Chapter 5: *Scymnus* (*Neopullus*) Lady Beetles from China

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INTRODUCTION

In 1995, we found our first *Scymnus* (*Neopullus*) lady beetle in China (*Neopullus* is a subgenus of *Scymnus*, the largest genus in the family Coccinellidae). At that time there were just a few known species in the subgenus and very little was known of their biology. By the end of the project, 14 years later, we had doubled the number of known *Scymnus* (*Neopullus*) species and thoroughly studied the biology of the three species that seemed the most promising candidates for biological control of *Adelges tsugae* Annand, the hemlock woolly adelgid (HWA) in the eastern United States. This discussion of the research on the three *Scymnus* (*Neopullus*) species is a good example of the process of classical biological control—(1) the discovery of potential agents and determination of their biology and host range in their indigenous environment; (2) importation and further study in quarantine of their biology and potential host range in the areas targeted for their release; (3) using sleeve cages to evaluate the response of natural enemies to their new environment and their impact on the pest; and (4) the free release of the biological control agents into the environment.

The "we" refers to the several teams that participated in the project. The context of the research can be better understood by recognizing the principals of the China team, with a few remarks about how and where we worked. The project's good luck was to have started with Yao Defu of the Chinese Academy of Forestry who was skilled in natural enemy research and forming partnerships. Yu Guoyue of the Beijing Academy of Agriculture and Forestry Science was indispensible in identifying or providing new descriptions for the more than 70 species of lady beetles we found on hemlock in China. In Sichuan Province, Jianhua Zhou provided excellent leadership as well as hands-on help in the lab and field. In Yunnan, Li Li proved to be a most proficient collector and became so involved that he earned an engineering degree for his research on HWA natural enemies. Wenhua Lu of the University of Rhode Island did the initial studies in quarantine and acted as a liaison with the team in China.

It is not possible to describe here the adventures and hardships this team experienced. Hemlock in China occurs in remote, rugged mountains; hence, access was a challenge (Havill and Montgomery 2008). These forests have high diversity and both the Chinese and "Lao-wei" (foreigners) were excited about exploring these areas. Our working relationship was exemplified by the fun the Chinese had in speaking of "Lao-Mike" ("lao" literally means "old," but depending on context it shows respect as an old friend or teacher or can be derogatory as a silly-old-fool) and the custom of sharing a meal and drink with local officials and forest workers who helped us with our research.

OVERVIEW OF THE GENUS *SCYMNUS*

Scymnus is the largest genus of lady beetles (Coccinellidae) with more than 600 described species. Adults are relatively small, less than 3 mm, compact, and pubescent. Larvae are characterized by a white waxy covering. Most of the species in the genus are aphidophagous, feeding on either aphids or adelgids. The genus *Scymnus* is divided into seven subgenera: *Scymnus* (*Scymnus*) Kugelann 1794, *Scymnus* (*Pullus*) Mulsant 1846, *Scymnus* (*Didion*) Casey 1899, *Scymnus* (*Neopullus*)

Sasaji 1971, *Scymnus* (*Parapullus*) Yang 1978, *Scymnus* (*Mimopullus*) Fursch 1987, and *Scymnus* (*Orthoscymnus*) Canepari 1997 (Kovar 2007). The subgenera *Pullus* and *Scymnus* are widespread, with the former having three-fourths of the species and the latter about 50 species. The other subgenera have less than 10 known species, except for the subgenus *Neopullus*, which has 22 known species (Kovar 2007) that are Palaeartic, except for *Parapullus* and *Didion*, which also occur in North America.

The subgenera can be separated by a combination of the number of antennal segments (10 or 11), the length of the postcoxal line (complete or incomplete) and the presence or absence of distinct carinae on the intercoxal projection of the prosternum. Species in *Scymnus* (*Neopullus*) can be distinguished from the *Scymnus* species indigenous to North American by a combination of 10-antennal segments, a complete postcoxal line, and distinct carinae on the intercoxal process. Using a key to North American Coccinellidae (Gordon 1985), *Scymnus* (*Neopullus*) would key to *Didion*, except that the latter is distinguished by its feeble intercoxal carina. (Note: Gordon placed *Didion* in a separate genus rather than a subgenus of *Scymnus*.) *Sasajiscymnus tsugae* (Sasaji & McClure), imported from Japan to the United States for biological control of the hemlock woolly adelgid, has 9-segmented antennae and an incomplete postcoxal line.

There have been 17 species of *Scymnus* lady beetles introduced to the United States, but only two are known to have established (Hagen et al. 1999). Both of the species that established are in the subgenus *Pullus* and attack adelgids (Gordon 1985). *Scymnus* (*Pullus*) *impexus* Mulsant, native to Europe, was introduced in the United States and Canada during 1959-1963 to control the balsam woolly adelgid, *Adelges piceae* Ratzeburg. Although *S. impexus* was initially promising, it may have died out since its last recovery was 1978 in British Columbia (Harris and Dawsen 1979). *Scymnus* (*Pullus*) *suturalis* Thunberg, also native to Europe, was introduced to Michigan in 1961 to control adelgids on pine and is now established in several northeastern States (Gordon 1985). It attacks *Pineus strobi* (Hartig), *P. pini* (Macquart) and *A. tsugae* in Connecticut (Lyon and Montgomery 1995). The abundance and seasonality of *S. suturalis* were examined on white pine and eastern hemlock, which had up to one larvae/branch (Montgomery and Lyon 1996). This lady beetle is now seldom collected in Connecticut (Montgomery, pers. obs.). There also is a *Pullus* lady beetle, *Scymnus* (*Pullus*) *coniferarum* Crotch, indigenous to the western United States, that feeds on both pine and hemlock adelgids (Whitehead 1967, Montgomery and McDonald 2010, chapter 10).

Worldwide there are 22 described species in the subgenus *Scymnus* (*Neopullus*) and the hosts are known for only a few of these. *Scymnus* (*Neopullus*) *hoffmanii* Weise is an important predator of aphids on crops in China and Japan (Yang and Zheng 1991, Kawauchi 1997). Seven *Scymnus* (*Neopullus*) species have been collected from hemlock in China (Yu et al. 2000) and three of these (Fig. 1) are the focus of this chapter: *Scymnus* (*Neopullus*) *camptodromus* Yu & Liu (*Sc*), *S.* (*N.*) *sinuanodulus* Yu &Yao (*Ss*) and *S.* (*N.*) *ningshanensis* Yu & Yao (*Sn*). Although many species of Coccinellidae and other families of predators were collected, these three species seemed the most promising for biological control of HWA.

DISTRIBUTION AND BIOLOGY IN NATIVE RANGE

Distribution

The search for natural enemies of the hemlock woolly adelgid in China focused on three provinces with extensive, widely distributed stands of hemlock—Yunnan and Sichuan in Southwestern China and Shaanxi in Central China (Fig. 2). These collection locations occur between 26.3°N and 33.3°N latitude and 1900 and 3200 meters elevation.

Habitat

The Sino-Himalayan region has three species of hemlock: *Tsuga chinensis*, which is widespread, occurring in 15 China provinces; *T. dumosa*, which occurs in a narrow zone across the southern Himalayan Range belt from Sichuan and Yunnan

Figure 1. Adults of three species of *Scymnus* (*Neopullus*): left to right, *S. camptodromus* (*Sc*), *S. ningshanensis* (*Sn*), and *S. sinuanodulus* (*Ss*). The beetles are about 2 mm in length.

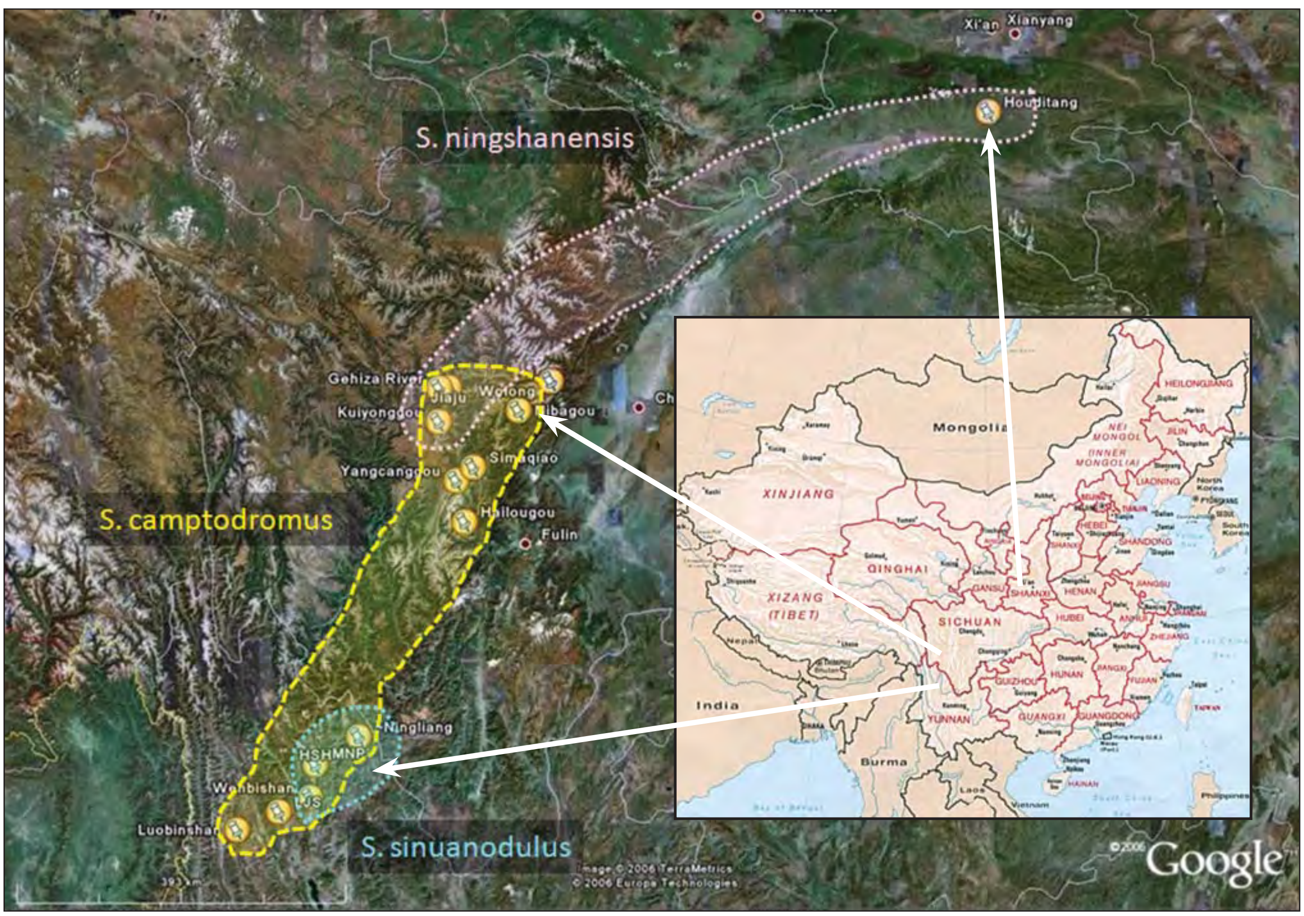

Figure 2. Map showing primary collecting area in China, with known distribution of three species of *Scymnus* (*Neopullus*) lady beetles outlined.

Provinces to Pakistan; and *T. forrestii*, which is limited to northwest Yunnan and southwest Sichuan (Fargon 1990). Like their eastern United States congeners, the hemlocks in China are shade tolerant and drought intolerant. Montgomery et al. (1999) provides additional information about the nomenclature and distribution of hemlock in China.

In China, hemlock occurs only in mountainous regions, especially fog-belts at elevations between 1,800 to 3,500 meters where moisture is plentiful during the growing season. Because the hemlock occurs in a limited elevation zone and the mountains are very steep and isolated, the hemlock often occurs in isolated "islands" (Wang 1961). This is a transitional area between broad-leaved deciduous forest and montane coniferous forest. These stands are extraordinarily diverse and have many of the same genera found in forests in the eastern United States. Besides *Tsuga*, there are species in the genera *Abies*, *Aesculus*, *Carpinus*, *Cercis*, *Chamaecyparis*, *Clethra*, *Corylus*, *Ilex*, *Juglans*, *Juniperus*, *Lindera*, *Magnolia*, *Malus*, *Prunus*, *Picea*, *Pinus*, *Pseudotsuga*, *Sorbus*, *Taxus*, *Tilia*, and *Ulmus*. There are also species in genera not present in eastern North America such as *Castanopsis*, *Cercidiphyllum*, *Schima*, *Cunninghamia*, *Keteleeria*, and *Lithocarpus*. This assemblage of a large number of tree species with the crown layer shared by several species is a characteristic of the mixed mesophytic forest type. This area of southwest China and the Southern Appalachian region of eastern North America are the only areas in the world where this forest type occurs (Wang 1961).

In China, hemlock occurs only as a forest tree—it is not grown as an ornamental or landscape tree. Often it is the tallest tree in the forest with characteristic flat tops above the canopy. Although HWA and their lady beetle predators occur in the crowns of these trees, the crowns of big trees are not accessible. The distribution of HWA in the crown of *T. dumosa* is uniform vertically and by quadrant (Zhou et al. 2007, Li and Lu 2008). Most collecting is done in the lower crown, reachable from the ground, and on small trees growing near wet areas. The forests where collecting was done are protected and managed forest farms or preserves in which livestock are sometimes allowed to graze.

Climate

The areas where *Scymnus* (*Neopullus*) lady beetles were collected in China generally are more southerly in latitude and higher in altitude than the target areas for biological control in the eastern United States. Seasonal temperatures can be similar, however, because a 1000 m increase in altitude has roughly the same effect on air temperature as a 7.5 degree increase in latitude (http://en.wikipedia.org/wiki/Alpine_climate). A comparison of average monthly temperatures for two of the collecting areas in China and two potential release areas in the United States provide examples of seasonal temperatures (Fig. 3). This shows that these four areas have similar winter temperatures, between -21 °C and -18 °C, but the areas in China have much lower summer temperatures. The lower summer temperatures are associated with the seasonal monsoon that occurs as warm tropical winds from the southeast collide with the rise of the Tibetan Plateau. Rainfall data, available for two of these areas (Fig. 4) shows the typical pattern of rainy summers and dry winters of the collecting areas in China and the more even pattern of rainfall in the Southern Appalachian area of the United States. Thus, the major climatic difference in the collecting area and the potential release area is the seasonal pattern of rainfall and cool summers.

Host Associations in Endemic Area

In addition to hemlock, other conifers and some angiosperms were sampled by beating limbs over an umbrella to determine the extent that alternate hosts may be used by the three *Scymnus* (*Neopullus*) species. Of the three species, only *Ss* was found on any host plant other than hemlock with a regularity that was not considered incidental (Table 1). During the first four years of collecting, the 5-needle white pine, *Pinus armandii* Franch, had heavy infestations of the adelgid *Pineus armandicola* Zhang, Zhong & Zhang, and the adelgid's egg-filled ovisacs were on the pine's needles in both spring and fall. In the spring and late fall, *Ss* was about one-fifth as abundant on the white pine as on hemlock, but *Sc* and *Sn* were not recovered from white pine. In Yunnan during September, when HWA was in diapause, *Ss* was more numerous on *P. armandi* than on hemlock. Numerous *Scymnus*

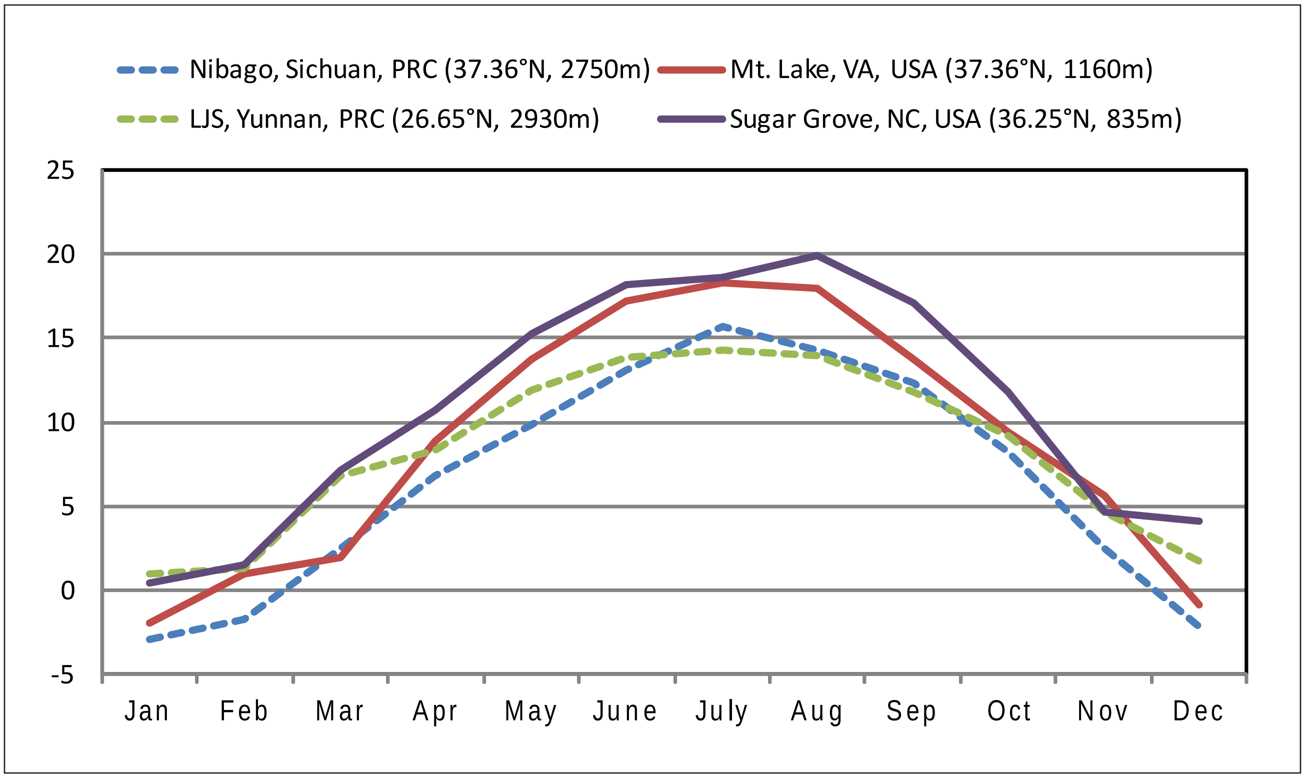

Figure 3. Average monthly temperatures (°C) of selected collecting locations in China and potential release sites in the United States. Latitude and meters altitude shown in brackets. Data collected by temperature recorders at the field sites for two or three year periods between 1999-2008.

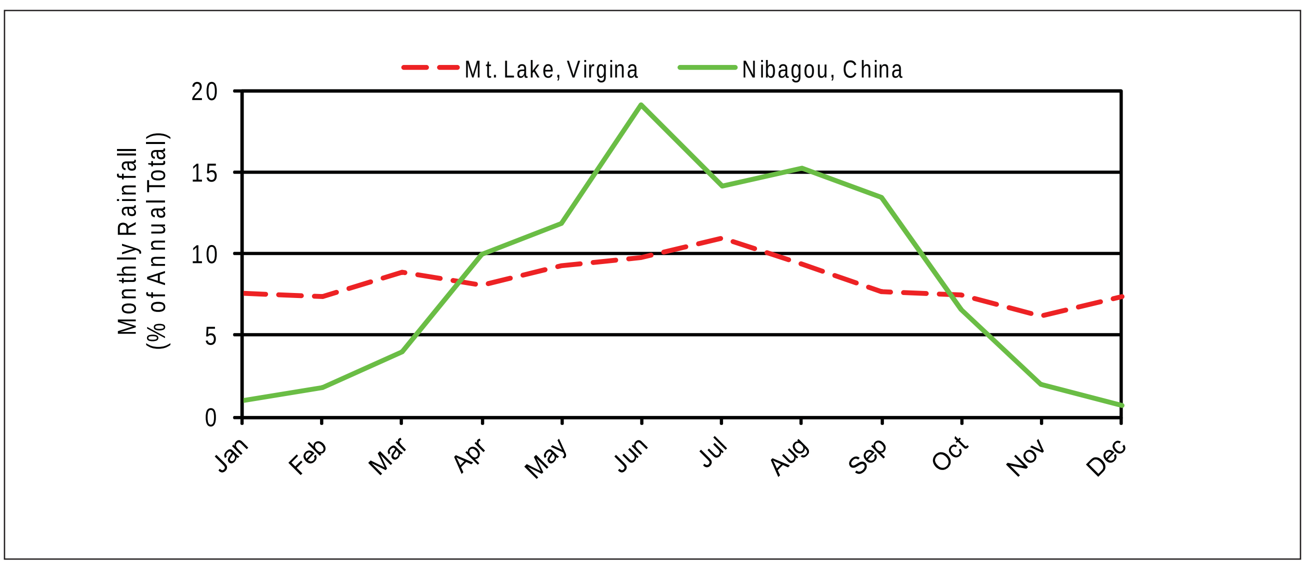

Figure 4. Average monthly precipitation as a percentage of total annual precipitation.

larvae collected from adelgid infested white pine at this time were identified as *Scymnus* (*Pullus*) *yunshanpingensis* Yu. Another *Pullus* species, *S.* (*P.*) *geminus* Yu and Montgomery, was abundant on white pine in the spring in Sichuan. Both of these *Scymnus* (*Pullus*) species will feed and oviposit on HWA, but their primary host seems to be pine adelgids. The other two species were not collected from white pine. It seems that the species in the subgenus *Pullus* may have pine adelgids as primary hosts whereas the three *Neopullus* species imported have HWA as the primary host, but may feed on pine adelgids to some extent.

HWA Phenology

Monthly sampling of HWA life stages and predators was done at three locations in Sichuan and Yunnan Provinces for one year. The number of ovisacs in each monthly sample was the most useful indicator of predator/HWA dynamics since the egg stage is targeted by most of its predators. As in the eastern United States, the onset of oviposition by HWA occurs earlier at more southerly latitudes. In Yunnan Province, the abundance of egg containing ovisacs peaks first in December and January and then in March whereas in colder Sichuan, the peak in abundance of ovisacs occurs in March and April (Fig. 5). In Sichuan, there is considerable overlap in eggs laid by the overwintering generation (sistens) and the spring generation (progrediens). In Sichuan, HWA ovisacs (and all nymphal stages) were found in low numbers throughout the summer and into early fall, especially in Nibagou, the coldest site. The cool summers of these regions may result in a longer period of survival and egg production by the progredientes. Other explanation for the presence of active stages throughout the summer include two progrediens generations or the progeny produced by migration of gallicolae from spruce. The difference in biology may also be related to the adelgids on hemlock in China being distinct genetically from HWA on other continents (Havill et al. 2006).

Predator Abundance and Phenology

Predators were collected by beating foliage over umbrellas. This technique is best suited for collecting adults. Usually 30 hemlock trees were beaten at each site every month. The closure of roads caused a switch to alternative sites in Sichuan; hence, the same three sites were not followed for

Table 1. Occurrence of adult *Scymnus sinuanodulus* by beating foliage in Lijiang Prefecture, Yunnan, People's Republic of China.

Tree Species	Occurrence	Hemiptera present
Tsuga dumosa Eichler	Frequent	*Adelges tsugae*, Diaspididae
T. forrestii Downie	Frequent	*A. tsugae*
Pinus armandii Franch.	Moderate	*Pineus* spp., Aphididae
P. yunnanensis Franch.	Rare	Diaspididae
Picea likiangensis Pritz.	Rare	Adelgid galls
Abies delavayi Franch.	Rare	*Adelges* sp., Aphididae
Larix potaninii Batalin	None	*Adelges* sp.
Taxus yunnanensis Chang et L.K. Fu	None	Pseudococcidae
Keteleeria evelyniana Mast.	None	Aphididae, Diaspididae
Quercus pannosa Hand.-Mazz.	None	Coccidae
Betula alnoidis Hamilt.	None	Aphididae
Populus yunnanensis Dode	None	None
Alnus ferdinandi-coburgii Schneider	None	None
Rhododendron spp.	None	None

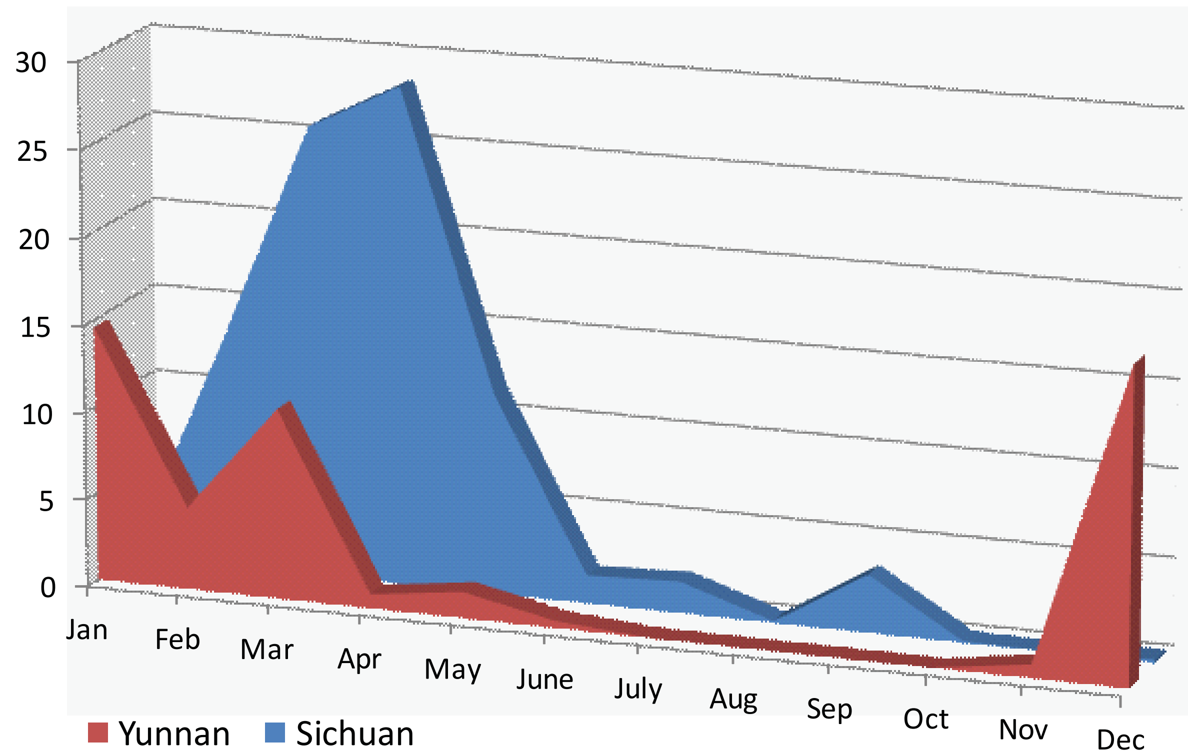

Figure 5. Seasonal presence of HWA ovisacs in Sichuan and Yunnan Provinces; values are the average number on a twig for 60 twigs from each of three sites in each province. Data collected by Jianhua Zhou in Sichuan and Li Li in Yunnan.

12 consecutive months. Overall 2,695 and 3,153 specimens were collected in Sichuan and Yunnan, respectively. This is an average of 2-3 predators per umbrella sample with a range of zero for all samples taken in January and February in Sichuan to 8-9 per sample for May in Yunnan and October in Sichuan. The overall abundance of categories of natural enemies in the two Provinces was dissimilar (Table 2). Only seven specimens of *Laricobius* sp. were collected, all in Sichuan Province; thus, based on abundance, *Laricobius* spp. do not appear to be important regulators of HWA in China. In both provinces, the majority of predators collected are in the family Coccinellidae. In Yunnan, 62% of the Coccinellidae were the species *Sc* and *Ss*. In Sichuan, 48% of the Coccinellidae were the species *Sc* and *Sn*. *Ss* was not found in Sichuan and only three *Sn* individuals were found in Yunnan. The two most abundant predators were the large, colorful coccinellid *Oenopia signatella* (Mulsant) in Sichuan and the anthrocorid, *Tetraphleps galchanoides* Ghauri, in Yunnan. Both the literature and laboratory host range studies indicate that these last two predators are generalists feeding on aphids, adelgids, and other Heteroptera; thus, they appear to be non-specific, opportunistic species.

Table 2. Total number of predators recovered from umbrella samples taken monthly in two provinces.

Species	Sichuan	Yunnan
S. camptodromus	240	232
S. ningshanensis	226	3
S. sinuanodulus	0	269
Oneopia spp.	549	23
Other Coccinellidae	746	436
Laricobius sp.	7	0
Anthocoridae	22	305
Other Predators	141	171

The seasonal abundance of the three predators in each province that seem to have the greatest role in HWA population dynamics is presented in Figure 6. Very few predators were collected during the coldest months of January and February in either province. There were two peaks in abundance of adult predators, one in the spring and another in the fall. In May and June, one site in Yunnan, which had the highest density of HWA, also had a high density of *T. galchanoides* nymphs and adults. Because this true bug pierces and sucks fluid from its prey and the HWA carcass remains attached to the stem, it was possible to assess, with the aid of a hand lens, that mortality of HWA exceeded 90 percent. The fall peak in predator abundance does not correspond well to HWA biology—HWA was still in diapause in Yunnan, but active stages of HWA were present in Sichuan—so its significance to HWA regulation is unclear.

The abundance of the adults of the *Scymnus* (*Neopullus*) species peak in early spring, then increase in late spring and early summer (Fig. 6).

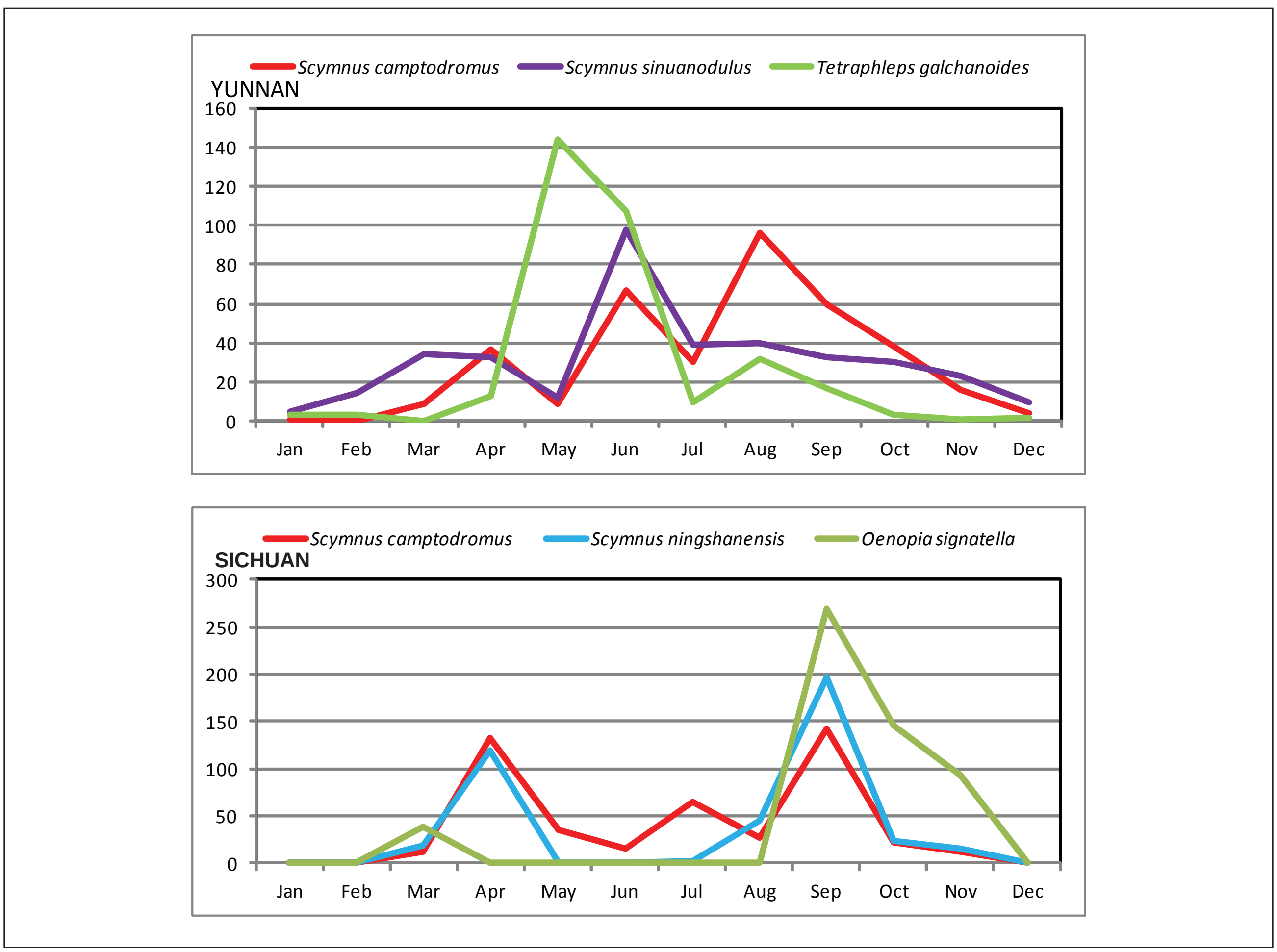

Figure 6. Seasonal presence of selected predator species recovered from monthly foliage beating samples.

This decrease in mid-spring likely corresponds to mortality of the overwintered lady beetles after egg laying and is followed by the appearance of the newly eclosed adults about two months later. The adults are abundant on hemlock in the fall and this is the best time to collect them for export. Adults of *Sc* collected in the fall or spring will immediately lay eggs in quarantine, but *Sn* and *Ss* collected in the fall will not begin to lay eggs until after a few months in cold storage. Because *Sn* and *Ss* lay eggs in the spring, adults collected then for export may have laid most of their eggs and thus may produce few eggs when brought into the quarantine.

Observations of immatures in the field (Yao and Hongbin 1999; Montgomery, pers. obs.) indicate that larvae were present during April and May in both provinces. At the end of May, larvae with waxy plumes were seen walking on the trunk and branches, and pupae were found under the bark on the bole of the tree (Fig. 7). Mating of *Ss* adults was observed in early April and they oviposited from then until late May. In Sichuan, oviposition by *Sc* was observed only during April, but eggs were found in the field in late summer. Based on the temperature data of the study locations (Fig. 3), development of the immature stages occurs when daily average temperatures are between 5 °C and 15 °C.

Figure 7. *Scymnus* (*Neopullus*) larva feeding on HWA (left) and pupae in bark crevices on the bole of a hemlock tree in Yunnan, China (photos by Guoyue Yu).

BIOLOGY STUDIES IN QUARANTINE

The *Scymnus* (*Neopullus*) lady beetles imported from China exhibit two different life history schemes that synchronize their development with that of HWA, including the approximately three month aestivation period of HWA. Two species, *Ss* and *Sn*, both have an extended pre-oviposition period; new adults mate soon after emergence but need some exposure to cool temperatures before females will initiate oviposition. They lay eggs only in the spring that quickly hatch. The other species, *Sc*, can begin to oviposit one month after emergence if mated, but these eggs enter diapause and do not hatch until the next spring. The only other record of an egg diapause in Coccinellidae is for another *Scymnus* species, *S.* (*Pullus*) *impexus* (Mulsant), which feeds on *Adelges piceae* (Ratzeburg) in Europe (Delucchi 1954). Details on these two strategies and the data that supports them are provided in the following sections.

Egg Deposition and Hatch

The females of all three species select concealed, protected places, near HWA to lay their eggs, but in the laboratory will lay eggs in exposed areas and white gauze (*Ss* and *Sn* only) if suitable oviposition sites are not available. Eggs are usually laid singly, but may be in groups if the adults are crowded. The eggs of all three species are first yellow-orange, but become darker in 2-3 days. As they near hatch, the outer shell (chorion) becomes transparent and reddish eye spots are visible. Just before hatching, the egg surface becomes iridescent as the chorion separates from the embryo. Eggs of *Sc* are generally deposited in more concealed locations and have a more leather-like surface than the other two species (Fig. 8). This may provide added protection because the eggs of *Sc* will not hatch until 4-8 months after being laid, whereas the eggs of *Sn* and *Sc* hatch in about two weeks after being laid.

The eggs of *Ss* and *Sn* will hatch in an average of 10 and 8 days, respectively when held at 18-20 °C. Egg hatch was about 90% for both these species. Storing eggs of these two species at 5 °C for two weeks does not affect hatch, but longer storage reduces percent egg hatch. Thus, the eggs of these two species are not able to overwinter.

Figure 8. *Scymnus camptodromus* egg inserted between bud scales of hemlock.

Larva and Pupa Development

The larvae have four instars, are elongate, yellowish to reddish brown, densely setaceous on the head and plates, and the body has tubercles, but lacks prominent spines (Fig. 9) (Lu et al. 2002). The newly-hatched larvae are transparent with the color of the hemolymph and recently consumed adelgid visible (Fig 10). The larvae grasp HWA crawlers with their mandibles and suck the prey contents and then expel it back and forth into the dead prey's exoskeleton to aid in digestion (Lu et al. 2002). By the last instar, the larvae produce a conspicuous waxy covering on the cuticle (Fig. 11). The pupae are naked, with the larval exuvium attached only to the last abdominal segment, but are covered with coarse setae with viscous droplets on the tips, which we believe are defensive (Fig. 12).

At temperatures ≥ 15 °C, the eggs of *Sc* remain yellow-orange and show no signs of embryo development until exposed to temperatures < 15 °C for 1-3 months (Keena and Montgomery 2010). There is some variation among individual eggs in amount of chill required to break diapause, and diapause seems to be broken most quickly when eggs are held at temperatures near 5 °C. Once diapause has been broken, the *Sc* embryo will begin to develop, even at temperatures near 0 °C, and the speed of development slowly increases with increasing temperatures up to 15 °C. At a constant 10 °C, the eggs will hatch after an average of 227 ± 32 days. Eggs will hatch after exposure to 5 °C and the percentage hatch increases with increasing time at 5 °C. The temperature regime for highest percentage hatch (90%) in the shortest time is 56 days at 5 °C followed by about 2 months at 10 °C. The optimal temperature for *Sc* egg hatch is near 10 °C. Thus, *Sc* eggs will spend the summer in diapause, develop only after exposure to the cool temperatures of fall, and hatch after HWA has begun laying eggs.

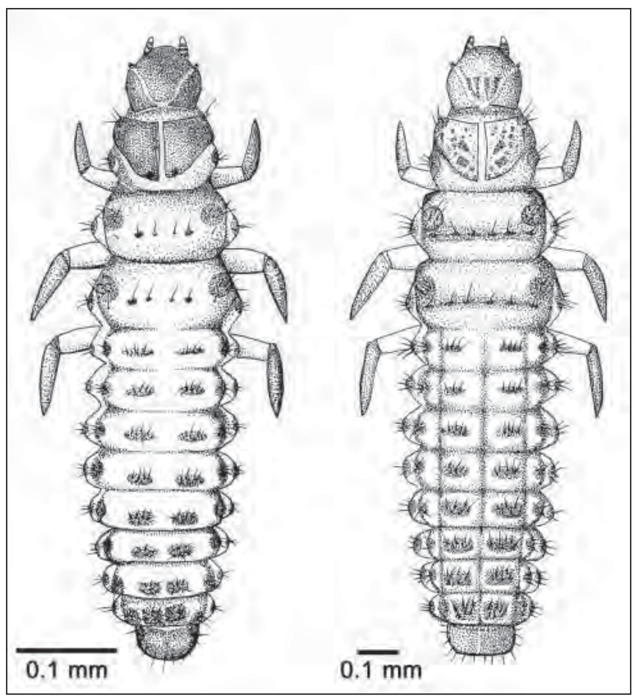

Figure 9. Drawings of *Scymnus sinuanodulus* larvae, first instar (left) and fourth instar (from Lu et al. 2002).

Figure 10. *Scymnus camptodromus* first instar larva.

Figure 11. Fourth instar *Scymnus* larva and HWA eggs.

Figure 12. Droplets at the tips of the setae covering *Scymnus* pupa.

The total time for development at 20 °C for the larval and pupal stages is about 20 days and 10 days, respectively, for *Ss* and *Sc*. *Sn* has a shorter period in the larval stages (Table 3). The fourth larval instar of *Sn* is shorter than the other two species, which spend about half of the last larval stage wandering or inactive (Lu et al. 2002). This behavior has also been observed in another lady beetle that feeds on adelgids, *S.* (*P.*) *impexus* (Delucchi 1954).

The larvae of *Sc* can develop at constant temperatures between 10 and 25 °C (Keena and Montgomery 2010). Larvae of *Ss* also complete development at temperatures between 15-25 °C, but rearing at lower temperatures was not attempted (Lu and Montgomery 2001). However, 10 °C and 25 °C may be sub-optimal, because survival to pupation for *Sc* larvae was only 50% at 10 °C, > 70% for 15-20 °C, and 25% for 25 °C (Keena, unpublished data). For *Ss*, survival from egg hatch to pupation was > 60% at 15-20 °C and only 5% at 25 °C (Lu and Montgomery 2001). The *Sc* larvae took an average of 90 ± 3 days and 20 ± 1 days to pupate, at 10 °C and 25 °C, respectively. In their indigenous environment, average temperatures range between 5 °C and 15 °C during the months the larvae and pupae are developing. The lab rearing indicates that these beetles should thrive at the warmer temperatures they would encounter in the Southern Appalachian Mountains (cf. Fig. 3).

Table 3. Development time (days) of larval and pupal stages of *Scymnus sinuanodulus* (*Ss*) and *S. camptodromus* (*Sc*) at 20 °C and *S. ningshanensis* (*Sn*) at 18-20 °C.

Stage	*Ss*[1]	*Sn*[1]	*Sc*[2]	*Sc*[3]
L1	3.1	2.8		
L2	2.5	2.5	20	20
L3	2.9	3.7		
L4	11.4	5.9		
Pupa	10.6	11.1	9.0	10
Total	30.5	26.0	29.3	30

Data sources: [1]Montgomery et al. 2002; [2]Keena and Montgomery 2010; [3]Lu and Montgomery 2000.

Mating and Oviposition

The adults of all three species are uniformly dull orange after emergence, with the elytral maculation that is distinctive for each species taking a few days to appear. After about three weeks, the adults of all three species become more active and are observed to mate and fly frequently (Fig. 13). Adult weights of the three species vary with larval food quality and rearing temperature, but *Sc* may be larger than *Sn* and *Ss* (Table 4). The greater weight of *Sc* may have been due in part to it being reared individually whereas *Ss* and *Sn* were reared in groups. Another factor may be that the weights of *Ss* and *Sn* were taken after they had been in lab culture for 12 and 11 generations, respectively, whereas the *Sc* colony was a mixture of populations established 5 to 4 generations ago. Measurements of the body size made on field collected specimens indicate that *Sc* and *Ss* are the same size, but *Sn* is a little smaller (Yu et al. 1997, Yu et al. 2000).

Female *Sc* first mate when about 3 weeks old and begin oviposition about a week later if active stages of HWA are available. Only a few eggs are laid if HWA nymphs are the only food and total egg production is better if females are held at < 10 °C until HWA eggs are available. When HWA eggs are available, oviposition will begin about 2 weeks after placement at > 10 °C. The females normally deposit eggs individually in concealed locations such as bud scales (especially ones that form a curl), but prefers the pollen cones of hemlock. When opened pollen cones are available, along with ample HWA eggs for food, they will average 10-14 eggs/female/week at 20 °C and often lay multiple eggs in a cone. If the food supply is not optimal, only 1-2 eggs are laid in a week.

Figure 13. *Scymnus sinuanodulus* with wings spread open as in flight (photo by Nathan Havill).

Table 4. Average adult weights (mg) of *Scymnus sinuanodulus* (*Ss*), *S. camptodromus* (*Sc*), and *S.ningshanensis* (*Sn*).

Sex	*Ss*	*Sn*	*Sc*
Male	0.90	0.96	1.30
Female	0.98	1.15	1.30

Females of the other two species (*Ss* and *Sn*) also mate at about 3 weeks post-eclosion, but have a prolonged pre-oviposition period that includes exposure to temperatures of 5-10 °C for at least two months (Lu and Montgomery 2001). After four months exposure to cool temperatures, they will lay eggs within 48 hours after being warmed to 20 °C and provided HWA eggs for food. Peak egg laying for *Sn* is 18 egg/female/week when held at 19 °C. The average number of *Sn* eggs produced per ovipositing female in the laboratory was 28 in China and 85 in the United States. This difference likely reflects the artificially extended laying period in quarantine in the United States and the availability of foliage with a higher density of ovisacs that had about 30% more eggs/sac. A reasonable expectation for oviposition in the laboratory is one to two eggs per day for a period of 5 to 10 weeks, if good quality and abundant food and oviposition sites are available. Both *Ss* and *Sn* oviposit single eggs in concealed locations such as bud scales and at the edge of ovisacs. If the ratio of oviposition sites to beetles is low or they are confined on the same foliage for several days, multiple eggs may be found in the same place and on the twigs, under dead needles on the bottom of the rearing container, and on substrates added to the cup such as gauze.

Two experiments showed that hemlock woolly adelgid eggs must be present for *Sn* to lay eggs (see Montgomery et al. 2002). Adults removed from cold storage were given foliage either with adelgid eggs or with only adelgid nymphs (third instar). Beetles provided adelgid eggs laid an average of

2.3 ± 0.8 eggs/week (N = 15) over a three-week period whereas the 15 lady beetles given only nymphs laid a total of three eggs for the entire three weeks. These three eggs were laid in the first week. When provided a diet simulating late winter conditions, with mostly adelgid nymphs and few hemlock woolly adelgid eggs, the lady beetle laid an average of only 6.42 ± 3.54 eggs during the 4 weeks following removal from cold storage, whereas 30.42 ± 8.98 eggs were laid by the beetles when they were provided adelgids at peak oviposition. Similar studies on *Ss* have shown that they will lay fewer eggs when provided only HWA nymphs than when provided HWA eggs (Lu and Montgomery 2001).

During the summer months when HWA is in diapause, the adults of all three beetle species feed little and are inactive during the summer. Survival is best during the summer when the adults are held at 10-15 °C, which is equivalent to the summer temperatures where they are indiginous. During the winter months, adults survive well at 5 °C and will feed occasionally on artificial diet. Both *Ss* and *Sn* adults have been found to have super cooling points generally between -12 and -20 °C so are well adapted to survive in cold climates (Costa et al. 2008). Since *Sc* eggs overwinter, it would be beneficial to know if *Sc* eggs can withstand sub-freezing temperatures as well as the adults.

Feeding Behavior on HWA

Scymnus adults prey on adelgid eggs by chewing them, often leaving smeared egg contents or partially consumed eggs. The beetles feed first on eggs exposed outside the ovisac, then gradually crawl into the ovisac until little of their body is visible. Often the adult adelgid is dislodged and subsequently dies. When adult beetles attack adelgid adults or older nymphs, they take a single bite, which causes dark brown hemolymph to ooze from the adelgid that the beetles then drink; they sometimes chew on the adelgid's body without entirely consuming it. The adult beetles feed little on the active crawlers, which easily escape when approached (Lu et al. 2002).

In China, adult *Ss* and *Sc* ate, respectively, an average of 22 and 31 HWA eggs daily, taking 60-80 seconds to consume an egg (Yao and Wang 1999). In quarantine at 20 °C, adult *Sn*, removed from cold storage, consumed 1.0 ± 0.29 nymphs, 0.8 ± 0.31 adults, and 5.5 ± 0.23 eggs/day when given a mix of stages (Montgomery et al. 2002). Both *Ss* and *Sn* adults fed at 0 °C and consumed 7-10 and 9-17 eggs in 20 hours when held at temperatures between 2.5 °C and 10 °C, respectively (Costa et al. 2008).

Scymnus larvae feed on all stages of adelgids, but mostly on eggs. When feeding on adelgid eggs, the larvae enter the adelgid ovisac and usually consume all of the eggs before leaving. Larvae suck the eggs, leaving the chorions, whereas adult beetles chew the eggs and do not leave the chorions. Although adelgid crawlers usually escape encounters with adult beetles, *Ss* and *Sc* larvae have been observed capturing and eating this active stage. The larvae appear to feed through a form of extraoral digestion on the crawlers. They bite and suck out the contents of the crawler, then regurgitate the contents back repeatedly into the crawler several times, before abandoning the empty corpse (Lu et al. 2002). When starved, 1st instar *Ss* larvae fed on crawlers of the hemlock scale *Fiorinia externa* Ferris, regurgitating in the same way (Lu et al. 2002). The 1st instar *Ss* beetle larva have also been observed attacking the aestivating 1st instar adelgid nymph by turning the prey on its side, piercing the underside of the thorax near the stylet, and sucking out the hemolymph. Large beetle larvae, starved for 1-2 days, were cannibalistic.

Larvae are voracious feeders. For example, *Sn* larvae in the third instar consumed 99.2 ± 11.7 adelgid eggs/day. Chinese colleagues reported that consumption of HWA ovisacs by each larval instar (I-IV) was 1.8 ± 0.6, 3.9 ± 1.5, 5.3 ± 2.0, and 11.2 ± 3.1, respectively (Montgomery et al. 2002). The adelgid ovisacs had an average of 31 ± 11 eggs/sac; thus, total consumption by a larva was 23 ovisacs, or 713 eggs. First instar larvae of all three *Scymnus* species do not survive if they do not have adelgid (or their own) eggs on which to feed.

HOST EVALUATION IN QUARANTINE

Methods for Host Preference Testing

The methods and results are a compilation and summary of previously reported experiments (Montgomery and Lyon 1996, Montgomery et al. 1997, Butin et al. 2002, Butin et al. 2004, Hoover et al. 2010), unpublished data of the authors, and personal communications from K. Hoover. Included in some of the tests for comparison purposes were two non-native coccinelid species established in the United States, *Harmonia axyridis* Pallas, a predator of arboreal aphids, and *Scymnus* (*Pullus*) *suturalis* Thunberg, a predator of pine adelgids. Feeding by adults was measured as it is the adults stage that make the choice of prey on which the larvae will feed. Tests were not conducted during the summer when the adults are normally inactive and feed little.

A series of sequential tests were used. First, no-choice tests are made where adult beetles are confined with a single type of potential prey—this indicates what they will not eat even when in a starved condition. Where warranted, this is followed by tests where the beetles are offered a choice between two to four prey items—this indicates relative preference. Unless indicated otherwise, prey was presented on a small section of host material in a Petri dish with a filter paper on the bottom. Prey were carefully counted before placement in the dish. Beetles usually were tested individually, and starved, but given water, prior to testing. The number of prey remaining was counted after a period of 20 hours or more, and the presence of fecal droplets on the filter paper was noted in no-choice tests.

The prey evaluated included insects that could be encountered on hemlock foliage (e.g. scales, psocids, and predatory dipteran larvae), tree feeding aphids, and other adelgids present in eastern North America. There are only five *Adelges* and seven *Pineus* species of adelgids reported from eastern North America; all of the *Adelges* are introduced species, while five of the *Pineus* species are native. One aphid tested, *Paraprociphilius tesselatus* (Fitch), is the primary prey of the only predaceous lepidopteran in the continental United States, *Feniseca tarquinius* F.; therefore, there was concern that the *Scymnus* beetles might attack this aphid.

Prey Acceptance (No-choice Test Results)

Four species of adelgids, five species of aphids, and representatives of non-aphidoid taxa were presented to the lady beetles (Table 5). *Harmonia axyridis*, a generalist predator, had a high acceptance rate for all the prey it was presented, except for *Fiorinia* elongate scale. It was the only predator that fed on the large aphid, *Cinara pinea*. This coccinellid is very active and capable of catching large, active prey. It also consumed the predaceous Diptera larvae that prey on aphids and adelgids. The *Scymnus* beetles were more restrictive in their prey acceptance. The aphids they were presented were all first instar nymphs, most of which were larger than HWA adults; only *Eucallipterus tiliae* and *Aphis gossypii* are comparable in size to first instar HWA nymphs. Aphids were not appreciably consumed by these *Scymnus* beetles, except for the consumption of *Aphis gossypii* by *Sc*. The first instar aphids were presented in a small container off its host and without filter paper, and may have been unable to escape the beetles. The other *Scymnus* species tested will also consume small aphids off host. The only species that preyed on all the adult adelgids was *Sc*. The pine adelgid, *Pineus strobi*, which is smaller than HWA, was consumed at rate equal to HWA.

Prey Preference (Choice Tests)

Because prey preferences among the adelgids were not clear based on single prey tests, HWA and another adelgid on their respective host plant were offered together in a Petri dish. Extensive choice tests by Butin et al. (2004) indicated that *Sn* prefers HWA over *Adelges laricis* and *A. cooleyi* and any aphid tested, but HWA and *P. strobi* were eaten equally.

When *Sc* females were presented with a choice of HWA and 2-3 other adelgids, they showed a strong preference for HWA. In the four-way choice tests, the relative preference was HWA > *Pineus strobi* >> *Adelges cooleyi* or *Adelges laricis*. The combination of other prey present, when only three were offered, resulted in significant differences in the relative feeding preference. *Sc* females were 6.6-fold more likely to eat HWA eggs over larch adelgid, 4-fold more likely to eat eggs of the combined group of *Adelges cooleyi*

Table 5. No-choice feeding[1] by adult lady beetles during 24-72 hour confinement with prey.

Prey Item	*Harmonia axyridis*	*Scymnus suturalis*	*Scymnus ningshanensis*	*Scymnus sinuanodulus*	*Scymnus camptodromus*
Adelgidae					
Adelges tsugae Annand					
egg	+++	+++	+++	++	+++
crawler	nt	nt	++	+	+
aestivating nymph	++	+	+	+	+
nymph III & adult	++	+++	+	++	++
Pineus strobi (Hartig)	+++	+++	+++*	++	+++
Adelges laricis	nt	nt	0*	nt	+++
Adelges cooleyi (Gillete)	nt	nt	++*	nt	+++
Aphididiae					
Cinara pinea (Mordwilko)	+++	0	0	0	0
Eucallipterus tiliae (L.) nymph	+++	+	+	++	nt
Prociphilus tessellatus (Fitch)	+++*	nt	+*	nt	0
Eriosoma lanigerum (Hausmann)	nt	nt	nt	nt	0
Aphis gossypii Glover					+++
Diaspididae					
Fiorinia externa Ferris	0	0	0	0	0
Pseudococcidae					
Pseudococcus sp.	+++	0	0	0	nt
Psocoptera					
Pseudocaecilidae	+++	0	0	0	nt
Diptera					
Syrphidae (larva)	++	0	0	0	nt
Chamaemyiidae (larva)	++	0	0	0	nt

[1] Codes refer to the percentage of predators tested that fed on the prey: 0 = none; + = <33%; ++ = 33 to 67%; +++ = >67%; and "nt" = not tested.

* Indicates score based on the proportion of alternative prey eaten in 2-way choice test between indicated species and HWA.

and *Pineus strobi* over *Adelges laricis*, and 1.6-fold more likely to eat HWA over the combined group of *Adelges cooleyi* and *Pineus strobi* (Hoover et al. 2011). Beetles only laid eggs on hemlock infested with HWA during these choice tests.

Choice tests using only the host plant found that significantly more time was spent by *S. suturalis* on white pine than on hemlock, whereas hemlock foliage was preferred by *Ss* and *Sn* (Table 6). Thus, it appears that tree foliage itself may vary in attractiveness to *Scymnus* species.

In summary, all three species of *Scymnus* imported from China are adelgid specialists, but when adelgids are not present, adults will minimally feed on slower moving aphids that are similar in size to HWA. Larvae seem to require HWA eggs to complete development, but will feed on other adelgids. They prefer HWA over other adelgids in choice tests and the adelgid's host plays a role in host choice. They appear to more readily accept adelgids on hemlock than on pine, and on pine than on other conifers. Adelgid eggs are required for larvae to complete development and for optimal oviposition. Both adults and larvae will locate an HWA ovisac and generally feed on it until it is exhausted before moving to the next. They will feed on crawlers, nymphs, and adults, often only injuring or partially consuming the later.

Table 6. Foliage preferences by adult lady beetles given a choice between pine and hemlock for 240 seconds.

Predator species	No. tests	Time on foliage (seconds) *Tsuga canadensis*	Time on foliage (seconds) *Pinus strobus*	Probability[1]
S. suturalis	7	24.1	55.2	<0.001
S. sinuanodulus	13	93.9	41.6	<0.001
S. ningshanensis	8	80.7	29.3	<0.001

[1]Probability that means are equal, paired t-test

Since these beetles are specialized on adelgids, of which seven of the 12 species in eastern North America are non-native, and they have a strong preference for HWA, they are not likely to have any appreciable impact on non-target prey. In addition, when *Sc* were presented with eggs of various adelgids without host or waxy coverings, they ate three times more eggs than when the same adelgid eggs were presented with the female parent on host material in a 48 hour time period. This indicates that prey acceptance is at least partially based on host characteristics. *Sc* adults also showed a significant preference for the eggs of the overwintering generation of the adelgids (both HWA and larch adelgids) when compared to the second generation, which may indicate some host quality differences between generations.

ESTABLISHMENT

Field Evaluation in Sleeve Cages

Information on the efficacy of biological control agents can be difficult to obtain when they are released freely into the environment because they disperse and both the agent and its target are affected by external factors that cannot be controlled. Field cage studies are an effective method to evaluate efficacy of natural enemies in a controlled setting and are more realistic than the laboratory, although the cages may alter microclimate and interfere with some enemy/prey interactions (Luck et al. 1999). Between 1999 and 2008, a series of experiments were conducted using sleeve-cages (Fig. 14) to confine *Sn*, *Ss*, and *Sasajiscymnus tsugae* on HWA infested branches of eastern hemlock.

Figure 14. Sleeve cages used to confine lady beetles on HWA infested hemlock branches.

The recommended procedure, which was refined over several years of use, is as follows: sites should be selected several months before the cages and beetles will be placed on the trees. They should have healthy hemlocks that have branches reachable from the ground with a density of HWA ranging from 100-300 per 0.5 meter of branch. The sleeve cages are made of light weight "No-see-um" knitted polyester fabric (nylon is more susceptible to UV rot) and are 1 m long × 0.65 m wide. Each treatment should have at least 30 replications, because variation within treatments is high. Just before the start of the test, branches are selected that appear to have between 150-350 ovisacs on the terminal 0.5 m. The number of ovisacs is estimated visually on each branch (it takes about 3 full days for two people to count 160 branches) and these are tagged where the open end of the bag will be closed over the branch. The branches are ranked by HWA density and the treatments assigned randomly within the sequential groups based on the number of treatments. (Initially, trees were considered blocks with complete sets of treatments assigned to each tree, but it was found that variation between trees was not a significant component of random variation.) Ideally, mature beetles held over winter in cold storage are used in the experiments. They may be removed from the cold and held as a group overnight for mating; however, mating just prior to placement in the field does not seem to increase fecundity. The beetles are sexed and placed individually in small containers that can easily be opened with one hand in the cage. Ideally, the sleeve cages are placed on the branches in April before HWA eggs have started to hatch and average daily temperatures are 5-7 °C, and removed in June, when the progredientes are just starting to lay eggs. The branch with the bag attached is cut from the tree and taken to the lab and placed in cold storage until the bags can be opened. The beetles and HWA are counted with the aid of a microscope. The statistic most useful in assessing impact on HWA is the per capita change in the population ($r=ln\ [Nt/No]$) where No was the initial population (sistens ovisacs) and Nt was the final population (progrediens ovisacs) in each sleeve cage.

In the first experiment, the sleeve cages were installed at two sites in Connecticut on 20 April and most were removed one month later. During this month, the eggs in the sistens ovisacs hatched and progrediens nymphs were present when the cages were removed. The HWA population increased in all the cages during the month but those with a single female *Ss* had, on average, lower HWA populations than the control bags without a beetle (Fig. 15). Note that there was a cage effect. HWA numbers were higher on the branch in the cage without a beetle than on the branch without a cage—the effect of the beetle was compared to the caged branch without a beetle. The branches where beetles produced progeny reduced HWA populations more than uncaged branches. Some of the cages in this experiment were allowed to remain on the branches until July 7. At this time, the HWA population in the cages with reproductive beetles was not significantly different, on average, than in the cages without beetles (Fig. 16). By July, the cages contained aestivating neosistentes and most of the beetles were dead, perhaps from the lack of suitable food. In cages without the beetle, the HWA populations increased in the first generation

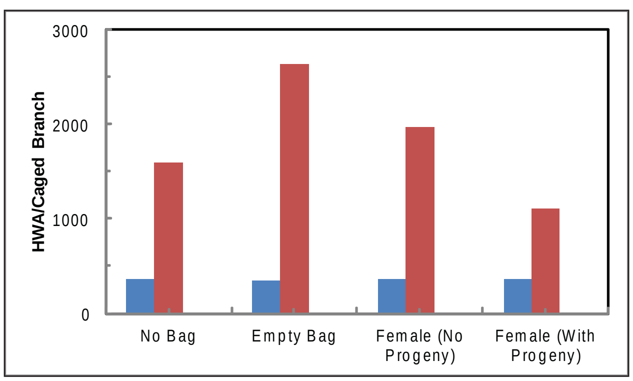

Figure 15. Population change of HWA in cages for one month (sistens ovisacs to progredien nymphs).

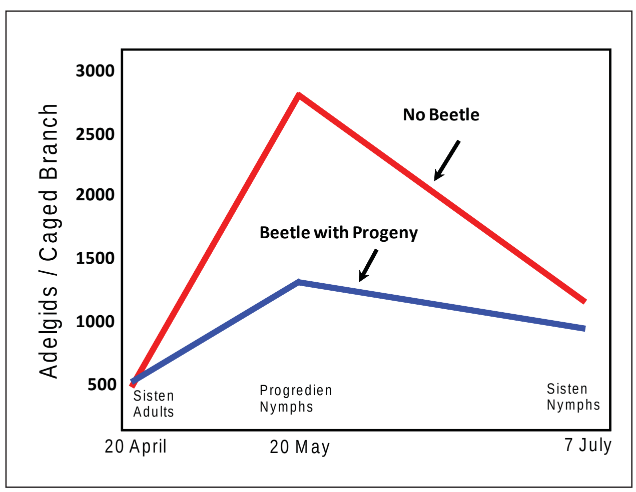

Figure 16. Population change of HWA in cages with or without beetles for two generations of HWA.

and then crashed in the next generation whereas bags with the beetle stabilized the population, but at a level still high enough to damage trees. Stabilization of pest populations below damaging thresholds is the goal of biological control.

In a similar caged study, the presence of *Sn* resulted in negative growth of HWA populations, whereas the population growth in the cages without the beetle was positive (Butin et al. 2003). This study also included *S. tsugae* as a treatment and this lady beetle also reduced HWA populations compared to the control, but not significantly. The *S. tsugae* were new beetles that had not been overwintered in the laboratory. The *S. tsugae* did not reproduce in the bags, whereas one-third of the *Sn* did. Butin also reported that, in the laboratory, the fecundity of *Sn* increased with increasing densities of HWA ovisacs. With both *Ss* and *Sn*, the beetle cages with the most beetle progeny were those with the highest number of HWA at the end of the experiment and most of the progeny were produced within the first week after the beetles were placed in the bags. These results indicated that plentiful, fresh food is needed for good beetle reproduction.

Other caged field trials, conducted from 2002 to 2006, were less successful. In some, the HWA population crashed in the control cages and neither the presence of *Ss* nor *Sn* was able to drive the HWA populations significantly lower (although many cages with beetles contained no HWA at the end of the feeding period). The beetles did not produce progeny on these declining populations. These trials showed the importance of "pre-conditioning" of the adult females placed in the cages. It was also found that degree day growth models could be used to predict when each stage of the lady beetle would be present in the cage and that the free released and caged beetles had the same development rates.

In 2007, three species of lady beetles (*Sn*, *Ss*, and *S. tsugae*) were evaluated in sleeve cages in northwestern North Carolina. All three species significantly reduced HWA compared to the cages without beetles (Table 7). However, there was not a significant difference in reduction in HWA populations among beetle species. For this test, all three species had been held over winter at 7 °C and short daylength at the Forest Service laboratory in Hamden. This test demonstrated that placement of overwintered, reproductively active lady beetles in the field, when day-night temperatures ranged from -2 °C to 12 °C, results in good oviposition by all three species and full development of their progeny, including maturation feeding of the new adults, before HWA enters aestivation.

In 2008, a sleeve cage evaluation focused on *S. tsugae*. For this trial, *S. tsugae* was obtained from the North Carolina Department of Agriculture rearing facility. These beetles are normally reared at 25 °C

Table 7. Populations of HWA (average/caged branch) when the cages were placed on the branches April 15 and removed June 19-22. The change in HWA population (r) is (*ln* Final – *ln* Initial). The number of progeny the female beetle produced in each cage is based on number of all beetle stages recovered at the end of the test, minus the initial female.

Treatments	Empty cage (no beetle)	*Scymnus ningshanensis*	*Scymnus sinuanodulus*	*Sasajiscymnus tsugae*
Sistentes (initial)	254.5	297.0	258.0	252.5
Progredientes (final)	132.7	49.5	1.5	17.6
HWA pop'n change (r)	-0.77	-3.79	-6.02	-5.85
HWA pop'n change (%)	-41.0	-84.1	-99.0	-95.0
Beetle progeny (avg)	na	4.8	3.2	7.9
Beetle progeny (max)	na	24	29	31
Replications (n)[1]	30	34	31	24

[1]Initally 30 controls and 34 cages for each treatment, but some cages were damaged.

and stored at 18 °C prior to release. The adults are usually less than one-month-old when field released. Thirty replicates of four different ages of *S. tsugae* were placed in cages on April 1. Complete sets of the four treatments were removed on a given day between July 5 and 16, and the progeny counted (Table 8). The two-week-old beetles did not produce any progeny and only one of the one-month-old beetles produced progeny. The beetles held for one month at 18 °C and then placed in cold storage for one month also did not produce progeny. The only beetles that reproduced well had been reared the previous year and had been producing progeny for field release prior to being placed in the bags. These beetles were noticeably smaller and much less active than the much younger beetles in the other treatments. The progeny of the oldest beetles were either adults or pupae, whereas the progeny from the one-month-old beetles were only in the larval stage, which indicates that they may not have been ready to oviposit when released. This experiment suggests that releasing mature, ovipositing *S. tsugae* beetles in the spring would be more successful than releasing beetles that became adults that same spring. With both *S. tsugae* and the *Scymnus* beetles, better reproduction occurred with adults that had been overwintered in the laboratory.

While sleeve cages are useful in defining release parameters and impact of lady beetles, the cages seem to inhibit their reproduction. In all the trials, the cages with the most progeny were the cages that had the most HWA at the end of the trial (control cages excepted). It seems that all of these lady beetles produce more progeny when more food is available.

Table 8. Production of progeny in sleeve cages by *Sasajiscymnus tsugae* varying in age.

Adult Age (Months)	Produced Progeny	Progeny (Avg. No.)	Progeny (max.)
0.5	0%	–	–
1	3%	16	16
1+1*	0%	–	–
>8	30%	32	64

*Held one month at 18 °C and then refrigerated one month

Environmental Releases

Free releases of *Ss* (Table 9) and to a limited extent of *Sn* (Table 10) have been made, but there is no record of recovery of these beetles in the years following their release. Following the early spring release of adult beetles that had been overwintered, both *Ss* and *Sn* larvae have been recovered and adults found until November, but not in the year following release. Thus, there is no evidence that either *Ss* or *Sn* are established in the eastern United States.

IMPLICATIONS FOR BIOLOGICAL CONTROL

The *Scymnus* (*Neopullus*) lady beetles that prey on HWA have either delayed reproductive maturation in the adult stage or an egg diapause. The periods of arrested development in all three *Neopullus* species coincide with the aestival diapause of the HWA neosistentes. The adults of these lady beetles prey on HWA during all periods when it is active, and their larvae are present in the spring when HWA eggs are present.

A key question is whether a species with a life history where adults exist for several months before they lay eggs that quickly hatch, or a species with a life history where eggs exist for several months before they hatch, is more suitable biological control agent for HWA. The adults of *Sn* and *Ss* spend eight months exposed to predators and the elements before they lay eggs that soon hatch in spring. On the other hand, *Sc* begins laying eggs soon after eclosion and has an extended oviposition period in which to stockpile eggs, but these eggs then remain in diapause for many months until they hatch in early spring. It is not possible to predict which survival strategy would result in better HWA control. However, in their native range in China, the abundance of *Sc* was more consistent among the sites and during the survey period than the other species.

Table 9. Environmental releases of adult *Scymnus sinuanodulus*.

Date	No. released	Location	Rearing Lab.	Condition
April, 2004	150	Rauben Co., GA	USFS, Hamden	10-wk-old adults
October, 2004	320	Rauben Co., GA	"	4-month-old adults
April, 2005	528	Fairfield Co., CT	"	reproductive adults
Spring, 2005	1,530	NJ	NJ Dept. Agric.	new adults
"	1,210	PA	"	"
"	750	NC	"	"
"	460	MD	"	"
"	460	WV	"	"
Spring, 2006	1,500	NJ	"	"
"	1,200	PA	"	"
"	1,000	NC	"	"
"	500	MD	"	"
"	1,000	WV	"	"
July, 2006	228	Watauga Co., NC	Sleeve cages	new adults
Spring, 2007	6,305	NJ	NJ Dept. Agric.	new and old adults
April, 2007	496	Avery Co., NC	USFS, Hamden	reproductive adults
May, 2007	45	Gt. Smoky Mtn. NP	UTK, Knoxville	post-reproductive
Late May, 2008	208	Chattahoochee NF, GA; 1 site	UGA, Athens	reproductive adults
Spring, 2008	7,480	Chattahoochee NF, GA; 9 sites	UGA, Athens	eggs & larvae
Spring, 2009	8,400	Chattahoochee NF, GA; 15 sites	UGA, Athens	eggs & larvae
Late May, 2009	165	Chattahoochee NF, GA; 1 site	UGA, Athens	reproductive adults
Spring, 2010	15,741	Chattahoochee NF, GA; 53 sites	UGA, Athens	eggs & larvae
Late May, 2010	100	Chattahoochee NF, GA; 1 site	UGA, Athens	reproductive adults
Spring, 2011	11,075	Chattahoochee NF, GA; 23 sites	UGA, Athens	eggs & larvae
Early Spring, 2011	800	Chattooga River Corridor; 7 sites	UGA, Athens	reproductive adults

The first predator imported from China for biological control of HWA was *Sc* in 1995. Unfortunately, this species has been very difficult to rear because of its egg diapause. The requirements for breaking its egg diapause have now been deciphered and colonies can now be maintained in the laboratory. This species has several characteristics that indicate it would make it a good biological control agent: (1) it has a true aestival diapause as does HWA; (2) it occurs over a broad geographic area and in diverse habitats in its native range; (3) its larvae are present at a key point in the life cycle of HWA; and (4) its adults feed on HWA throughout most of the year.

Table 10. Environmental releases of reproductive adult *Scymnus ningshanensis* reared at the USDA Forest Service lab in Hamden, CT.

Date	No. released	Location
April, 2007	300	Hampshire Co., MA
April, 2007	300	Hampden Co., MA
April, 2007	300	Hartford Co., CT
April, 2009	500	Avery Co., NC

The other species of *Scymnus* (*Neopullus*) imported from China have been environmentally released, but appear not to have established. *Ss* has been released in considerable numbers in several areas and does not seem to merit further effort at establishment. The other species, *Sn*, has been released only in very low numbers and may merit further effort. A significant problem with this species is that the colony was founded on a single collection of a low number of specimens, which declined to a colony of less than ten individuals before being multiplied to sufficient numbers for release.

In addition to the *Scymnus* (*Neopullus*) lady beetles imported, there were other predators in China that appear when HWA is at its highest densities. These predators are mostly opportunists that feed on a broad range of prey. This non-specificity makes them unsuitable as biological control agents, but the significance of opportunistic predators in driving down high HWA populations where it is indigenous needs to be recognized. It is not surprising that native, HWA specific predators are lacking in the eastern United States considering than HWA is a relatively recent introduction; what is surprising is the lack of native opportunistic predators preying on HWA.

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