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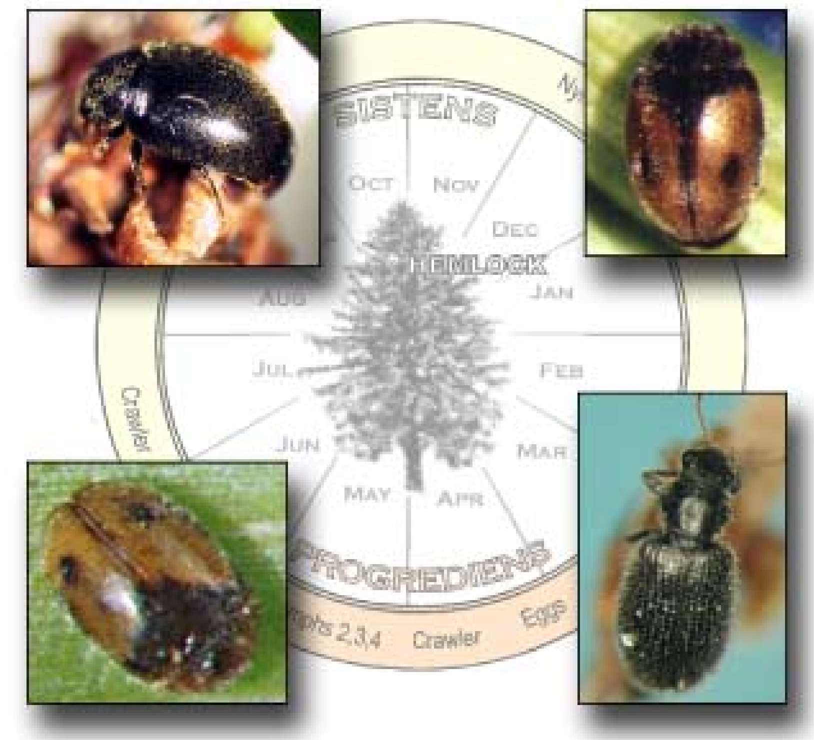
TECHNOLOGY
TRANSFER

Biological Control

BIOLOGICAL CONTROL OF HEMLOCK WOOLLY ADELGID



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On the cover

Clockwise from top left: adult coccinellids *Sasajiscymnus tsugae, Symnus ningshanensis,* and *Scymnus sinuanodulus*, adult derodontid *Laricobius nigrinus*, hemlock woolly adelgid infected with *Verticillium lecanii*.

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Biological Control of Hemlock Woolly Adelgid

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About this publication

This publication covers the distribution, biology, damage, and biological control of hemlock woolly adelgid (HWA) and is a substantial revision of FHTET-2000-08, *Biological control of Hemlock Woolly Adelgid in the Eastern United States*, by Mark McClure, 2001.

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DEDICATION

We dedicate this publication to Mark McClure – recently retired from the Connecticut Agricultural Experiment Station – who conducted many of the early investigations into the hemlock woolly adelgid. His efforts provided a foundation for all the work now being carried out.

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INTRODUCTION

Overview

Figure 1. Hemlock woolly adelgid. a) Ovisacs on the underside of a hemlock branch (UGA1276001). b) Adult and eggs inside an ovisac (UGA1276002). c) Adult with wax removed (UGA1276003).

The hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Homoptera: Adelgidae), is a non-native pest and has become the single greatest threat to the health and sustainability of hemlocks (*Tsuga* species) in eastern North America. The defoliation and mortality of hemlock forests equates to the loss of distinctive habitat and microclimates, and degradation of biodiversity. Also, it poses a significant threat to ornamental hemlocks, which are widely used as landscape trees and provide valuable biodiversity to the urban environment.

The HWA is an insect 1 to 2 mm long (about the size of this letter "o") with piercing/sucking mouthparts, which it inserts at the base of hemlock needles to feed on nutrients stored in young twigs (McClure et al 2001). The tree's vitality depends on these nutrients; depleting the nutrient reserves causes needle loss and a reduction in shoot growth, and can seriously impair tree health. As the adelgid matures it produces increasing amounts of white woolly wax, with which it protects itself and its eggs (Fig. 1).

Populations of HWA in eastern North America fluctuate greatly in response to various factors, such as cold winter temperatures, declining tree health, and drought. Controlling HWA on ornamental hemlocks is relatively easy compared to controlling it in forested and woodland settings. Individual trees can be treated either by drenching foliage with insecticidal soaps or horticultural oils, or by injecting the trunk or soil with chemical insecticides (e.g., imidacloprid). Regardless of the application method, care must be taken, because insecticide applications can unintentionally lead to an increase in secondary pests, such as spider mites and scales. In the forest, and especially in those situations in which insecticide use is not appropriate, biological control with predators and pathogens is the preferred way to manage HWA populations.

Distribution

HWA is native to Asia (Japan, India, Nepal, southwestern China, and Taiwan), where it is a common but harmless inhabitant of several hemlock (*Tsuga*) species. It was first observed in Oregon and British Columbia in the 1920s on western hemlock (*T. heterophylla* [Ref.] Sargent) and mountain hemlock (*T. mertansiana* [Bong.]) Carr. It can now be found from northern California to southeastern Alaska, where it is relatively harmless. Natural enemies account for some HWA mortality, but apparently western and mountain hemlock, as well as hemlock species found in Asia, have host defenses against HWA.

Hemlock woolly adelgid was first collected from hemlock in the eastern United States in 1951 near Richmond, Virginia. The pathway and source of the introduction are undetermined but thought to be nursery stock from Asia. It quickly established itself in natural stands and spread rapidly. By the 1980s it was causing widespread mortality. It has since spread into 16 states on the eastern seaboard, from northeastern Georgia to southeastern Maine, where it is a serious pest of eastern hemlock (*T. canadensis* [L.] Carr.) and Carolina hemlock (*T. caroliniana* Engelm.). Since 1993, the main front of the HWA infestation has been advancing at a rate of approximately 17 kilometers (10 miles) per year (Fig. 2), and isolated infestations have been discovered far ahead of the main front.

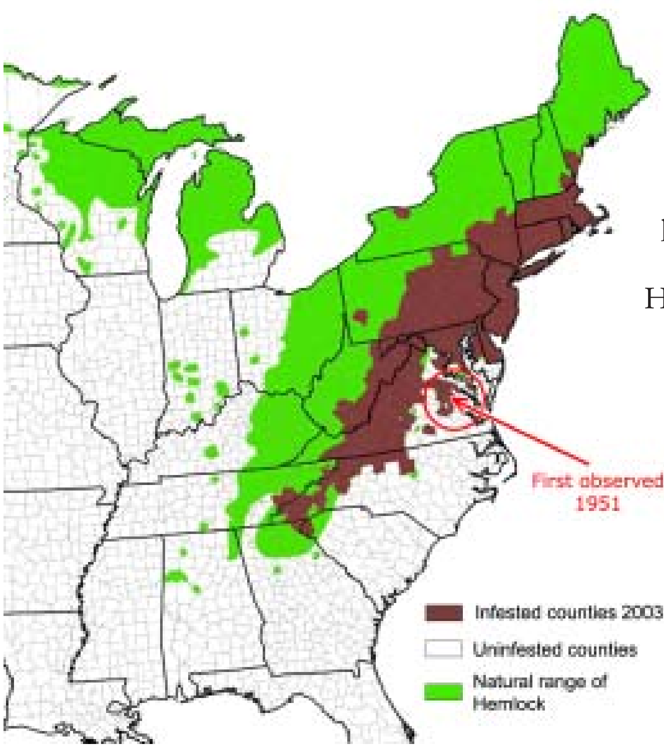

Figure 2. Native range of hemlock (green) and range of hemlock woolly adelgid (brown) in 2003.

Hemlock woolly adelgid spreads mainly as eggs or "crawlers," the mobile first instar nymphs of the insects that hatch from the eggs, which are carried by wind, birds, other forest animals, or people. In the eastern United States, HWA is established in much of cold hardiness zone 6 (mean annual minimum temperature -25° to 18°C [-13° to 0°F]) and parts of zone 5 (-30° to -23°C [-20° to -10°F]). It is likely to continue to spread and threaten eastern and Carolina hemlocks across much of their natural ranges.

Biology and Damage

In North America, hemlock woolly adelgid is parthenogenetic (only females occur, reproducing without males) and produces two generations a year. One, the **sistens**, is wingless, hatches in late spring, overwinters, and survives about nine months. The other, the **progrediens**, hatches in early spring, is comprised of both wingless and winged (sexupara) offspring, and survives for about three months. Sexupara fly from hemlock in search of a species of spruce (*Picea*) on which to deposit eggs (Fig. 3). However, a suitable species of spruce is not present in North America, so this portion of the populatin dies before sexual reproduction occurs.

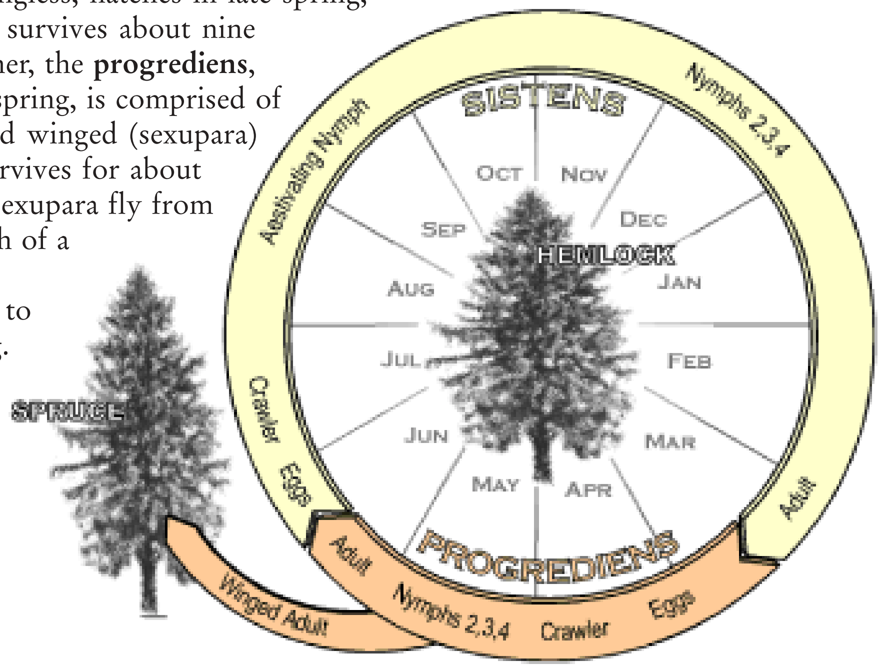

Figure 3. Hemlock woolly adelgid annual life cycle on hemlock in North America.

The lifecycle of HWA promotes a rapid increase in population (Fig. 3). Shortly after the sistens eggs hatch, the first instar nymphs relocate to the bases of needles and immediately become dormant (aestivation). As temperatures cool in the fall, the sistens nymphs break dormancy and begin to feed and develop throughout the winter when temperatures are moderate. Adults begin to lay eggs in March – earlier in southern states or during mild winters. During late fall and early spring, few natural enemies are active and hemlocks produce abundant quantities of sugar and amino acids, which provide good nutrition in the twigs where adelgids feed. This results in a high level of egg production. A single sistens female typically lays between 50 and 175 eggs (as many as 300 have been observed) (McClure et al. 2001). The progrediens generation lays fewer eggs, typically between 25 and 125, but offspring mature rapidly after hatching. Egg production in early spring and again in early summer has a multiplier effect on the population, which if unchecked by natural enemies or other factors results in exponential population growth.

Hemlock woolly adelgid feeding on eastern and Carolina hemlocks cause the needles to desiccate (dry up) and the buds to stop growing. Within a few months of heavy infestation, the tree looks grayish green, needles begin to drop off, and little or no new foliage is produced. Foliage loss and dieback of major limbs become visible in 2 to 4 years. An infested hemlock may survive for many years, but its foliage is usually sparse at the branch tips and very top of the crown (Fig. 4, page 4). Weakened trees often succumb to diseases and attacks from other insects, such as hemlock borer *Melanophile fulguttata* (Harris), elongate hemlock scale *Fiorinia externa* Ferris, and are easily broken and thrown by wind.

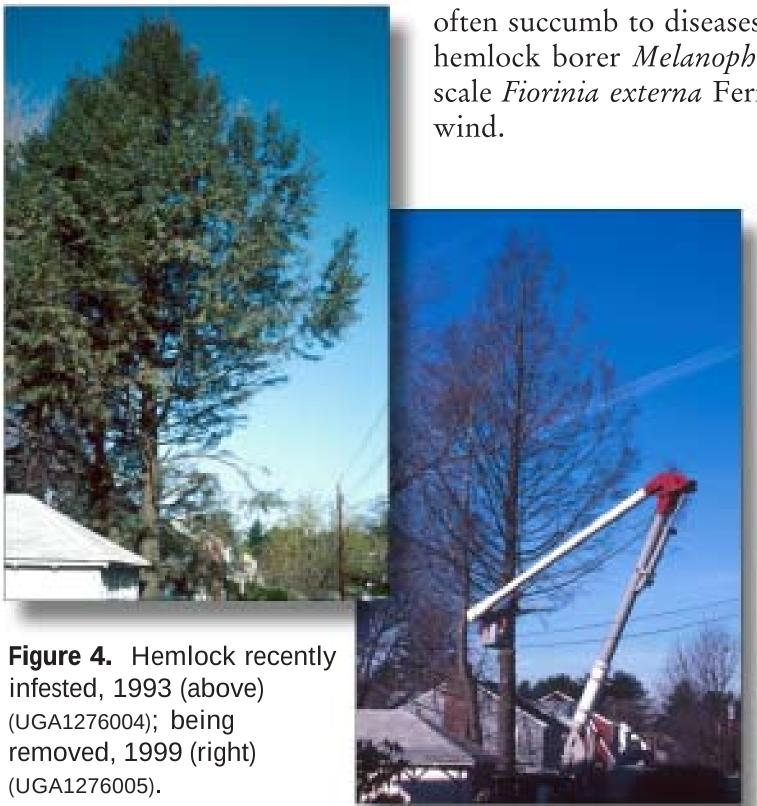

Figure 4. Hemlock recently infested, 1993 (above) (UGA1276004); being removed, 1999 (right) (UGA1276005).

Focus on Biological Control

Biological control uses natural enemies (predators, parasitoids, or pathogens) to lower pest populations. There are no known parasitoids of HWA, worldwide, and there are only a few predators of HWA in the eastern United States. Most of the predators tend to be generalists (McClure 1987, Montgomery and Lyon 1996, and Wallace and Hain 2000). Some non-host-specific, native fungi have been found to infect HWA, but naturally occurring epizootics have not been observed (Reid et al 2002).

Native predators and pathogens in North America cannot maintain HWA populations at low enough densities to prevent them from damaging hemlock forests, so in 1992, studies began on biological control agents imported to the United States from Japan, China, and Canada (Fig. 5).

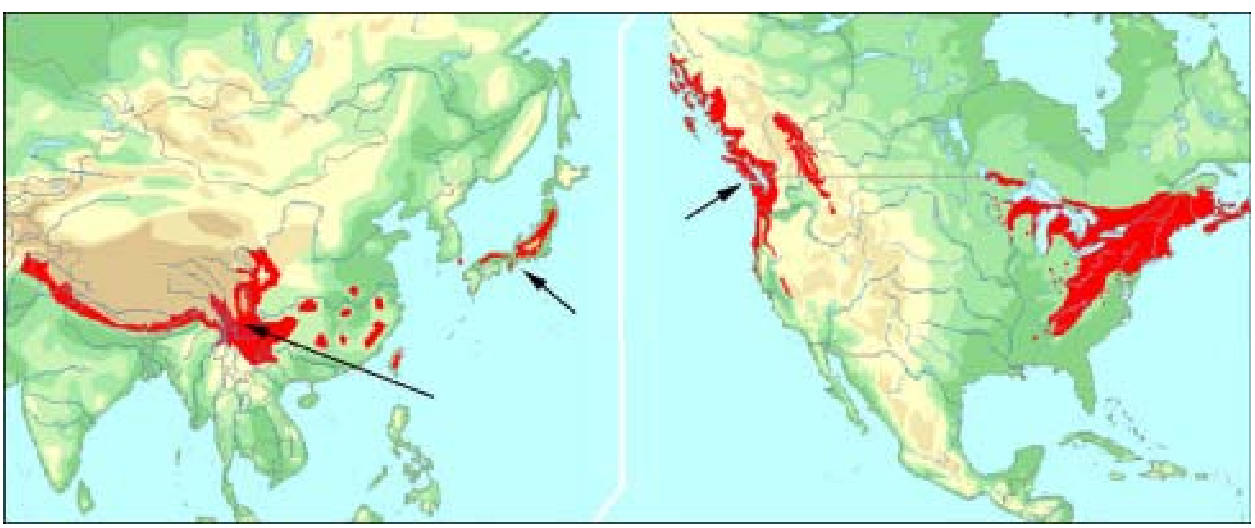

Figure 5. Worldwide occurrence of Hemlock (red) with arrows showing where biological control agents were collected for importation to the United States.

CHAPTER 1: BIOLOGICAL CONTROL AGENTS

Sasajiscymnus Lady Beetle from Japan -- Carole Cheah

Figure 6. Life stages of *Sasajiscymnus tsugae.* a) Egg inserted in budscale (UGA1276006). b) Larva (UGA1276007). c) Pupa (UGA1276008). d) Adult (UGA1276009).

Explorations for natural enemies of HWA began in Japan in 1992. Of the several predators collected from adelgid-infested Japanese hemlocks, a tiny lady beetle, *Sasajiscymnus tsugae* (Sasaji and McClure) (formerly *Pseudoscymnus tsugae* Sasaji and McClure [Coleoptera: Coccinellidae]), from Osaka prefecture (approximately 34° N) on the northern island of Honshu (Sasaji and McClure 1997) proved to have the greatest potential for biological control of HWA (Fig. 6). (***Note:*** The lady beetle, *Pseudoscymnus tsugae*, was recently reclassified as *Sasajiscymnus tsugae* by Vanderberg [2004].)

Sasajiscymnus tsugae belongs to the Tribe Scymnini, a group of small coccinellids, less than 3mm long, that are specialist predators of aphids, scales, mealybugs, and adelgids. The adult is black, about 2mm long, and both larvae and adults feed on all stages of HWA. Females lay eggs singly or in small clusters in concealed locales on hemlock foliage, buds, cones, and stem crevices.

There are four larval instars and a pupal stage, with 70 percent of the total pre-adult development time taking place during the mature fourth instar. Eggs hatch in 6 days at 25°C (77°F) and 10 days at 20°C (68°F); maturation to adult takes 24 days at 25°C and 40 days at 20°C (Cheah and McClure 1998.)

Sasajiscymnus tsugae prefers adelgids to aphids, and can complete development on HWA as well as the balsam woolly adelgid, pine bark adelgid, and Cooley spruce gall adelgid. A lab colony of *Sasajiscymnus tsugae* was established in Windsor, Conn., in 1994; *S. tsugae* were free-released in North America in 1995. Subsequent studies at The Connecticut Agricultural Experiment Station showed it to have a high lifetime fecundity. Females lay between 64 and 513 eggs (avg. 280) over a period of 5 to 30 weeks (avg. 14 weeks) (Cheah and McClure 1998). Adults have long life spans (longer than one year with overwintering). *Sasajiscymnus tsugae* is bivoltine, producing two generations per year in the northeastern United States (Cheah and McClure 2000).

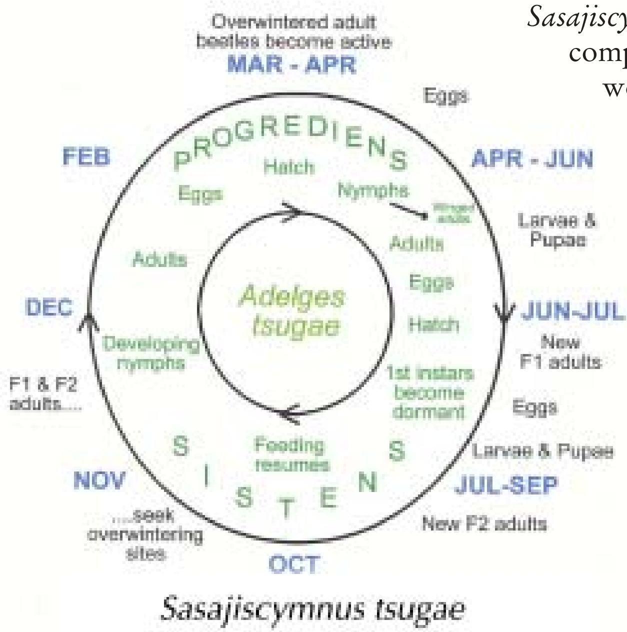

Figure 7. Seasonal synchrony between *Sasajiscymnus tsugae* and its prey, hemlock woolly adelgid.

Other field studies have shown that *S. tsugae* can reproduce after release, disperse locally, survive heat waves, overwinter, and establish in a variety of different hemlock habitats in Connecticut and other states (Cheah and McClure 2000). It responds well to laboratory mass rearing on HWA-infested foliage collected from the field between fall and mid-summer.

Field releases of *S. tsugae* began in Connecticut in 1995 and elsewhere in 1999. Since 1995, over 1-million *S. tsugae* have been released on more than 100 sites in 15 eastern states, from South Carolina to Maine. The releases were made possible largely through the mass rearing efforts of the Phillip Alampi Beneficial Insects Laboratory of the New Jersey Department of Agriculture, and EcoScientific Solutions, LLC, the North Carolina Department of Agriculture, and Clemson University.

Studies conducted in Connecticut have established that a significant synchrony exists between the life cycles of *S. tsugae* and HWA (Cheah and McClure 2000) (Fig. 7). Adult beetles emerge from overwintering sites in hemlock forests in March and April. Females generally mate before the onset of winter and begin oviposition on the HWA sistens generation in April, when daytime temperatures average 15°C (59°F). Females lay eggs throughout the spring into mid-summer on both the sistens and progrediens adelgid generations. A smaller second generation of *S. tsugae* is also produced on progrediens, with new adults emerging from mid-August into September. Incubation periods and larval development times vary and depend on seasonal spring temperatures. Larvae feed on all stages of HWA (Cheah and McClure 1996) and the first generation of adults generally emerges in June or July. Adult *S. tsugae* can survive the late summer period by feeding on dormant settled adelgid nymphs and can be found in hemlock forests during the late summer and early fall. Adults were found on hemlock foliage during mild winters in the northeast (Cheah and McClure 2000). In sleeve cages, *S. tsugae* survived minimal daily winter lows of -21.6°C (-7°F) in northern Connecticut, and -21°C (-5.8°F) in north central Maine (Cheah and Donahue 2003).

Field Impacts

At four forested sites in Connecticut and Virginia, initial field releases of *S. tsugae* on un-bagged branches have shown good potential for producing localized reductions in pest levels. Between 2,400 and 3,600 adults were released, 30 to a branch per site, onto established HWA infestations in May, 1997. In October, HWA densities were significantly lower on release branches than on non-release control branches, 500 meters away. Adelgid densities were 47-83 percent lower on exposed, un-bagged branches in the release area, compared to both bagged branches, which previously had been enclosed to exclude both native and introduced predators, and un-bagged branches in control areas (McClure et al 2000). Sampling for *S. tsugae* with a beating sheet, following augmentative releases in subsequent years, indicated successful overwintering, reproduction, and local dispersal, both laterally and vertically into the upper canopy (Cheah and McClure 2000).

In Connecticut it appears the long-term impact of *S. tsugae* releases on hemlock health varies with the type of site. Long-term monitoring (4 to 7 years) of *S. tsugae* release sites in Connecticut shows that hemlock recovery on some sites can be hampered by several factors, including soil characteristics (rocky or shallow), drought stress, and infestations of either elongate hemlock scale or hemlock borer. Also, the probability of effective prey reduction within 3 years after a predator release is influenced by the magnitude of the adelgid infestation prior to the release. In sites with stressed trees and initially high adelgid and scale infestations, *S. tsugae* appears less able to establish itself rapidly enough within a few years of release to slow hemlock decline. However, in some sites previously infested hemlocks with dieback have demonstrated a remarkable ability to recover and produce new shoots, especially under favorable environmental conditions, such as abnormally moist or cool springs and summers (Cheah and McClure 2000).

In better quality woodland sites, adelgid densities have remained low and the trees have shown recovery after 4 to 7 years. Recent winter extremes (2000 and 2003) have dramatically reduced adelgid populations, which has favored predator impact. It is evident that the winter mortality of the sistens generation plays a significant role in adelgid population dynamics in spring and summer.

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Scymnus Lady Beetles from China -- Michael E. Montgomery

Exploration for natural enemies of HWA was undertaken from 1995 through 1997 in the Chinese provinces of Yunnan, Sichuan, and Shannxi, at the edge of the Tibetan Plateau (Yu et al. 1997, Wang et al. 1998, Zhao et al. 1999) (Fig. 8). Three species of hemlock grow in this steep, rugged, mountainous terrain at altitudes between 2,500 and 3,000 meters (8,000-10,000 feet). The hemlocks usually occur as a scattered, minor component in forests of mixed deciduous and coniferous species (Mongtomery et al. 1999).

Figure 8. Map of China showing three provinces (red arrows) from which *Scymnus* spp were collected.

More than 54 species of lady beetles were collected with more than half of the species new to science (Yu et al. 2000). Two-thirds of the lady beetles collected are in the coccinellid subfamily Scymninae, which, with more than 600 species, is the largest subfamily of lady beetles. Its members are small, pubescent, and dull colored. They are fairly prey specific and usually feed only on one family in the order Homoptera. Several species have been used successfully for biological control (Hagen et al. 1999). The two groups of lady beetles in the Scymninae subfamily that seem most promising for biological control of HWA are in the genera *Sasajiscymnus* and *Scymnus* (subgenus *Neopullus*). Both are endemic to Asia and have not been found in North America.

Figure 9. *Scymnus* lady beetles from China. a) *Scymnus camptodromus* Yu and Liu (UGA1276010). b) *Scymnus sinuanodulus* Yu and Yao (UGA1276011). c) *Scymnus ningshanensis* Yu and Yao (UGA1276012).

At each of three collection sites in China, the most abundant lady beetles feeding on HWA were previously unknown species of *Scymnus* (*Neopullus*) (Coleoptera: Coccinellidae). (These lady beetles were also observed to feed on an adelgid found on *Pinus armandii* [Yu 1999]). The beetles were subsequently identified as *Scymnus camptodromus* Yu et Liu, *Scymnus sinuanodulus* Yu et Yao, and *Scymnus ningshanensis* Yu et Yao (Fig. 9).

The biology and host range of each of the three *Scymnus* species were studied in China and, after importation to the United States, in quarantine.

Host range tests showed *Scymnus* adults prefer to feed on adelgids, but will feed to a limited extent on aphids (Montgomery et al. 1998, Butin et al. 2002). *Scymnus* adults will feed on all life stages of HWA; however, *Scymnus* larvae (first instar) will survive only if they can feed on HWA eggs.

The life stages of the three lady beetles are similar (Fig. 10). In nature, all three beetles lay many single eggs in bud-crevices, beneath the ovisac wool, or in other concealed locations. The eggs of *S. sinuanodulus* and *S. ningshanensis* hatch in about 10 days; the eggs of *S. camptodromus* enter diapause and do not hatch until the following spring. Diapause makes it difficult to rear *S. camptodromus* in the laboratory, making it less attractive as a candidate for biological control than either *S. sinuanodulus* or *S. ningshanensis*. All three beetles pass quickly through four larval instars and a pupal stage (Lu et al. 2002), with total development time from egg hatch to adult between 25 and 30 days at room temperature, 20°C (68°F) (Wang et al. 2000, Montgomery et al. 2002). Generally, newly matured adults overwinter one season before laying eggs, and unlike *Sasajiscymnus tsugae*, they produce only one generation per year.

Figure 10. Life stages of *Scymnus* lady beetles, represented by *S. ningshanensis*. a) Egg inserted in bud scale (UGA1276013). b) Larva (UGA1276014). c) Pupa, masked with defensive secretion at tip of setae (UGA1276015). d) Mating adults (UGA1276016).

Egg laying by the *Scymnus* beetles is dependent on the availability of prey. Beginning within one week after emergence from overwintering, the beetles lay one to two eggs per day for 4 to 6 weeks, followed by a decline of approximately one month due to a lack of prey. However, if prey should happen to be available, the egg-laying period can last for 7 months (Zhao et al. 1998, Lu and Montgomery 2001, Mongtomery et al. 2002).

Figure 11. Sleeve cage used for field study of *Scymnus* lady beetles (UGA1276017).

Sleeve cages are being used to evaluate the impact of *S. sinuanodulus* and *S. ningshanensis* on the population dynamics of HWA. Adelgid woolly masses on a hemlock branch are counted in April, when the overwintering sistens generation of HWA starts to lay eggs. After counting, either a lone female beetle or a male/female pair is placed in a mesh bag, which is then affixed to the branch tip infested with around 300 adult adelgids, far more than the beetles are expected to eat (Fig. 11). Bags with no beetles serve as controls. In late June, when the progeny of the sistens are mature, the bagged branches are brought to the laboratory; the adelgids and lady beetles are counted and compared to the original counts (Butin et al. 2003).

In these field studies, the HWA populations were lower in the bags containing *Scymnus* beetles than in the control bags. However, the extent of the impact of the lady beetles appears to be affected by the condition of the adelgid population prior to the branches being bagged. If the adelgid population is low and rapidly increasing, the beetles dramatically reduce the rate at which the HWA population increases; if the population is declining because of a reduction in host nutritional value, the impact of the lady beetles on the HWA population is relatively small. By preventing low HWA populations from increasing, the *Scymnus* beetles stabilize the population, which in turn prevents the adelgids from damaging hemlock. Preconditioning the beetles for several weeks with simulated winter conditions prior to placement in the field might influence the beetles effectiveness against HWA.

The Connecticut Agricultural Experiment Station and the Phillip Alampi Beneficial Insects Laboratory, New Jersey Department of Agriculture, are cooperating with the USDA Forest Service to mass produce *Scymnus* beetles. As of this writing, a mid-summer (2004) release of newly reared, non-overwintered adult *S. sinuanodulus* beetles is underway in the Southern Appalachians.

Acknowledgments

Thanks and appreciation for assistance in collecting, rearing, and evaluating *Scymnus* lady beetles is extended to: Defu Yao, Houping Liu, Hongbin Wang, Guoyue Yu, Xueqin Xu, Hengxiao Guo, Carol ODell, Wenhua Lu, Julie Slowik, Jenny Ogrodnick, Lynn Jones, Nathan Havill.

Laricobius Derodontid Beetle from British Columbia -- Scott Salom

Dr. Lee Humble, a scientist with the Canadian Forest Service in British Columbia who searches for natural enemies of HWA and the balsam woolly adelgid, observed that the small, little-known beetle, *Laricobius nigrinus* Fender (Coleoptera: Derodontidae), consistently feeds on HWA in western hemlock seed orchards. Drs. Scott Salom and Loke Kok (Virginia Tech) visited these seed orchards and, between 1997 and 2003, imported the beetles to Virginia for study under quarantine. They determined *L. nigrinus* produces one generation per year and undergoes diapause at the same time and for the same duration as HWA (Zilahi-Balogh et al. 2003 a, b). The predator will feed on other adelgids, but prefers to feed on HWA, and will complete development only on HWA (Zilahi-Balogh et al. 2002).

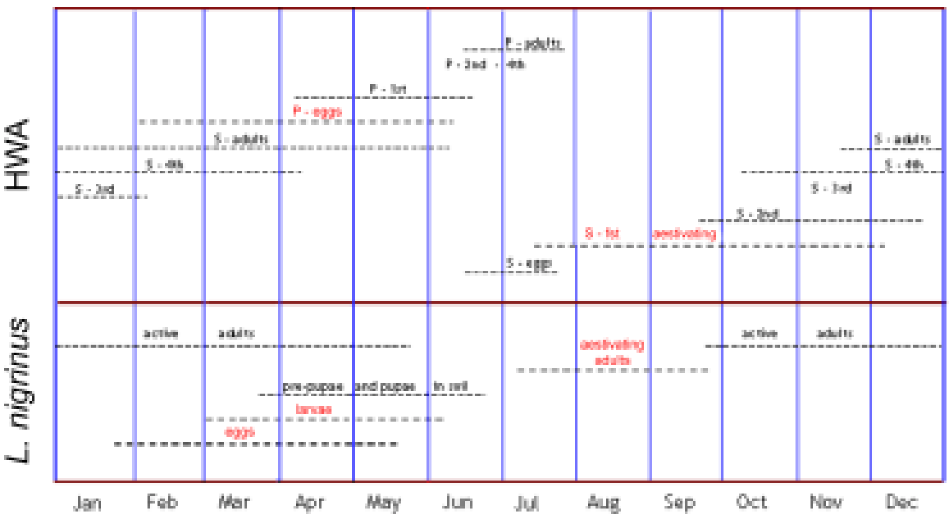

Figure 12. Lifecycles of HWA and *Laricobius nigrinus* from field data collected in Victoria, British Columbia (Zilahi-Balogh et al. 2003b). Life stages in red text denote synchrony between predator and prey.

Lab studies show that *L. nigrinus* prefers temperatures between 12° and 15°C (54° to 59°F), and field studies in British Columbia and Virginia show it is active in the winter – a critical point, because HWA is also active during the winter (Fig. 12). Adults feed on all HWA life stages present from November to May, and begin laying eggs in HWA ovisacs (one per ovisac) in February (Fig. 13). Larvae feed exclusively on HWA eggs.

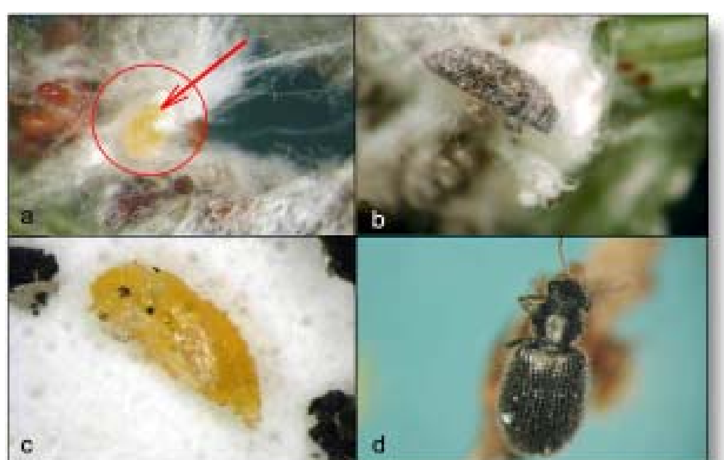

Figure 13. Life stages of *Laricobius nigrinus*. a) Yellow egg amidst red hemlock woolly adelgid eggs (UGA1276018). b) Late instar Larva (UGA1276019). c) Pupa (UGA1276020). d) Adult (UGA1276021).

Laricobius nigrinus was removed from quarantine in September, 2000. The following year, Virginia Tech began conducting field evaluations and exploring ways to rear the insect on a large scale. In

Figure 14. Eastern hemlocks with sleeve cages for *Laricobius nigrinus* (UGA1276022).

general, rearing HWA predators in the lab is labor intensive, mostly due to the enormous amount of fresh food required to maintain and build colonies. For *L. nigrinus* specifically, there are additional complications: They need cold temperatures; both the pupae and diapausing adults must live in soil; and the diapause period in lab-raised *L. nigrinus* must be in synch with the diapause period of HWA in the field. Complications aside, tremendous progress has been made, and the mass production of sufficient quantities of beetles needed for operational release appears achievable.

Field evaluations of *L. nigrinus* have been promising. Adults survive the Virginia winters in sleeve cages, and feed voraciously on HWA sistens. In the first field-release study of this predator, progeny produced by ovipositing adults in sleeve cages killed 50 percent more HWA progrediens than died naturally on untreated branches (Fig. 14). These experiments utilized 144 adults (one to three per branch) for only 10 days. Yet, during that time they yielded close to 12,000 predator eggs. Sampling to determine the establishment of *L. nigrinus* at this first release site began in fall 2003 and will continue for several years. In November and December 2003, 300 adult *L. nigrinus* were released at each of seven sites within the mid-Atlantic region. Releases will continue at increasing frequency over the next several years in an attempt to establish *L. nigrinus*.

Figure 15. *Laricobius* new species (UGA1276023).

A study was initiated in 2003 to evaluate the potential competitive interaction among two host-specific predators, *L. nigrinus* and *S. tsugae*, and the generalist, *Harmonia axyridus*. First-year results show the majority of *L. nigrinus* activity occurs earlier than that of both the other predators; both *L. nigrinus* and *S. tsugae* will feed on each others' eggs, but only when HWA density is very low; and *H. axyridus* might not be as formidable a predator on the other two as was expected.

A worldwide search for additional *Laricobius* species began in 2002. As a result, two new species were discovered in China, one of which is currently being reared and studied under quarantine at Virginia Tech (Fig. 15).

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Pathogens -- Bruce L. Parker, Scott Costa, Margaret Skinner

Insect pathogens (entomopathogens) can dramatically reduce insect populations (e.g., declines in gypsy moth populations in North America due to the fungus *Entomophaga maimaiga*). Most entomopathogens are microorganisms and include fungi, bacteria, viruses, protozoa, rikettsia and microsporidia.

Insect-killing Fungi

Insect-killing fungi differ from most entomopathogens because they can penetrate directly through the body wall of an insect (Fig. 16). Following the fungus-induced death of an insect, large numbers of fungal spores are released from the cadaver to continue the infection cycle throughout the insect population.

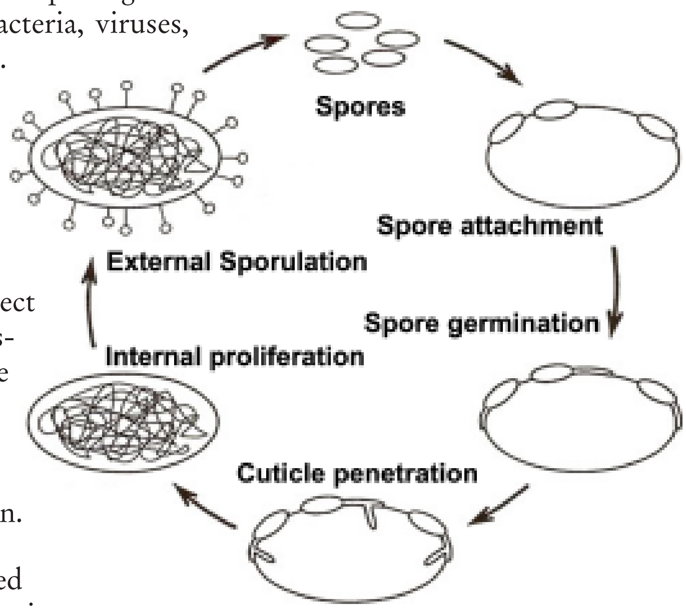

Figure 16. Generalized infection cycle of insect-killing fungi.

There is a long history associated with the use of insect-killing fungi for insect pest management. Four notable species, *Beauveria bassiana*, *Metarhizium anisopliae*, and *Verticillium lecanii* (Fig. 17, page 14), and *Paecilomyces* sp., have a nearly ubiquitous, terrestrial, worldwide distribution. Although these fungi are generalists in their host range, a variety of biological, ecological, and behavioral factors serve to limit their effects on non-target species. Also, certain species and some isolates of a given species tend to be virulent to insects within a given order.

Thousands of HWA, many showing signs of fungal infection, were collected from hemlock forests along the eastern seaboard of the United States and from southern China (Reid et al. 2002). From those collected, 79 different insect-killing fungal isolates were recovered, established in pure culture, and identified to species. Pure cultures have been placed in long-term storages at the University of Vermont Entomology Research Laboratory (ERL), Burlington, Vermont, and at the USDA, Agriculture Research Service (ARS), Ithaca, New York.

Extensive laboratory studies have been done to verify that these and other isolates from the ERL collection do indeed infect HWA (Reid 2003). Several of the more virulent isolates were selected for further testing. The selected isolates readily germinated and infected HWA at temperatures normally found throughout HWA's range and where hemlocks commonly grow. Field tests have been conducted to determine optimal formulations and spray delivery systems, rates,

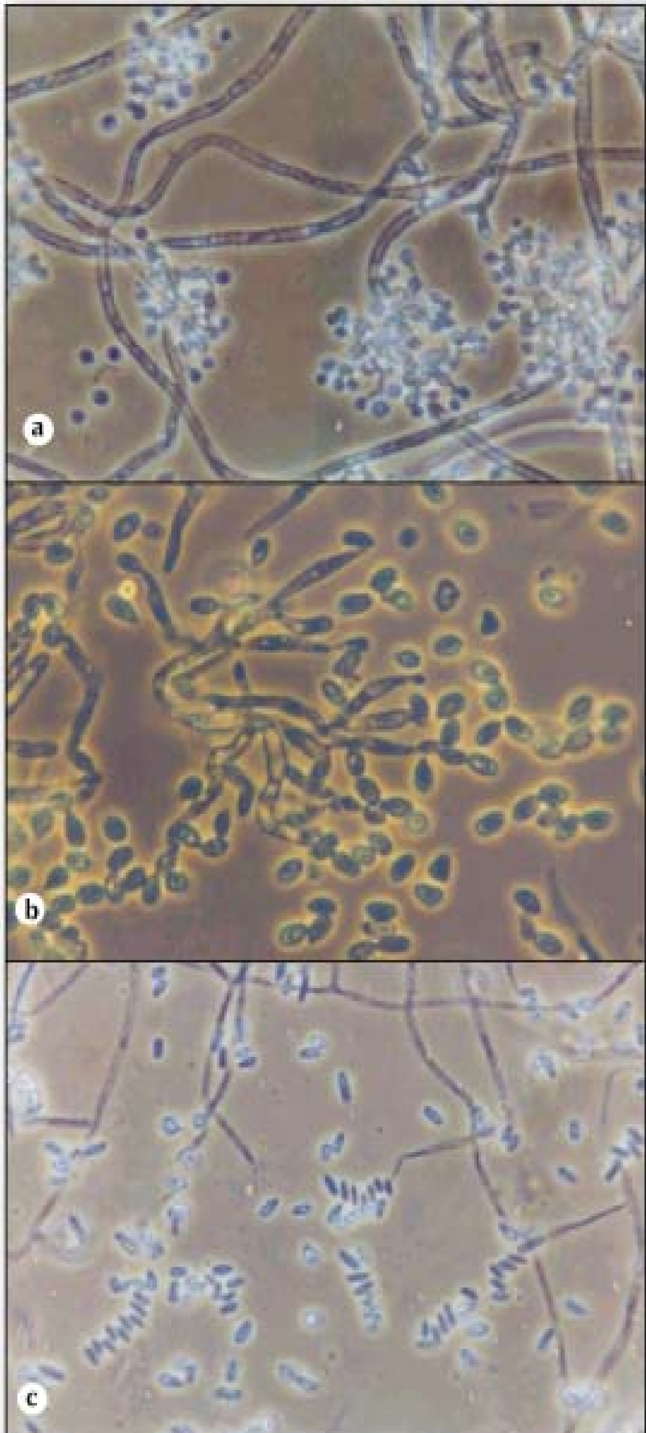

Figure 17. Spores. a) *Beauveria bassiana* (UGA1276024). b) *Metarhiz-ium anisopliae* (UGA1276025). c) *Verticillium lecanii* (UGA1276026).

and timings (e.g., spring *vs*. fall). The effects of fungi on *S. tsugae*, a non-target predator of HWA, and the development of technology for mass production of fungi for field-testing also are being studied. Although research is on-going, considerable progress has been made and results indicate fungi have good potential as a biological control agent for managing HWA.

Field Efficacy of Insect-Killing Fungi

Between spring 2001 and fall 2003, various field trials were conducted at Mount Tom Reservation, Holyoke, Massachusetts, to select the most virulent fungal isolates and determine the best timing and concentration for fungus applications. Fungi were applied to HWA-infested branches using hand-held and pressurized, and ULV sprayers (five to six branches per treatment). Both single and multiple applications of conidia and blastospores were tested at concentrations ranging from 5 x 10^7 to 2 x 10^8 spores per milliliter. Non-treated and formulation blank treated controls were used. Horticultural oil (1.0 percent) was used as a positive control. Survival and density of HWA were examined 3 to 4 weeks after application.

A reduction in HWA populations was selected for gauging fungal efficacy, because it is more reliable than observed mortality, and it best reflects our goal of suppressing HWA populations. During 2002 and 2003, HWA populations were significantly reduced by a single fall application of ARSEF 6010 (*V. lecanii*) at 1 x 10^8 spores per milliliter (Fig. 18, page 15). The presence of fungal outgrowth on an HWA cadaver killed by *V. lecanii* suggests the fungus could reinfect additional insects (Fig. 19, page 15). A second isolate, CA 603 *(B. bassiana)*, had significant activity only during 2002, and was also effective when applied using a ULV sprayer (data not presented).

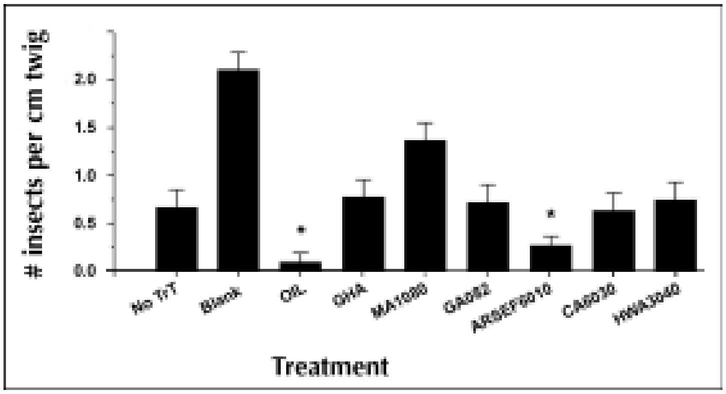

Figure 18. The density (#/cm) of HWA sistens in field trials in 2003, 4 weeks after treatment with insect-killing fungi at 1 x 10^8 sp/ml. *Means significantly different from blank control (GLM-ANOVA and Dunnett's). There were no significant differences among pre-treatment counts.

Horticultural oil also caused substantial population reductions, demonstrating the utility of the population-based approach for evaluating fungal efficacy. Similar applications of either fungi or oil during spring HWA population build-up were not as effective. The reduced effectiveness might be due to the fact that, in spring, HWA are covered with a waxy wool that might prevent the spray from making direct contact with the insect. The wool is absent during late summer and fall when HWA are aestivating as first instar sistens. Targeting HWA during late summer and fall also provides a wider window of opportunity for applying fungi.

Non-target Effect on HWA Predators

The management of a pest using biologically based strategies often includes more than one component in the management system. If insect-killing fungi are to be deployed in conjunction with predators it is important that these organisms be mutually compatible. During 2001, a petri dish assay system was developed at ERL that provides for both contact and residual exposure of *S. tsugae* to entomopathogenic fungi. Building on the assay development, laboratory testing for non-target effects against *S. tsugae* was completed during 2002. Evaluations were done in the forest in 2003 and several fungal isolates were exposed to *S. tsugae* in sleeve cages on HWA infested hemlock branches similar to those in Figure 11 (page 10). Adult *S. tsugae* for these trials were provided by the Phillip Alampi Beneficial Insect Lab, New Jersey Department of Agriculture.

Figure 19. Outgrowth of *Verticillium lecanii* from an HWA cadaver (UGA1276027).

In the laboratory assay, a concentration of 2 x 10^8 conidia per milliliter – twice the current field application rate – did not cause any significant decrease in survival rate for *S. tsugae* for any of the fungi examined, and none of the treated *S. tsugae* showed signs of fungal outgrowth. However, on day 15 there was some indication of reduced survival at the highest concentration of fungal isolates MA-1080 and GA-082. When tested under field conditions, no reduced survival was found with isolates that had significant field activity against HWA (Fig. 20). However, an isolate of *M. anisopliae*, with limited field activity against HWA (MA-1080), did result in reduced *S. tsugae* survival. This result was expected, because *M. anisopliae* is noted for activity against beetles. Consequently, this isolate was removed from further development.

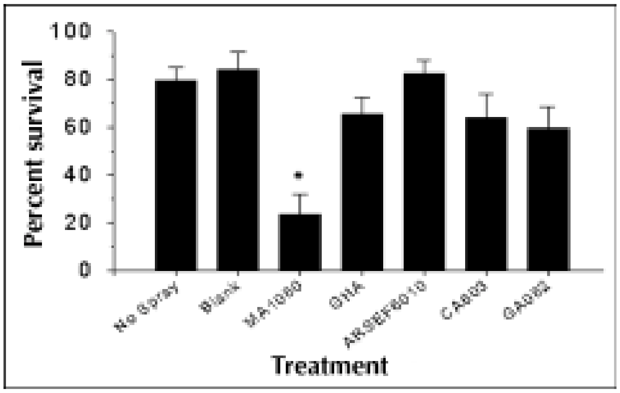

Figure 20. The survival of *Sasajiscymnus tsugae* in the forest after exposure to insect-killing fungi. *Means significantly different from blank control.

Further replicates of the field study are needed, and should include tests against *Scymnus* spp., *Laricobius* spp., and other predators being reared for release against HWA.

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CHAPTER 2: OUTLOOK

Developing a successful biological control program for HWA is a difficult task. The performance of natural enemies is rigorously density-dependent on the quality of both the HWA population and health of the tree. For example, predators are most effective against HWA if introduced to and allowed to become established on, small, healthy, developing HWA populations that have yet to have a severe impact on their hemlock hosts. Conversely, fungi might be most effective if applied to stressed HWA populations. In effect, predators and fungi could work in concert to maintain HWA populations at levels less damaging to hemlocks.

Half the range of hemlock in the eastern United States is now infested with HWA, and the entire range is at risk. It appears that a classical biological control program that uses both predators and pathogens will be required to maintain HWA populations at non-damaging levels. To be considered in such a program, candidate species must:

- show a significant impact and close association with the target pest;
- have a host range limited to that of HWA (or similar pest);
- originate in a climate similar to the proposed area of release;
- be tolerant of a wide range of environmental variables;
- be phenologically synchronized with HWA's life cycle.

The predators, *Sasajiscymnus tsugae*, *Scymnus sinuanodulus*, *Scymnus ningshanensis*, and *Laricobius nigrinus,* as well as several fungi, meet many of these attributes.

However, neither individually nor as a complex, should they be considered an immediate remedy to the HWA problem, but rather as parts of a long term solution.

The rearing of predators in laboratories must continue, along with the release of predators into numerous geographical areas to establish and promote their natural spread. Field insectaries need to be established in an effort to supplement the release of laboratory-reared individuals with “wild” individuals, which are more adapted to climate and other local variables.

The development of new isolates of insect-killing fungi is a long term process. The most active isolates of such fungi require further field testing in larger-scale trials and in multiple geographical areas. The methodology to mass produce these fungi for field trials has been developed and will support research on identifying optimal delivery strategies.

Integrating these and other biological approaches is an underlying theme that must be continuously considered while developing an HWA management strategy. Efforts must continue in Asia and along North America’s west coast to identify more natural enemies of HWA. The addition of species to the natural enemy complex will improve chances for successful biological control of HWA as it spreads throughout the range of eastern hemlock.

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