



Biological control of hemlock woolly adelgid in North America: History, status, and outlook

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HIGHLIGHTS

- The invasive insect *Adelges tsugae* threatens ecologically important conifer species.
- Classical biological control in North America has been pursued for three decades.
- Efforts with coccinellid predators have not yielded a successful biocontrol agent.
- Two Derodontid predators are established and spreading but do not prevent host decline.
- Two Chamaemyiid predators show promise for complementing existing agents.

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ABSTRACT

The hemlock woolly adelgid (HWA, *Adelges tsugae*, Hemiptera: Adelgidae) is an invasive insect that threatens the ability to maintain eastern hemlock (*Tsuga canadensis*) and Carolina hemlock (*Tsuga caroliniana*) as ecologically functional components of eastern North American forests. Since the early 1990 s, a classical biological control program for HWA has been pursued using insect predators from regions of Asia and western North America where the adelgid is native. Early efforts to establish ladybird beetles (Coleoptera: Coccinellidae) resulted in the establishment of *Sasajiscymnus tsugae* from Japan, but consistently poor field recovery of this species suggests it has not been an effective biocontrol agent in the introduced range. Two additional ladybird beetles, *Scymnus ningshanensis* Yu & Yao, and *S. camptodromus* Yu & Liu, were released but did not establish. Subsequent introduction, widespread establishment and spread of *Laricobius nigrinus* and *La. osakensis* (Coleoptera: Derodontidae) has provided substantial predation on winter stages of HWA, but has not provided overall regulation of adelgid populations below levels that cause hemlock shoot growth loss and mortality. Recently, efforts have focused on prey-specific genetic lineages of *Leucotaraxis argenticollis* and *Le. piniperda* (Diptera: Chamaemyiidae), which co-occur with *La. nigrinus* in their native range of the Pacific Northwest and show promise for eventual establishment in eastern North America. This review provides a brief introduction on the ecology, evolution, and population dynamics of HWA and its hosts, detailed coverage of the predators that have been (and are being) evaluated as biological control agents, and a discussion of the overall biological program with considerations for its future implementation.

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1. Introduction

For at least 70 years, the hemlock woolly adelgid (HWA, *Adelges tsugae* Annand) has been present as a non-native invasive insect pest in eastern North America. Historically, it ranks among the 10 most destructive forest insects or pathogens in the coterminous United States (US) in terms of increasing the baseline mortality rate of its host trees (Fei et al. 2019). A variety of negative economic, aesthetic, and ecological impacts (Aukema et al. 2011, Vose et al. 2013, Abella et al. 2014) are caused by HWA, especially in natural forests where eastern hemlock (*Tsuga canadensis* (L.) Carrière) is considered a foundation species that drives ecosystem structure, composition, and processes (Ellison et al. 2018). To date, HWA has invaded the entire range of Carolina hemlock (*T. caroliniana* Engelm.) and more than half the range of eastern hemlock, and is poised to continue spreading through the Great Lakes region, northern New England, and southeastern Canada (Ellison et al. 2018, USDA Forest Service 2022).

In 2001, the Hemlock Woolly Adelgid Initiative was formalized by the US Department of Agriculture (USDA) Forest Service, the USDA Animal and Plant Health Inspection Service (APHIS), the National Association of State Foresters, and the National Plant Board. This represented a coordinated commitment by these agencies to accelerate the development of research, management tactics and technology transfer to help reduce the spread and impact of HWA (Onken and Reardon 2011, HWA Initiative 2021). A central component of the HWA Initiative has been the development of a robust biological control program, the seeds of which were planted by work beginning in the early 1990s. Since then, the strategy to develop biological control options for HWA in eastern North America has grown and evolved as new information about the system has been gained. Although biological control efforts to date have not resulted in population regulation of HWA sufficient to alleviate its negative impacts, much has been learned about the ecology and evolution of HWA, its hosts, and its natural enemies, and substantial progress has been made toward the ultimate goal of regulating HWA populations via a community of introduced predatory agents.

The purpose of this review is to convey the current scientific knowledge about HWA biological control, with emphasis on progress that has been made since the last comprehensive review of the program 12 years ago (Onken and Reardon 2011). Other review papers have focused on the biology and impacts of HWA since it emerged as a pest in eastern North America (e.g., McClure 1987, Havill et al. 2011, Havill et al. 2016a, Limbu et al. 2018, Ellison et al. 2018). These papers provide many of the details about HWA biology, host relations, and impact, so our review will only highlight the aspects that are most relevant to biological control. The review is timely given the recent spread of HWA into new geographic regions of the US and Canada, and a current summary of program history and status is of benefit to hemlock resource managers and scientists who may lack extensive experience with HWA and its biological control. We first provide a brief introduction to the global ecology, evolution, and population dynamics of HWA and its hosts, followed by detailed coverage by family (Coleoptera: Coccinellidae, Coleoptera: Derodontidae, and Diptera: Chamaemyiidae) of insects that have been, or are currently being evaluated, as biological control agents. Finally, we provide a discussion of the overall HWA biological control program and considerations for its future implementation.

1.1. HWA diversity and invasion history

The approach that has dominated HWA biological control research has been importation biological control, also called classical biological control, which involves the release of exotic natural enemies to suppress the abundance and negative impact of an exotic pest (Heimpel and Mills 2017). For this approach, natural enemies are most often sourced from the native geographic range of the pest, so a thorough understanding of the origin of the pest is needed. Shortly after HWA was found to be impacting trees in the mid-Atlantic states and Connecticut in the 1970s

and 1980s, it was postulated that it invaded from Asia (McClure 1987). At the time, HWA was known to be present in Japan (Inouye 1953) and Taiwan (Takahashi 1937), as well as in western North America, where it was first described (Annand 1924). Many of the early publications after the discovery of HWA in the eastern United States reasonably assumed that it was also exotic in western North America (e.g., McClure 1992, McClure 2001). At the time, HWA was not reported to be in mainland China, but Dr. Michael E. Montgomery from the USDA Forest Service surmised that it was likely to be there because of the presence of three hemlock species and its proximity to Japan and Taiwan (M.E. Montgomery, personal communication). In 1994, Dr. Sean T. Murphy, from the International Institute of Biological Control, made a scouting trip to Taiwan and mainland China, where he confirmed the presence of HWA in Sichuan Province. Within this timeline, the earliest explorations for potential biological control agents focused on Japan (McClure 1995), followed by southwestern China (Yu et al. 2000), the Pacific Northwest of North America (Zilahi-Balogh 2001), and Taiwan (Yu and Montgomery 2007).

It eventually became clear that populations of HWA endemic to different regions or on different host hemlock species have genetic and biological differences, and these differences could inform HWA biological control. Worldwide, there are ten species of hemlock: two in Japan [*T. sieboldii* Carrière and *T. diversifolia* (Maxim.) Mast.], three in mainland China [*T. chinensis* (Franch.) Pritzl ex Diels, *T. forrestii* Downie, and *T. dumosa* (D. Don) Eichler], with *T. dumosa* extending through the Himalayas to northeastern India, and *T. chinensis* also present on Taiwan, one on Ulleung Island, South Korea (*T. ulleungensis* G.P. Holman, Del Tredici, Havill, N.S. Lee & C.S. Campb.), two in western North America [*T. heterophylla* (Raf.) Sarg. and *T. mertensiana* (Bong.) Carr.] and two in eastern North America (*T. canadensis* and *T. caroliniana*) (Farjon 2010, Holman et al. 2017). In addition to these accepted species, phylogenetic analyses suggest that *T. chinensis* could be split into three species representing populations in eastern China, western China, and Taiwan (Havill et al. 2008, Feng et al. 2021). Havill et al. (2006) published the first evidence that HWA that feed on these different hemlock species in different regions were genetically distinct. Mitochondrial DNA sequences showed deep evolutionary divergences among adelgids collected from Japan, Taiwan, western China, and western North America. Havill et al. (2006) found evidence that adelgids feeding on *T. sieboldii* in southern Honshu, Japan were the source of the exotic population in the eastern United States, and that there was a separate mitochondrial lineage of adelgids in Japan at higher elevations feeding on *T. diversifolia*. Populations of HWA from western China and Taiwan were deeply diverged from those in North America and Japan. Surprisingly, HWA from western North America did not genetically match the other lineages and had higher diversity than in eastern North America, suggesting that it might actually be indigenous to the region.

A subsequent phylogeny of Adelgidae that used mitochondrial and nuclear DNA sequence data (Havill et al. 2007) provided additional support for HWA being endemic to western North America, and used molecular dating to estimate that the western lineage split from Japanese HWA approximately 6 million years ago. A follow-up study with additional samples, and using microsatellite markers in addition to mitochondrial DNA (Havill et al. 2016b), reinforced the results of Havill et al. (2006) and uncovered new details. This work confirmed that the Japanese lineage specializing on *T. sieboldii* was the source of the eastern North American introduction, and found that there was a single clone of HWA throughout all of the eastern US, suggesting a single introduction event. This study also found evidence for additional endemic lineages in central China, Ulleung Island, and Taiwan. Havill et al. (2016b) also performed molecular dating analysis which produced a more likely timeframe for the split between HWA in western North America and southern Japan occurring during one of the last two glacial periods (14–29 thousand years ago or 57–71 thousand years ago). High microsatellite heterozygosity in western North America also provided additional evidence that HWA is endemic to the region and has been

reproducing asexually for a very long time.

Different HWA lineages also vary in their life cycles (Havill and Footitt 2007, Havill et al. 2016b). In Japan, the same HWA lineage that was introduced to eastern North America alternates between generations on *T. sieboldii* and *Picea torano* (Siebold ex K. Koch) Koehne, on which it produces galls and a sexual generation. In China, HWA alternates between *T. chinensis*, *T. forrestii*, or *T. dumosa* and *Picea likiangensis* (Franch.) E.Pritz. or *P. brachytyla* Franch. Pritz. In Taiwan, it alternates between *T. chinensis* and *P. morrisonicola* Hayata (Chen et al. 2014), and in Bhutan, between *T. dumosa* and *Picea spinulosa* (Griff.) A. Henry (N. Havill, personal observation). Locally, populations can continue an asexual life cycle with two generations per year on *Tsuga* when their *Picea* host species are not available. The lineage in western North America only rarely produces the winged migrants (sexuparae) that would migrate to *Picea*, presumably because it has nearly lost that trait over thousands of years without a spruce species to sustain them. In eastern North America, many sexuparae are produced but cannot complete the life cycle on eastern *Picea* species, so they die, sometimes in spectacular fashion as mass depositions on beaches (Havill et al. 2022). This leaves two asexual exulis generations of HWA in eastern North America: one long generation that consists of sistens individuals that diapause in the summer and develop through the winter, and another that consists of progrediens individuals that develop quickly in the spring and early summer (Fig. 1).

There is also some limited evidence that different lineages have different host preferences. Havill et al. (2016b) found distinct genetic lineages that specialize on *T. sieboldii* and *T. diversifolia*, despite these host species' proximity at intermediate elevations in Japan. It also appears that the *T. sieboldii* lineage will not develop readily on *T. chinensis* (Del Tredici and Kitajima 2004). Otherwise, it is not known how well the different HWA lineages can complete development on the *Tsuga* or *Picea* host species from other regions.

This information about the diversity of HWA and its hosts has helped to guide the biological control effort in eastern North America. Most importantly, it has focused the evaluation of natural enemies to agents from Japan and western North America because these regions harbor the HWA lineage that was introduced to eastern North America and its closest relative. This information has also provided an awareness of how different the endemic hemlock adelgid lineages and host plants are from those in eastern North America, so it is possible that natural enemies will

behave differently after introduction. For example, if predators use chemical or visual cues from *T. heterophylla* to find western HWA in western North America (Arsenault et al. 2015), will this translate to using cues from *T. canadensis* to find Japanese HWA in eastern North America? Such questions may prove to be important as predator establishment and impact continue to be evaluated in the introduced range.

1.2. Population dynamics of HWA

McClure (1991) provided the first comprehensive description of HWA population dynamics. He described a biannual boom-bust cycle on the naive North American host *T. canadensis*, whereby the density of newly-established HWA populations increases quickly causing infested hemlock branches to fail to produce much, if any, new shoot growth in year 1 of infestation. Since the next generation of HWA infests previously-uninfested hemlock needle bases, HWA suffers density-dependent mortality and population density declines abruptly in year 2. Subsequently, the hemlock branches recover and produce new shoot growth that same year. HWA densities thereby increase again in year 3 and the cycle repeats itself in years 3-4, although in McClure's (1991) study, all trees died in the fourth year of infestation. Paradis (2011) also observed this boom-bust cycle, but the study trees survived and the cycle persisted into the future.

In the dynamics described by McClure (1991), HWA density in its introduced range on *T. canadensis* is determined by the availability of space (needle bases) on the hemlock branches where HWA crawlers insert their stylets to feed (Young et al. 1995). This is an example of a bottom-up effect (Hunter and Price 1992) in contrast to the top-down regulation of western HWA on western hemlock, *Tsuga heterophylla*, in the Pacific Northwest (Crandall et al. 2022). Adelgid densities are thus maintained near carrying capacity and pronounced density-dependent mortality occurs across the HWA life stages, because HWA fecundity is high and the number of offspring produced far exceeds the space available for settling on hemlock twigs. In a life-table study on hemlock branches inoculated with varying densities of HWA, Sussky and Elkinton (2014) documented three separate density-dependent causes of mortality in both the progrediens and sistens generations. These were density-dependent dispersal of neonates in both generations and density-dependent mortality of settled progredientes. Furthermore, progrediens fecundity declines with density, and sexuparae production

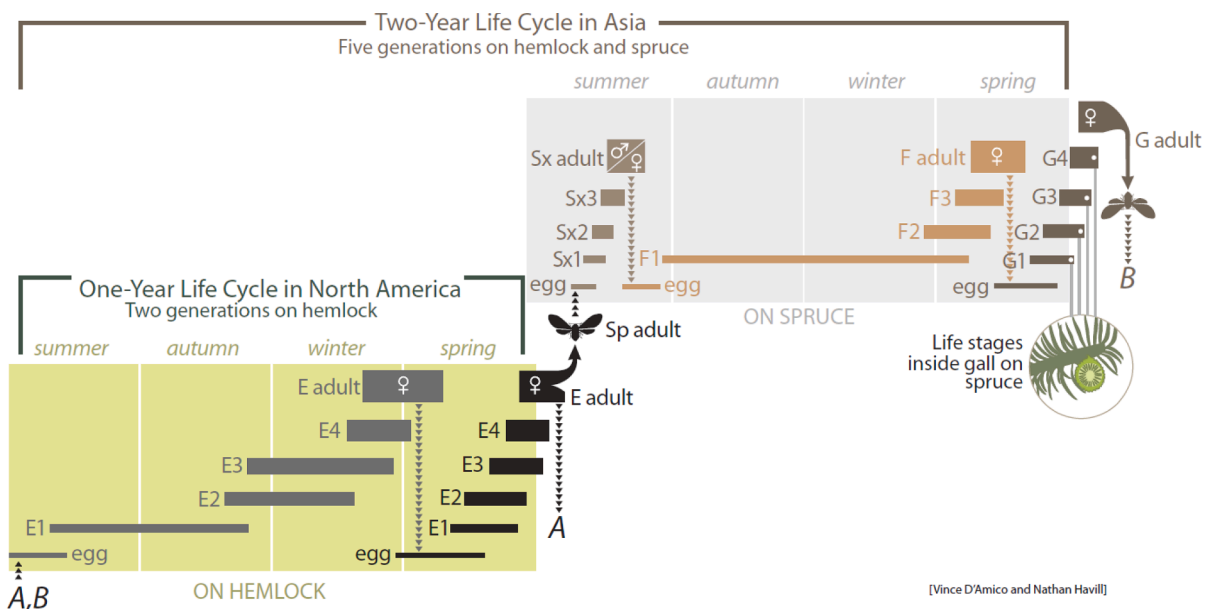


Fig. 1. Life cycle of hemlock woolly adelgid (HWA, *Adelges tsugae*) in its native range in Japan and its introduced range in eastern North America. Abbreviations for the generations: E = exulis, Sp = sexupara, Sx = sexualis, F = fundatrix, G = gallicola. Diagram by Vince D'Amico and Nathan Havill, USDA Forest Service.

increases with density (McClure 1991, Paradis et al. 2008, Sussky and Elkinton 2014). As explained above, sexuparae are a source of mortality because the *Picea* hosts they seek in Asia are not naturally present in North America. The existence of HWA-populations near carrying capacity in eastern North America, coupled with the high fecundity of HWA individuals exceeding 100 offspring per individual present a challenge to implementing biological control (Elkinton et al. 2011). Crandall et al. (2020) demonstrated that density-dependent survival in the progrediens generation compensated for high mortality caused by *Laricobius nigrinus* beetles feeding on the overwintering ovisacs from the previous generation (Jubb et al. 2020), so that the resulting HWA density was largely restored to that of the previous year.

In terms of natural enemies, there are no known parasitoids that attack the Adelgidae (Havill and Footitt 2007). Previous studies of naturally occurring predators that attack HWA in its introduced range concluded that these enemies are likely to cause minimal impact (Montgomery and Lyon 1996, Wallace and Hain 2000). Chandler et al. (2022) documented a substantial decline of HWA populations in Pennsylvania and New England in the summer of 2018 associated with high levels of rainfall in July and August of that year. They presented evidence that it was caused by fungal pathogens, such as those documented in earlier studies in Vermont and Massachusetts (Gouli et al. 1997, Reid et al. 2010). Although preliminary efforts to employ formulations of insect-killing fungi against HWA on a landscape scale (Costa 2011) have not been realized in practice, mortality caused by naturally-occurring entomopathogens may be worthy of additional attention for their effects on HWA population dynamics.

Following its discovery near Richmond, Virginia in the early 20th century, HWA spread northward and invaded New England in the late 1970s (McClure 1987, McClure 1991). HWA spread more rapidly to the northeast than west or south (Morin et al. 2009), possibly because birds migrating north in the spring may carry progrediens crawlers on their bodies (McClure 1990, Russo et al. 2019), but also because hemlocks comprise a much greater proportion of the forest canopy in northern vs southern states (Morin et al. 2009). Population spread stalled, however, in southern Vermont and New Hampshire in the 1990s, presumably limited by cold winter temperatures (Parker et al. 1998, Orwig et al. 2012). Winter temperatures in this region are far lower than in southern Japan, where HWA in the eastern United States originates (Havill et al. 2006, 2016b). Various studies have documented significant overwintering mortality of HWA (Parker et al. 1998, Shields and Cheah 2005, Trotter and Shields 2009). Paradis et al. (2008) and Ellison et al. (2018) mapped the future expected spread of HWA based on climate warming models and concluded that HWA would continue to spread north into northern regions where survival is currently limited by cold winter temperature. Shifts of 221–468 km northwards and 110–164 km eastwards have been projected for the North American range of HWA by the period 2050–2070 (Kantola et al. 2019).

Skinner et al. (2003) showed that overwintering HWA from southern locations in the US suffered higher mortality when exposed to cold temperatures than HWA from northern locations. Butin et al. (2005) and Lombardo and Elkinton (2017) collected HWA from different locations in the northern and southern US and reared them through one generation in common garden experiments in Massachusetts. HWA originating from southern sites had higher cold-induced mortality (Butin et al. 2005) and warmer supercooling points (Lombardo and Elkinton 2017) than adelgids originating from northern locations, confirming a genetic basis for cold hardiness in HWA. Elkinton et al. (2017) showed that the supercooling point of HWA decreased in response to several days of prior exposure to cold temperatures both in the field and the laboratory, demonstrating that cold-hardiness can also be induced. Sudden declines in temperature did not allow HWA populations sufficient time to develop cold hardiness. Thus, rapid declines in temperature associated with polar vortex events cause high adelgid mortality as reported by Tobin et al. (2017) and explain why variation in winter temperature is a better predictor of winter mortality than minimum winter temperature

(Paradis et al. 2008). Various studies suggest that polar-vortex cold events may become more frequent as the climate changes (Limpasuvan et al. 2004, Cohen et al. 2018), which may limit the northward spread of HWA despite the expected warming climate. Such events may also have a direct impact on winter active predators, such as *Laricobius* species, because larvae of these beetles feed on the overwintering ovisacs in late winter (Sumpter et al. 2018). Because *Laricobius* species have lower fecundity than HWA (Mausel et al. 2008), these beetles may be more influenced by polar vortex cold events than HWA. The high fecundity of HWA enables it to rebound quickly from high winter mortality (McAvoy et al. 2017).

Several studies show that high temperatures in summer months can also cause significant mortality of HWA. Sussky and Elkinton (2015) reported higher mortality of HWA in Massachusetts on sunlight-exposed branches than on branches shaded from sunlight. Mech et al. (2018) reported relationships between mortality of summer aestivating HWA and high temperature extremes in both the laboratory and the field. Weed et al. (2016) showed that unusually warm temperatures in spring in the Pacific Northwest induced a summer-long aestivation of the HWA progrediens generation in that region.

Another factor affecting the population dynamics and impact of HWA includes its interaction with the elongate hemlock scale, *Fiorinia externa* Ferris, another invasive herbivore than infests hemlock needles and has spread across the northeastern US from an introduction to New York City in 1908 (Preisser et al. 2008). Preisser and Elkinton (2008) showed that the scale actually helps reduce HWA-induced hemlock mortality by robbing hemlock twigs of nutrients that HWA requires. Combined infestations of elongate hemlock scale and HWA are less damaging to hemlock than infestations with HWA alone (Preisser and Elkinton 2008, Preisser et al. 2008).

This section has summarized the most important known biotic and abiotic factors that govern the population dynamics of HWA. The important finding of Crandall et al. (2022) that HWA populations in the Pacific Northwest are kept at low density by a suite of predators, including those that prey on the summer generation of HWA, supports the ongoing biological control effort to introduce and establish these predators to the eastern US. For the purposes of this paper, we define establishment as the ability to recover predator larvae or adults after reproducing for three or more generations after release (Mausel et al. 2010).

2. Coccinellid predators

Several species of lady beetles (Coleoptera: Coccinellidae) have been evaluated as biological control agents of HWA. One species from Japan, *Sasajiscymnus tsugae* Sasaji & McClure, was the first predator evaluated and released, and three species of *Scymnus* from China were evaluated, two of which were released. Of these, only *Sa. tsugae* became established, but as discussed below, recovery of this species has been low and inconsistent despite a long history of release.

More than 70 species of Coccinellidae have been found on hemlocks in China (Yu et al. 2000). *Scymnus* is the largest genus of lady beetles worldwide with more than 600 described species, most of which feed on either aphids or adelgids (Gordon 1985). *Scymnus* subgenus *Neopullus* has 22 species, seven of which were found feeding on hemlock woolly adelgid in China (Yu et al. 2000). Three of the most common coccinellid species on hemlocks in China, *Scymnus sinuanodulus* Yu & Yao, *S. ningshanensis* Yu & Yao, and *S. camptodromus* Yu & Liu, were collected and imported to the US for further study to determine if they were good candidates for HWA biological control. There have been 17 species of *Scymnus* previously introduced into the US, but only two are known to have established. The two established species are from the *Pullus* subgenus and they feed on adelgids (Hagen et al. 1999, Gordon 1985). *Scymnus impexus* Mulsant was imported from Europe to control balsam woolly adelgid, *Adelges piceae* Ratzeburg, in the 1960's, but its last reported recovery was in 1978 (Harris and Dawson 1979). *Scymnus*

suturalis Thunberg was imported from Europe in 1961 to control adelgids on pine (Gordon 1985). This species attacks *Pinus strobi* (Hartig), *Pinus pini* (Marcquart), and *A. tsugae* (Lyon and Montgomery 1995). There is one *Scymnus* (*Pullus*) native lady beetle, *Scymnus conferrarum* Crotch that feeds on both pine and western hemlock adelgids (Montgomery and McDonald 2010) that recently received some attention to see if it might be a good candidate for control of hemlock woolly adelgid in the eastern US. This species was deemed unsuitable for HWA biological control because it is collected more often on pine adelgids than HWA in western North America, and its taxonomy was uncertain because of deep interspecific DNA barcode divergence (Darr et al. 2018). Only *Sa. tsugae* from Japan, and the three *Scymnus* species imported from China, will be discussed in more detail below.

2.1. *Sasajiscymnus tsugae*

Sasajiscymnus tsugae (Fig. 2) is a small (~2 mm) black lady beetle (Coleoptera: Coccinellidae) that is native to Japan (Sasaji and McClure 1997). It was the first predator species deployed for HWA biological control, with releases in 1995 in Connecticut (Cheah and McClure 1996). To date, about 3.4 million have been released throughout the eastern United States (Hakeem et al. 2013, Table 1). It has become established in some locations, but recoveries are inconsistent and in low numbers (Cheah et al. 2005, Grant 2008, Hakeem et al. 2011, 2013, Jones et al. 2014). In a 2008–2012 survey of 65 release sites in the Great Smoky Mountains National Park, where more than 500,000 *Sa. tsugae* had been released over the previous 6 years, Hakeem et al. (2013) found that the likelihood of recovery (achieved at 13 sites) was positively related to the number of years since release, suggesting the importance of long-term (greater than 5 years) monitoring to document establishment. Anecdotal reports have suggested impacts on adelgid density and hemlock health (Cheah et al. 2005, Cheah 2011, Cheah 2018, 2021), but to date, no field impacts due to *Sa. tsugae* have been conclusively demonstrated. Consequently, this species is no longer a major focus of research or management beyond continued monitoring of its occurrence

and impacts (Havill et al. 2016a, but see Cheah 2018, 2021).

There are still open questions about this species, which if answered, could help explain why it has not resulted in a lasting reduction in HWA populations after its release. *Sasajiscymnus tsugae* was reported to be effective at regulating HWA numbers in Japan (McClure 1995) and in early field observations in the US (McClure and Cheah 1998), and it is possible to produce large numbers in rearing colonies (Palmer and Sheppard 2002, Jubb 2011), so why corresponding numbers are not encountered in the field after release is not clear.

One possible explanation is that the laboratory colonies of *Sa. tsugae* harbored low genetic diversity leading to inbreeding depression, which has impacted its fitness in the field. The rearing colonies of *Sa. tsugae* originated from two shipments from Osaka Prefecture, Japan of 58 and 74, mostly immature individuals, in 1994 and 1995, respectively (USDA Agricultural Research Service GRIN database). A subset of these were reared to the adult stage to begin the first colony at the Connecticut Agricultural Experiment Station (C. Cheah, personal communication). In 1997, 100 beetles from this colony were used to seed a backup colony at the Phillip Alampi Beneficial Insect Laboratory at the New Jersey Department of Agriculture (Palmer and Sheppard 2002). Material from this New Jersey colony was used to establish at least eight additional colonies at various academic, state, and private institutions. The transfer of beetles to establish new colonies using a small number of founding individuals likely resulted in low genetic diversity.

Due to their poor performance in caged field trials (Butin et al. 2003, Flowers et al. 2006), and observations that beetles in rearing colonies were behaving less robustly and had lower fecundity over time, efforts were made to augment them with new genetic material from Japan. In 2008, 330 beetles from Osaka and Nara Prefectures were received by the North Carolina Department of Agriculture rearing laboratory, and 60 mating pairs were used to augment the colony (North Carolina Department of Agriculture 2009). Likewise, in 2010, 43 *Sa. tsugae* adults from Osaka and Kyoto Prefectures were received for the colony at the University of Tennessee (P. Parkman, personal communication). These beetles were kept separate for research purposes until 2013 when they

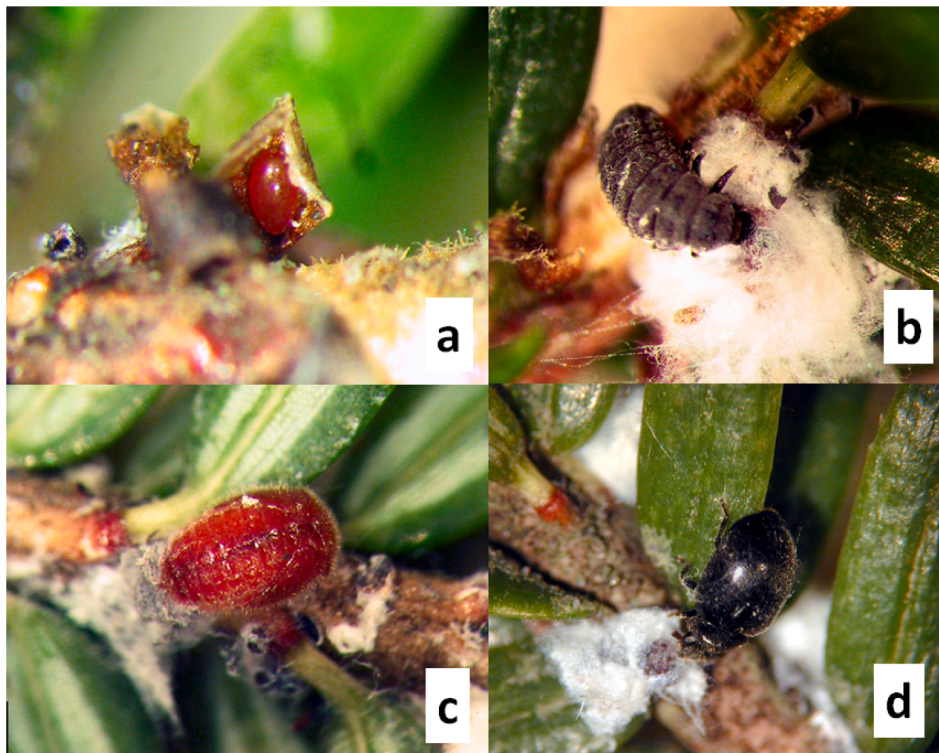


Fig. 2. Life stages of *Sasajiscymnus tsugae*: (a) egg inserted in bud scales, (b) fourth instar larva, (c) pupa, (d) adults mating. Photos by Carole Cheah, Connecticut Agricultural Experiment Station.

Table 1
Conservative (minimum) estimates¹ of release and recovery data for predatory insects released for biological control of hemlock woolly adelgid in the eastern United States through January 2023.

Predator species	Years laboratory reared and released	No. of release sites	No. of release events	No. of predators released		No. of predators recovered		No. of sites with recoveries ²	Established ⁴
				Adults	Eggs or larvae	Adults ²	Larvae ³		
<i>Sasajiscymnus tsugae</i>	1995 – 2017	574	1,373	3,392,766	294,420	3,986	0	86	Yes
<i>Scymnus sinuonodulus</i>	2004 – 2011	65	171	18,955	42,696	0	0	0	No
<i>Scymnus ningshanensis</i>	2007 – 2009	4	4	1,400	0	0	0	0	No
<i>Laricobius nigrinus</i>	2003 – 2020 ⁵	433	1,164	366,484	125,820	32,686	14,023	189	Yes
<i>Laricobius osakensis</i>	2012 - present	88	154	76,538	3,000	1,877	597	22	Yes
<i>Leucotaraxis</i> spp. ⁶	2015 - present	65	130	46,127	0	1	0	1	TBD
Total			2,996	3,902,270	465,936	34,564	14,620		

¹ Data summarized from voluntary entries in the Hemlock Woolly Adelgid Predator Database (Virginia Tech 2022) made by numerous federal, state, local, university, and private cooperators, and from Montgomery and Keena (2011). Data are conservative estimates due to releases and recoveries that have not been entered into the database.

² Indicates F1 generation or older, with the exception of data for *Sasajiscymnus tsugae*, which could include recovery of F0 adults because some recoveries were reported during the same season of release to investigate dispersal.

³ Recovery of *Laricobius nigrinus* larvae could also include *Laricobius rubidus* and *La. rubidus* x *La. nigrinus* hybrids.

⁴ Indicates F3 or older generation recovered. TBD = to be determined.

⁵ Although laboratory-rearing of *La. nigrinus* for operational release has been largely phased out, releases representing redistribution of field-collected individuals continues.

⁶ Includes both *Leucotaraxis argenticollis* and *Leucotaraxis piniperda*.

were merged with the existing colony. Material from both augmented colonies was shared with other rearing facilities. Despite attempts to add genetic stock to the colonies, the low number of beetles used to initiate the original colony, the series of bottlenecks as new colonies were initiated, and the small number of additional wild beetles used to augment the colonies may have limited the genetic diversity released into the field. In addition, continuous rearing in the laboratory over many generations may have resulted in adaptation to rearing conditions at the expense of fitness in the field. The laboratory colonies routinely reared *Sa. tsugae* at uniformly warm (24–25 °C) temperatures to accelerate development (Jubb 2011). This may have selected for beetles that preferred these temperatures, at the expense of survival in field conditions.

Another factor that may have affected the ability of *Sa. tsugae* to regulate HWA populations is its host specificity. Cheah and McClure (1998) noted that *Sa. tsugae* could develop from egg to adult on balsam woolly adelgid, *A. piceae* (Ratzeburg), Cooley spruce gall adelgid, *A. cooleyi* (Gillette), and pine bark adelgid, *P. strobi*. Later, Butin et al. (2004) tested the feeding preference of *Sa. tsugae* on HWA versus *P. strobi*, *A. cooleyi*, the larch adelgid, *A. laricis* Vallot, and the woolly alder aphid, *Paraprocephalus tessellatus* (Fitch). *Sasajiscymnus tsugae* showed a preference for feeding on HWA adults versus the other species and preferred the eggs of HWA to those of *A. laricis*, but not the other adelgid species. Larvae of *Sa. tsugae* were unable to develop on *P. tessellatus*. In a second host range study, Jetton et al. (2011), compared feeding preference, oviposition preference, and development on HWA versus *A. piceae*. This study did not find a preference for one prey species over the other but did find that more *Sa. tsugae* survived to the adult stage feeding on HWA than on *A. piceae*. In addition to being collected on HWA in Japan, *Sa. tsugae* has also been collected on grasses and shrubs in marshes in Fukui prefecture (Sasaji and McClure 1997), from *Pinus thunbergii* Parl. infested with the European line adelgid, *Pinus pini* (Goeze), in Chiba, Kanagawa, and Shizuoka prefectures (Matsubara 2000). Recently, Seki (2021) reported collecting *Sa. tsugae* from *Pinus strobus* L. and *P. parviflora* Siebold & Zucc. infested with unidentified aphid-like insects in Hokkaido, where hemlock does not naturally occur. Given that *Sa. tsugae* can develop on other adelgid species, and has been found in Japan on plants other than hemlock (sometimes in regions where hemlock does not grow), there remain questions about its realized host range in both its native and introduced geographic ranges. If it is seeking prey other than HWA, this may have implications for its ability to regulate HWA.

An additional factor that could explain low recovery of *Sa. tsugae* in the field is that it may not be well synchronized with HWA in North America. *Sasajiscymnus tsugae* feeds on all stages of HWA, but developing nymphs, adults and eggs are required to stimulate beetle oviposition (Cheah and McClure 1998, Palmer and Sheppard 2002). HWA undergoes a prolonged period of aestivation from late summer to early winter (Salom et al. 2001, Fig. 1), and *Sa. tsugae* larvae cannot survive only on a diet of aestivating first instar adelgids (Palmer and Sheppard 2002). Cheah and McClure (2000) reported that it is possible for *Sa. tsugae* to produce a second generation in Connecticut in some years, depending on the mean daily temperature, but that the majority of the population undergoes a single generation. Correspondingly, in rearing colonies it is possible to produce two generations per year, but only by bringing HWA into the lab in the winter to force early oviposition, or by holding HWA in a cooler to provide a continuous supply of HWA eggs to support larval development. Therefore, the environment in the introduced range may not be able to sustain large numbers of *Sa. tsugae* during the period when HWA is aestivating.

2.1.1. Summary and Outlook: *Sasajiscymnus tsugae*

1. *Has the agent established in the field?* Yes, but with inconsistent recovery in low abundance.

2. In areas where established, is it reducing HWA population density? Not investigated.
3. In areas where it is reducing HWA density, is it improving hemlock health? Not applicable.

Although more than 3 million *Sa. tsugae* have been released since 1995, there is no scientific evidence demonstrating effects of this predator on field populations of HWA. Concerns exist about its genetic diversity, host specificity, and synchrony with HWA in North America. As such, the focus of the HWA biological control program has shifted to other candidate agents.

2.2. *Scymnus sinuanodulus* and *Scymnus ningshanensis*

2.2.1. Origin and biology

Scymnus sinuanodulus was first described from specimens collected in Yunnan, China in 1995 (Yu et al. 1997) and *Sc. ningshanensis* (Fig. 3) was first described from specimens collected in Shaanxi, China in 1998 (Yu et al. 2000). These species of lady beetles were found feeding on hemlock woolly adelgid on *T. chinensis*, *Tsuga dumosa*, and *Tsuga forrestii* in mountainous regions between 1,900 and 3,200 m at locations between 26.3 and 33.3°N latitudes. Work to understand the biology of these two species and determine their potential as biocontrol agents for HWA began in 1995 in China and in 1996 in the US after importing adults to the USDA Forest Service Quarantine facility in Ansonia, CT under USDA APHIS permit. Multiple further imports were made to establish genetically diverse colonies. Yu et al. (1997, 2000) provided full morphological descriptions of the adults of each species.

Eggs are laid singly, usually laid under bud scales, under HWA egg sacs, or in other concealed locations (Lu et al. 2002). When first laid, eggs are yellow orange but become dark brown after a day or two as the embryos develop. The larvae are fusiform, yellowish to reddish brown, densely setaceous, with tubercles, and have a waxy covering, in all but the first instar (Lu et al. 2002). Larvae go through four instars, feed mostly on HWA eggs but also will feed on other HWA stages. Larvae feed extra-orally (suck the body contents then regurgitate them back into the egg or nymph before sucking them up again) and adults chew their prey

and suck up liquids.

When eggs are held at 18–20 °C, they hatch in an average of 10 (*Sc. sinuanodulus*) and 8 (*Sc. ningshanensis*) days (Montgomery and Keena 2011). Eggs can be held temporarily at 5 °C but cannot overwinter. Each of the first three larval instars of both species are completed in about 3 days at 18–20 °C, but *Sc. sinuanodulus* larvae spend 11 days and *Sc. ningshanensis* spend 6 days in the fourth instar (Montgomery and Keena 2011, Lu and Montgomery 2001). The fourth instar of *Sc. ningshanensis* is shorter because it spends less time wandering and is inactive before it pupates (Lu et al. 2002). The pupae of both species complete development in about 11 days at 18–20 °C. Total development of *Sc. sinuanodulus* from egg to adult took 73, 40, and 35 days at 15, 20, and 25 °C, respectively, but only 5% of the individuals could complete development at 25 °C (Lu and Montgomery 2001). Neither species can complete development on HWA nymphs alone. Third instar *Sc. ningshanensis* larvae consume about 100 HWA eggs per day.

Mating occurs when adults are about 3 weeks old, but egg laying does not begin until the following spring and requires cold exposure as prerequisite (Lu and Montgomery 2001). Female *Sc. ningshanensis* must consume HWA eggs in order to oviposit, and can produce an average of 30 eggs per week during their peak oviposition period when high-quality food is present (Montgomery and Keena 2011). To survive during the summer when HWA is diapausing, the adults either need to find another food source or can be chilled in the laboratory to 10–15 °C, which is the typical range of summer temperatures in China where they are found. The adults, however, are adapted to survive cold winters because their supercooling points are generally between –12 and –20 °C (Costa et al. 2008). Both species go through only one generation per year.

In host range tests, these two *Scymnus* species were found to be adelgid specialists that will minimally feed on some slow-moving aphids that are similar in size to HWA. *Scymnus sinuanodulus* prefers HWA over *A. laricis* and *A. cooleyi* and any aphid tested but will consume equal numbers of HWA and *P. strobi* (Butin et al. 2004). In choice tests to compare host plant attractiveness, both species preferred hemlock foliage over pine foliage. Thus, neither species is likely to have any appreciable impact on non-target prey, even other adelgid species, of

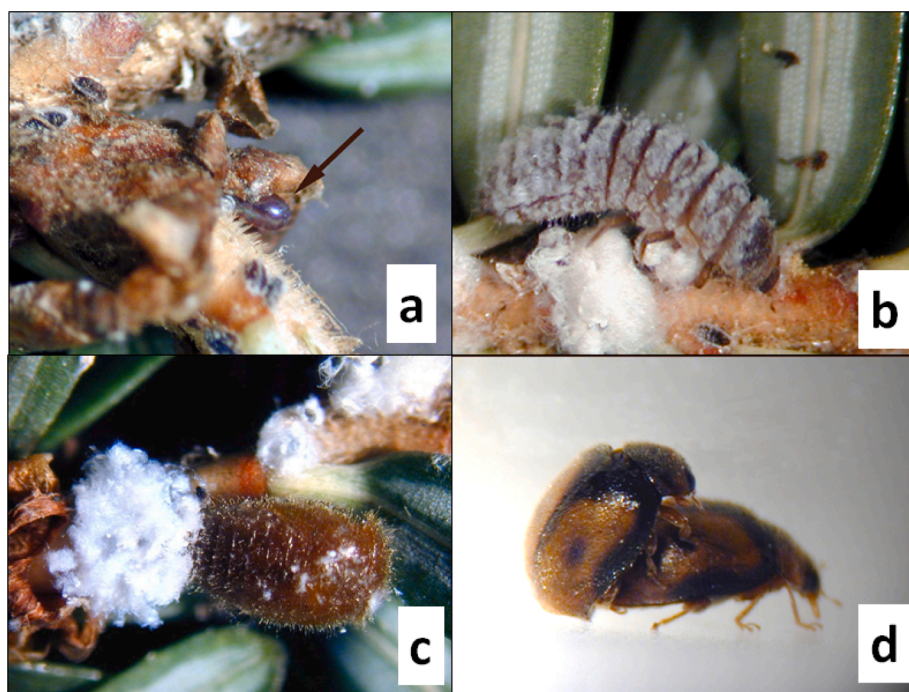


Fig. 3. Life stages of *Scymnus ningshanensis*: (a) egg inserted in bud scales (see arrow), (b) fourth instar larva, (c) pupa, (d) adults mating. Photos by Michael Montgomery, USDA Forest Service, Northern Research Station.

which seven of the 12 in North America are non-native (Havill and Foottit 2007). Permission from APHIS to release clean colonies of these species from quarantine for further research in an insectary in Hamden, CT was obtained in 1998 for *Sc. sinuanodulus* and 2000 for *Sc. ningshanensis*.

2.2.2. Rearing, release, establishment, and spread

In the laboratory, adults were overwintered for 4 months (at 15°, 10°, 5°, 10°, and 15 °C for durations of 2, 4, 6, 2, and 2 weeks, respectively) in plexi-glass cages with HWA-infested hemlock foliage (inserted into floral foam blocks) and supplemental honey and wheat. In early March, adults were moved to 17 °C and 12:12 L/D and monitored for oviposition through the end of July. Oviposition occurred in 473 ml paperboard cups with 2 or 3, 15 cm pieces of infested foliage, 2 or 3 females and 1 male per cup. Infested foliage was changed weekly and the foliage with eggs on it was inserted into floral foam blocks and held in large cages at 20 °C and 16/8 L/D with fresh foliage added weekly. After a minimum of 45 days, adults were collected using an aspirator and then stored at 20 °C with infested foliage until released or used for colony maintenance.

Field evaluation of both species in sleeve cages occurred between 1999 and 2008 on branches of eastern hemlock infested with HWA. Cages with beetles present had significantly fewer HWA than those without beetles (Butin et al. 2003, Montgomery and Keena 2011). The cage studies provided useful information on how to precondition beetles before release and how heavy an HWA infestation was needed to see good reproduction. Free releases of *Sc. sinuanodulus* and to a limited extent of *Sc. ningshanensis* were made between 2004 and 2011, but there is no record of recovery of these beetles in the years following their release (Table 1). Most free releases were made in the spring with 150–15,000 *Sc. sinuanodulus* adults and 300–500 *Sc. ningshanensis* adults released per site. Releases of *Sc. sinuanodulus* were made in CT, NJ, PA, MD, WV, NC, and GA. Releases of *Sc. ningshanensis* were only made in MA, CT and NC.

2.2.3. Summary and Outlook: *Scymnus sinuanodulus* and *Sc. ningshanensis*

1. Has the agent established in the field? No.

2. In areas where established, is it reducing HWA population density? Not applicable.
3. In areas where it is reducing HWA density, is it improving hemlock health? Not applicable.

Scymnus sinuanodulus and *Sc. ningshanensis* are no longer being reared in North America. The lack of establishment by *Sc. sinuanodulus* following large releases over a wide geographic range suggests that further effort to release this species may not be merited. The fact that fewer numbers of *Sc. ningshanensis* were released may have reduced the chances of successful establishment, but the similarity of the biology of these two species suggests it also does not merit further study.

2.3. *Scymnus camptodromus*

2.3.1. Origin and biology

Scymnus camptodromus (Fig. 4) was first described from specimens collected in Yunnan and Sichuan, China in 1995 (Yu et al. 1997). Yu et al. (1997) provides full morphological description of the adults of this species, and a key to distinguish this species from the other *Scymnus* (Neopullus) species is available (Yu et al. 2000). This species was found feeding on HWA on *T. chinensis*, *T. dumosa*, and *T. forestii* in the same mountainous regions of China where *Sc. sinuanodulus* and *Sc. ningshanensis* were found. Work to understand its biology and determine its potential as a biocontrol agent for HWA began in 1995 in China and continued in the US after importing adults to the USDA Forest Service Quarantine facility in Ansonia, CT under USDA APHIS permit. Multiple further imports were made to establish a genetically diverse colony.

Eggs are usually laid in concealed spots and have a more leather-like surface than eggs of the other *Scymnus* species. Multiple eggs may be laid in bud scales, dry hemlock pollen cones, or in other concealed locations. Eggs remain yellow-orange in color at temperatures ≥ 15 °C and don't develop until experiencing a 1–3 month exposure to cool temperatures < 15 °C (Keena et al. 2012). The optimal temperature for *Sc. camptodromus* egg hatch is 10 °C. Thus, the summer diapause of its eggs coincides with the HWA diapause; eggs develop after exposure to cool temperatures in the fall and hatch when the HWA progrediens generation begins to lay eggs.

Larvae go through four instars, look similar to other *Scymnus* larvae,

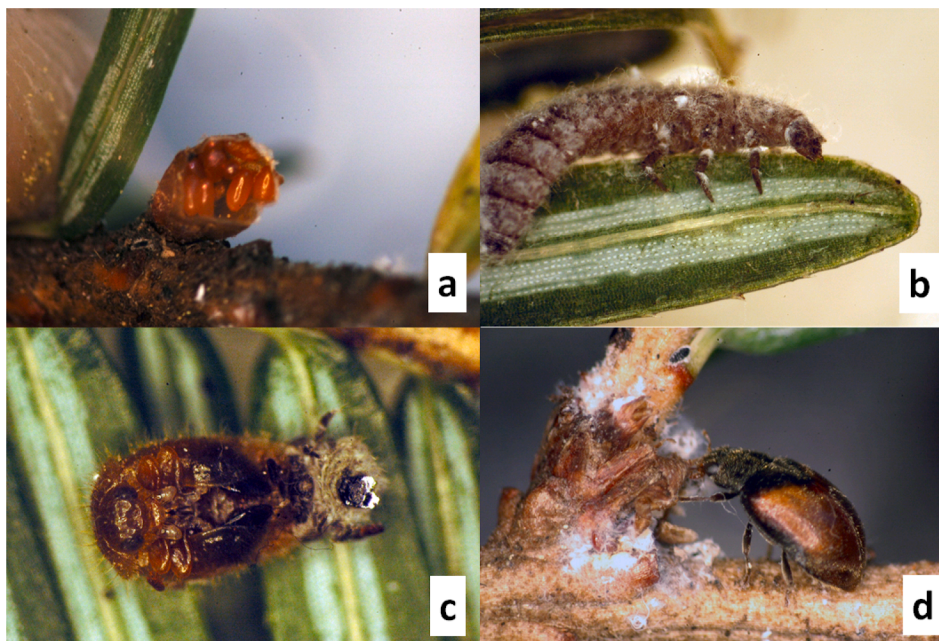


Fig. 4. Life stages of *Scymnus camptodromus*: (a) eggs inserted in hemlock pollen cone, (b) third instar larva, (c) pupa, (d) adult feeding. Photos by Melody Keena, USDA Forest Service, Northern Research Station.

and feed extra-orally, primarily on HWA eggs but also on other stages. Larvae consume an average of 23–31 eggs per day from neonate to pupation (Limbu et al. 2015). The larvae can develop at temperatures between 10 and 25 °C but survival to pupation is reduced at 10 and 25 °C (Limbu et al. 2015). At 20 °C, *Sc. camptodromus* individuals spend an average of 3 days in each of the first three instars, 9 days in the fourth instar, and 11 days as a pupa. The lower developmental threshold for larvae was estimated to be 5 °C and 50% of the individuals could develop from hatching to adult in 424 days. Pupae and new adults of *Sc. camptodromus* look the same as those of the other *Scymnus* species. Females mate after about 3 weeks old and lay an average of 10–14 eggs per week at 20 °C when abundant HWA is available.

In host range tests, *Sc. camptodromus* was found to be an adelgid specialist that would feed on some slow moving aphids that are similar in size to HWA like first instar *Aphis gossypii* Glover (Limbu et al. 2016). Adults will feed on North American adelgid species but strongly prefer HWA. Females will not oviposit on any host material except HWA-infested hemlock. A small proportion of *Sc. camptodromus* larvae given only pine bark adelgid (*P. strobi*) (Hemiptera: Adelgidae) and larch adelgid (*A. laricis*) (Hemiptera: Adelgidae) were able to complete development. Permission from APHIS to release clean colonies of these species from quarantine for further research was obtained in 2000. Additional host range testing and a full evaluation of the genetics of this species were requested when a request for unconfined field release was made in 2014.

2.3.2. Rearing, release, establishment, and spread

In the laboratory, adults are held in groups of 10–15 females and 5–10 males at 15–20 °C and 14:12 L:D with HWA-infested foliage to induce oviposition. Foliage is removed every other week and all eggs are carefully removed. Eggs are held singly in 1.5 ml micro-centrifuge tubes with a small air hole in the lid. Eggs are exposed to one of several regimes (this extends the possible hatch window) that can break the diapause (Keena et al. 2012). Eggs must be removed or can grow mold if left on the dead hemlock branches. Larvae are reared individually at 15–20 °C in specialized rearing cages made from 355 ml clear plastic cups. Foliage (about 9 cm long) with a high density of HWA females actively producing eggs is added to the cages and changed every 7–10 days depending on larval size and available HWA eggs. Group rearings are less successful since the larvae of this species are very aggressive predators and so can be cannibalistic.

Sleeve cage studies (i.e., confined release) were conducted in 2012 and 2013 in Pennsylvania (S. Limbu, K. Hoover, and M. Keena, unpublished data). After two months, branches caged with *Sc. camptodromus* adults inside had reduced HWA populations when compared to branches caged without beetles. Females laid eggs in the sleeve cages demonstrating they could reproduce under natural conditions. No studies to determine if they could overwinter as eggs outdoors have been conducted. No free releases have been made since permission has not yet been obtained.

2.3.3. Summary and Outlook: *Scymnus camptodromus*

1. *Has the agent established in the field?* No; it has not been released.
2. *In areas where established, is it reducing HWA population density?* Not applicable.
3. *In areas where it is reducing HWA density, is it improving hemlock health?* Not applicable.

The laboratory colony of *Sc. camptodromus* was lost in 2015 due to a chamber malfunction that raised the temperature too high, so any further work on this species would require new importation of adults from China. This species has several characteristics that indicate it has potential as a biocontrol agent for HWA: (1) its aestival egg diapause coincides with HWA diapause; (2) it is found in high numbers across a broad geographic range in China; (3) its larvae are present during the

active egg laying times of HWA; and (4) its adults are long lived and could feed on HWA most of its active period. There are, however, still several unanswered questions about this species, its ability to survive year-round in the eastern US, and the difficulty of mass rearing it for potential releases. Field insectaries would likely be needed to build substantial populations for release if a colony were restarted and permission for free release were sought and granted.

3. Derodontid predators

Laricobius spp. (Coleoptera: Derodontidae) are predatory beetles that specialize in feeding on Adelgidae (Franz 1958; Clark and Brown 1960; Lawrence and Hlavac 1979). The most recent review of the genus by Leschen (2011) described 21 species worldwide, with three native to North America: *La. nigrinus* Fender, associated with HWA on western *Tsuga* spp., *T. heterophylla* and *T. mertensiana* (Zilahi-Balogh et al. 2006); *La. laticollis* Fall, associated with *Adelges cooleyi* (Gillette) on *Pseudotsuga menzesei* (Mirb.) Franco; and *La. rubidus* LeConte, associated with *Pinus strobi* on *Pinus strobus* L. (Leschen 2011, Clark and Brown 1960, Wantuch et al. 2019). Following the introduction and spread of balsam woolly adelgid, *Adelges piceae*, a significant effort was made to introduce the European species, *La. erichsonii* Rosenhauer, into North America as a biological control agent (Montgomery and Lyon 1996). This insect may have established (Brown and Clark 1956), although this is uncertain due to the difficulty in distinguishing it from *La. rubidus* (Clark and Brown 1960). In British Columbia, *La. erichsonii* was released between 1960 and 1968 and was considered established as of 1978, but in a formal survey by Humble (1994), it was not collected, and has not been found in any North American surveys since 1978 (Montgomery and Lyon 1996).

Because *Laricobius* spp. specialize on adelgids, species associated specifically with HWA have been targeted as potential biological control agents. *Laricobius* species associated with HWA that have been considered for biological control include *La. baoxingensis* Zilahi-Balogh & Jelinek and *La. kandingensis* Zilahi-Balogh & Jelinek from China (Zilahi-Balogh et al. 2007), *La. osakensis* Montgomery and Shiyake (Montgomery et al. 2011) and *La. naganoensis* Leschen from Japan (Leschen 2011), *La. taiwanensis* Yu & Montgomery from Taiwan (Yu and Montgomery 2007), *La. nigrinus* Fender from western North America (Zilahi-Balogh et al. 2003a), and *La. rubidus* LeConte from eastern North America (Zilahi-Balogh et al. 2005, Mausel et al. 2008). Due to the remoteness and difficulty in collecting many of the Asian *Laricobius* spp., the biological control program has focused on the study, rearing, release, recovery, and impact assessment of *La. nigrinus* and *La. osakensis* for HWA (Mausel et al. 2010, Toland et al. 2018). *Laricobius rubidus* is also studied due to its presence on HWA in eastern North America and its ability to hybridize with *La. nigrinus* (Havill et al. 2012).

3.1. *Laricobius nigrinus*

3.1.1. Origin, biology, and ecotypes

The first description of *La. nigrinus* (Fig. 5) by Fender (1945) was from specimens collected years earlier from Bear Springs, Oregon and then Creston, British Columbia. The insect was reported from the east side of the Cascade mountain range on HWA on *T. heterophylla* and on *Adelges laricatus* (Patch) on *Larix occidentalis*. Nutt., and in the interior Rocky Mountains on *Pinus strobi* on *Pinus monticola* Douglas ex D. Don. Havill et al. (2012) found weak but significant genetic differentiation between *La. nigrinus* collected from coastal and interior sites. Additionally, coastal and interior populations may differ in host range, with coastal populations found only on HWA, and interior populations found on HWA as well as other adelgid species such as *A. laricatus* and *Pinus coloradensis* (Gillette) (Mausel et al. 2011a, Havill et al. 2012, C. Jubb personal communication).

Canadian Forest Service scientist Dr. Leland Humble consistently found *La. nigrinus* on HWA-infested *T. heterophylla* in seed orchards on southern Vancouver Island, prompting author S.M.S. to visit those sites

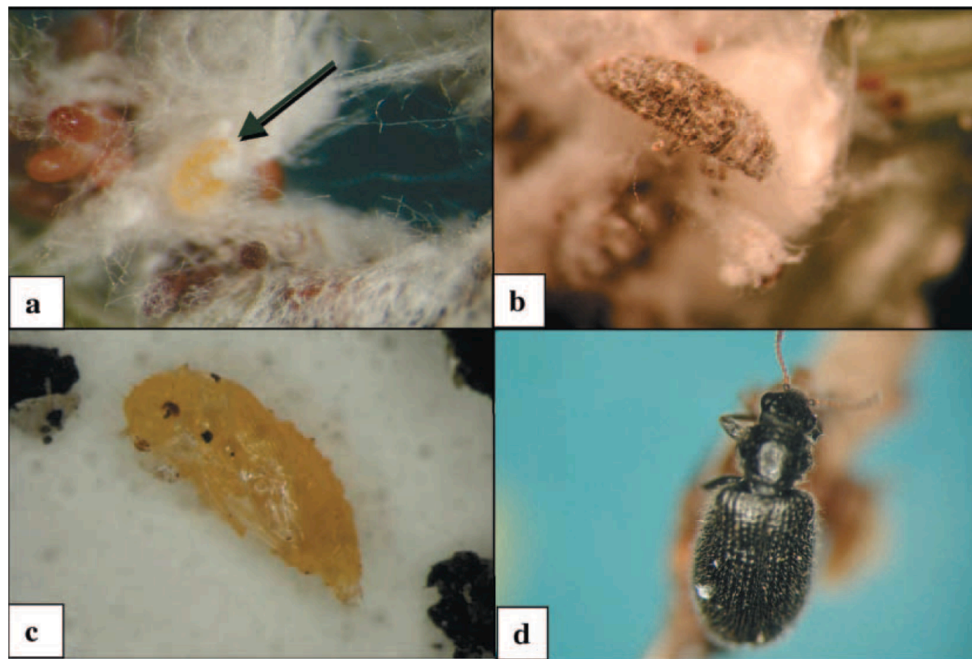


Fig. 5. Life stages of *Laricobius nigrinus*: (a) yellow *L. nigrinus* eggs amidst red *Adelges tsugae* eggs, (b) late-instar larva (4th instar), (c) pupa, (d) adult (after Cheah et al. 2004). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

in 1997. Once permits with USDA APHIS were approved, S.M.S. arranged to have beetles collected and shipped to the Beneficial Insects Containment Facility (BICF) at Virginia Polytechnic Institute and State University (Virginia Tech). Simultaneous field studies were initiated near Victoria, BC and lab studies were conducted at the BICF, to gain a better understanding of the insect's biology and determine its potential as a biocontrol agent for HWA. Shepherd et al. (2014) reported a method for sexing live adults by pressing lightly on the abdomens of chilled specimens to extrude their terminal segments.

Laricobius nigrinus adults actively feed on winter developing sistens nymphs and are present from October through early April (Zilahi-Balogh et al. 2003a). Females oviposit usually one egg per adult sistens ovisac (Zilahi-Balogh et al. 2003b) and the eggs are present from February through March (Zilahi-Balogh et al. 2003a). Larvae feed on progrediens eggs and are present from March through April. The occurrence of *La. nigrinus* egg and larval stages with the presence of HWA eggs is highly synchronous. Upon completing development, fourth instar larvae drop from the tree into the soil and pupate. They eclose to adults within two weeks and then enter aestival diapause, where they remain until the fall when adults emerge from the soil (Lamb et al. 2007). Adult emergence from the soil (relative to the number of larvae entering the soil) typically averages 30–40% in the lab (Foley et al. 2021) but is closer to 17% in the field (Foley et al. 2022a).

Results from a life cycle field study of *La. nigrinus* (Zilahi-Balogh et al. 2003a) are consistent with results obtained in laboratory development studies. The minimum developmental temperature thresholds for *La. nigrinus* eggs, larvae, pre-pupae, and pupae were 5.4, 3.2, 2.9, and 3.1 °C, respectively (Zilahi-Balogh 2003c). Fastest development for all life stages occurred at 18 °C. Only eggs and larvae were able to survive at 21 °C, but this temperature resulted in significantly lower survival for larvae. Development from oviposition to adult eclosion required a minimum of 3.7 °C and 666.7°-days. Mean lifetime fecundity of *La. nigrinus* was 100.8 eggs over a 13.2 week oviposition period and mean larval consumption was 225.9 and 252.3 HWA eggs at 12 and 18 °C, respectively (Zilahi-Balogh 2003c).

Supercooling points have been used as a proxy to better understand climate matching between insects and their new introduced environments (Anderson et al. 2015). Supercooling studies of the two *La.*

nigrinus ecotypes (coastal and interior) revealed that interior beetles from Coeur d'Alene, ID had a significantly lower mean supercooling point of -19.2 °C compared to coastal beetles from Seattle, WA (-16.9 °C). Interior beetles collected from Moscow, ID had a mean supercooling point of -18.6 °C, which was not significantly different from the Coeur d'Alene or Seattle populations (Mausel et al. 2011a). Significantly greater survivorship was observed for Coeur d'Alene (interior) vs. Seattle (coastal) populations after 18 h exposure to -15.4 °C in outdoor cages. These results suggested that the interior ecotype may be better suited for colder climates.

How *La. nigrinus* adults search for and find their prey is not well understood, but several laboratory studies have provided insight. Mausel et al. (2011b) demonstrated that adult beetles are attracted to a vertical silhouette, and beetles were observed walking towards seedling stems and climbing them under lighted conditions compared with dark conditions (observed with red light). Once on the seedlings, adults climbed and searched the branches until they found adelgids, where they would then evaluate and begin feeding on their prey. On uninfested seedlings, the insects went through a cycle of grooming, crawling, and pre-flight exercise followed by flight. Eventually 90% of the beetles would fly off the needle tips of uninfested branches. No flying was observed from infested branches. Broeckling and Salom (2003) identified olfactory sensilla on *La. nigrinus* antennae with no sexual dimorphism observed. This led to studies on the use of olfaction by adults to find habitat and prey. Wallin et al. (2011) demonstrated that *La. nigrinus* adults in an olfactometer were responsive to volatiles from HWA host trees, but not to HWA alone. In subsequent studies, a significantly higher proportion of walking *La. nigrinus* chose HWA-infested eastern hemlock over eastern hemlock alone (Arsenault et al. 2015a), but in a separate experiment, the difference in response to infested vs. uninfested foliage was not significant (Arsenault et al. 2015b). Shepherd et al. (2016) found no difference in *La. nigrinus* antennal responses to volatiles from infested vs. uninfested hemlock foliage, but detected antennal responses to a number of natural and synthetic volatiles common to conifer species. These studies suggest that foliage volatiles are important olfactory stimuli when *La. nigrinus* adults are searching for host plants of their prey, but that other cues are likely involved when locating HWA on the branches.

Host-range studies demonstrated that coastal *La. nigrinus* could not fully develop on any insect tested other than HWA, and in paired-choice tests greatly preferred ovipositing in HWA ovisacs (Zilahi-Balogh et al. 2002b). The data from these studies led USDA APHIS in 2000 to write a Finding of No Significant Impact (FONSI) letter for this species, allowing it to be removed from quarantine. Moving the insect into the Virginia Tech Insectary allowed for the development of rearing procedures that have been used in multiple laboratories (Salom et al. 2012, Foley et al. 2021).

3.1.2. Rearing, release, establishment, and spread

Laricobius nigrinus requires live prey for development, necessitating the location, collection, and transportation of large amounts of HWA-infested hemlock foliage to support the laboratory rearing effort. Through a series of detailed studies, Salom et al. (2012) described how to optimally rear *Laricobius* beetles in quantities sufficient to make operational releases throughout the eastern US. Rearing *La. nigrinus* is a complicated process that requires specialized facilities. The Virginia Tech Insectary has two water cooling rooms where temperatures are kept between 13 and 19 °C, depending on the time of year. With one generation per year, there is an annual sequence of rearing activities employed that are fully described in Foley et al. (2021). Virginia Tech and the University of Tennessee Insectaries reared 116,046 and 112,348 *La. nigrinus* beetles, respectively between 2004 and 2021.

The first seven releases of *La. nigrinus* took place in 2003. By 2005, 22 releases were made, as far south as GA (plant hardiness zone 7a) and as far north as MA (plant hardiness zone 5a). These initial efforts were designed to learn what factors were most critical for successful beetle establishment. Mausel et al. (2010) evaluated numbers of adults released, season of release, and plant hardiness zone as a proxy for minimum winter temperature. Within three years of release, establishment was observed in 13 (59%) of the sites, and establishment was positively correlated with minimum winter temperature and release size. High probability of establishment was predicted to occur for zones 7a, 6b, and 6a. Mausel et al. (2010) also determined that detection of low *La. nigrinus* populations was more reliable by sampling for larvae in the spring, by placing branch clippings in buckets or funnels and waiting for larval drop, than by sampling for adults from fall through spring using beat sheets. Wiggins et al. (2016a, 2016b) demonstrated that emergence traps placed on the ground beneath the dripline of hemlock trees can also be used to monitor and collect *Laricobius* adults as they emerge in the fall.

Since 2003, operational releases have relied on a mixture of lab-reared and field-collected adults redistributed from the Pacific Northwest or from well-established sites in the eastern US. An HWA predator database (Virginia Tech 2022) provides a record of releases and recoveries (albeit incomplete) of HWA predators in the eastern US. Through January 2023, at least 366,000 adult *La. nigrinus* have been released at 433 sites (Table 1). Widespread establishment of *La. nigrinus* has been well documented. Jubb et al. (2021) visited 24 release sites in VA (with releases dating from 2003 to 2014) and recovered beetles from 82% of the sites. Foley et al. (2019) was able to collect adults from HWA-infested hemlocks wherever they sampled within a 10-mile radius between Blacksburg and Radford, VA, even though beetles were not released in either town. Forest health specialists throughout the eastern US have recovered at least 32,000 adults and 14,000 larvae from 189 sites (Table 1). Crandall et al. (in press) documented establishment of *La. nigrinus* at 18 of 49 release sites from northern Pennsylvania through New England. Establishment occurred more frequently at sites with relatively warmer winter temperatures; these sites typically have much colder winters than the native range of *La. nigrinus* in the Pacific Northwest.

Success in recovery of *La. nigrinus* has led to the identification of localized areas of high predator abundance, where beetles can be recovered in numbers sufficient to collect and redistribute them to new locations. This has reduced the need to rear *La. nigrinus* in the lab,

creating an operational shift whereby forest health specialists from multiple states can field-collect beetles for new releases in selected sites.

3.1.3. Hybridization and predator impact

Although the preferred host of *La. rubidus* is pine bark adelgid, *P. strobi* (Clark and Brown 1960), it also preys on HWA (Montgomery and Lyon 1996, Wallace and Hain 2000, Mausel et al. 2008), especially at sites where eastern white pine, *P. strobus*, and *T. canadensis* co-occur. Zilahi-Balogh et al. (2005) demonstrated that HWA is a suitable host for *La. rubidus*, in that it was able to complete development and survive similarly on HWA and PBA. Davis et al. (2011) found low sequence genetic divergence between *La. nigrinus* and *La. rubidus*, and Montgomery et al. (2011) confirmed that they are recently diverged sister species. After *La. nigrinus* began to establish in the eastern US, *La. nigrinus* and *La. rubidus* were observed mating in the field (Mausel et al. 2008), leading to investigation of possible hybridization between the two species. Havill et al. (2012) used six microsatellite markers plus mitochondrial COI haplotypes to examine genetic structure of both of these *Laricobius* species across North America, and found widespread hybridization with asymmetrical introgression towards *La. nigrinus* on hemlock in the eastern US. Subsequent field studies showed that following release of *La. nigrinus* in mixed hemlock-pine stands, the proportion of *La. nigrinus* increased while the proportion of *La. rubidus* decreased on hemlocks over time (Fischer et al. 2015). The proportion of hybrids collected from hemlocks remained stable (10–15%) 2–9 years following the release of *La. nigrinus*. At the same time the proportion of *La. nigrinus* and hybrids on white pine remained low. Additional monitoring of *La. nigrinus* and hybrid recoveries at release sites has produced remarkably similar results regarding the stability of hybridization rates (Mayfield et al. 2015, Wiggins et al. 2016b), with the exception of the most recent study that found just 2% hybrids on hemlock (Jubb et al. 2020). Additional studies could determine whether this lower rate of hybridization over time might be the result of reinforcement of barriers to hybridization developing due to decreased fitness of hybrids.

Field studies examining relationships between *La. nigrinus* predation and HWA densities have been conducted in both the native and introduced ranges. On *T. heterophylla* at urban forest sites in Seattle, Washington, Mausel et al. (2017) documented strong positive numerical responses of *La. nigrinus* (predator aggregation and reproduction) to increasing HWA densities and observed high proportions of predated ovisacs associated with low HWA survivorship rates. A similar numerical response of *La. nigrinus* was also observed on young, field-planted *T. canadensis* in Virginia, where beetles were initially released only 2–3 years prior (Mausel et al. 2008). In that study, branches exposed to established *La. nigrinus* populations had significantly lower mean HWA sistens density (0.6 vs. 1.7 adelgids/cm) and a higher proportion (35% vs. 2%) of ovisacs disturbed (i.e., ovisacs torn open as larvae consume eggs) compared to branches from which *La. nigrinus* were excluded via closed mesh cages (Mausel et al. 2008). Similarly, in a cage exclusion experiment using mature *T. canadensis* at forest site in Georgia, branches exposed to *La. nigrinus* had significantly lower HWA sistens density (3.1 vs. 6.6 adelgids/cm) and higher ovisac disturbance (70% vs. 39%) compared to caged branches from which *La. nigrinus* were excluded. Both studies noted that other adelgid mortality factors were likely involved (Mausel et al. 2008, Mayfield et al. 2015). These single-site impact studies were followed by a more geographically comprehensive effort at nine sites (from Georgia to New Jersey) across the known established range of *La. nigrinus*. A cage-exclusion approach at the branch level was used, HWA sistens mortality caused by both low winter temperatures was distinguished from mortality caused by *La. nigrinus* predation. Across all sites over four years, 46% of the sistens populations died due to winter temperatures and 38% of the sistens ovisacs were disturbed by *La. nigrinus* (Jubb et al. 2020).

Elkinton et al. (2011) developed a population dynamics model to predict what impact predators and winter mortality in the sistens generation would have on subsequent generations of HWA. They predicted

that even a 90% reduction in sistens populations is compensated for by reduced density-dependent mortality in the next (progrediens) generation, leading to high winter sistens populations the following year. This prediction was supported in field studies reported by Crandall et al. (2020), who followed the recolonization of HWA branches by the progrediens generation following the impacts on the sistens generation reported in Jubb et al. (2020). The results strongly suggested that predator pressure on developing progrediens and sistens eggs (present in late spring and early summer, when *Laricobius* is aestivating) is needed to reduce HWA populations below levels that cause substantial reduction in shoot growth (<4 HWA/cm, McClure 1991).

To date, few studies have attempted to quantify impacts of *Laricobius nigrinus* predation based on changes in hemlock tree or stand health. In a multi-year, three state study where *T. canadensis* crown condition was compared between predator-release plots and non-release plots, *La. nigrinus* ultimately failed to establish due to a cold-weather event that drastically reduced or eliminated adelgid prey after predator release (Sumpter et al. 2018), prohibiting conclusions about biological control impact on tree and stand health. Using an alternative approach, Preston et al. (2023) evaluated branch-level responses of eastern hemlock (in the form of new shoot production, photosynthetic rate, transpiration rate, and stomatal conductance) to treatments in which *La. nigrinus* adults were either included or excluded from branches. *Laricobius nigrinus* predation reduced the density of live sistens ovisacs during the winter, and by the end of the hemlock growing season, branches on which the highest density of *La. nigrinus* were included produced more new shoots than branches with no predators (Preston et al. 2023). However, branch physiology measurements did not significantly differ between treatments with and without predators, and after two adelgid generations, the final HWA sistens density exceeded previously-reported damage thresholds (McClure 1991) meaning that new shoot growth would likely be compromised the following year (Preston et al. 2023). This further

illustrates the “progrediens rebound” phenomenon described by Elkin-ton et al. (2011) and Crandall et al. (2020), which makes *La. nigrinus* predation alone insufficient to regulate HWA densities from year to year.

3.1.4. Summary and Outlook: *Laricobius nigrinus*

1. *Has the agent established in the field?* Yes, over a wide geographic range.
2. *In areas where established, is it reducing HWA population density?* Yes, in the winter and early spring stages (sistens adults, progrediens eggs), but the effect is offset by a rebound in population density in subsequent adelgid stages (progrediens adults, sistens eggs).
3. *In areas where it is reducing HWA density, is it improving hemlock health?* There is evidence to suggest branch health is improved by *Laricobius* predation (Preston et al. 2023), but the effect is temporary. Whole tree and whole-stand health has not been sufficiently evaluated.

Due to abundant field populations, laboratory rearing of *La. nigrinus* has been de-emphasized over the last decade but continues to be part of the overall rearing effort, largely through by-catch of eggs and developing larvae when collecting HWA for *La. osakensis* colony food. Emphasis on field-collection and redistribution of established adults, to enhance dispersal or supplement previous releases, will likely continue. The successful establishment, widespread dispersal, and impact of *La. nigrinus* suggests that this insect can play an important role in overall predation, but that additional predators and management strategies will be necessary. Periodic re-assessment of the impacts of *La. nigrinus* on HWA regulation and hemlock health, particularly in combination with other predator species, will help determine the long-term outcome *La. nigrinus* in eastern forests.



Fig. 6. Life stages of *Laricobius osakensis*: (a) yellow *La. osakensis* egg next to red *Adelges tsugae* egg, (b) *La. osakensis* larva covered in *A. tsugae* wool, (c) adult male, (d) adult female. Photo credits: Virginia Tech (a), and Ligia Vieira, Virginia Tech (b, c, d). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.2. *Laricobius osakensis*

3.2.1. Origin and biology

Upon discovery that HWA in the eastern US originated from an adelgid lineage in Japan associated with *Tsuga sieboldii* (Havill et al. 2006), additional efforts to survey and assess natural enemies of HWA in Japan were initiated. *Laricobius osakensis* (Fig. 6) was collected from HWA in natural forests and ornamental plantings in 11 prefectures throughout Japan (Montgomery et al. 2011). Although *La. osakensis* occupies the same temporal niche as *La. nigrinus*, additional work on this species was considered worthwhile based on potential differences in its biology, adaptability to the eastern North American climate, and potential for successful biological control of HWA. Jubb et al. (in press) provided a guide for distinguishing adults of *La. osakensis* from *La. nigrinus* and *La. rubidus* using characteristics of pronotal morphology.

The general timing of the HWA generations on hemlock in Japan is similar to what has been reported at numerous locations in the U.S. (McClure 1989, Zilahi-Balogh et al. 2003a, Joseph et al. 2011a), with expected deviations resulting from different site-specific climates (Vieira et al. 2013a). In Japan, strong synchrony between HWA and *La. osakensis* was observed, similar to that found between HWA and *La. nigrinus* (Zilahi-Balogh et al. 2003a). Some differences with *La. osakensis* in Japan include later emergence of adults and earlier oviposition (Vieira et al. 2013a). Despite these differences, the presence of *La. osakensis* larvae closely coincides with the presence of sistens eggs (from January to June), peaking in March (Vieira et al. 2013a).

In lab-based developmental rate studies, *La. osakensis* was able to complete development from egg to adult between 11 and 22 °C, with maximum survival of 38% at 15 °C (Foley et al. 2022b). The lower minimum developmental threshold from egg to adult was 5.5 °C for *La. osakensis* compared with 4 °C for *La. nigrinus*. Based on survivorship data, optimal temperatures for *La. osakensis* were 10 °C for oviposition, 15 °C for larval growth, and 15–18 °C for pre-pupal and pupal life stages. Overall, *La. osakensis* completed development and was able to survive at higher constant temperatures than *La. nigrinus*, suggesting the former may be better suited for more southern or warmer portions of the HWA introduced range. Mean supercooling points, however, did not differ between *La. nigrinus* and *La. osakensis* during monthly measurements from November through February (Toland et al. 2019). This may indicate that *La. osakensis* could tolerate northern climates in a manner similar to *La. nigrinus*.

3.2.2. Evaluation, rearing, and release

Three adelgid and three non-adelgid hemipteran species were evaluated in choice and no-choice feeding, oviposition and development tests for assessing the host-range of *La. osakensis* (Vieira et al. 2012). In no-choice feeding tests, females consumed significantly more adults and eggs of HWA than of *P. strobi* and *A. piceae*. In two separate years of no-choice testing, significantly more *La. osakensis* eggs were oviposited on HWA ovisacs than on the other adelgids and non-adelgids. In two-choice tests, more eggs were oviposited on HWA than the alternate prey. Development was completed only on HWA. Development ended at the 3rd instar on other adelgids and 1st instar for non-adelgids. With support provided by these data, *La. osakensis* was approved for removal from quarantine by USDA APHIS in 2010 (USDA 2010, Mooneyham et al. 2016).

In laboratory studies of feeding and oviposition for both *La. osakensis* and *La. nigrinus*, larval attack rates for both species were similar, but handling time was less for *La. osakensis*, leading to a greater functional response (Vieira et al. 2012). Numerical response was also greater for *La. osakensis* than for *La. nigrinus*, with more eggs laid by the former at all prey densities tested (Vieira et al. 2012). These results supported efforts to release and establish *La. osakensis*.

Methods for rearing *La. osakensis* mirrored efforts described for *La. nigrinus* (Salom et al. 2012, Foley et al. 2021). Founding colonies for rearing relied on repeated field collections from Japan. Collections were

made in fall 2010, 2012, 2015, and 2019 from the mountains of Honshu Island with the intention of collecting potentially more cold-hardy beetles for rearing. Upon receiving APHIS approval to release *La. osakensis*, rearing labs in the eastern US shifted focus from *La. nigrinus* to *La. osakensis*. Between 2011 and 2021, the two major rearing labs (Virginia Tech and Univ. Tennessee) combined to produce a total of 152,483 adult *La. osakensis*.

The *La. osakensis* rearing effort was complicated, and initial releases were delayed, due to the discovery of a small number of individuals of a cryptic and previously undescribed species, *La. naganoensis* Leschen, among the field collected adults from Japan (Fischer et al. 2014). A purification protocol was developed to ensure that no *La. naganoensis* contaminated the *La. osakensis* colonies used for releases in the eastern US. To accomplish this, colonies were divided into separate cohorts, each containing 20 adults. The progeny of each cohort was kept separate through the rearing process. The first year of purification found that six of the eight cohorts contained at least one male and female *La. naganoensis*. The progeny from these cohorts were not used for operational rearing. Additionally, one cohort contained 1 female *La. naganoensis*, and that cohort was removed as well, as it was possible the female had mated in the field prior to separating into cohorts in the lab. The remaining cohort was used to start the next generation for use in operational rearing and release (Fischer et al. 2014). This purification process was repeated for every new wild collection of *La. osakensis*.

In 2017, the original 2010 FONSI from USDA APHIS was amended to dismiss the requirement that colony purification be carried out under quarantine conditions (USDA 2017). Because *Laricobius* spp. are all adelgid specialists and cannot survive on any other insects, an accommodation was made by way of this amendment for allowing *La. naganoensis* to be reared and released with *La. osakensis*. However, the petitioner's intention for requesting this accommodation was to allow rearing labs the opportunity to purify field-collected *La. osakensis* colonies in less-constraining facilities than in ill-suited quarantine facilities. The 2019 collection of beetles from Japan was subjected to the same purification process as all previous field collections, and no releases of *La. naganoensis* have been made in North America to date.

3.2.3. Establishment, spread, and impact

The first four releases of *La. osakensis* were made in Virginia and West Virginia in 2012 and 2013 (Mooneyham et al. 2016) followed by six more in 2014 and 2015. All sites were sampled for evidence of establishment between 2015 and 2017, and *La. osakensis* larvae were recovered from five sites (Toland et al. 2018). The greatest number of larvae were recovered from the northernmost release site in Pennsylvania (Plant Hardiness Zone 6a). As of 2021, at least 76,000 adults and about 3,000 eggs have been released at 88 sites ranging from Tennessee to Maine. Over 1,800 adults and nearly 600 larvae have been recovered from at least 22 of those sites (Table 1). In fall 2021, 1,684 adults were collected from a single release site in Pennsylvania and were redistributed to four new sites in Pennsylvania and Maryland. Field collection and redistribution of *La. osakensis* is also occurring in the mountains of western North Carolina.

The only published impact studies on *La. osakensis* conducted to date demonstrated that adults actively feed on HWA sistens nymphs and adults. For the entire duration of the study, adult beetles fed on an average of 1.2 sistens adults per day, whereas larval predation was estimated at 1.3 ovisacs per day, resulting in use of more than 40 ovisacs per individual during their development (Vieira et al. 2013b). Lamb et al. (2005) observed overall predation rate numbers for *La. osakensis* that were lower than those reported for *La. nigrinus*, but this was attributed to lower densities of HWA in the *La. osakensis* cages and the predators running out of prey. Exclusion cage studies conducted in Japan demonstrated that *La. osakensis* greatly impacted HWA sistens populations (Lamb et al. 2008). At this time, more conclusive exclusion cage studies with *La. osakensis* established in the eastern US have not been initiated; thus, it is uncertain whether *La. osakensis* are more

impactful on HWA than *La. nigrinus*, as predicted by [Vieira et al. \(2012\)](#). As the release and recovery program matures, this line of investigation will likely be pursued.

3.2.4. Summary and Outlook: *Laricobius osakensis*

1. *Has the agent established in the field?* Yes.
2. *In areas where established, is it reducing HWA population density?* Not sufficiently evaluated.
3. *In areas where it is reducing HWA density, is it improving hemlock health?* Not evaluated.

Laboratory rearing of *La. osakensis* is ongoing and will eventually be phased out as the number of established sites increases and re-distribution of field collected beetles becomes routine. Additional collections from Japan to replenish laboratory colonies may not be necessary as collections are starting to be made from established sites in the eastern US. The successful establishment and population growth of *La. osakensis* is encouraging, and periodic re-assessment of the impacts of biological control agents on HWA and hemlock health would help determine its overall efficacy. Although *La. osakensis* occupies the same temporal niche as *La. nigrinus*, it is unknown if one species is superior to the other in terms of impacts on HWA in different parts of the introduced range of HWA in the eastern US. Efforts to evaluate effects of *La. osakensis* on HWA population densities and hemlock health in different portions of the introduced range would help determine where establishment would be most beneficial.

4. Chamaemyiid predators

4.1. Importance of Chamaemyiidae and Leucotaraxis in biological control

Silver flies (Diptera: Chamaemyiidae) are a family of predators, almost all of which feed as larvae on Aphidoidea and/or Coccoidea ([Gaimari 2010](#)). Many species are commonly found feeding on adelgids, so have been studied as potential biological control agents. [Zilahi-Balogh \(2002a\)](#), [Ross et al. \(2011\)](#), and [Montgomery and Havill \(2014\)](#) provide reviews of previous efforts to use silver flies for biological control of adelgids. The efficacy of using silver flies to control adelgids has been mixed. Species of *Cremifania*, *Leucopis*, and *Neoleucopis* failed to control balsam woolly adelgid after introduction from Europe and India to North America ([Montgomery and Havill 2014](#)). However, species of *Neoleucopis* (Blanchard) are reported to have controlled *Pineus pini* (Macquart) in Hawaii ([Culliney et al. 1988](#)), and *Pineus boernerii* Annand in New Zealand ([Zondag and Nuttall 1989](#)) and Chile ([Francke-Gros-mann 1962](#)). For the attempts that resulted in successful control, the host pine (*Pinus*) trees of the adelgids were also non-native. In Hawaii, pines are considered by many to be invasive pests themselves ([Oppenheimer 2002](#)), so from some perspectives, controlling the adelgids may not have been the most desirable outcome.

Previous to their use against HWA, flies in the genus *Leucotaraxis* had not been used as biological control agents. *Leucotaraxis argenticollis* (Zetterstedt) and *Le. piniperda* (Malloch), the two species discussed here, were previously in the genus *Leucopis*. A taxonomic revision by [Gaimari and Havill \(2021\)](#) placed these species, plus *Le. atrifacies* (Aldrich) and *Le. sepiola* (Gaimari and Havill), into the new genus because of their morphological and evolutionary distinction from the other members of *Leucopis*. These four species form a distinct monophyletic group that feeds only on adelgids. [Gaimari and Havill \(2021\)](#) produced a key to all silver fly species known to feed on adelgids; *Leucotaraxis* can be distinguished from *Leucopis* by features of the scutum and frons.

In addition to belonging to an evolutionarily divergent genus that feeds only on adelgids ([Gaimari and Havill 2021](#)), two additional studies found evidence that *Le. argenticollis* and *Le. piniperda* each contain genetically distinct lineages specific to different adelgid species. In western North America, specimens of both species collected from HWA

are phylogenetically divergent from those collected from adelgids on pine and spruce in the eastern U.S. ([Havill et al. 2018](#)). This suggests that within each species, there are distinct lineages that specialize on different adelgid prey in the different regions ([Havill et al. 2018](#)). In a follow-up study, [Havill et al. \(2023\)](#) analyzed additional samples with microsatellite markers which are capable of picking up fine scale patterns of differentiation. This study, which included more flies collected from other adelgid prey species in western North America, confirmed the east–west divergence within each species that was reported in [Havill et al. \(2018\)](#), as well as differentiation associated with feeding on different adelgid prey species in western North America. For both species, there were distinct lineages specific to feeding on HWA versus feeding other adelgid prey species that use *Abies* and *Pinus* as host plants. This genetic differentiation may be due to adaption to using different host plant cues to locate adelgids on different hosts, but this trait has yet to be studied. This study suggests a genetic basis for prey specificity and attests to the importance of continuing to collect flies from HWA and not from other adelgid species in the Pacific Northwest to maximize the chances of controlling HWA in eastern North America.

4.2. HWA and Leucotaraxis predators in western North America

Western hemlock, *T. heterophylla*, is distributed from coastal southeast Alaska, southward through coastal and interior British Columbia, Washington, Idaho, western Montana, Oregon, and coastal northern California ([Little 1971](#)). In the past, detection of HWA infestations on western hemlock was mostly by chance because it posed no economic threat. Indeed, HWA was not even mentioned as a pest of *T. heterophylla* in the USDA Forest Service Silvics of North America ([Burns and Honkala 1990](#)). The search for predators for use in eastern North America for biological control has spurred in earnest the exploration for HWA in western North America, but with limited resources, vast areas of the western hemlock range have yet to be evaluated.

Interest in the natural enemy complex of HWA on *T. heterophylla* increased after the discovery and subsequent research on *La. nigrinus* as a possible biocontrol agent in eastern North America ([Zilahi-Balogh et al. 2003a, 2003b, 2003c](#)) and accelerated after it was determined that the HWA lineage in western North America is native to that region ([Havill et al. 2007, 2016b](#)). One of the difficulties of working on HWA in western North America is that populations regularly fluctuate over the span of a few years at any one site, requiring constant survey work to locate infestations for research. [Kohler et al. \(2008a\)](#) surveyed natural enemies associated with HWA on *T. heterophylla* at 16 sites in Oregon and Washington in 2005 and 2006. Sampling infested foliage with a beat sheet, they found 55 predator species in 14 families with most identified as generalists. Of the specialist predators they found, *La. nigrinus* was the most numerous, but they also found the chamaemyiid flies, *Le. argenticollis* and *Le. piniperda*, to be abundant. This was the first record of either of these *Leucotaraxis* species pre-dating HWA ([Kohler et al. 2008a](#)). Sampling with a beat sheet, the abundances of *Le. argenticollis* and *Le. piniperda* were likely underestimated relative to *La. nigrinus*, as reflected in the ratio of immatures to adults collected, which was three times higher for the chamaemyiids ([Ross et al. 2011](#)). Later studies that examined HWA infested foliage under a microscope, favoring detection of larvae, found these *Leucotaraxis* species to be the most abundant predators ([Kohler et al. 2016, Rose et al. 2019](#)). This pattern does not always hold at individual sites, as [Dietschler et al. \(2021\)](#) found predator abundance to vary by site and year, with instances where *La. nigrinus* or either species of *Leucotaraxis* were most abundant at a particular site.

When pooled together, the larval abundance of the *Leucotaraxis* species was found to be synchronous with adults producing progrediens eggs in spring and sistens eggs in early summer ([Kohler et al. 2008a, Grubin et al. 2011, Kohler et al. 2016](#)), but these studies did not distinguish the dynamics of each species separately because molecular diagnostics to separate immatures of the species were not yet available. [Rose et al. \(2019\)](#) used DNA barcoding to distinguish between larval

Leucotaraxis species and found no difference in the phenology of the two species, but sample size was low and data from multiple sites was combined. A short, 29-day study was made of adult emergence using DNA barcoding to distinguish species with collections from Washington and Oregon beginning at the end of March and reared in a lab at 20–25 °C (Neidermeier et al. 2020). With a robust sample size, they were able to barcode a 20% subsample of emerging adults and found emergence peaks of the species were temporally partitioned, with *Le. piniperda* emerging prior to *Le. argenticollis*.

A pattern of species partitioning was also found by Dietschler et al. (2021) who used DNA barcoding and morphological identifications to track emergence for two seasons of each *Leucotaraxis* species and *La. nigrinus* from three sites in the northern Puget Sound, Washington. For this study, monthly sampling was initiated in early March, and they found that rearing the immature flies in the lab (range 13–20 °C, Celis et al. 2022) was accelerating insect development and that observed emergence patterns were a byproduct of sampling the same cohort multiple times at each study site. By incorporating accumulated heating degree days at the time of adult emergence, they were able to compare temporal emergence patterns between sites, independent of abundance, and found the same pattern was repeated: *Le. argenticollis* adults emerged first in spring, then *La. nigrinus* pre-pupae dropped to their pupation sites in the soil, followed by *Le. piniperda* adults emerging, and finally a second group of *Le. argenticollis* adults emerged in early summer.

The dropping of the univoltine *La. nigrinus* prepupae during the progrediens egg stage (Zilahi-Balogh et al. 2003a) in lab rearing containers was key to understanding emergence timing, the number of generations of the *Leucotaraxis* spp., and potential for competition between these three predators. *Le. argenticollis* adults emerging just prior to *La. nigrinus* larval drop indicates they may be laying eggs while *La. nigrinus* larvae are feeding on HWA progrediens eggs. The life span of the flies in the wild is unknown and it is possible that they may be laying eggs over an extended period. Emergence of *Le. argenticollis* prior to *Le. piniperda* is contradicted by Neidermeier et al. (2020), but their study started a month later, potentially missing the first emergence peak of *Le. argenticollis* found by Dietschler et al. (2021). The two emergence peaks for *Le. argenticollis* found by Dietschler et al. (2021) indicate either that there could be two generations per year, or some individuals are undergoing a facultative diapause to delay development until later in the season. The possibility exists that *Le. argenticollis* may be using a strategy of multiple developmental periods to increase its chances of eclosing in more favorable conditions (Tammeru et al. 1999). In contrast, Dietschler et al. (2021) found only a single adult emergence peak for *Le. piniperda*, suggesting that this species is univoltine. This result is contradicted by Neidermeier et al. (2020), who found two emergence peaks for *Le. piniperda* over a 29-day period, but as described above, they may have sampled the same cohort twice, and accelerated development caused by lab rearing conditions could have resulted in two emergence peaks.

4.3. Collection, rearing, and release

Collections of western *Leucotaraxis* spp. for research and for field release have mostly been made in the Puget Sound region of Washington state and western Oregon (Kohler et al. 2008a, 2008b, Motley et al. 2017, Neidermeier et al. 2020, Dietschler et al. 2021, Celis et al. 2022). More recent collections have also been made in southwestern British Columbia (Celis et al. 2022) and northern California (Havill et al., 2023). Due to both *Le. argenticollis* and *Le. piniperda* being present in both eastern and western North America (Havill et al. 2018), and on other adelgid hosts in western North America (Havill et al. 2023), only flies collected from HWA-infested western hemlock foliage are imported for biological control.

Western hemlocks are home to large numbers of arthropods, including parasitoids that target *Leucotaraxis* (Kohler et al. 2008a, 2008b, Celis et al. 2022), therefore specialized training and quarantine

facilities are needed to safely rear *Leucotaraxis* adults from western foliage in the east coast. Adult western *La. nigrinus* can be readily collected in its native range with beat sampling (Zilahi-Balogh et al. 2002b, Kohler et al. 2008a), but the more fragile *Leucotaraxis* spp. adults can only be obtained in large numbers by collecting immature stages on infested western hemlock branches for rearing to the adult stage. These *Leucotaraxis* species pupariate on the hemlock branches near the larval feeding sites, so eggs, larvae, and puparia can all be collected with infested foliage. The genetically distinct western lineage of HWA is not present in eastern North America (Havill et al. 2006, 2016b), so western foliage must be carefully handled and packaged for shipping to eastern quarantine facilities to prevent accidental release of western HWA, as well as parasitoids of *Leucotaraxis* (Celis et al. 2022) and any other undesired species.

To rear *Leucotaraxis* adults, western hemlock branches (0.5 – 1 m in length) with moderate to heavy densities of HWA are clipped from the trees and are shipped overnight (Dietschler et al. 2021, Celis et al. 2022). Branches are carefully packaged to prevent escape of arthropods, are sent to authorized quarantine facilities, and containers are carefully opened, all in accordance with USDA APHIS permit guidelines. At the Sarkaria Arthropod Research Laboratory greenhouse (Cornell University, Ithaca, NY), containers are opened by personnel wearing full Tyvek suits with attached booties and nitrile gloves sealed to the suit sleeves with masking tape. The greenhouse has natural lighting and is temperature controlled between 13 and 20 °C (Celis et al. 2022) to moderate the rate of insect development and slow foliage decline. Infested foliage is cut into 30–50 cm pieces and placed inside custom-fabricated acrylic cages with 120- μ m mesh to allow ventilation and prevent the escape of HWA crawlers and other insects. Infested hemlock branches are inserted in saturated floral foam in plastic shoeboxes to keep branches hydrated. Cages are checked daily in the afternoon for insect emergence. Adult *Le. argenticollis* and *Le. piniperda* are collected using an aspirator. Flies are grouped each day by foliage collection date and site of origin. Adult *Leucotaraxis* are housed together temporarily in containers with a water source and honey-wheat to allow for mating to occur prior to release. Daily, at least two male flies from each collection period and site are identified to species (using methods described in Dietschler et al. 2021 and in section 4.6 below) to track species composition over time. During times when a transition of species emergence is expected, more flies are screened for species identification. This process allows personnel to efficiently prepare flies for research or release purposes with a high degree of confidence in the species composition.

Laboratory rearing for research purposes has been used to investigate *Leucotaraxis* spp. biology. Rearing *Leucotaraxis* from egg to adult (Fig. 7) is possible with access to a steady supply of eastern hemlock infested with ovipositing HWA (Dietschler et al. 2023) and provides access to immature stages for research that are unavailable from western collections. This approach could be modified to scale up rearing multiple generations for mass release purposes, but their lengthy life cycle and our incomplete understanding of developmental cues may present challenges for phenological matching of colony offspring with available prey.

4.4. Observations on development and behavior

Leucotaraxis eggs are usually laid on, under, or inside of HWA ovisacs, which is similar to oviposition behavior seen in some other Chamaemyiids (e.g., Gaimari and Turner 1997). The egg chorion does not deflate upon hatch, but hatching can be verified under the microscope. First-instar larvae are clear to whitish and develop yellow coloration after a period of feeding. Larval exuvia are nearly impossible to locate as they are always white and are shed within HWA ovisacs, which are also white. Because of this, it has not been possible to model development rate for each of the three larval instars individually. Larvae feed primarily on HWA eggs but have been observed on occasion attempting to feed on a crawler or a settled nymph. Adult HWA are often killed by

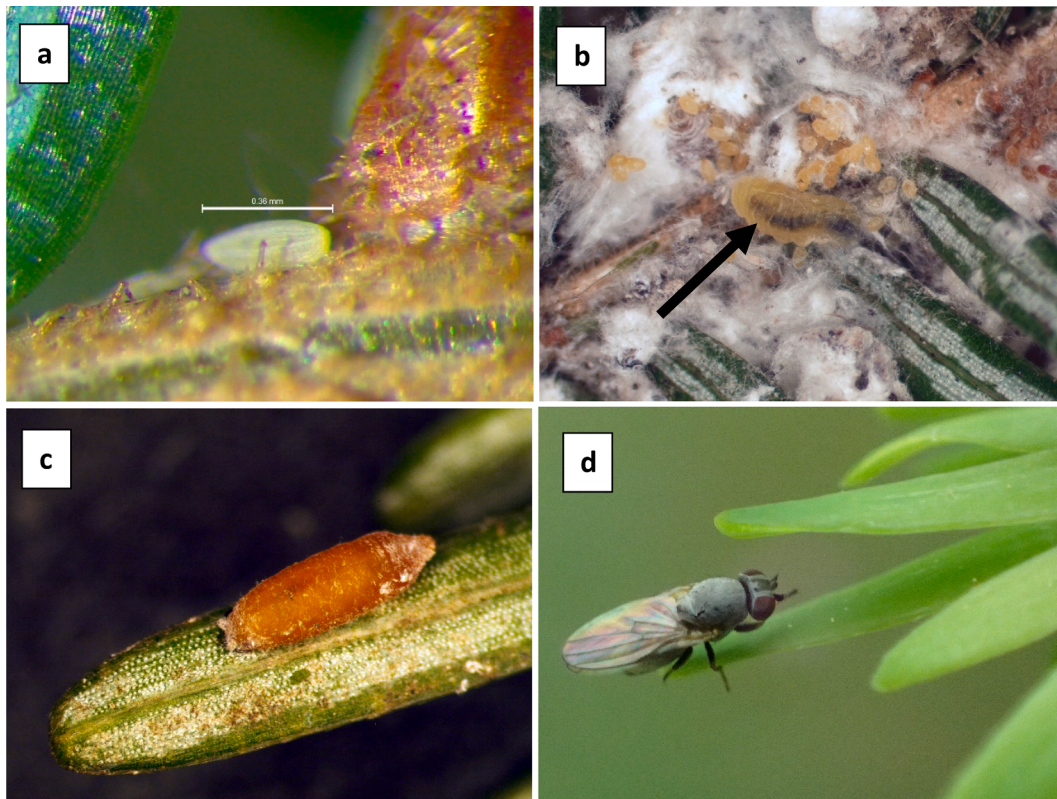


Fig. 7. Life stages of *Leucotaraxis* spp. that prey on *Adelges tsugae* in western North America: (a) egg, which has been removed from the wool of an *A. tsugae* ovisac to increase visibility, (b) larva next to *A. tsugae* eggs, (c) puparium, (d) adult. Photo credits: Tonya Bittner, Cornell University (a), Nathan Havill, USDA Forest Service (b, c), and Bryan Mudder, USDA Forest Service (d).

Leucotaraxis larvae, but it is not clear if this is the result of predation or, more likely, from the adelgids being physically dislodged from their feeding sites. Larvae excrete a sticky, glossy black frass that increases in quantity with larval size and which is either the same or visually indistinguishable from a black substance used to adhere the puparium to the plant, similar to that seen in *Leucopis* predators of wheat aphids (Gaimari and Turner 1997). Like other cyclorrhaphous flies, they have a postfeeding larva stage, which is a third-instar larva that has ceased feeding (Fraenkel and Bhaskaran 1973) and either sits still or locomotes, presumably in search of a pupation site. We have observed adult flies appearing to feed on large droplets of honeydew that accrue on ovisacs when foliage is stored in a cold room. Species of silver flies have been observed “milking” aphid honeydew (e.g., Gaimari and Turner 1997), but we have not observed this behavior in these species.

The potential for parasitism in this group was reviewed by Ross et al. (2011) and confirmed for these species by Kohler et al. (2008b) and Celis et al. (2022). Both studies found solitary parasitoids in the genus *Pachyneuron* (Hymenoptera: Pteromalidae) and *Melanips* (Hymenoptera: Figitidae) emerge from *Leucotaraxis* puparia. Celis et al. (2022) noted that *Melanips* were more closely associated with *Le. piniperda* and *Pachyneuron* with *Le. argenticollis*, and also found that the rate of parasitism increased through the growing season. Because larvae and puparia can contain immature parasitoids that will emerge during the puparial stage, it is imperative to rear flies to the adult stage prior to release.

4.5. Release history

A few small releases were made in 2015, with groups of 36 and 20 *Leucotaraxis* spp. released in New York, and 123 flies released in Tennessee. The first sizeable field releases with the goal of establishing western *Leucotaraxis* spp. populations for HWA management in the

eastern US began in 2017. A total of at least 42,000 adult *Le. argenticollis* and *Le. piniperda* (species combined) have been released in eight states throughout the infested range of eastern hemlock (Table 1). From 2015 to 2019, all field releases of *Leucotaraxis* were conducted using identifications at the genus level (Virginia Tech 2022), because no methods were available to determine the species of living flies in bulk, and *Le. argenticollis* and *Le. piniperda* are very similar morphologically. Prior to 2021, genetic identification was the only reliable published method to separate species (Havill et al. 2018). This proved to be slow and difficult to implement, however, when making real-time release decisions with relatively short-lived organisms. New methods have led to expanded capabilities to efficiently separate living adult *Leucotaraxis* by species for field release and research (Dietschler et al. 2021) leading to a shift toward single species releases since 2021.

4.6. Release technique

To date, all free field releases of *Le. argenticollis* and *Le. piniperda* have been performed with adults reared from wild-collected HWA infested western hemlock branches. The potentially high prevalence of parasitoids in wild *Leucotaraxis* collections highlight the importance for trained individuals to process all adult flies prior to release, due to the currently unknown distribution of these parasitoids on the east coast (Celis et al. 2022). Prior to 2021, adult emergence patterns were unknown, with no techniques to quickly identify living adults, leading to *Le. argenticollis* and *Le. piniperda* often being combined for release. Dietschler et al. (2021) demonstrated that living adult male *Leucotaraxis* can be identified without chilling them using the unique external morphology of genitalia. Female flies are identified by differences in postpronotal setae (Gaimari and Havill 2021), but it may be necessary to chill the flies to see this under the microscope. The propensity for species to emerge in temporally-separate species clusters, coupled with 50:50 sex ratios at

eclosion (Dietschler et al. 2021), enables the separation of species for research and release examining unique genitalia characters in a sample of males from each cluster.

Identifying the correct phenological stage of the prey necessary for the predators to feed and reproduce is crucial for effective establishment. *Leucotaraxis* spp. are most abundant during the egg laying stages of both generations of HWA in the western North American range, indicating this is the primary stage when they are laying eggs and early instar larvae are feeding (Grubin et al. 2011). Therefore, release of adult *Leucotaraxis* in eastern North America should coincide with these phenological stages of HWA development to increase their probability of establishment. Development of HWA can be variable by location and year (McClure 1987, Gray and Salom 1996, Zilahi-Balogh et al. 2003a, Mausel et al. 2008, Joseph et al. 2011a), meaning that close observation of prey development is needed to time releases with the appropriate stage of prey. Detailed research on the development of HWA has made tracking development possible (Salom et al. 2002, Limbu et al. 2022), leading to phenological models to help predict development (Tobin and Turcotte 2018).

Information on the biological needs for successful *Leucotaraxis* development and field establishment is limited (i.e., prey resource density, abiotic conditions, etc.). Currently, release protocols rely on conditions shown to optimize *La. nigrinus* establishment while data for *Leucotaraxis* is gathered. Suitable release sites for *La. nigrinus* have been defined as having greater than 50% hemlock composition, high density of HWA ($\geq 20\%$ trees infested), and healthy trees with high live crown ratio and foliage density (Mausel et al. 2010, Mayfield et al. 2020). Research indicates that *La. nigrinus* has a lower probability of establishment in colder regions (Mausel et al. 2010), but recent research shows *Le. argenticollis* to be tolerant of cold winters throughout much of the HWA-infested range (Dietschler et al. 2023). Two primary methods for releasing adult flies have been used: 1) confined release using mesh insect bags and 2) free release of adults into an infested stand. Confined bag releases consist of placing a large nylon mesh bag over an HWA-infested branch, securing the bag to the branch with a zip tie and foam pipe insulation, then releasing a known quantity of adult male and female flies into the bag (Motley et al. 2017). Field releases using bag enclosures are ideal for research and/or smaller quantity releases for establishment. As field collection and lab sorting methods have improved (Neidermeier et al. 2020, Dietschler et al. 2021, Celis et al. 2022), free releases of mostly single-species populations have enabled free release of larger quantities of adult *Leucotaraxis* spp., similar to methods used for *Laricobius* spp. (Mausel et al. 2010, Mayfield et al. 2020).

4.7. Monitoring approaches

Insects released as biological control agents targeting other insects have a historical success rate (achievement of satisfactory control of the pest) of about 10% (Cock et al. 2016) and it can take many years to determine whether control is being achieved. Although establishment of a reproducing population does not equate to successful biocontrol, it is a first positive indication toward that goal. Monitoring of release sites for any early signs of successful reproduction and potential establishment can provide confirmation that the biocontrol effort is worth continuing and may provide clues to improve the release strategies along the way. Currently, researchers are pursuing monitoring programs targeting the collection of various predator life stages as well as environmental DNA signals to look for early signs of establishment for both *Leucotaraxis* spp. and *Laricobius* spp.

Foliage collections are one way to determine if *Leucotaraxis* spp. and *Laricobius* spp. life stages are present in the field. A simple setup consists of collecting HWA-infested foliage, with the cut ends submerged in a water source, housed in a cage capable of containing small insects and a light source to attract them. However, the efficiency of collecting from multiple samples can be greatly increased using a custom container such

as the one described in Mayfield et al. (2021), with collecting vessels at the top and bottom to collect both adult flies (and adult beetles) above and pre-pupal drop of beetles below. Researchers have modified these “Lari-Leuco” containers to hold larger amounts of foliage and are actively using them to monitor multiple sites. The timing of foliage collection and trapping is critically important to maximize success. Ideally at least two collections per site should be attempted, the first during the period when sistens adults are laying progrediens eggs, and the second when progrediens adults have laid sistens eggs. The first collection targets both *Le. argenticollis* adult emergence and larval drop of *Laricobius* spp. The second collection could yield *Le. piniperda* and potentially a second emergence of *Le. argenticollis*. Site-specific release history and phenology should be used to refine the sampling scheme.

Another monitoring approach for adult predators involves the use of prism-shaped, bright yellow sticky traps. These have been deployed in western North America to verify their efficacy; traps hung from infested western hemlock in sunny locations perform best (Troy Kimoto, unpublished data). These traps target adult predators and are less reliable than foliage collections, but they capture adults of both *Leucotaraxis* spp. and *La. nigrinus* (N. Dietschler, unpublished data).

Environmental DNA is an emerging tool for detecting the presence of a variety of species, both aquatic and terrestrial. Environmental DNA, or eDNA, is defined as a complex mixture of genomic DNA from many different organisms found in an environmental sample and includes both intracellular and extracellular DNA fragments in various states of degradation (Taberlet et al. 2018). After collecting this mixture (template) from the environment, specific primers can be used to detect species of interest and quantify their DNA using qPCR. Species-specific qPCR assays were designed and tested for the detection of HWA, *Le. argenticollis* (eastern and western lineages), *Le. piniperda* (eastern and western lineages), and the beetles *La. nigrinus* and its sister species *La. rubidus* (Kirtane et al. 2022). Kirtane et al. (2022) obtained environmental samples by filtration of water from rinsing foliage samples, followed by a standard DNA isolation/purification kit and then qPCR. Using foliage samples of HWA infested *T. heterophylla* from the western range of *Leucotaraxis* spp., they detected eDNA of western *Leucotaraxis* lineages on twigs after immature flies had been counted and removed. This provided evidence that the method should work when western flies are present on infested eastern hemlock for biocontrol. Ideally, positive eDNA signal would be paired with physical evidence of specimens to confirm recent occupancy at a site. Current work is focused on targeted site collections paired with one or more of the previously mentioned collecting approaches to look for a correspondence of eDNA signal and physical specimens.

4.8. Summary and Outlook: *Leucotaraxis argenticollis* and *Le. piniperda*

1. *Has the agent established in the field?* Evaluation for establishment is currently ongoing.
2. *In areas where established, is it reducing HWA population density?* Not yet applicable.
3. *In areas where it is reducing HWA density, is it improving hemlock health?* Not yet applicable.

Researchers in the Pacific Northwest have noted the abundance of the two specialist predators, *Le. argenticollis* and *Le. piniperda*, during the progrediens generation, when *La. nigrinus* is not active, and recognized their potential for biocontrol of HWA in eastern North America (Kohler et al. 2008a, Grubin et al. 2011, Kohler et al. 2016, Rose et al. 2019, Dietschler et al. 2021). Research has demonstrated that the western lineages of these *Leucotaraxis* species can feed and reproduce on eastern HWA (Motley et al. 2017), as well as survive the winter in some of the coldest locations of the eastern hemlock range (Dietschler et al. 2023a). Research in western North America is aimed at understanding the ecology of *Leucotaraxis* to optimize efficacy of their introduction in eastern North America. Exploration for western HWA populations

continues so that the availability of predators for release can be maintained. Post-release monitoring continues to evaluate *Leucotaraxis* population establishment and spread at release sites in eastern North America. A single western *Le. argenticollis* larvae was recently recovered in the Lower Hudson Valley of New York State, and while researchers were unable to confirm establishment (due to an augmentative release one month prior), this does provide evidence of successful reproduction from an unconfined release (Crandall et al. in press). Environmental DNA is a promising tool being developed to speed the assessment of *Leucotaraxis* introductions (Kirtane et al. 2021) to help guide the most efficient use of limited predator numbers released for biocontrol of HWA.

5. Discussion of overall program status and outlook

To date, the classical biological control program for HWA has not prevented hemlock mortality and decline. Whether the program will ultimately be successful in this matter is uncertain. Throughout its tenure, the program has pursued the long-term objective of establishing a diversity of natural enemies that collectively regulate HWA populations in the introduced range. This goal for multiple agents is based in the natural enemy diversity that exists in regions where the adelgid is native (Yu et al. 2000, Kohler et al. 2008a, Montgomery and Keena 2011) and characteristics of the pest life cycle (high fecundity, multiple generations, prolonged period of asexual reproduction) that make HWA difficult to control with a single agent. As the preceding sections demonstrate, substantial progress has been made in the last three decades toward achieving this objective. Seven different insect predator species from Asia and western North America representing four genera (*Scymnus*, *Sasajiscymnus*, *Laricobius*, and *Leucotaraxis*) have been evaluated and released, and three of these (*Sa. tsugae*, *La. nigrinus* and *La. osakensis*) are now established in eastern North America. Since the initial releases of *La. nigrinus* and *La. osakensis* (20 and 11 years ago, respectively), these species have become widely established throughout the range of eastern and Carolina hemlock (Mausel et al. 2010, Toland et al. 2018, Foley et al. 2019, Jubb et al. 2021) and can be field-collected in numbers sufficient to redistribute populations to new localities. Furthermore, western lineages of the two species of *Leucotaraxis* (*Le. argenticollis* and *Le. piniperda*, released since 2015) are able to reproduce on the Japanese lineage of HWA on eastern hemlock (Motley et al. 2017), and recent research shows that western *Le. argenticollis* can overwinter in the eastern US (Dietschler et al. 2023). These developments are reason for optimism.

In general, biological regulation of HWA populations below acceptable damage thresholds in eastern North America has yet to be achieved. There are demonstrable impacts on winter stages of the adelgid by the well-established *Laricobius* species (Jubb et al. 2020), but density-dependent population rebound in the progrediens generation, coupled with lack of predation on sistens eggs, compensates for these losses to *Laricobius* predation (Crandall et al. 2020, Preston et al. 2023). Although the established coccinellid predator *Sa. tsugae* can prey on both generations of HWA (Cheah 2011), conclusive evidence linking this predator to measurable field impacts on HWA has been elusive. The most immediate prospects for natural enemies that could effectively complement the *Laricobius* species, by preying on both adelgid generations, are the western lineages of *Le. argenticollis* and *Le. piniperda*. The western genetic lineages of these species are extremely prey-specific (Havill et al. 2023), are found in high abundance with *La. nigrinus* in the Pacific Northwest (Kohler et al. 2016) and exhibit temporal niche differentiation that appears to complement *La. nigrinus* activity (Rose et al. 2019, Neidermeier et al. 2020, Dietschler et al. 2021). Furthermore, data presented by Crandall et al. (2022) suggest that HWA populations in the Pacific Northwest are more strongly regulated by top-down effects associated with predation by the native natural enemy complex than by bottom-up factors mediated through the host.

As such, continued release, monitoring and research associated with

the *Laricobius* and *Leucotaraxis* species will be needed to determine if their combined effect can regulate HWA below host-damaging levels in eastern North America. Similar to the approach for *Laricobius*, releases of *Leucotaraxis* should focus on areas where hemlock is abundant and highly valued, and where there is a commitment to monitoring for establishment and abundance. Recently, guidelines for monitoring *Laricobius* and *Leucotaraxis* have been combined into a single survey approach (Dietschler et al. 2023b), utilizing carefully-timed foliage sampling and specialized rearing containers that help separate individuals of the different genera (Mayfield et al. 2021). Careful specimen preservation and molecular identification of field-recovered insects is key to this effort, as it enables immature insects to be differentiated by species, and *Leucotaraxis* species to be further differentiated by their original geographic, prey-associated lineages (Havill et al., 2023). This ensures that rare recoveries of eastern lineages of *Le. argenticollis* or *Le. piniperda*, usually associated with adelgids of non-hemlock hosts, are not mistaken as evidence of establishment of western-lineage populations released for biological control. Committed and consistent use of the online HWA Predator Database (Virginia Tech 2022) by stakeholders to track releases and recovery efforts for all predator species will also help develop a comprehensive picture of how well various agents are establishing and spreading. The database can also be used to strategically identify sites appropriate for new releases, augmentation of established populations, or pursuit of research questions.

Field insectaries are anticipated to be an integral component of the HWA biological control program as it continues to mature. Field insectaries are natural or planted stands of hemlock where production and maintenance of both prey and predator populations are encouraged, and from which predators can be periodically harvested and redistributed (Salom et al. 2011). Although laboratory rearing has been essential to the early phases of production for each predator species, it is technically challenging, labor and space intensive, and expensive to maintain (Foley et al. 2021). Ironically, the natural spread of established *Laricobius* populations has complicated laboratory rearing efforts, because HWA-infested foliage collected from the field to feed predators in the lab often contains *Laricobius* life stages from wild populations (Foley et al. 2021). Given the establishment success of *La. nigrinus* and *La. osakensis* to date, a gradual shift toward more field-insectary production of *Laricobius* makes sense operationally and makes available limited laboratory resources for rearing the newer *Leucotaraxis* agents.

Operational shifts toward field-based production of predators, as well as repurposing existing lab infrastructure to accommodate the newer candidate agents (i.e., *Leucotaraxis*), should help decrease the overall cost-to-benefit ratio of the biocontrol effort. To our knowledge, no formal economic cost-benefit analysis of the HWA biological control program (or any other component of HWA management) has been conducted, and economic and ecological benefits of associated with HWA biological control may still be years in the making. Assessments of predator impacts on hemlock health and survival in the introduced range will be important to future demonstration of program benefits. This is a clear research gap in the program, as very few studies (Sumpter et al. 2018, Preston et al. 2023) have been designed to assess biological control impacts on hemlock health or survival.

The biological control program for HWA represents one component of a multifaceted integrated pest management strategy that also includes chemical control, silviculture, resistance breeding, gene conservation, monitoring, and research (HWA Initiative 2021). In the near term, biological control of HWA may be enhanced if implemented in tandem with one or more of the other field management tactics. For example, chemical control with systemic insecticides has been an important stop-gap measure to curtail hemlock mortality and preserve high value tree and stand health within the invaded range (Vose et al. 2013, Abella 2014). Several studies have demonstrated the potential to improve future prey abundance and quality by temporarily preserving foliage health on select hemlocks via chemical treatments with imidacloprid

(Joseph et al. 2011b, Eisenback et al. 2014, Mayfield et al. 2015). Mayfield et al. (2015) demonstrated that as previously-treated trees lost chemical protection, they produced a higher proportion of new shoots, had a lower proportion of dead shoot tips, harbored more adelgid prey, and were as frequently colonized by *Laricobius* predators as compared with untreated hemlocks. Sumpter et al. (2018) attempted to assess the integration of chemical and biological control using these tactics at a broader scale, but a cold temperature-induced, HWA mortality event in 2014–2015 thwarted *La. nigrinus* establishment and prevented expression of any predator-related effects. Nonetheless, successful establishment of both *La. nigrinus* and *La. osakensis* on hemlocks previously treated with imidacloprid continues to be observed by the authors, and established *Laricobius* populations are spreading naturally into areas containing insecticide-treated hemlocks (Foley et al. 2019). A suggested strategy for integrating chemical and biological control in forest stands was recently developed for resource managers and landowners (Mayfield et al. 2020).

Potential also exists to enhance biological control through integration with silvicultural tactics that manipulate sunlight on hemlock trees. Elevated light levels have been associated with reduced HWA densities and superior plant growth in several studies utilizing artificial shade on potted or planted young eastern hemlock seedlings (Hickin and Preisser 2015, Brantley et al. 2017, Mayfield and Jetton 2020, McAvoey et al. 2017). In a southern Appalachian field study, infested understory eastern hemlocks released in small canopy gaps had greater net photosynthesis, high foliar carbohydrate concentrations, and superior shoot and radial growth compared to unreleased trees, despite similar numbers of adelgids (Miniat et al. 2020). Mayfield et al. (in review) recently demonstrated that in the years following implementation of small canopy gaps, improvements in crown condition and percent basal area growth of HWA-infested hemlocks were directly related to the amount of tree competition removed. These studies suggest that silvicultural practices could be used to enhance the physiological tolerance of eastern hemlock to HWA infestation, resulting in trees and stands that provide a more consistent source of prey for biological control agents. They also suggest that infested hemlocks with elevated sunlight exposure may be preferable trees to choose for releases and repeated monitoring of predators.

In contrast to the narrow geographic range occupied by the Carolina hemlock in the southern Appalachians (Jetton et al. 2008), eastern hemlock is widely distributed from Nova Scotia west to Minnesota and south to Alabama, spanning elevations from sea level to 1500 m, USDA plant hardness zones 3 through 7, and a wide variety of soil and habitat types (Godman and Lancaster 1990, Potter et al. 2012, USDA ARS 2023). With such environmental diversity in the invaded host range, it is reasonable to expect that the success of HWA biological control, and the most effective combination of predators, will vary regionally (Mausel et al. 2010). As discussed above, there is evidence that the inland ecotype of *La. nigrinus* may be more suited to colder climates than the inland ecotype, and that *La. osakensis* may be particularly well suited for warmer parts of the invaded range. Research on environmental factors affecting the survival of *Le. argenticollis* and *Le. piniperda* in the eastern US has begun (e.g., Dietschler et al. 2023a); building on this line of research could help optimize the *Leucotaraxis* release strategy. Although no negative ecological nor operational consequences of hybridization between *La. nigrinus* and *La. rubidus* have yet been reported (Fischer et al. 2015), the degree of hybridization could vary regionally by forest type (i.e., as the relative abundance of *Pinus strobus* and *Pineus strobi* changes). Monitoring regional and temporal patterns in this hybridization phenomenon would help determine if it is having any influence on the effectiveness of HWA biological control. Consideration should also be given to the possibility that regionally optimal strategies may shift as projected changes in climate alter the behavior and distribution of organisms at multiple trophic levels in this complex system.

6. Conclusion

In the three decades since initial exploration for natural enemies began, the biological control program for HWA has both grown and evolved. Sustained public interest in the value of eastern and Carolina hemlock, committed support from government agencies, and a strong collaborative network of managers, scientists, and other stakeholders, has made the program productive. Initial focus on coccinellid predators (*Sa. tsugae* and *Scymnus* spp.) from China and Japan, which have not performed as hoped, has shifted to deronontid predators (*Laricobius* spp.) from the Pacific Northwest and Japan. The latter have established widely, show clear impacts on key portions of the adelgid life cycle, and are spreading naturally on the landscape. If lineages of the silver fly predators (*Leucotaraxis* spp.) from the Pacific Northwest can establish and effectively prey on the phases of the adelgid life cycle that are currently unaddressed by *Laricobius*, the HWA biological control program may well reach its goal of population regulation in the invaded range. If they cannot, there are few viable biocontrol options remaining. One option would be to renew work on *Scymnus camptodromus* by starting a new colony in quarantine and completing additional host range testing and genetic evaluation requested by USDA APHIS. Another option would be to explore for other silver fly species that feed on HWA in regions other than western North America. Results of research, release, and monitoring efforts on *Leucotaraxis* spp. in the next five to ten years are likely to reveal much about the ultimate direction and outcome of the biological control program. Success of the program is likely to be enhanced through integration with other management tactics, consideration of variable environmental conditions across regions, and recognition of an ever-changing climate.

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