in Japan where winter temperatures commonly drop below -35°C (-63°F), it should continue to spread in eastern North America until it occupies the entire range of eastern hemlock (see Appendix 1).

Existing management options for HWA are ineffective, expensive, temporary, and have non-target impacts. For these reasons, there is a need to identify an effective, host specific biological control organism and release it into the environment for the control of HWA.

II. Purpose and Need for the Proposed Action

The United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS) is proposing to issue permits for the release of a predatory beetle, *Laricobius naganoensis* (Coleoptera: Derodontidae). Bi-annual field collections of the already approved *L. osakensis* are necessary to maintain the vigor and genetic diversity in rearing colonies that produce beetles for release as a biological control agent for HWA. *L. naganoensis* is sympatric with *L. osakensis,* where both species co-occur in high altitude areas in the Nagano Prefecture on *Tsuga diversifolia*, at an approximate ratio of < 1:5 (Fischer et al. 2014).

The morphological similarity between the two species and variation within *L. osakensis* make it difficult to differentiate these species. *Laricobius naganoensis* is distinguished from *L. osakensis* by having pale tibiae, more vivid orange-red coloration of the elytra, and an acute median lobe of the male genitalia (Leschen 2011). Females cannot be reliably differentiated using morphology. Males can be differentiated by their genitalia, but this identification requires dissection of dead specimens. Accurate species separation requires molecular testing involving mitochondrial DNA (Fischer et al. 2014).

L. osakensis has become a principal biological control agent for operational releases in the eastern U.S. Since *L. osakensis* is currently approved for release and *L. naganoensis* is not, the quarantine lab they are shipped to are required to conduct a 2-year or longer purification process to ensure that no *L. naganoensis* are released. This significantly constrains the rearing effort for *L. osakensis.* The arduous rearing technique described by Lamb et al (2005) puts a severe bottleneck on the operation when you add the purification process.

To date, 21 species of *Laricobius* have been described (Leschen 2011). Previous records describing species of this genus (Clark and Brown 1958, Lawrence 1989, Lawrence and Hlavac 1979) support more recent experimental studies demonstrating that *L. nigrinus* and *L. osakensis* are adelgid-specific predators with greatest preference to HWA, which they have co-evolved with. *Laricobius erichsonii* was introduced from Europe into North America during the mid-20th century as a biological control agent for the non-native balsam woolly adelgid, *Adelges balsamea* ((Clark and Brown 1958). There is no evidence in the literature that beetles for any *Laricobius* species feed on any prey other than insects in the family Adelgidae). Host-range testing of live *L. naganoensis* is not possible since we do not know what species each beetle is until it dies. And since the ratio of *L. naganoensis* to *L. osakensis* is so low, it would require following hundreds of beetles through experiments that typically require 30. Two factors make experimental assessment of *L. naganoensis* impossible: 1. Not enough beetles are collected in the wild to have a sufficient sample size for host feeding tests; and 2. If there were enough beetles, the logistical aspects of conducting the trials would be beyond most lab's abilities.

This environmental assessment[1] (EA) has been prepared, consistent with USDA, APHIS' National Environmental Policy Act (NEPA) implementing procedures (Title 7 of the Code of Federal Regulations (CFR), part 372). It examines the potential effects on the quality of the human environment that may be associated with the release of *Laricobius naganoensis* included in *L. osakensis* releases used to control infestations of hemlock woolly adelgid within the continental United States. This EA considers the potential effects of the proposed action and its alternatives, including no action.

III. Alternatives

[1] Regulations implementing the National Environmental Policy Act of 1969 (42 United States Code 4321 *et seq.*) provide that an environmental assessment "[shall include brief discussions of the need for the proposal, of alternatives as required by section 102(2)(E), of the environmental impacts of the proposed action and alternatives, and a listing of agencies and persons consulted." 40 CFR § 1508.9.

This section will explain the two alternatives available to APHIS; no action and to issue permits for environmental release of *Laricobius naganoensis*. Although APHIS' alternatives are limited to a decision on whether to issue permits for release of *L. noganoensis*, release of other predators for use in biological control are described and are not under APHIS jurisdiction. The current biological control program will continue regardless of APHIS decision here.

Releases of *L. osakensis* began in 2012 (Mooneyham et al. 2016), and through 2015, 16,195 beetles were released at a total of 23 locations in VA, MD, PA, WV, and OH. A colony of the southern strain of *L. osakensis* was started at the University of Tennessee in 2012, and since 2013, over 2,000 beetles have been TN and NC. Recoveries of the progeny of *L. osakensis* released has so far been documented at three sites in VA and two sites in TN. Because *L. osakensis* comes from the same location as the *A. tsugae* in eastern North America comes from (Havill et al. 2006, Montgomery et al. 2011), and because there is evidence that this predator has the potential to be more effective than *L. nigrinus* (Vieira et al. 2012), there is tremendous motivation in continuing to release, monitor, and evaluate its effectiveness.

A. No action

Under the no action alternative, APHIS would not issue permits for the field release of *L. naganoensis*. The means that the following options are in play: 1. Continue purifying wild collected colonies and 2. Since the purification effort is not sustainable, stop rearing and releasing *L. osakensis*.

<u>The Purification Process:</u> In September of 2011, it was discovered through genetic analysis, that a previously undescribed *L. naganoensis,* was inadvertently brought back to Virginia Tech in the fall of 2010 while collecting parent *L. osakensis* beetles for the 2011 rearing season. From the initial 86 beetles sent to Dr. Nathan Havill (USDA Forest Service, Northern Research Station), genetic testing found that five of the beetles were *L. naganoensis*. This meant we could not carry out the first planned release of *L. osakensis*. A purification procedure was developed and approved by APHIS (Permit # P526P-02688 - Appendix), where all *L. osakensis* beetles were moved from the insectary to the quarantine facility. Since the quarantine facility is not set up for large-scale rearing, many beetles were killed to accommodate for the reduced space. The plan proposed and carried through has beetles being held in separate groups of 20. Offspring from each of these groups were tracked and held together, separate from other groups. At the end of the rearing season, founding adults are tested for the presence of *L. naganoensis.* If any were discovered, all offspring from that group were removed from the colony. The colony is not considered clean until the group is free of any *L. naganoensis* offspring. For some groups, this requires 1 year for a few groups but at least 2 years for most groups. This adds another constraint to the rearing effort. Historically, the predator rearing labs have shown decline in productivity in just a few years when wild beetles are not added to the founding colonies. To maintain colony vigor, collections from Japan are required every 2-3 years. Under this scenario, we

would have to purify colonies in quarantine continuously for as long as we rear *L. osakensis*. Such an effort would not be sustainable.

Since the purification is unsustainable, shipments of *L. osakensis* will no longer take place and rearing colonies will wither away. The predator rearing and release program for *L. osakensis* will end and the release and establishment of an effective biological control agent to control *A. tsugae* will not occur.

B. Issue permits for environmental release of *Laricobius naganoensis*

Under this alternative, APHIS would issue permits for the field release of *L. naganoensis* for the control of HWA. These permits would contain no special provisions or requirements concerning release procedures or mitigating measures.

1. Description and Taxonomy of *Laricobius naganoensis*

Laricobius naganoensis (new species) was first collected in 2008 in Nagano Prefecture, Japan on *Tsuga diversifolia* hosting *A. tsugae*, and both identified and described by Leschen (2011). *L. naganoensis* and *L. osakensi* occur sympatrically and are more closely related to each other than to any other species in the genus. Due to the variability in coloration among populations of *L. osakensis* and *L. naganoensis*, the described morphological characteristics cannot be used to accurately separate live specimens. Leschen (2011) recommended dissection of male specimens. There is no way to separate females other than through mitochondrial DNA testing. Voucher specimens of the holotype are currently located at the Osaka Museum of Natural History. Paratypes are located at this same museum as well as the Yale Peabody Museum, the British Museum of Natural History, and the U.S. Museum of Natural History.

2. Life history

There are no biological studies on *L. naganoensis*, although based on the purification efforts, they can survive and develop in the laboratory on HWA. There is no evidence of hybridization between *L. osakensis* and *L. naganoensis* (Fischer et al. 2014). As a relevant point of reference, the life cycles of *Laricobius nigrinus* and *L. osakensis* are presented. Both species have life cycles that are highly synchronous with HWA (Zilahi-Balogh et al. 2003, Vieira et al. 2013). There is one generation per year. Adults emerge in the fall from the soil, and are active during the winter feeding on developing HWA sistens. They will oviposit in sistens ovisacs from February to March, as sistens adults oviposit progrediens eggs. Predator eggs hatch and larvae feed on adelgid eggs, and as later instars, feed on developing progrediens. From April to May, the larvae drop to the soil and pupate, eventually eclosing to adult. They then go into aestival diapause until the onset of cooler fall temperatures, where upon the adults emerge. The native *L. rubidus* has a similar life cycle even though its host, the pine bark adelgid is not active in the winter (Wantuch, Ph.D. dissertation in progress)

3. Native geographical range

It has only been collected in Nagano, Tochigi and Gunma Prefectures in Japan.

4. Impact on Hemlock Woolly Adelgid

Laricobius naganoensis has been collected from HWA infested hemlock trees in Japan and has been reared at Virginia Tech for several years and generations exclusively on *A. tsugae*. Indicating that *L. naganoensis* is a primary host of HWA. While the feeding rate of adult and larvae of *L. naganoensis* is unknown, it is very similar in size to *L. osakensis* and *L. nigrinus*, suggesting its feeding rate and impact on the HWA would likely be very similar to that of *L. osakensis* and *L. nigrinus*.

IV. Affected Environment

A. Native North American Species

Species related to the Hemlock woolly adelgid

There are 13 described species of Adelgidae known to be native to North America (Havill and Foottit, 2007), and at least three introduced species, including the balsam woolly adelgid and the eastern spruce gall adelgid, known pests (Johnson and Lyon, 1991). Known hosts of these 16 species are spruce, pine, fir, Douglas-fir, larch, and hemlock. The HWA is the only adelgid species in North America, and possibly the world, which utilizes hemlock.

Adelgidae have complex life cycles involving either a parthenogenetic (no sexual reproduction) cycle or both parthenogenetic and sexual cycles, these are termed holocyclic species. Havill and Foottit (2007) provide the following description of adelgid life cycles and habits: Five generations make up the typical adelgid holocycle. Three are produced on the primary host, where sexual reproduction and gall formation occur; two are produced on the secondary host that supports a series of asexual generations. The entire cycle takes two years to complete. Spruce (*Picea* spp.) is always the primary host and another conifer genus (*Abies*, *Larix*, *Pseudotsuga*, *Tsuga*, or *Pinus*) is always the secondary host. Adelgids are highly host specific: A given species can survive and reproduce only on certain tree species within a single primary and secondary host genus. For example, *Pineus orientalis* can alternate between *Picea orientalis* and *Pinus silvestris*, but it cannot survive on *Picea abies, Pinus strobus*, or *Pinus cembra.*

Hemlock woolly adelgid lacks its needed species of spruce in eastern North America, so HWA cannot complete the sexual cycles in the eastern US. In Connecticut, *A. tsugae* completes two wingless parthenogenetic generations on *T. canadensis.* One, termed the sistens, is present from July through April, and the second, termed the progrediens, is present from April through June. The sistens mature in February, then from March through May each female from the sistens generation produces a single white cottony ovisac containing up to 300 eggs (McClure 1987, 1989). Egg production is inversely density-dependent (McClure 1991). Some of these eggs hatch to become progrediens and the remainder become a migratory parthenogenetic generation, the sexuparae. Nymphs of both types begin hatching in April, quickly develop through four instars, and mature in June. The winged sexuparae adults that mature on hemlock in June migrate to spruce, and in July each lays up to 15 eggs beneath its folded wings. Adult sexuparae produce eggs only on spruce (*Picea* spp.); those which do not migrate to spruce, die on hemlock without producing eggs. (McClure, 1996). The progeny of sexuparae produced on spruce represent the sexual generation of A. *tsugae* called the sexuales. The sexuales nymphs that hatch and begin to feed on spruce in July all die within a few days; none develop beyond the first instar on any of 15 different North American spruce species (McClure 1987, 1991).

Native range of hemlock woolly adelgid

HWA is native to Asia and western North America (Havill et al. 2006). Annand (1924) first described HWA from the Pacific Northwest of North America (PNW) on western hemlock. It is present throughout this region of western North America, but at typically harmless densities (Furniss and Carolin 1977, Tait et al. 1985). Havill et al. (2006) determined that this population is molecularly distinct from populations in Asia and the eastern U.S., and thus likely native to the region.

Present distribution in North America

HWA presently is known to occur in 21 states from Maine to Georgia (see Appendix 1). Approximately 60% of the *T. canadensis* range within the U.S. and 100% of the *T. caroliniana* range is infested (USDA Forest Service 2015)

Species related to *Laricobius naganoensis*

The genus *Laricobius* (Coleoptera: Derodontidae), is represented in North America by four species, of which two *L. rubidus* (eastern NA) and *L. nigrinus* (western NA) have been noted feeding on HWA. *Laricobius* species only feed on the Adelgidae. The remaining species of Derodontidae in North America feed on various species of fungus during their entire life cycle. *Laricobius rubidus* completes development, and survives well on a HWA (Zilahi-Balogh et al. 2005). It is present in Connecticut (Montgomery and Lyon 1996), North Carolina, Virginia (Wallace and Hain 2000), Maine, New Hampshire, Massachusetts, District of Columbia, Pennsylvania, New York, Michigan, Quebec, Ontario, and New Brunswick (Lawrence 1989, Downie and Arnett 1996). The primary host of *L. rubidus* is the pine bark adelgid, *Pineus strobi* Hartig (Hemiptera: Adelgidae) on white pine, *Pinus strobus* L. (Clark and Brown 1960).

Of the 55 species of predators collected from HWA infested western hemlock in the Pacific Northwest, *Laricobius nigrinus*, *Leucopis argenticollis* Zetterstedt, and *Leucopis atrifacies* (Aldrich) (Diptera: Chamaemyiidae) comprise 59% of all the predators collected, with *L.*

nigrinus being the most abundant (Kohler et al. 2008). There are 21 known *Laricobius* spp. worldwide and three are native to North America (Leschen 2011), the third species, *L. laticollis* is much less common and was only one of 756 *Laricobius* specimens collected in the survey.

The known distribution of *L. nigrinus* ranges from northern California to British Columbia and in interior British Columbia, Alberta, and northern Idaho (Furniss and Carolin, 1977; Lawrence, 1989). *Laricobius nigrinus* has one generation per year with good synchronization with HWA throughout the year (Zilahi-Balogh et al. 2003). Both the predator and prey are active in the fall, winter, spring, and diapause in the summer. Adults emerge from the soil in the fall, disperse to hemlock branches, and feed on HWA sistens nymphs, adults, and progrediens eggs (available in early-mid spring) then die. In the laboratory tests conducted at Virginia Tech, *L. nigrinus* completed development on HWA but not on several other eastern adelgids and aphids tested, therefore, gaining release from quarantine by USDA-APHIS in 2000 (Zilahi- Balogh et al. 2002). *L. nigrinus* has subsequently been released in all eastern states where HWA is present.

B. Hemlock Resources in North America

Eastern hemlocks are long-lived, late successional climax trees that, if left undisturbed, eventually dominate stands. Canadian hemlock, *Tsuga canadensis* may take 250-300 years to reach maturity and live for 800 years, and attain heights of 150-175 feet. Eastern hemlock is the most shade tolerant tree species in North America, capable of surviving underneath a shaded forest canopy for as long as 350 years (Quimby, 1996). Because it is so shade tolerant and long-lived, hemlock is a late successional (or "climax") tree that can dominate a forest for centuries.

Eastern hemlock forests create distinctive microclimates and provide important habitat for a wide variety of wildlife. In the Northeast, 96 bird and 47 mammal species are associated with hemlock forests (Yamasaki et al. 2000). Of these, at least eight species of birds and ten species of mammal have strong ecological linkages with hemlock forest habitat. In Connecticut alone, almost 90 species of birds use hemlock as a food source, nesting site, roost site, or winter shelter (Havill et al. 2014). Examples of birds include black-throated green warbler, blackburnian warbler, blue-headed vireo, winter wren, red-breasted nuthatch, ruffed grouse, and the northern goshawk. Examples of mammals include black bear, bobcat, snowshoe hare, red squirrel, and southern red-backed vole. Hemlock is well known as important winter habitat for white-tailed deer. Many native plant species thrive in hemlock stands, including leatherwood, rattlesnake plantains, bunchberry, goldthread, bluebeard, Canada mayflower, wood sorrels (Evans et al. 1996, Havill et al. 2014).

Hemlock forests can also be a critical factor in supporting native brook trout populations, by maintaining cool stream temperatures and stable flows. Small streams in hemlock forests are three times more likely to support native brook trout populations than similar streams in hardwood forests in the park (Snyder et al. 2001). Snyder et al. (2001) showed that small streams in hemlock forests are typically 1 - 2^0C (2 - 3.5^0F) cooler in the summer than similar streams in hardwood forests in the study area (Delaware Water Gap NRA). Furthermore, hemlock forest streams typically support about 65 species of aquatic insects, compared to only about 35 species in hardwood forest streams. About 15 species of aquatic insects seem to occur almost exclusively in hemlock forest streams in the park (Snyder et al. 2001).

For these and other reasons hemlock stands are very popular recreational sites for fishing, hiking, hunting and bird watching. Although hemlock is not a valuable timber species, it is used widely for pulpwood and utilitarian uses. Hemlocks are valued landscape plants: with 274 cultivars of eastern hemlock, it is one of the most cultured and cultivated landscape trees in the United States (Havill et al. 2014).

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