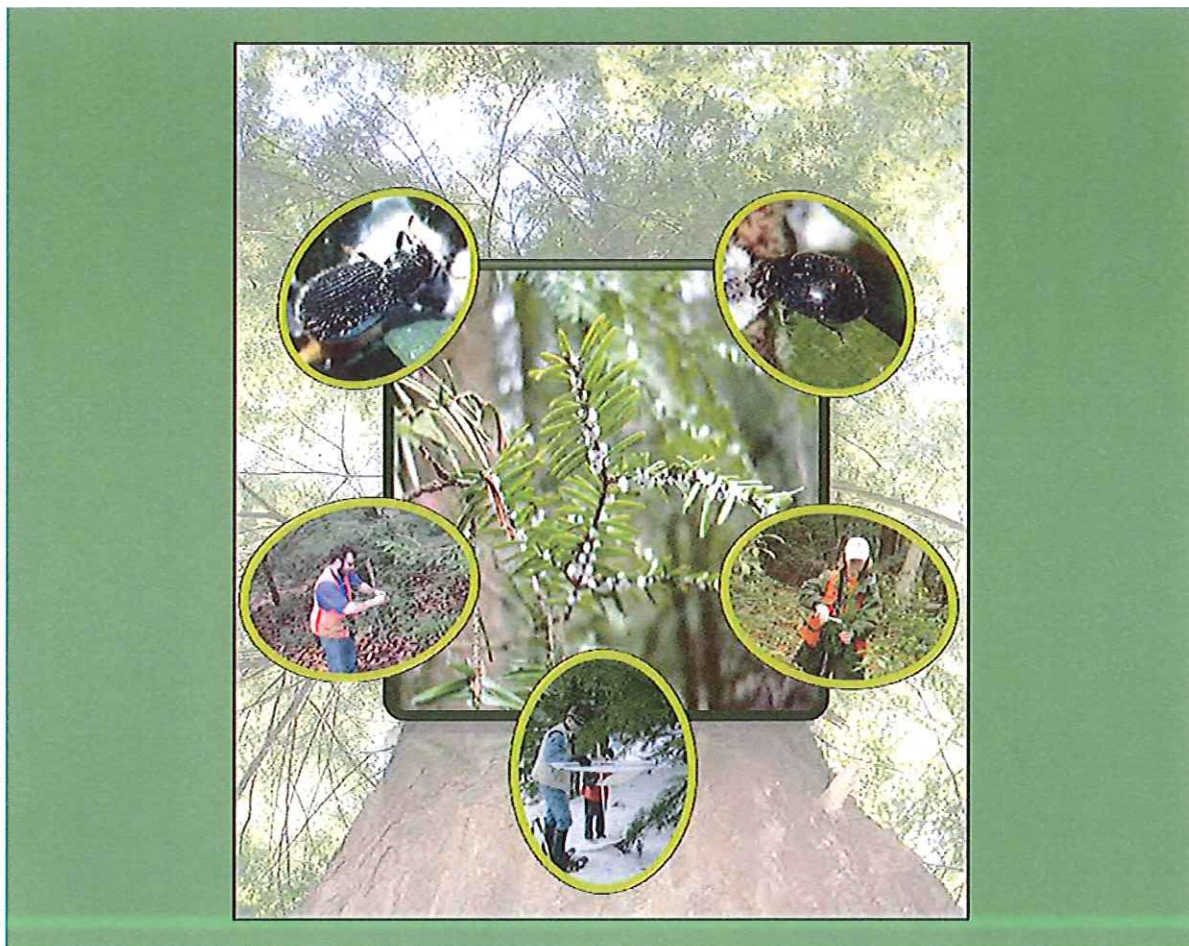


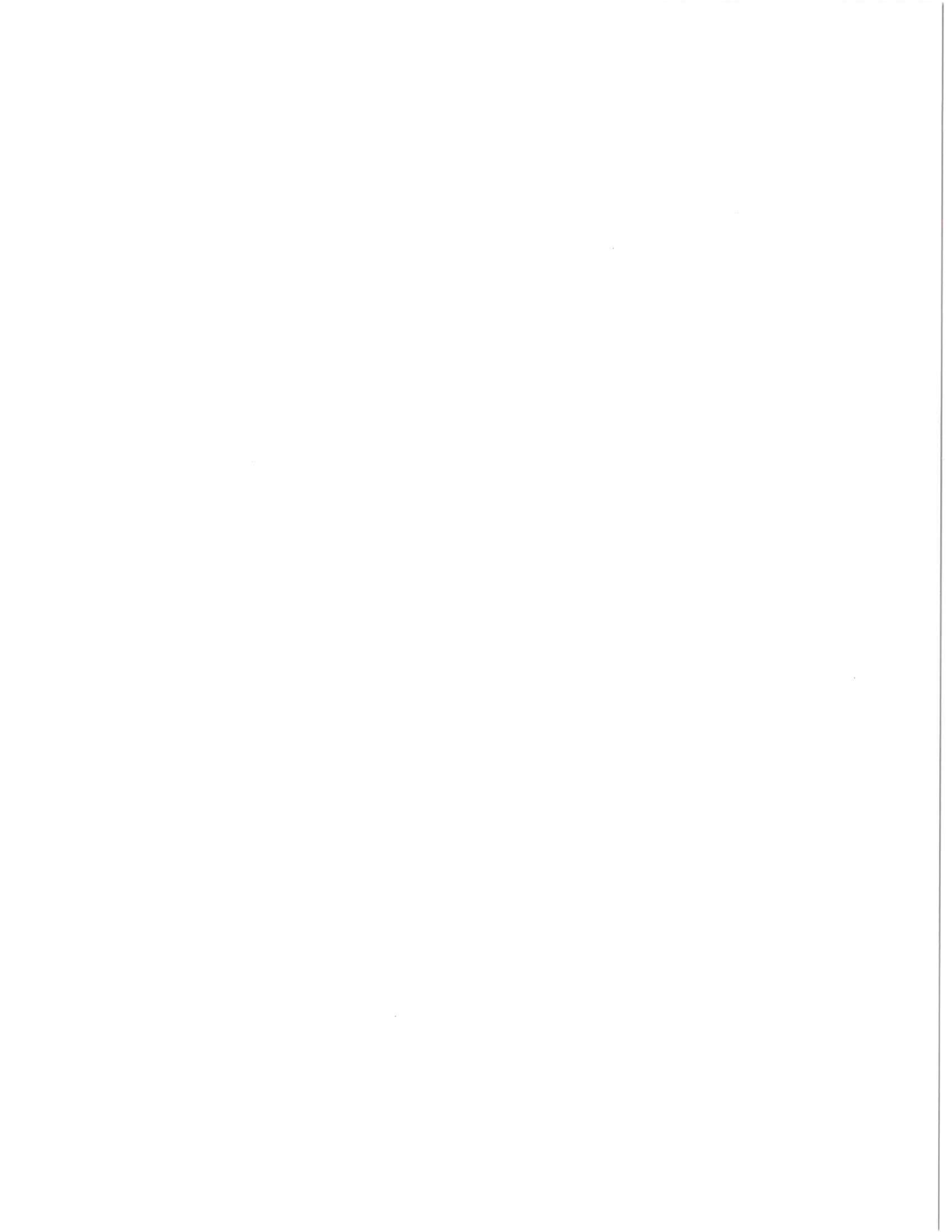
Forest Health Technology Enterprise Team

TECHNOLOGY
TRANSFER

*Biological
Control*

IMPLEMENTATION AND STATUS OF BIOLOGICAL CONTROL OF THE HEMLOCK WOOLLY ADELGID





CHAPTER 4: SASAJISCYMNUS (=PSEUDOSCYMNUS) TSUGAE, A LADYBEETLE FROM JAPAN

Carole Cheah

The Connecticut Agricultural Experiment Station, Valley Laboratory, Windsor, CT

ORIGIN, DESCRIPTION, AND HISTORY OF INTRODUCTION

The first exploration for native natural enemies of hemlock woolly adelgid, *Adelges tsugae* (HWA) in Japan, conducted by The Connecticut Agricultural Experiment Station, began in 1992. Several predators were collected from adelgid-infested Japanese hemlocks, but it was a tiny ladybeetle which proved to have the most potential for biological control in subsequent laboratory and field evaluations. This ladybeetle was collected from adelgid-infested Japanese hemlocks, *Tsuga sieboldii* and *Tsuga diversifolia*, between mid-May and late June 1992, in 13 of 37 forests and at 11 of 37 ornamental sites in 12 prefectures throughout Honshu, Japan from sea-level to 1,980 m elevation (Sasaji and McClure 1997). In Japan, McClure determined that *S. tsugae* was responsible for 86% adelgid mortality in landscaped sites and 99% adelgid mortality in forest sites. In 1997, Sasaji and McClure named this new coccinellid species *Pseudoscymnus tsugae* (Coleoptera: Coccinellidae). In 2004, the genus was renamed *Sasajiscymnus* as a replacement for a name already in use for a genus of shark, and *P. tsugae* is now known as *Sasajiscymnus tsugae* (Vandenberg 2004). North American colonies of *S. tsugae* originated from field collections made in 1994 and 1995 from Takatsuki, Osaka prefecture, Japan (approximately 34° N), and were much later diversified with more recent collections from the Kansai district, central Honshu, Japan (Shiyake et al. 2008). *Sasajiscymnus tsugae* belongs to the Tribe

Scymnini, a group of small coccinellids, less than 3mm in length, which are specialist predators of aphids, scales, mealybugs, and adelgids. The adult is entirely jet black, on average 2 (1.5-2.5) mm in length, with dorsal pubescence and 9-segmented antennae (Sasaji & McClure 1997) (Fig. 1a). Amber-colored eggs are laid singly or in small clusters in concealed locales on hemlock foliage, buds, cones and stem crevices. Eggs, measuring 0.48 mm on average (Fig. 1b), hatch in 6-10 days, and development from egg to adult takes 24 and 40 days at 25 °C and 20 °C, respectively (Cheah and McClure 1998). There are four larval instars and a pupal stage, with the mature fourth instar (Fig. 1c) consuming > 70% of the total adelgid stages required for completion of development to adult (Cheah and McClure 1996). The mature fourth instar larva is on average 2.25-3.30 mm in length and dark grey or reddish brown in color while the pupa is a reddish brown (Fig. 1d) (Cheah and McClure 1998). *Sasajiscymnus tsugae* highly prefers adelgids to aphids and can also complete development on other adelgid species such as balsam woolly adelgid, *Adelges piceae*, pine bark adelgid, *Pineus strobi*, and Cooley spruce gall adelgid, *Adelges cooleyi* (McClure and Cheah 1998, Cheah and Donahue 2003). Host range tests showed that *S. tsugae* preferred *A. tsugae* to *Pineus strobi* on *Pinus strobus*, *Adelges laricis* on *Larix deciduas*, *Adelges cooleyi* on *Pseudotsuga menziesii* and the woolly alder aphid, *Paraprociphilus tessellatus* on *Alnus serrulata* (Butin et al. 2004). This species has no reproductive diapause and is, therefore, amenable

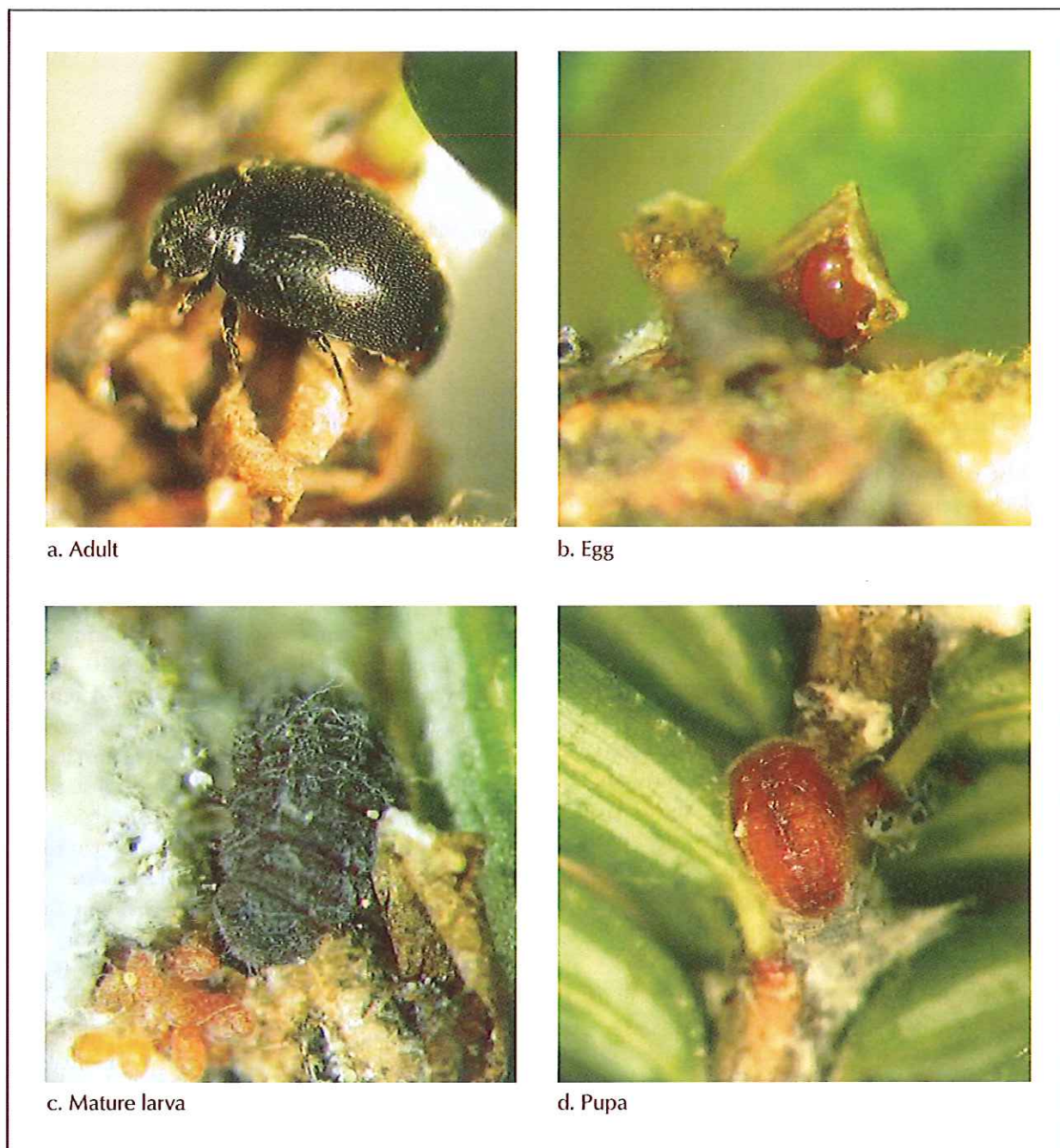


Figure 1. Stages of *Sasajiscymnus tsugae* (photos by C. Cheah).

to laboratory mass rearing on field collections of *A. tsugae* infested foliage from fall to mid-summer. Mass rearing magnitude is primarily limited by healthy adelgid prey availability. A federal permit for the release of *S. tsugae* in Connecticut was issued in April 1995 (Hennesy 1995). The first field release of *S. tsugae* was made in a town park in Windsor, Connecticut in May 1995. A starter colony of *S. tsugae* was transferred to the Philip Alampi Beneficial Insects Laboratory (PABIL), New Jersey Department of Agriculture in 1997, where mass rearing for the release in multiple states was initiated. Releases of *S. tsugae* were then expanded to other states in 1999, largely through the early mass rearing efforts of PABIL and EcoScientific Solutions LLC (Scranton, PA). More insectaries rearing *S. tsugae* and other HWA predators were later established in the southern states, greatly expanding releases in the southern range of HWA. Since 1995, over two million *S. tsugae* have been released in more than 400 sites on federal and non-federal lands to combat hemlock woolly adelgid in 16 eastern states from South Carolina to Maine.

BIOLOGY AND SYNCHRONY OF LIFE CYCLE WITH PREY

Scientists at The Connecticut Agricultural Experiment Station investigated the biology, life cycle and potential of *S. tsugae* for biological control of HWA. *Sasajiscymnus tsugae* has a high lifetime fecundity, and females lay an average of 280 (64-513) eggs over 14 (5-30) weeks (Cheah and McClure 1998). The adult has a long life span (> 1 year with overwintering), and exhibits excellent field synchrony with the both sistens and progrediens generations of *A. tsugae*. This species is the only multivoltine introduced HWA predator, producing two generations in the northeast (Cheah and McClure 2000). One hypothesis examined the relative developmental time of the predator in relation to its prey (Kindlmann and Dixon 1999). Kindlmann and Dixon predicted that predators that have a longer developmental time than their prey are unlikely to be successful biological control agents. Generation time ratio is defined as the

ratio of predator to prey developmental times (Kindlmann and Dixon 1999). Three year field studies in Connecticut indicated that temperature regulated *S. tsugae* F1 generation time is about 5 weeks in late spring and early summer and is similar for the F2 generation in mid to late summer (Cheah and McClure 2000). In contrast, generation time for the sistens *A. tsugae* is about 32 weeks, and 10 weeks for the summer progrediens generation (McClure, 1987). Generation time ratios for the *S. tsugae*-*A. tsugae* relationship are very favorable and between 0.16 and 0.5, conferring an advantage on *S. tsugae*. Comparison of these relative development times indicates the effective predatory impact of *S. tsugae*. In addition, successive, overlapping F1 cohorts of *S. tsugae* also span the second progrediens generation of *A. tsugae* and *S. tsugae* is the primary introduced predator with impact on this second adelgid generation. Furthermore, adults continue to feed and survive on aestivating first instar adelgid nymphs throughout the summer, augmenting the predation impact on its adelgid prey. Adults and larvae of *S. tsugae* are highly mobile and voraciously feed on all life stages of *A. tsugae*, from eggs and first instars to adults. Each beetle larva consumes about 500 adelgid eggs or 50 to 100 adelgid nymphs, depending upon their size, to complete development to adult. Adults can live for more than one year and may consume about 50 adelgid nymphs each week during times of peak reproductive and feeding activities (Cheah and McClure 1996).

The synchrony between life cycles of *S. tsugae* and *A. tsugae* studied in Connecticut is shown in Fig. 2 (Cheah and McClure 2000). 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 egg masses of the *A. tsugae* sistens generation in April, when daytime temperatures average 15 °C. Eggs of *S. tsugae* are laid throughout the spring into mid-summer during periods of both adelgid generations. Incubation periods and larval developmental times are variable and dependent on seasonal spring temperatures. The first field generation of adults generally emerges in June and July, but the timing is seasonally dependent on ambient temperatures.

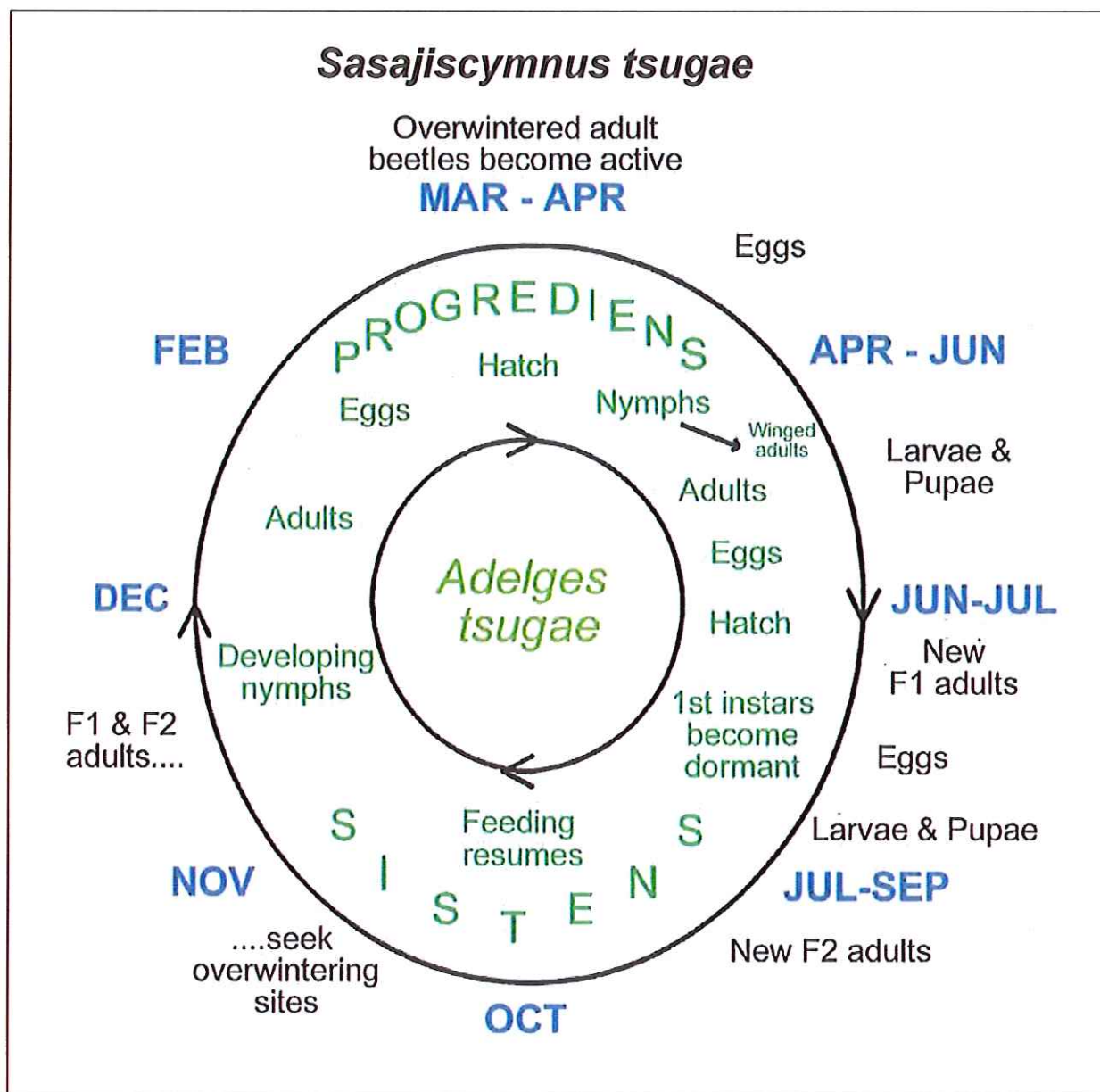


Figure 2. Seasonal synchrony between *Sasajiscymnus tsugae*, and its prey, the hemlock woolly adelgid, *Adelges tsugae* in Connecticut (adapted from Cheah and McClure 2000).

A smaller second generation of *S. tsugae* is also produced on the progrediens generation of *A. tsugae*, with new adults emerging in mid-August into September. Adult *S. tsugae* can survive the late summer period of dormant settled adelgid nymphs and were found in hemlock forests during the late summer and early fall. Sampling at various different sites in Connecticut and Virginia over 4 years showed that *S. tsugae* remained with its adelgid prey year round. In Connecticut, in the northern end of the adelgid distribution, *S. tsugae* adults were detected throughout the year during warm winter years, with larvae occurring from May to September in the field. Parallel sampling at two forest sites in Virginia, in the southern range of adelgid distribution, showed that *S. tsugae* adults were present from April to November (Cheah and McClure 2000). During milder winters in the northeast, adults overwinter on hemlock foliage (Cheah and McClure 2000). *Sasajiscymnus tsugae* has been documented in field cages in 2003 to survive minimum daily winter lows of -7 °F in northern Connecticut and -5.8 °F in 2002 in north central Maine during studies of adaptation to balsam woolly adelgid (Cheah & Donahue 2003).

FIELD RECOVERIES

Field studies and recoveries of the beetle have documented the ability of *S. tsugae* to reproduce after release, locally disperse, survive heat waves, survive mild and severe Connecticut winters, and establish in a variety of different hemlock habitats in Connecticut between 1995-2005 (Cheah and McClure 2000, 2002; Cheah et al. 2005). Recoveries of overwintered beetles (larvae and adults) were made in Connecticut release sites in years following severe Connecticut winters in 1996, 2000, 2003, and 2004 (McClure et al. 1999, Cheah and McClure 2002, Cheah et al. 2005). All stages from larvae, pupae, to adults of *S. tsugae* could be found through intensive forest ground sampling after the year of release in 65% of Connecticut release sites from 1996-2001 (Cheah et al. 2005). However, the persistent challenge in predator field sampling efforts continues to be the use of

the limiting beat sampling technique, which only samples accessible lower canopy foliage of hemlocks and results in the consequent misinterpretation of the establishment of *S. tsugae*. Bucket tree sampling of mature hemlocks in Connecticut and New Jersey has shown that the beetle is highly mobile and dispersed to upper canopies after release. Adults and larvae have been retrieved at 5-20 m heights on the outer hemlock branches in the forest canopy while concurrent ground sampling yielded no recoveries, indicating that the beetle was not evenly distributed in the hemlock canopy (Cheah et al. 2005). However, bucket truck sampling is expensive and not suitable for many release sites. In Japan, sweep net sampling up to 5 m in the canopy in a landscape setting in Osaka was effective in recovery of *S. tsugae* every month from March to December (Shiyake et al. 2008). Shiyake and colleagues recovered 27 predacious species from adelgid-infested *T. sieboldii* during 2007 sampling and found that *S. tsugae* was observed on Japanese hemlocks for more months than any other predator; only absent in samples during January and February. Recoveries of *S. tsugae* have also been documented in western North Carolina, including in areas where *S. tsugae* was not known to have been released (McDonald et al. 2008). More recent detailed studies in the Great Smoky Mountains National Park using pole pruners and beat sampling techniques recovered adults and larvae of *S. tsugae* in 21.2% of sites sampled in 2008 and 2009 and these recoveries were significantly associated with older release sites (5-7 years after release) (Hakeem et al. 2010).

FIELD IMPACT AND HEMLOCK RECOVERY IN CONNECTICUT

In Connecticut, *S. tsugae* releases from 1995-2007 have occurred in hemlock forests statewide over three climatic divisions and in a wide variety of soil types and habitats. This was also a period in which severe droughts occurred in 1995, 1999, and 2002. Initial hemlock mortality was recorded on marginal hemlock sites on ridge-tops, resulting from hemlock borer infestations and on the heels of devastating outbreaks of hemlock looper in the

early 1990s. Concurrent with the expansion of HWA infestations in Connecticut in the 1990s, hemlocks were also stressed by the elongate hemlock scale, *Fiorinia externa*. Populations of this scale have significantly increased in density and range in Connecticut during the past 5 years, resulting in the decline of hemlock stands. *Sasajiscymnus tsugae* is the major introduced predator for biological control in Connecticut and efficacy of these releases on hemlock crown health has been monitored annually in a comparative approach since 2003. In the decade of the 1990s, severe winters punctuated the climate of Connecticut, and while the adelgid suffered major population reductions, populations tended to rebound to damaging levels in subsequent milder years. Ten years after the first release of *S. tsugae*, a period in which around 170,000 adult beetles were released in Connecticut for biological control of HWA, dramatic recovery of adelgid-

impacted, declining hemlocks was recorded in many of the older established release sites, beginning in 2005, after successive extreme winters in 2003 and 2004 significantly reduced adelgid populations (Cheah 2006, Cheah 2011 in press). Recent winters in 2006-2008 and 2010 have been mild in comparison, but adelgid populations in the forests of Connecticut have not rebounded to original widespread damaging levels. Annual foliage transparency trends and other hemlock crown indicators such as new shoot production and tip dieback in *S. tsugae* release sites showed that even declining hemlocks recovered in one year when there was ample precipitation and a reduction in adelgid densities (Fig. 3). A comparative survey in Connecticut of 14 non-release sites, which were matched to *S. tsugae* release sites climatically, topographically and in HWA infestation history, was performed in 2005 to compare hemlock crown conditions in release



Figure 3. Recovery of an adelgid-infested hemlock in one year in Connecticut where *S. tsugae* was released in 1999 (photos by C. Cheah).

and non-release stands. Hemlock health assessments were also made in eight baseline hemlock sites which had no infestations of either adelgid or elongate hemlock scale. Crown health of hemlocks were rated (n = 15 trees/site) using standard U.S. Forest Service Forest Inventory Analysis (FIA) crown health assessment procedures, which has also been the method used to assess the conditions of hemlocks in release sites. A total of 287 hemlocks in release sites, 210 in non-release sites, and 90 hemlocks in baseline sites were compared statistically. In 2005, hemlock foliage transparency was significantly lower in the 14 annually monitored *S. tsugae* sites, as compared to that in paired non-release matches (Cheah 2011 in press). Mean foliage transparency in 6-11 year release sites was also similar to that in the baseline sites, located at high elevation in the colder northwest corner of the state, indicating that recovery of hemlocks had approached that in non-

infested sites. An increase or stabilization of foliage transparency readings is interpreted to be due to the concurrent abundant new shoot production on previously infested hemlocks, an indication in itself that adelgid populations in the whole crown have been depressed. In addition, HWA crown levels in 2006 and 2007, measured in classes of <10%, 11-50%, 51-75% and >75% showed that in the majority of release sites, average levels of adelgid in the site have been reduced from the initial pre-release levels. Annual hemlock crown health ratings from 2006-2010, show that this overall recovery has persisted to the present in *S. tsugae* release sites (Fig. 4; Cheah 2011 in press). This recovery has continued even in southern most sites, which have not had significant recent winter mortality of HWA until the winter of 2010-2011. With the reduction of adelgid populations during severe winters, abundant precipitation and cool growing seasons,

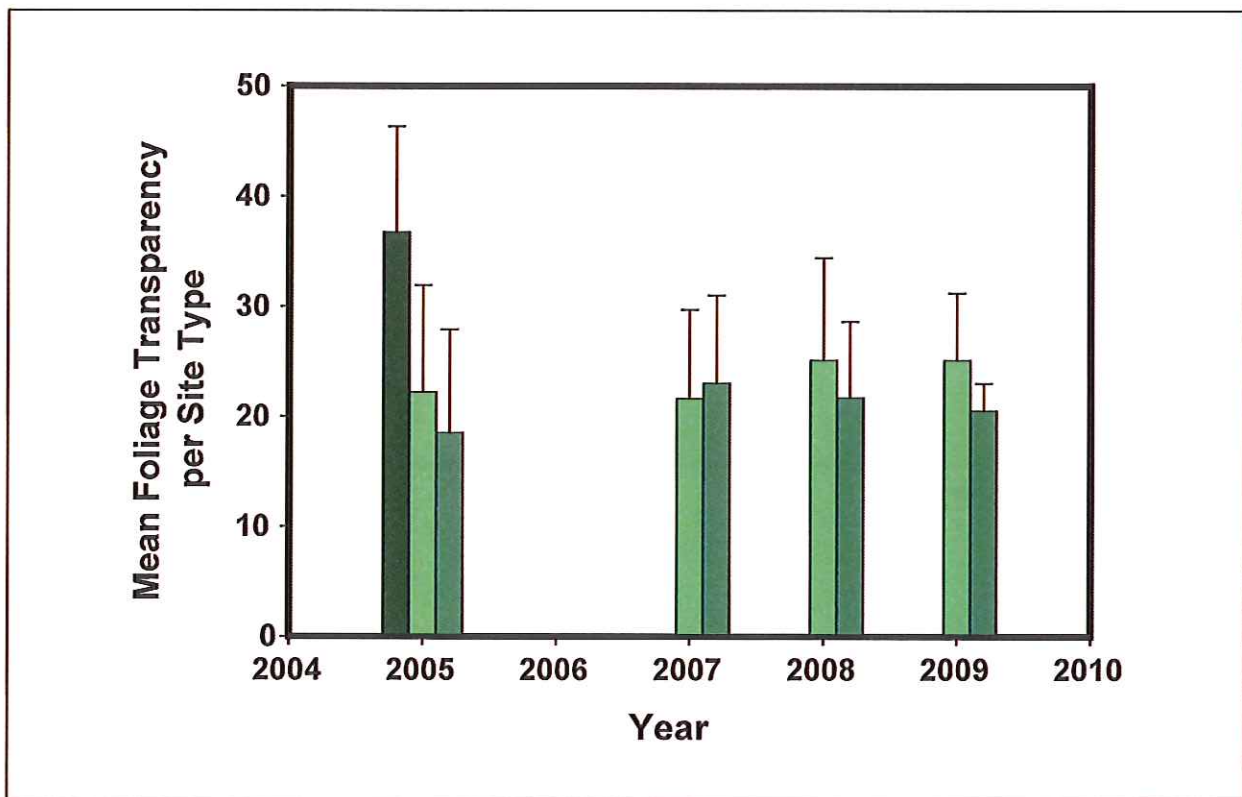


Figure 4. Comparison between *S. tsugae* release (light green), non-release (dark green), and uninfested baseline hemlock forest sites in Connecticut (Cheah 2011 in press).

hemlock recovery in monitored release sites has been recorded in Connecticut in all types of soil types and sites, from rocky ridge tops to riparian, ravine to level habitats since 2005 to date (Fig. 5; Cheah unpublished). There has been negligible hemlock mortality in release sites since 2001. But in 2010,

Connecticut sites which have had concurrently high infestations of elongate hemlock scale, had thinner crowns and higher foliage transparencies than sites with low or negligible scale infestations, indicating the deleterious impact of uncontrolled elongate hemlock scale populations (Cheah unpublished).

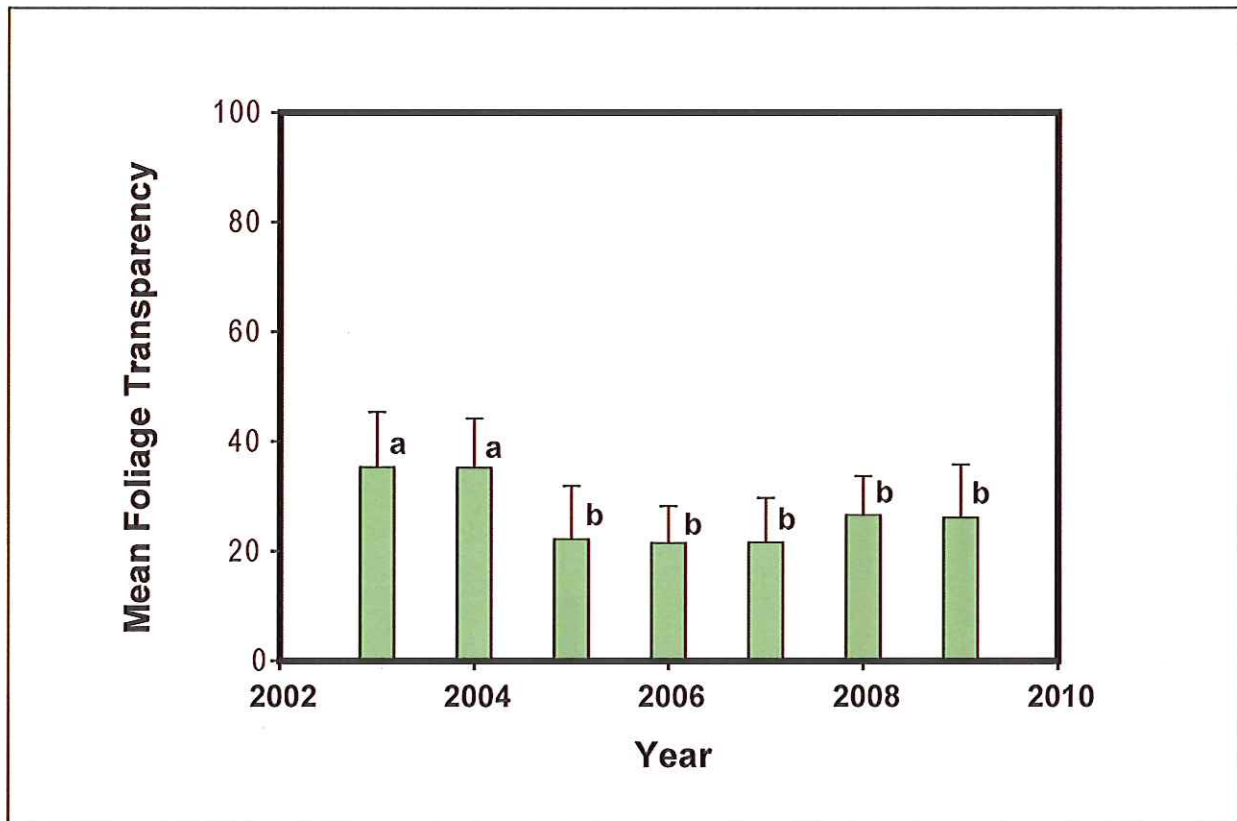


Figure 5. Mean annual foliage transparencies of previously adelgid-damaged hemlocks in 16 Connecticut *S. tsugae* release sites (9-15 years from year of first release), showing sustained crown recovery from 2005-2009. Bars are followed by different letters showing significant differences at the $p < 0.05$ level (Cheah unpublished).

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