AN ABSTRACT OF THE THESIS OF

Maggie K. Byrkit for the degree of Master of Science in Forest Science presented on June 11, 2004.

Title: Predators of Hemlock Woolly Adelgid in Northwestern Oregon.

Abstract approved:

Darrell W. Ross

Hemlock woolly adelgid, *Adelges tsugae* Annand, is a serious threat to eastern hemlock, *Tsuga canadensis* Carriere, in the eastern United States. Infestations of *A. tsugae* are spreading rapidly and tree mortality caused by the insect is increasing yearly. As part of an integrated management approach, a classical biological control program is being implemented in eastern forests impacted by *A. tsugae*. Two insect predators have been released as control agents, but their impact on *A. tsugae* to date is uncertain. A priority of the USDA Forest Service Forest Health Technology Enterprise Team (FHTET) is to identify additional potential control agents. The Pacific Northwest, where *A. tsugae* inhabits western hemlock (*T. heterophylla*), was identified as a region to survey for adelgid predators. Thirty-one *T. heterophylla* infested with *A. tsugae* in northwestern Oregon were sampled monthly for predatory arthropods from April 2003 to April 2004. *Adelges tsugae* development was monitored. In September 2003 and March 2004, trees

were sampled to determine *A. tsugae* density. Development of adelgids on study trees was similar to development observed in the eastern U.S., with differences in timing between 2003 and 2004. Trees infested with *A. tsugae* had only a small amount of defoliation. Small numbers of several predatory arthropod taxa were collected from *A. tsugae* infested branches, including *Laricobius nigrinus* Fender, coccinellid beetles, mirids, other hemipterans, pseudoscorpions, earwigs and lacewings. The abundance of *L. nigrinus* appeared to be positively correlated to the density of *A. tsugae*.

Predators of Hemlock Woolly Adelgid in Northwestern Oregon

by
Maggie K. Byrkit

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Maggie K. Byrkit, Author

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DEDICATION

This thesis is dedicated to my father, David Kelly, who raised me to know the value of the woods, learning, and myself.

Chapter 1: Introduction and Literature Review

Hemlock woolly adelgid in the U.S.

Hemlock woolly adelgid (*Adelges tsugae* Annand) is a serious forest pest threatening eastern U.S. forests. *Adelges tsugae* is native to several parts of Asia (McClure 1987). It was introduced into the northwest United States by unknown means and was first reported in several locations in Oregon in the 1920's by Annand (1924). Populations of *A. tsugae* were reported in Richmond, VA in 1953 or 1954 (Souto et al. 1996). The origins of the eastern and western infestations are not known. Currently, about one-third of the natural range of eastern hemlock (*Tsuga canadensis* Carriere) is infested with *A. tsugae*, including counties in 15 eastern states (USDA Forest Service 2002).

Infestations of *A. tsugae* are spreading rapidly to new areas. Forests across New England have been infested by *A. tsugae*, independent of site factors such as latitude or elevation (Orwig and Foster 1999). *Adelges tsugae* caused mortality is widespread and increasing yearly (Orwig and Foster 1999). Some heavily infested stands in central southern Connecticut have a rising mortality rate that is over 60 percent for all hemlocks (Orwig and Foster 1999). In New Jersey, *A. tsugae* caused mortality is above 90 percent in some stands (Mayer et al. 2002). A large proportion of the hemlocks in the Delaware Water Gap National Recreation Area in eastern Pennsylvania and New Jersey are dead or in severe decline (U.S. Department of Interior 2000).

There are official efforts to limit the spread of *A. tsugae* to uninfested areas. Maine, Michigan, New Hampshire and Vermont have instituted quarantines that ban the import of hemlock nursery stock from any states known to have *A. tsugae* infestations, and have imposed regulations on transport of hemlock logs and other hemlock products. As of December 2002, populations of *A. tsugae* inhabit one county in New Hampshire (USDA Forest Service Forest Health Protection 2002).

The USDA Forest Service, the National Association of State Foresters and the National Plant Board identified biological control as an area of emphasis for management of *A. tsugae* in 2004 (USDA Forest Service et al. 2004). Native predatory arthropods do not significantly impact eastern *A. tsugae* populations (McClure 1987, Wallace and Hain 2000). Therefore, a classical biological control program for *A. tsugae* in the eastern United States was initiated and several predatory insects are currently being investigated (Zilahi-Balogh et al. 2002b).

Hemlock woolly adelgid biology and phenology

Adelgids, members of the Homopteran family Adelgidae, are small, soft-bodied insects with piercing/sucking mouthparts. Adelgids feed by inserting a stylet bundle into tree xylem ray parenchyma cells. The primary hosts for all Adelgidae are spruce trees; other conifers act as secondary hosts (Mitchell 1962). In the United States, *A. tsugae* lives and reproduces successfully only on hemlock (McClure 1987). In the eastern U.S., *A. tsugae* is found on eastern hemlock and Carolina hemlock (*T. caroliniana* Engelmann). In Oregon and Washington, *A. tsugae* inhabits western hemlock (*T. heterophylla* Sargent) and mountain hemlock (*T. mertensiana* Carriere) (Montgomery 1996).

Hemlock woolly adelgids are oval in shape and purple or dark brown in color. Adult *A. tsugae* measure slightly less than 1 mm in length and developing nymphs are smaller. During its development *A. tsugae* produces a white, fibrous exudation that covers its body and eggs. The masses of woolly fibers are 1-4 mm in width, and have the appearance of tiny snowballs on infested branches. The woolly masses of dead *A. tsugae* adults remain attached to the branch after adelgid eggs have hatched and the larvae have dispersed, until they are dislodged by rain, snow, or wind. Eastern hemlock trees that are heavily infested and covered with white woolly masses look "flocked" (McClure 1995b).

In its native range there are three forms of *A. tsugae*. Two of the forms, sistens and progrediens, are parthenogenic, wingless, and complete development on hemlock. McClure (1989) recorded that in Connecticut, sistens lay approximately 50 eggs in February. Adult adelgids may lay as many as 300 eggs each (McClure et al. 2001). These eggs hatch in April into mobile first instar larvae, or crawlers. Crawlers are mobile for 1-2 days, then settle at the base of a hemlock needle, insert a feeding stylet bundle and do not move again until they become adults. Over about 4 weeks these larvae develop through 4 larval instars into adult progrediens (McClure 1989). Progrediens lay about 25 eggs, which hatch in June or July as sistens (McClure 1989). First instar sistens nymphs disperse, settle at a feeding site and begin an aestival diapause. It was determined by Salom et al. (2001) that the aestival diapause is facultative, dependent on temperature and photoperiod cues. Diapause continues until October or November, when the nymphs resume development. Sistens then develop through the winter, reaching adulthood and laying eggs in February (McClure 1989).

Sexuparae are winged males and females that develop concurrently with progrediens in the spring (McClure 1987). In Japan, sexuparae disperse to a spruce host, where they reproduce sexually. In North America there are no suitable spruce hosts, and sexuparae do not survive (McClure 1989). In western North America, the sexuparae form has not been observed (A. Lamb, personal communication).

Adelges tsugae crawlers are dispersed by wind, across short and long distances (McClure 1987). Birds and deer may also act as long-range dispersal agents of *A. tsugae*, if crawlers fall into their plumage or fur when they visit infested trees. Individual *A. tsugae* crawlers can survive cold temperatures and several days without food, aiding dispersal (McClure 1987). Humans can disperse *A. tsugae* in similar fashion. Moving bark chips or other woody materials from infested areas to un-infested sites is also thought to be an anthropogenic cause of spread (McClure 1987).

Effects of hemlock woolly adelgid on hemlock trees

An infestation of *A. tsugae* begins with an individual or a few adelgid nymphs settling on a branch, feeding and reproducing parthenogenically. As the population of adelgids on a tree increases, so does the area of infested crown. Infestations on eastern hemlock start on one or more branches, spread throughout the canopy, and eventually cover the entire tree. The youngest growth on each branch is infested first, and then *A. tsugae* will move to older needles.

Adelges tsugae inserts a feeding stylet bundle composed of 4 stylets into the base of a needle to feed (Young et al. 1995). The stylet bundle takes a primarily intercellular path and usually ends in xylem ray parenchyma cells (Young et al. 1995). While developing through its four instars, the adelgid may cause the needle to yellow and fall off. It has been hypothesized that in addition to feeding on the xylem ray parenchyma cells (primarily storage cells that do not transport solutes) the adelgid injects a toxin into the plant, causing further injury (McClure 1991, Young et al. 1995).

With the loss of live needles, the photosynthetic capacity of the tree decreases (McClure 1991). Hemlocks that are stressed by disease, drought or another pest insect are likely to be killed by *A. tsugae* infestation more rapidly than healthier trees (McClure 1991). In Connecticut trees that became heavily infested with *A.tsugae* died in as few as 4 years (McClure 1991).

Effects of hemlock woolly adelgid on hemlock stands and forests

The successional dynamics of forests may be changed by *A. tsugae*. As trees die from the damage caused by *A. tsugae*, canopy gaps are produced, increasing light levels and allowing other species to grow in the open space. Early successional species such as black birch, red maple and oak may capitalize on the removal of competition from hemlocks, and prevent the natural return of hemlocks to the area (Orwig and Foster 1999).

Salvage logging, one response to *A. tsugae* damage, can also have severe impacts on vegetation structure. Orwig and Kizlinski (2002) looked at vegetation response in stands that had been salvage-logged in response to *A. tsugae* spreading through Connecticut and Massachusetts forests. A high percentage of residual hemlock stems in logged stands were dying, probably as a result of changed environmental factors caused by the logging (Orwig and Kizlinski 2002).

Soil water chemistry is affected by the rapid loss of hemlock trees. Yorks et al. (2003) simulated *A. tsugae* caused hemlock mortality in four stands in New York and measured the ensuing changes in soil water chemistry. Their results showed rapid and significant increases in NO_3- levels in soil water after hemlock mortality in a stand, compared with control stands (Yorks et al. 2003).

Hemlock and balsam woolly adelgids in the northwest

In the northwestern United States, *A. tsugae* are commonly found on ornamental hemlocks, in seed orchards, and on some forest trees (D. Overhulser personal communication, D.L. Mausel personal communication). The adelgid seems to spread easily even to isolated trees. In Oregon, populations may persist for many years but rarely reach outbreak densities and have not been observed to cause tree mortality.

Balsam woolly adelgid (*Adelges piceae* Ratzeburg), another introduced adelgid, is a serious pest in Canada and the western United States, infesting true firs (*Abies* spp.) and Douglas fir (*Pseudotsuga menziesii* Mirbel) (Mitchell 1962). A classical biological control program to control populations of *A. piceae* was implemented starting in the 1930's.

Control of hemlock woolly adelgid

Controlling populations of *A. tsugae* is difficult and has been approached in several ways. Individual ornamental trees infested with *A. tsugae* can be treated effectively with horticultural oils or pesticide sprays, so long as branches are completely drenched with spray (McClure 1997). In a hemlock stand, drenching branches with insecticide is not a realistic option. Improving the health of hemlock through management may slow the effects of adelgid infestation. Spread of the infestation to other ornamentals can be limited by careful cultural practices, such as preventing the transport of wood chips or other materials from around the infested tree (McClure 1995b).

Eradication of hemlock woolly adelgid from all trees in an infested stand is not a viable option (Pais and Polster 2002). Therefore, forest managers may take an integrated approach that involves evaluation, treatment, management and monitoring to minimize *A. tsugae* outbreaks and tree mortality (Pais and Polster 2002). This approach may include pesticide sprays, silvicultural management such as removal of trees, and classical biological control.

Biological control by natural enemies

Classical biological control programs involve manipulation of natural enemies in a system affected by a pest organism. In all natural systems, natural enemy populations can regulate populations of host or prey organisms. Some natural enemies may regulate prey populations more effectively at low prey densities; others are more effective when prey are abundant (Debach and Rosen 1991). A variety of biological, physical and environmental factors may limit the efficacy of regulation by natural enemies. Sometimes populations of organisms reach levels that are considered economically damaging, even while regulated by natural controls, including natural enemies (Debach and Rosen 1991). Exotic insect herbivores introduced into areas where there are no evolved natural enemies may reach outbreak proportions and become economically important pests.

Debach and Rosen (1991) define biological control, in an ecological sense, as the "regulation by *natural enemies* of another organism's population density at a lower average than would otherwise occur." Biological control through introduction or augmentation of natural enemies has been implemented for control of organisms that have reached economically damaging population levels.

Importation and release of exotic enemies has been repeatedly used as a means of biological control of pest arthropods. When it is determined that native natural enemies do not give the desired level of control, exotic enemies can be used to reduce pest populations. Currently there are no known parasites of hemlock woolly adelgid, so biological control efforts have focused on predators.

Once predators are located, they must be evaluated carefully before release. Seasonal synchrony between predator and prey is important, as is testing for the ability of the predator to survive in the climate of the release area. In a review of 148 biological control releases of natural enemies, Stilling (1993) found that the most common causes of failure were related to climate. Poor matching of agent to release climate resulted in deaths due to direct climate effects such as high temperatures, or indirect climate effects such as disruption of predator-prey life cycle synchrony (Stilling 1993).

Biological control of balsam woolly adelgid

Balsam woolly adelgid is a European insect that infests true firs and Douglas fir in the United States and Canada. In the northwestern United States, *A. piceae* was found damaging grand fir (*Abies grandis* Lindley) in the 1930's and, since 1955, has damaged

Pacific silver fir (*A. amabilis* Forbes) and subalpine fir (*A. lasiocarpa* Nuttall) (Mitchell and Buffam 2001). Natural enemies were released in several areas in the Pacific Northwest to control *A. piceae* populations.

A variety of native natural enemies of the balsam woolly adelgid have also been identified. Over three years of study, Mitchell (1962) found 26 species of predatory arthropods associated with *A. piceae* in Oregon and Washington. These included dipterans in the families Syrphidae, Chamaemyidae and Itonididae, neuropterans, coleopterans, one hemipteran species and several spiders (Mitchell 1962).

Thirty-one natural enemies were imported from Europe, Asia and Australia and released in Canada or the U.S. as biological control agents for *A. piceae* (Zilahi-Balogh et al. 2002b). Several predators became established, including two coccinellid beetles, a derodontid beetle, and several dipterans (Zilahi-Balogh et al. 2002b). To date there is no evidence that introduced predators have reduced any balsam woolly adelgid populations (Mitchell and Buffam 2001).

Biological control of hemlock woolly adelgid

There are no predatory or parasitic species naturally found in eastern North America that are able to keep *A. tsugae* populations below economically damaging thresholds (McClure 1997). A survey of arthropods on *A. tsugae* infested hemlock in North Carolina and Virginia showed a low diversity and low density of native and established predators associated with the adelgid in that region. The authors surveyed lower crown branches on 62 trees at three sites with beat nets over the warm months of

1997 and 1998 and recovered 169 predatory specimens belonging to only three families (Wallace and Hain 2000). The effect of these native predators, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), green lacewings (Neuroptera: Chrysopidae); and brown lacewings (Neuroptera: Hemerobiidae), on *A. tsugae* was tested by excluding predators from infested branches. There was no evidence of any effect of predators on adelgid survival (Wallace and Hain 2000).

Because native predators exerting control on *A. tsugae* could not be found, other areas have been explored for natural enemies. Areas surveyed for potential biological control agents have included Japan (McClure 1995a, 1997), China (Montgomery et al. 1999) and western North America (Zilahi-Balogh et al. 1999, D.L. Mausel personal communication).

McClure (1995a, 1997) has described some of the native predators of hemlock woolly adelgid in Japan. The oribatid mite *Diapterobates humeralis* (Hermann) was found to consume woolly filaments of *A. tsugae* and in the process dislodge the adelgid eggs and nymphs (McClure 1995a). The coccinellid beetle *Pseudoscymnus tsugae* was common at some Japanese sites infested with *A. tsugae* (McClure 1997). *Pseudoscymnus tsugae* feeds on all life-stages of *A. tsugae*, and has two generations per year, which are synchronized with those of *A. tsugae* (McClure 2001).

Biological control using imported natural enemies has been implemented in *A. tsugae* infested eastern hemlock forests in Connecticut since 1995. Hundreds of thousands of *P. tsugae* have been reared and released in several forest locations (McClure et al. 1999). Surveys in years following releases showed that *P. tsugae* were able to successfully overwinter in Connecticut, and that beetles were able to reduce populations of *A. tsugae* on release branches (McClure and Cheah 1999). Although *P. tsugae* may have established in some areas, there is no evidence that the beetle has impacted populations of *A. tsugae* at the tree or stand level. A recent evaluation of the status of hemlocks and densities of *A. tsugae* at 5-year-old *P. tsugae* release sits in Connecticut showed high mortality of overwintering *A. tsugae* and a great deal of hemlock recovery in the form of new shoot growth (Cheah and McClure 2002). However, this high adelgid mortality and hemlock recovery was likely due to abnormally cold winters and above normal spring and summer precipitation, which favored hemlock growth. The recovery cannot be attributed to any impact of *P. tsugae* (Cheah and McClure 2002).

A beetle native to western North America, *Laricobius nigrinus* (Coleoptera: Derodontidae), has also been released as a biological control agent in the eastern U.S. *L. nigrinus* is native to the Pacific Northwest, and was in quarantine in a seed orchard in Blacksburg, VA (Zilahi-Balogh et al. 2002a). Active, feeding stages of the beetle were found to coincide with suitable prey stages of *A. tsugae* (Zilahi-Balogh et al. 2002a). In laboratory tests, *L. nigrinus* was found to prefer *A. tsugae* to other adelgids as a food source (Zilahi-Balogh et al. 2002a). USDA APHIS approved *L. nigrinus* for field release in 2000 (Zilahi-Balogh et al. 2002a).

Sites in China have been explored for potential biological control agents of *A. tsugae*. A survey by Montgomery et al. (1999) in three central Chinese provinces found a large diversity of Coccinellidae on native hemlocks, including nine beetles that are known to feed on *A. tsugae*. Three of the beetles, *Scymnus (Neopullus) sinunodulus* Yu et Yao, *S. (N.) camptodromus* Yu et Liu and *Scymnus (Neopullus) n. sp.*, were brought to quarantine facilities in Connecticut for rearing and evaluation of development (Lu and Montgomery 1999).

It has been suggested that chamaemyiid flies, gall gnats (Diptera: Cecidomyiidae) and anthocorid bugs all feed on *A. tsugae* in the Northwest (R.G. Mitchell, personal communication). In a study in western Washington, *L. nigrinus* larvae as well as unidentified syrphid fly larvae were observed feeding on *A. tsugae* on hemlock trees in an urban arboretum (D.L. Mausel personal communication).

Conclusion

The damage caused by hemlock woolly adelgid in eastern U.S. forests is increasing. Though cold winters and wet summers that favor hemlock growth may slow the rate of spread or impact of the adelgid, many more hemlocks are likely to die as a result of *A. tsugae* infestation. Native predators in the East have not impacted *A. tsugae* populations. The introductions of *P. tsugae* and *L. nigrinus* have not yet impacted *A. tsugae* populations. Continued releases may allow these beetles to establish, to impact populations of *A. tsugae*, and to reduce mortality rates of eastern hemlock, or the beetles may continue to have no effect on the adelgid. Augmenting the two agents already released for control of *A. tsugae* will require identifying additional predators from the natural and introduced ranges of *A. tsugae*, including Asia and the Pacific Northwest.

Chapter 2: Predators of Hemlock Woolly Adelgid (Homoptera: Adelgidae) on Western Hemlock in Northwestern Oregon

Maggie K. Byrkit and Darrell W. Ross

Abstract

Thirty-one western hemlocks, *Tsuga heterophylla* (Raf.) Sarg., infested with hemlock woolly adelgid, *Adelges tsugae* Annand, in northwestern Oregon were sampled monthly for predatory arthropods from April 2003 to April 2004. *Adelges tsugae* development was monitored. In September 2003 and March 2004, trees were sampled to determine *A. tsugae* density. Development of adelgids on study trees was similar to development observed in the eastern U.S., with differences in timing between 2003 and 2004. Trees infested with *A. tsugae* had only a small amount of defoliation. Small numbers of several predatory arthropod taxa were collected from *A. tsugae* infested branches, including *Laricobius nigrinus* Fender, coccinellid beetles, mirids, other hemipterans, pseudoscorpions, earwigs and lacewings. The abundance of *L. nigrinus* appeared to be positively correlated to the density of *A. tsugae*.

Keywords

Adelges tsugae, adelgid development, predator survey

Introduction

Hemlock woolly adelgid, *Adelges tsugae* Annand, is a serious threat to eastern hemlock, *Tsuga canadensis* Carriere, in the eastern United States. Infestations of this Asian insect were first observed in the U.S early in the 20th century (Annand 1924). Currently, about one-third of the natural range of eastern hemlock is infested with *A. tsugae* and infestations are spreading rapidly to new areas (USDA Forest Service 2002). Mortality of eastern hemlock due to *A. tsugae* is increasing yearly (Orwig and Foster 1999). In New Jersey, *A. tsugae* caused mortality is above 90 percent in some stands (Mayer et al. 2002).

In the northwestern United States, *A. tsugae* are commonly found on ornamental western hemlock (*T. heterophylla* Sargent), in seed orchards, and less commonly on hemlock growing in forests (D. Overhulser personal communication, D.L. Mausel personal communication). The adelgid is able to spread to isolated urban and rural trees. Private landowners report that populations of *A. tsugae* persist on ornamental *T. heterophylla* for many years without causing any apparent damage to the trees. In Oregon, *A. tsugae* rarely reach outbreak densities like those observed in the eastern U.S. (McClure 1987, McClure and Cheah 1999), and do not cause tree mortality.

The USDA Forest Service has identified biological control, surveys, and monitoring among its current priorities for control of *A. tsugae* (USDA Forest Service et al. 2004). Areas surveyed for potential biological control agents of *A. tsugae* have included Japan (McClure 1995, 1997), China (Montgomery et al. 1999) and western North America (Zilahi-Balogh et al. 1999, D.L. Mausel personal communication).

Two beetle species have been released as biological control agents for eastern *A. tsugae* populations. Hundreds of thousands of the Japanese coccinellid, *Pseudoscymnus tsugae* Sasaji and McClure, have been reared and released in several forest locations since 1995 (McClure and Cheah 1999). Surveys in years following releases showed that *P. tsugae* has established in some areas, but there is no evidence that the beetle has impacted *A. tsugae* populations at the stand level. The second beetle, *Laricobius nigrinus* Fender, is native to western Canada and the Pacific Northwest. *Laricobius nigrinus* beetles were cleared for release by APHIS in 2000 (Zilahi-Balogh et al. 2002).

Native predatory arthropods do not significantly impact eastern *A. tsugae* populations (McClure 1987, Wallace and Hain 2000). Currently there are no known parasites or pathogens of *A. tsugae*. Continuation of the biological control program for *A. tsugae* will require identifying additional natural enemies and matching them by climate suitability and preference for the adelgid. The objective of this study was to identify predatory species associated with *A. tsugae* in northwest Oregon. Predatory species were located through long-term sampling of *T. heterophylla* trees infested with *A. tsugae* in the Willamette Valley, Oregon.

Materials and methods

A survey of arthropods on *A. tsugae* infested hemlocks was conducted in four Oregon counties during 2003 and 2004. Between February and August 2003, urban and rural *T. heterophylla* supporting populations of *A. tsugae* were located. Thirty-one trees were sampled over the duration of the study for predatory arthropods (Table 1). Twenty of the *T. heterophylla* sample trees were located in urban settings on roadsides, in private yards, in one public park, and on the Oregon State University campus. These trees were located in high-use areas near auto or foot traffic. Eleven trees were located in a rural seed orchard with minimal auto and foot traffic. None of the trees were treated with pesticides during the study, or in the preceding two years.

In Clackamas County, sampling began March 25, 2003. In April 2003, sampling began at the J.E. Schroeder Seed Orchard, Marion County. In May 2003, five trees in Benton County were added to the study. Five additional trees in Multnomah County were sampled beginning in August 2003. Arthropods were collected from each study tree monthly through April 2004. Trees in Clackamas and Multnomah Counties were not sampled in January 2004 due to an ice storm.

Table 1. Location and description of trees sampled for *A. tsugae* predators in northwestern Oregon in 2003 and 2004.

City, County	Latitude (N)	Longitude (W)	Elev. (m)	Site Type	Height (m)	DBH(cm)	Initially Defoliated	First visit
Sandy, Clackamas	45°24.014	122 17.474	300	roadside	4.6	6	yes	3/25/2003
	45°24.014	122 17.474	300	roadside	16.2	60	no	
	45°24.018	122 17.503	300	roadside	12.2	23	no	
	45°24.026	122 17.615	300	roadside	9.8	17	no	
	45°23.986	122 15.588	300	roadside	11.6	23	no	3/27/2003
	45°23.993	122 15.568	300	roadside	12.2	13	no	
	45°22.088	122 13.781	400	yard	9.4	11	no	3/28/2003
	45°22.088	122 13.781	400	yard	10.1	65	no	
	45°26.199	122 12.868	500	yard	11.6	28	no	
	45°26.178	122 12.850	500	yard	12.2	37	no	
St. Paul, Marion	45 08.693	122 59.022	130	orchard	13.7	26	no	4/24/2003
	45 08.693	122 59.022	130	orchard	13.7	20	no	
	45 08.693	122 59.022	130	orchard	13.7	27	no	
	45 08.693	122 59.022	130	orchard	13.7	19	no	
	45 08.693	122 59.022	130	orchard	13.7	24	no	
	45 08.693	122 59.022	130	orchard	13.7	24	no	
	45 08.693	122 59.022	130	orchard	13.7	22	no	
	45 08.693	122 59.022	130	orchard	13.7	25	no	
	45 08.693	122 59.022	130	orchard	13.7	28	no	
	45 08.693	122 59.022	130	orchard	13.7	24	no	
	45 08.693	122 59.022	130	orchard	13.7	31	no	

Table 1, continued. Location and description of trees sampled for *A. tsugae* predators in northwestern Oregon in 2003 and 2004.

City, County	Latitude (N)	Longitude (W)	Elev. (m)	Site Type	Height (m)	DBH(cm)	Initially Defoliated	First visit
Corvallis, Benton	44°34.064	123 16.563	85	campus	9.4	17	no	5/1/2003
	44°32.330	123 15.470	80	yard	6.4	10	no	
	44 32.330	123 15.470	80	yard	8.5	16	yes	
	44 35.331	123 16.661	80	yard	2.1	3	no	
	44 35.331	123 16.661	80	yard	14.6	34	no	
Portland, Multnomah	45 32.409	122 37.818	70	park	20.7	65	no	8/3/2003
	45 32.420	122 37.817	70	park	22.6	47	no	
	45 32.439	122 37.775	70	park	25.6	60	no	
	45 32.454	122 37.759	70	park	22.9	60	no	
	45 32.630	122 35.852	130	yard	13.4	39	no	

For consistency, all trees were sampled in the morning. Temperature extremes were avoided. Most sampling occurred when the temperature was between 50° and 75° F, except November through February, when sampling was conducted at temperatures as low as 40° F. Sampling in heavy rain, high winds or other extreme weather conditions was avoided.

A region was selected for sampling on the lower crown of each tree based on accessibility of branches and distribution of the adelgid infestation (e.g., the east side). On some trees only a single lower crown branch was accessible. Branches sampled were between 1 and 3 m in height. A branch from the selected region was sampled on each visit, but not necessarily the same branch each visit. Sampling one branch per visit and, when possible, alternating the branch used, minimized the impact of the branch beating method on the *A. tsugae* populations, which were often very small.

At each visit foliage was examined with a hand lens to confirm the presence of living *A. tsugae* and to look for signs of predation on the adelgids, such as dislodged wool. Changes in the adelgid population or the condition of the tree from the previous visit were recorded, as was the life stage of the adelgids present.

Prior to June 2003, a nylon beating sheet was held directly underneath the distal 1 meter of a selected branch, and the branch was struck 15-20 times with a section of 2 cm diameter plastic pipe to dislodge arthropods. After June 1, 2003, a large fine weave insect net replaced the beating sheet. The net was slipped over the distal 1 m of a branch and the branch was struck 15-20 times (Figure 1). Arthropods on the sheet or in the net were collected with an aspirator into a vial.

Figure 1. Beating a *T. heterophylla* branch enclosed in a nylon insect net to collect arthropods. December 2003.

Vials containing arthropods were placed on ice for transport to the laboratory and placed in a freezer, usually within a few hours. Ethyl acetate was used to kill specimens when it was not possible to rapidly place them into a freezer. Approximately one-fourth of specimens collected were killed with ethyl acetate. Freezing or killing collected arthropods prevented damage or loss of specimens due to predation by spiders or other generalist predators within the vial.

Beat samples were sorted in the laboratory within a few days of collection. Specimens were identified using several insect keys and guides (Arnett 2000, Borror et al. 1989, Hodek 1973, Jaques 1951, Schuh 1995, Slater and Baranowski 1978, Stehr 1987). Specimens were placed in 80-95% ethanol for storage, or pinned. Voucher specimens of predatory species were prepared and placed in the collection at the Oregon State University Arthropod Collection, Corvallis, Oregon.

A 20-30 cm long *A. tsugae* infested branch tip was bagged and clipped from a branch on each study tree in June 2003. Foliage samples were examined under a dissecting microscope for the presence of predators and for signs of predation on the adelgids. Because *A. tsugae* populations were often very small, it was not possible to collect a second branch tip from every study tree without removing the adelgid population altogether. Instead, branch tips were collected from 1-3 trees per county during sampling visits in October 2003 and February 2004. All branch tips were examined under a dissecting microscope in the laboratory, and any arthropods present were removed and identified.

Some adult predators collected from January through April 2004 were given the opportunity to feed on *A. tsugae* in the laboratory. Each predator specimen was placed in a Petri dish arena with a hemlock twig, some *A. tsugae*, water-soaked cotton, and a supplemental dry food mixed with honey. The supplement, produced by Planet Natural®, consisted of nutritional yeast and dried whey. After one week the twig and *A. tsugae* were removed and a fresh twig was added. Fungal growth in the dishes often made it necessary to place the predator in a new dish when replacing the food. Predators were maintained in these arenas until their death, with fresh *A. tsugae* added regularly. Hemlock twigs removed from predator arenas were examined under a microscope for evidence of predation on *A. tsugae*.

In September 2003 and March 2004, *A. tsugae* were counted on a portion of a randomly selected branch in the sampling region of each study tree. Three 10 cm deep by 1 m wide bands were randomly selected from ten adjacent 10 cm divisions of the distal 1 m of branch (measured from the branch tip inward). For example, the bands 0-10 cm, 40-50 cm and 70-80 cm could be selected. The woolly masses within these three bands were counted and totaled. Because each adelgid produces a single mass of wool as it develops, this count identified the number of developing or adult *A. tsugae* within the selected bands. The totals from these counts provided an indication of relative *A. tsugae* density for two *A. tsugae* generations on the study trees; progrediens in September 2003 (sisten nymphs were present at this time but were in aestivation and not covered in wool, and therefore not counted), and sistens in March 2004 (progredien nymphs present were not counted).

At the J.E. Schroeder Seed Orchard a lift was used in November 2003 and March 2004 to examine the canopies of *A. tsugae* infested hemlocks. The lift provided access to hemlock branches up to 10 m in height. Branches were examined at 10 and 6 m on each infested tree to characterize the level of *A. tsugae* infestation. On March 26, 2004, adelgids were counted on the distal portion of a random branch of each study tree at a height of approximately 6 m using the procedure described above.

Results

Adelgid density and development

Infestations of *A. tsugae* in the lower crowns of study trees ranged from fewer than 5 adelgids in the entire lower crown to several hundred adelgids per branch. Most *A. tsugae* populations were limited to the youngest foliage on the distal 0.5 m of the branch.

Table 2 shows the densities of adelgids counted on study branches, placing the trees in low, medium and high-density population categories and indicating changes in category between the progredien and sistens generations. During the first sample collection, all study trees had at least one living adelgid in the lower crown. By the end of sampling, four study trees had no living adelgids in the lower crown. Six trees had a total population of less than 10 adelgids in the lower crown for the duration of the study. An ice storm in the Willamette Valley in January 2004 apparently had no effect on the size of adelgid populations.

Defoliation of lower crown branches was observed on three trees over the course of the study. A yard tree in Benton County had gradual needle loss from several lower crown branches throughout the study. A single branch on a Marion County tree was partially defoliated during the first sample collection, and completely defoliated by the last. One small (4.5 m tall) roadside tree in Clackamas County was completely defoliated and dead at the end of the study. Lower crown branches on all other trees were apparently undamaged from the beginning to the end of the study. No relationship between *A. tsugae* density and apparent tree condition was observed.

Table 2. Adelgid density on 30x100 cm of branch area during progredien and sistens generations, 2003-04.

Adelgid Density	No. Trees
STABLE	
Low, 0-30	20
Medium, 31-60	2
High, > 60	2
INCREASING	
Low -> Medium	1
Medium -> High	2
Low -> High	1
DECREASING	
High -> Medium	2
Medium -> Low	0
High -> Low	1
Total	31

On the 11 study trees in the J.E. Schroeder Seed Orchard, Marion County, *A. tsugae* density in the mid-canopy (approximately 6 m height) ranged from 0 to 28 adelgids per 30x100 cm of branch area.

Table 3 shows the timing and stages of adelgid development observed in this study, and the development of *A. tsugae* in southern Connecticut (McClure 1989). In 2003 development of *A. tsugae* in northwestern Oregon was similar to adelgid development observed in Connecticut, and in Virginia (Gray and Salom 1996). In the spring of 2004 the progrediens matured nearly two months earlier than in 2003. Sistens first-instar nymphs were observed in late April 2004: in 2003, sistens did not appear until mid-June (Table 3). Average monthly high temperatures in the mid-Willamette valley in March and April 2004 were higher than March and April 2003 (Table 4). Sexuparae were not observed on study trees in either year.

Predator survey

Branch beating yielded 0 to 34 arthropods per sample. Most were spiders or herbivorous insect species. Psocopterans (barklice) were extremely abundant in some beat samples (as many as 30 individuals per sample) and in the interest of collecting all potentially predatory specimens some were allowed to escape. Most spiders were between 1.0-5.0 mm in length. The most common spider taxa collected were *Philodromus rufus* Jenningsi, *Metaphidippus aeneolus* Curtis, and an unidentified species in the family *Microphantidae*. Predatory insects including coleopterans, hemipterans, neuropterans and dermapterans comprised 14 percent of all specimens collected. Table 5 lists predatory taxa, numbers and months collected and potential predators. Potential predators are taxa that are at least partially entomophagous. All other insects listed are capable of feeding on *A. tsugae* and may feed on eggs, developing nymphs or adults.

Table 3. Life stages of hemlock woolly adelgid observed in Connecticut (McClure 1989) and in northwestern Oregon, April 2003-May 2004.

Month	McClure 1989, CT	This study, OR, 2003	This study, OR, 2004
January			
February	Adult Sistens Eggs		Adult Sistens Eggs
March	Sexuparae & Progrediens		Progrediens (*maturing*)
April	(*maturing*)		Adult Progrediens Eggs
May		Adult Progrediens	Sistens (*aestivating*)
June	Adult Progrediens Eggs	Eggs Sistens (*aestivating*)	
July	Sistens (*aestivating*)		
August			
September			
October			
November	(*end aestivation*) Sistens (*maturing*)	(*end aestivation*) Sistens (*maturing*)	
December			

Table 4. Average monthly high/low temperatures (°F) for Salem, OR, February-April 2003 and February-April 2004, recorded by the Oregon Climate Service.

	2003	2004
February	52.7/34.5	51.4/35.7
March	**56.4**/41.6	**60.2**/37.8
April	57.7/41.3	**65.3**/39.8

Predatory beetles from two families, Coccinellidae and Derodontidae, were collected. Ten species of coccinellid beetle were collected in small numbers from all counties. One derodontid species, *Laricobius nigrinus*, was found on trees in all four counties (Table 5). In addition to those collected, large numbers of *L. nigrinus* adults and larvae were observed on a study tree on the Oregon State University campus, Benton County, throughout fall, winter and spring. Few *L. nigrinus* were found on trees with low adelgid densities (Figure 2). Larval *L. nigrinus* were observed curled up inside *A. tsugae* egg masses in April and May 2003 and April 2004. Larvae collected were typically covered in adelgid wool.

Table 5. Adult and larval insect predators and potential predators collected from *A. tsugae* infested hemlock in northwestern Oregon, April 2003-April 2004.

Predator taxa	Number	Months Collected	Predator taxa	Number	Months
<u>Class Insecta</u>					
Coleoptera: Coccinellidae			**Hemiptera: Reduviidae**		
Mulsantina picta minor L.	13	May-July, Dec., Mar.,Apr.	Unidentified spp. (nymph)	9	July, Sept.-Jan., Mar.
Coccinella septempunctata Casey	6	July-Oct.			
Strethorus spp. (probably *picipes* Casey)	6	May, June, Feb., Mar.	**Neuroptera: Hemerobiidae**		
Cycloneda polita Casey	3	Oct., Dec., Mar.	*Hemerobius* spp.	5	Nov., Jan.
Harmonia axyridis Pallas	2	May, Nov.			
Scymnus marginicolis Pullus	1	Sept.	**Neuroptera: Chyrisopidae**		
Unidentified spp.	3	Feb., Apr.	*Chrysopa* spp.	3	Sept., Feb., Mar.
Unidentified spp.	2	July			
Unidentified spp.	1	April	**Neuroptera: Unknown Larvae**	24	May, July, Aug., Apr.
Unidentified spp.	1	May			
			<u>Potential Predators</u>		
Coleoptera: Derontidae					
Laricobius nigrinus Fender (adults)	34	Apr., May., Oct.-Apr.	**Hemiptera: Berytidae**		
Laricobius nigrinus Fender (larvae)	100	May, Mar., Apr.	*Neides muticus* Say	4	May, Aug., Sept.
			Unidentified spp.	8	Sept.
Coleoptera: Unknown Larvae	7	May, Mar., Apr.			
			Coleoptera: Cantharidae	7	May, Apr.
Dermaptera: Forficulidae					
Forficula auricularia L.	30	May-Oct., Jan., Feb.	**Total Insect Predators**	**285**	
Diptera: Unknown Larvae	1	July	<u>Class Arachnida</u>		
			Pseudoscorpionidae	11	June, Sept., Oct.
Hemiptera: Miridae					
Ceratocapsus apicatus	1	Aug.			
Deraeocoris incertus Knight	11	July-Aug., Feb.	**Total Predators**	**296**	
Deraeocoris brevis Uhler	3	Sept., Mar., Apr.			

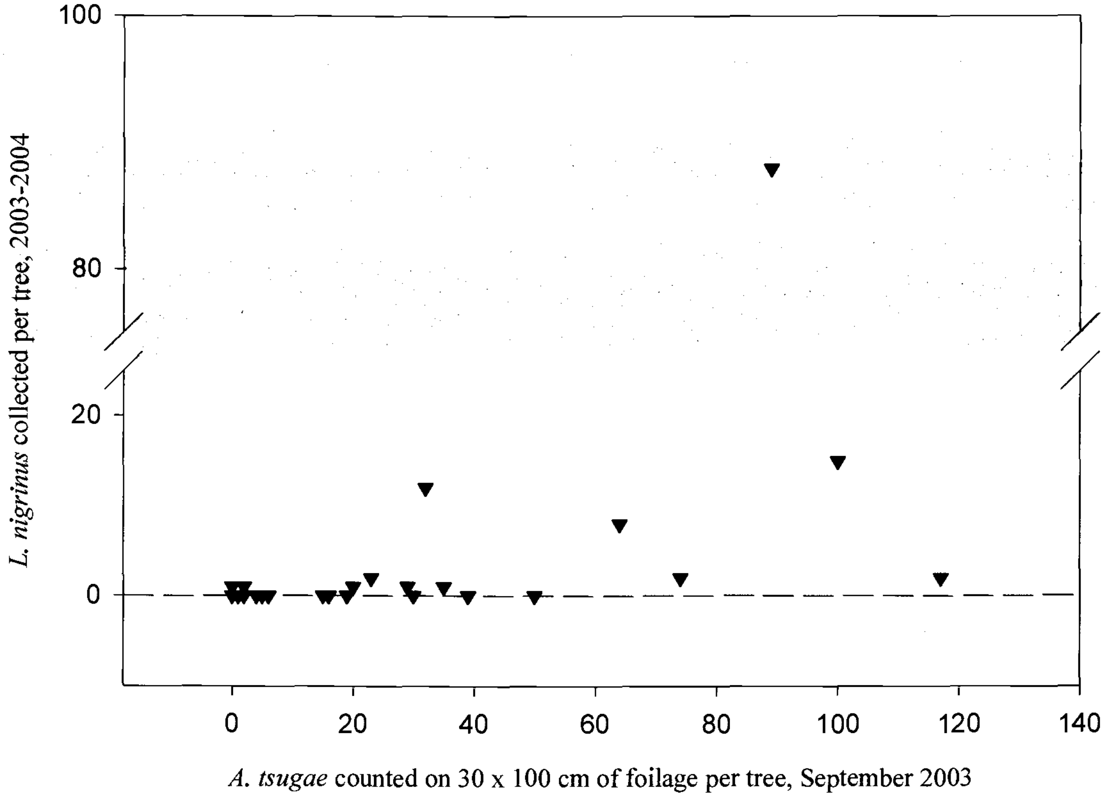

Figure 2. Densities of *L. nigrinus* and *A. tsugae* on *T. heterophylla* in northwestern Oregon, 2003-2004.

True bugs in the genus *Deraeocoris*, (Hemiptera: Miridae) were collected from all counties throughout the duration of sampling. One unidentified assassin bug species (Hemiptera: Reduviidae) was collected from Marion and Benton Counties (Table 5).

Green lacewings (Neuroptera: Chrysopidae) were collected from Marion, Benton and Multnomah Counties. Brown lacewings (Neuroptera: Hemerobiidae) were collected from trees in Benton County. Neuropteran larvae were collected from all counties throughout the study (Table 5).

Other predators collected include European earwigs (*Forficula auricularia* L.), pseudoscorpions (Pseudoscorpionida: Pseudoscorpionidae), unidentified coleopteran larvae and one unidentified dipteran larva. Potentially predatory insects collected were stilt-bugs (Hemiptera: Berytidae) and soldier beetles (Coleoptera: Cantharidae) (Table 5).

Between 0 and 9 arthropods were found on each foliage sample collected in May or June 2003. Spiders, mites and herbivorous species (mostly psocopterans) were removed from these samples. In June, one *Coccinella septempunctata* Linnaeus beetle was found on a foliage sample clipped from a Corvallis tree. No other predatory insects were found on foliage samples. Small numbers of spiders, mites and seed bugs (Hemiptera: Lygaeidae) were observed on foliage samples clipped in October and February.

Feeding observations

The duration of the feeding observations varied from 1 to 6 weeks. One mirid (*Deraeocoris* spp.) was found to puncture and partly consume adelgids. Three *L. nigrinus* beetles ate adelgids completely, in many cases tearing the woolly masses into several pieces and dislodging them from twigs. One European earwig also tore apart and consumed the adelgids within the woolly masses. Other predators observed, including additional mirids, green and brown lacewings and one assassin bug did not appear to disturb adelgids. Many of these predators died shortly after placement in the arenas, sometimes after becoming stuck in the honey-whey-yeast alternative food.

Discussion

Hemlock woolly adelgids are apparently able to disperse to isolated urban and rural *T. heterophylla* in northwestern Oregon, since infested trees were found scattered throughout the region. Populations of adelgids occur on stressed and healthy trees. Populations are usually too small to cause serious damage to the host tree. Although three study trees had some visible defoliation, it is unlikely that feeding by *A. tsugae* was the primary cause of the defoliation. In two cases it appeared that stress, caused by the proximity of a road might have caused branch die-off. In the third case, defoliated branches were hanging over a residential yard, and no cause could be determined.

Development of *A. tsugae* in northwestern Oregon was similar to that of adelgids in the eastern U.S (McClure 1989, Gray and Salom 1996), with some differences.

Sexuparae were noted in both Connecticut (McClure 1989) and Virginia (Gray and Salom 1996), but not in British Columbia (Zilahi-Balogh et al. 2003) or in Oregon. Early maturation of the progredien generation and appearance of sistens nymphs in April 2004 was possibly due to higher daytime temperatures during March and April 2004 than in the previous year (Table 4). Variation in the timing of adelgid stages was also observed in British Columbia, where maturation of sistens occurred as early as late October (Zilahi-Balogh et al. 2003). It was determined by Salom et al. (2001) that the aestival diapause of *A. tsugae* is facultative, dependent on temperature and photoperiod cues. Maturation of the two adelgid generations may also be dependent on temperature cues. Emergence of eastern *A. tsugae* sistens in Connecticut is generally timed with hemlock bud break (McClure 1989), and, in Oregon, in both 2003 and 2004 the beginning of the emergence of sistens nymphs coincided with the appearance of new needles.

Few predatory arthropods were collected from *A. tsugae* infested hemlock trees and only one species is likely using *A. tsugae* as a regular food source. Coccinellid beetles listed in Table 5 are capable of feeding on soft-bodied insects like adelgids, but the small numbers collected suggest that none of these species is an important predator of *A. tsugae*. Similarly, mirids are unlikely to be important predators of *A. tsugae* in Oregon. The genus *Deraeocoris* are known to specialize on aphids (Clausen 1940). Members of this genus have been observed eating *A. tsugae* in laboratories in Oregon and Connecticut (M. Montgomery, personal communication). However, only fourteen *Deraeocoris* and 15 predatory mirids total were collected in this survey.

Laricobius nigrinus was present on all but three trees with adelgid densities greater than 20 during the September 2003 count (Figure 2). In contrast, *L. nigrinus* was present on only two trees with an *A. tsugae* density of fewer than 20 (Figure 2). Both of these trees were located in the J.E. Schroeder Seed Orchard, near trees with larger adelgid infestations. The beetle is apparently able to colonize isolated *A. tsugae* populations throughout the study counties, unless the populations are extremely small. *Laricobius nigrinus* has been shown to specialize on *A. tsugae* (Zilahi-Balogh et al. 2002).

Other predators collected were either generalists, including pseudoscorpions, earwigs and spiders, or were collected in small numbers and therefore are unlikely to impact *A. tsugae* populations. Feeding tests indicated that under laboratory conditions *L. nigrinus*, *F. auricularia* and *Deraeocoris* spp. will consume *A. tsugae.*

The only predator observed feeding on *A. tsugae* in the field was larval *L. nigrinus*. The larvae were observed curled up inside *A. tsugae* egg masses in April 2003 and 2004 and May 2003. Larvae collected were typically covered in adelgid wool. Low predator densities and low adelgid densities on most study trees suggest that adelgids are not used by predators other than *L. nigrinus* as a regular food source. Some trees had populations of adelgids that never amounted to more than 10 individuals, but persisted throughout the study without any disturbance by predators. No relationship between *A. tsugae* density and the abundance of predatory insects was observed, except that *Laricobius nigrinus* was present in greater numbers on trees with greater adelgid densities (Figure 2).

Most *A. tsugae* populations monitored in this study were initially small and did not increase over two to three adelgid generations. Private landowners reported that populations of *A. tsugae* on their ornamental trees fluctuate from year to year, but do not appear to harm the trees. High-density populations of *A. tsugae* are rarely observed in northwestern Oregon. When *A. tsugae* populations are larger, or occur within hemlock stands, such as a seed orchard, *L. nigrinus* is able to find and feed on those *A. tsugae*. While *L. nigrinus* is an effective predator, it is unlikely that *L. nigrinus* is the sole mechanism preventing *A. tsugae* populations from growing to high densities.

One possible factor limiting the expansion of adelgid populations in northwestern Oregon is tree resistance. Pontius et al. (2002) suggest that foliar chemical differences between native North American hemlock species are associated with differential levels of adelgid infestation and hemlock decline. Foliar chemical differences could make some hemlock species less palatable to adelgids, or the differences could indicate a defensive reaction of some trees to adelgid infestation. Differential levels of palatability or defensive abilities between eastern and western hemlock would be consistent with data from this and prior *A. tsugae* research.

Future surveys for predators of *A. tsugae* in the Pacific Northwest should concentrate on larger populations of adelgids and seed orchards, where *A. tsugae* is likely to infest a large number of hemlocks. Surveys of populations of balsam woolly adelgid, *A. piceae*, and other adelgids in the Northwest could identify additional adelgid predators for evaluation as potential biological control agents. Identification of additional specialists on hemlock woolly adelgid will most likely require further exploration in the regions where *A. tsugae* is native.

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Chapter 3: Conclusion

The loss of eastern hemlocks caused by *A. tsugae* shows no sign of stopping. Intervention to prevent the spread of the adelgid and reduce its damage to trees will require continued integrated management. Thus far, quarantines and silvicultural treatments have been used in efforts to reduce spread. In addition, systemic and foliar pesticide treatments and biological control releases have been used to manage populations of the adelgid.

Pseudoscymnus tsugae and *L. nigrinus* were selected as biological control agents for *A. tsugae* based host specificity and other desirable characteristics. Both were collected from hemlock where they used *A. tsugae* as a food source. Field tests of *L. nigrinus* have shown synchronicity between its life cycle and that of *A. tsugae* (Zilahi-Balogh et al 2003). It is not yet known whether these agents, alone or in combination, can reduce damage to *T. canadensis* by *A. tsugae* on the stand level. Any future potential biological control agents should be carefully evaluated prior to release to maximize efficacy and reduce the chances of non-target effects. Locating additional host-specific predators will require surveys of persistent populations of the adelgid.

This study looked at predators present on trees hosting small, isolated populations of *A. tsugae*. Sampling was carried out successfully over an entire year, an unprecedented duration for a survey of *A. tsugae* predators. A large diversity of arthropod groups was collected from study trees, including a variety of predatory species, however, few were considered important predators of the adelgid. In northwestern Oregon, a complex of host-specific predators associated with *A. tsugae* was not observed.

Of the predators recovered, only *L. nigrinus* is likely to be an important predator of the adelgid.

Observations made in this study and in British Columbia (Zilahi-Balogh et al. 2003) suggest that emergence and maturation of the two yearly generations of *A. tsugae* are variable in their timing from year to year and between regions. The differences observed in Oregon between 2003 and 2004 coincided with warmer temperatures in the late winter and spring of 2004 than in the previous year. Future observations may show variations in adelgid development between and within regions that coincide with temperature regimes.

Hemlock woolly adelgid is not a threat to hemlock in northwestern Oregon. While it is unclear why populations of *A. tsugae* persist but do not cause damage to trees, one possibility is that western hemlock is a less suitable host for the introduced insect. Future work on the relationship between the adelgid and its hemlock hosts could explain why the damaging outbreaks in the east are not mirrored in the west.

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