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Establishment, hybridization and impact of *Laricobius* predators on insecticide-treated hemlocks: Exploring integrated management of the hemlock woolly adelgid

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An integrated management approach is needed to maintain eastern hemlock (*Tsuga canadensis* (L.) Carrière) in eastern North America and to minimize tree damage and mortality caused by the invasive hemlock woolly adelgid (*Adelges tsugae* Annand). This study examined the hypothesis that chemical control with low rates of insecticide and biological control can be combined in the same stand to impact adelgid populations, prolong crown health, and allow predator proliferation. Sixty *T. canadensis* trees in northern Georgia were individually treated via soil injection with 0%, 10%, or 25% of the label rate of imidacloprid insecticide, and the biological control predator *Laricobius nigrinus* Fender was released in the stand, two and four years later. By year seven, hemlocks treated with the 25% imidacloprid rate lost their insecticide protection, had significantly better crown health and higher adelgid densities than untreated trees, and supported as many *Laricobius* predator larvae as untreated trees. In year seven, no residues of imidacloprid were detected in *Laricobius* larvae feeding on previously-treated hemlocks. Most (77%) of the predators collected on study trees were identified as *L. nigrinus*, 12% were the native congener *Laricobius rubidus* LeConte, and 11% were hybrids between the introduced and native species. The hybridization rate remained stable over time. The density of undisturbed *A. tsugae* ovisacs was twice as high on branches protected from predators as compared with branches exposed to predators. Results suggest that chemical and biological control of *A. tsugae* can be successfully integrated to help prolong hemlock health, although additional predators may be necessary to protect hemlock trees in the southern Appalachians.

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1. Introduction

Eastern hemlock (*Tsuga canadensis* (L.) Carrière) is an ecologically important North American conifer distributed from Nova Scotia, west to Minnesota, and south through the Appalachian Mountain region into northern Georgia and Alabama (Godman and Lancaster, 1990). This long-lived, shade tolerant evergreen is considered a "foundation" species due to its robust influence on the vegetative structure, faunal species assemblages, and ecological processes in the ecosystems in which it occurs (Ellison et al., 2005; Vose et al., 2013). Due to its crown architecture, potential size, and ease of cultivation, eastern hemlock also has high aesthetic value in parks, recreational areas, and residential landscapes (Holmes et al., 2010; Havill et al., 2014). The sustainability of both eastern hemlock and the southern Appalachian species Carolina hemlock (*Tsuga caroliniana* Engelm.) are threatened by the invasive hemlock woolly adelgid (*Adelges tsugae* Annand), an insect native to Asia and western North America but which was introduced to

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the eastern U.S. from Japan by 1951 (Havill et al., 2006). Hemlock woolly adelgid has caused widespread decline and mortality in all age classes of *T. canadensis* in forests from New England to the southern Appalachians, with resulting or expected impacts on forest stand dynamics (Orwig, 2002); hydrologic processes (Ford and Vose, 2007), carbon and nutrient cycling (Knoepp et al., 2011), and vertebrate and invertebrate communities (Tingley et al., 2002; Adkins and Rieske, 2013).

In North America, *A. tsugae* produces two asexual generations per year: an overwintering "sistens" generation and a spring "progrediens" generation. First instar "crawlers" of both generations disperse either actively by crawling or passively by wind, birds or other animals. Crawlers insert a thin bundle of stylet mouthparts into the twig and feed on carbohydrates stored in the ray parenchyma cells. Shortly after settling on new shoots in early summer, sistens nymphs enter an inactive aestivation period but resume development in the fall, and secrete a waxy, wool-like substance that eventually serves as an ovisac for the adult female. Brood produced by sistens adults hatch in the spring and develop into either sessile progrediens adults or winged sexuparae. In Japan, sexuparae migrate and initiate a sexual generation on spruce (*Picea* sp.) but this phase of the life cycle is unsuccessful in North America and does not contribute to adelgid population growth (McClure, 1989, 1990; Havill et al., 2014).

A number of management strategies to minimize the impacts of *A. tsugae* on *T. canadensis* are being pursued, including chemical control (Cowles et al., 2006), biological control (Onken and Reardon, 2011), silvicultural manipulations (Fajvan, 2008), host gene conservation (Jetton et al., 2013), and enhancement of host resistance (Montgomery et al., 2009). The systemic neonicotinoid insecticide imidacloprid has been used widely to provide temporary protection to individual hemlock trees and can persist at efficacious concentrations within twigs for multiple years (Cowles et al., 2006; Coots et al., 2013). Imidacloprid is metabolized within the tree to produce at least two other compounds with insecticidal properties: olefin-imidacloprid and 5-hydroxy-imidacloprid. The olefin-imidacloprid metabolite is up to ten times more toxic than imidacloprid against some species of aphids (Nauen et al., 1998) and persists in treated hemlock trees for three or more years, whereas 5-hydroxy-imidacloprid is less toxic and less persistent (Coots et al., 2013). Although use of insecticides has been a critical short-term method for saving hemlock trees, it is not a viable stand-alone management strategy for several reasons, including: (1) treatments must be applied on an individual tree basis, (2) environmental concerns limit the location and amount of insecticide that can be applied per hectare, and (3) the time, cost, and labor associated with re-treating trees at regular intervals is prohibitive.

Biological control of *A. tsugae* has focused on several predator species from the native ranges of *A. tsugae* in Asia and western North America (Onken and Reardon, 2011). One of these predators, *Laricobius nigrinus* Fender (Coleoptera: Derodontidae) is native to the northwestern U.S. and western Canada where it feeds on *A. tsugae* on western hemlock (*Tsuga heterophylla* (Raf.) Sarg.). *L. nigrinus* adults feed on the sistens generation during the fall and winter and lay their eggs in the wool of sistens ovisacs. The larvae feed on *A. tsugae* sistens adults and progrediens eggs in the spring before dropping from the tree to pupate in the duff layer (Zilahi-Balogh et al., 2002, 2003). Since 2003, *L. nigrinus* has been released and become established at numerous locations across the range of *T. canadensis*, and in some areas has reached population densities sufficient for collection and redistribution of beetles to other locations (Mausel et al., 2010; Onken and Reardon, 2011). Significant reductions in *A. tsugae* sistens densities caused by *L. nigrinus* have been documented in controlled predator–prey enclosure studies (Lamb et al., 2006) and in young hemlock plantations (Mausel et al., 2008), but data quantifying the impact of *L. nigrinus* releases on *A. tsugae* densities in natural forests are lacking. Unexpectedly, *L. nigrinus* has subsequently hybridized with *Laricobius rubidus* LeConte, the only native congener in eastern North America and a predator of pine bark adelgid (*Pineus strobi* (Hartig)) on eastern white pine (*Pinus strobus* L.). These *Laricobius* congeners and their hybrids are morphologically similar and cannot be confidently distinguished without genetic analysis (Havill et al., 2012).

Despite the successful establishment and proliferation of *L. nigrinus* in the eastern U.S., rapid decline and mortality of *T. canadensis* has occurred at a number of sites where this predator has been released (Mausel et al., 2011). Operationally, only a few hundred to a few thousand *L. nigrinus* beetles have typically been released at one time in a given forest stand, due to practical limits on the number of beetles that can be field-collected or produced in rearing laboratories. Dispersal of *L. nigrinus* populations from release points is initially slow, extending only a few hundred meters after 5 years (Davis et al., 2012). It is therefore likely to take several years before established populations of *L. nigrinus* can increase to levels capable of substantially reducing the millions of *A. tsugae* that occur annually on individual trees. By that time, however, *T. canadensis* health may deteriorate past the point of potential recovery. Without management, decline and mortality in *T. canadensis* stands can be extremely rapid, particularly in the southern Appalachians, where average crown loss and percent tree mortality have exceeded 80% as quickly as four and seven years post-infestation, respectively (Elliott and Vose, 2011; Ford et al., 2012).

These operational and biological challenges indicate a need to integrate multiple types of *A. tsugae* control strategies in the same hemlock stands. One proposed management approach is to prolong hemlock health on select trees through temporary protection with insecticide, while simultaneously establishing *L. nigrinus* on the adelgids of nearby untreated trees (Salom et al., 2011) or unprotected portions of trees treated with low rates of insecticide (Joseph et al., 2011; Eisenback et al., 2014). In the theory of this approach, by the time insecticide protection diminishes and *A. tsugae* infests the previously-treated trees, the predator population is abundant and has a better chance of protecting those trees because they are in superior health and have better potential longevity than untreated trees. Furthermore, such previously-treated trees should be a better source of prey because they have more new shoots for *A. tsugae* to infest. Such an integrated management approach has yet to be implemented widely, partly due to concerns that *L. nigrinus* might experience lethal or sublethal effects of imidacloprid exposure upon eating adelgids settled on treated trees (Eisenback et al., 2010). However, *A. tsugae* is effectively controlled on *T. canadensis* at very low foliar concentrations of imidacloprid (>120 ppb, Cowles et al., 2006) indicating that exposure to insecticidal residues on branches where adelgids are feeding may be minimal. Field studies are needed to test these assumptions and determine whether *L. nigrinus* can successfully colonize, reproduce, and impact *A. tsugae* populations on trees previously treated with imidacloprid.

In previous work at our study location, Joseph et al. (2011) demonstrated that soil injection with low rates of imidacloprid (10% and 25% of label rate) could improve hemlock crown health relative to untreated trees, while also permitting a low density of adelgids two years after treatment. In the current study we subsequently released *L. nigrinus* in the same forest plot to determine if this predator would establish and proliferate on insecticide treated trees, and also addressed the following questions:

1. *Imidacloprid effects on predator and prey densities and hemlock health:* What are the longer-term (5–7 years post treatment) effects of imidacloprid treatment rate on *Laricobius*