Forest Health Technology Enterprise Team

TECHNOLOGY TRANSFER

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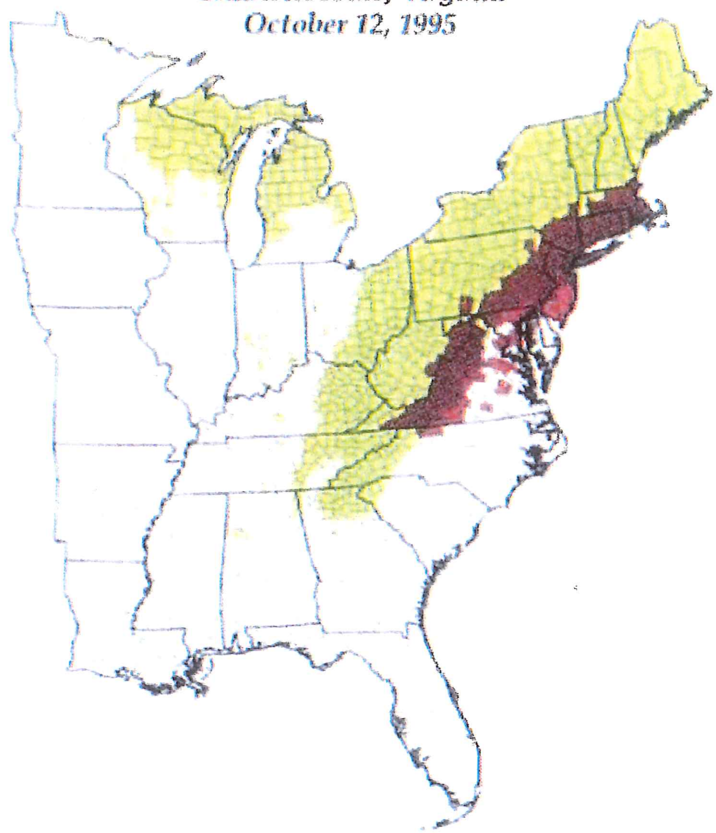

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EXOTIC NATURAL ENEMIES OF *ADELGES TSUGAE* AND THEIR PROSPECT FOR BIOLOGICAL CONTROL

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ABSTRACT

The natural enemy complex of the hemlock woolly adelgid, *Adelges tsugae* Annand (Homoptera:Adelgidae) in its native Japan is described, and the two most promising candidates for biological control in North America are identified. These are the oribatid mite, *Diapterobates humeralis* Hermann (Oribatei:Ceratozetidae) and the coccinellid beetle *Pseudoscymnus* n. sp. (Coleoptera:Coccinellidae). Studies in Japan showed that *D. humeralis* fed on the woolly material surrounding the adelgid egg mass and, in so doing, dislodged the eggs that were then subject to dessication or predation. However, the inherent low fecundity of *D. humeralis* poses a real challenge for mass rearing and release. The life cycle, development, predation rates, longevity, and fecundity of *Pseudoscymnus* n. sp. are reported. There are at least two overlapping generations of the coccinellid during the spring and early summer, which are well synchronized with the two adelgid generations. Predation by *Pseudoscymnus* n. sp. larvae and adults are of all adelgid stages available, for maximum impact. These attributes, together with a substantial fecundity and lifespan, point favorably to the potential success of *Pseudoscymnus* as a biological control agent of *A. tsugae*.

INTRODUCTION

The destruction of eastern hemlock, *Tsuga canadensis*, and Carolina hemlock, *T. caroliniana*, by the hemlock woolly adelgid, *Adelges tsugae* Annand, in eastern North America is due largely to a combination of host plant susceptibility (McClure 1992) and the lack of effective natural enemies in the adelgid's introduced range (McClure 1995a). In response to this threat, the Connecticut Agricultural Experiment Station initiated the exploration for native natural enemies of the adelgid in Japan in 1992. The investigation yielded two important findings: *A. tsugae* populations in Japan are regulated by the endemic natural enemy complex and the inherent host resistance of Japanese hemlocks, *T. diversifolia* and *T. sieboldii* (McClure 1992, 1995b,1995c,1996). As a result of the discoveries in Japan, the most promising candidates for introduction into North America were selected for further study and evaluation of their potential as classical biological control agents. Shipments of natural enemies have been received from colleagues in Japan to start laboratory colonies in Windsor, Conn. In 1995, after intensive laboratory investigations to supply information for a federal environmental assessment of the coccinellid predator, a permit allowing field releases was granted.

THE NATURAL ENEMY COMPLEX IN JAPAN

Adelgid population densities in 33 of 37 forest sites and 34 of 37 ornamental sites in Japan were kept at low levels by the native natural enemy complex (McClure 1995b, 1996). The latter consists of a hitherto undescribed coccinellid beetle (*Pseudoscymnus* new species; Sasaji and McClure in prep.), an oribatid mite (*Diapterobates humeralis* Hermann), a chrysopid lacewing (nr. *Mallada prasina* [Burm.]), a cecidomyiid fly (*Lestodiplosis* sp.), and an unidentified syrphid fly. Insect predators alone killed 96 - 99% of adelgids at forest sites and 69 - 86% at ornamental sites (Table 1; McClure 1995b). Studies in Japan revealed that the oribatid mite was not preying on the adelgid but rather consuming the woolly material surrounding the adelgid egg mass (McClure 1995c). This feeding action resulted in the dislodgement of adelgid eggs to the forest floor where the eggs were subject to either predation or dessication. At 17 forest and 23 ornamental sites where *D. humeralis* was found, the mite caused 91% and 98% mortality of adelgid eggs, respectively (McClure 1995c). The other four species were observed to prey on adelgid eggs, although larger immatures and adult adelgids were also attacked, particularly by the coccinellid and the lacewing (McClure 1995a,1996). Of the complex of natural enemies observed in Japan, both the oribatid *D. humeralis* and the coccinellid *Pseudoscmynus* n. sp. appeared to hold the most potential for biological control in the United States.

DIAPTEROBATES HUMERALIS (ORIBATEI: CERATOZETIDAE)

D. humeralis is a chiefly arboreal, oribatid mite which has a subarctic, boreal, and temperate distribution, ranging from North America, Northern Europe and Asia (Danks 1981, Aoki 1982, Behan-Pelletier 1986, Marshall et al. 1987). Previous studies reported that *D. humeralis* feeds mainly on the hyphae and spores of parasitic higher fungi (Tarras-Wahlberg 1960, 1961; Behan and Hill 1978). Oribatid mites generally feed on decaying plant tissues, fungi, algae and lichens (Woolley 1960, Butcher et al. 1971, Krantz and Lindquist 1979, Norton 1986, 1994) but only very occasionally have been reported to feed on other plant and animal material. The feeding behavior of *D. humeralis* on the woolly material surrounding the adelgid egg mass is a unique observation to date and constitutes the only example of an oribatid functioning as a control agent (McClure 1995c).

Biology and Potential for Biological Control. *D. humeralis* has at least two broadly overlapping generations per year; the life stages consist of the egg, larva, protonymph, deutonymph, tritonymph, and adult. Adult mites can live for 10 months; females each produce about 20 eggs at a time. Oribatid mites pose a challenge in rearing and study, and *D. humeralis* is no exception due to its extremely small size, long generation time, lack of knowledge of its nutritional requirements for reproduction, and ease of fungal contamination of the colony. Another obstacle that had to be surmounted was that of distinguishing between *D. humeralis* and another native arboreal oribatid, *Humerobates arboreus* Banks (Norton pers. comm.) which is almost identical in appearance to the former. In addition to adelgid wool, *D. humeralis* feeds on algae and lichens, but fecundity has been at best unpredictable and there is a long interval of at least six weeks prior to egg hatch. Its low fecundity combined with the difficulties encountered in maintaining a viable laboratory colony for field releases have hampered the evaluation of the mite for biological control. The situation is further complicated by the occurrence of native North American *D. humeralis,* which has been found in Connecticut but not necessarily in adelgid-infested areas due to the absence of previous sampling. A survey was conducted in 1995 in Connecticut to determine the distribution of native populations of the mite in relation to adelgid infestations; processing of the samples has yet to be completed. To date, there have been several releases in small quantities of *D. humeralis,* with some preliminary indication of overwintering during the severe winter weather of 1993-1994. However, it is still too early to determine if populations of *D. humeralis* have been established in significant numbers at these sites for evaluation of their impact on *A. tsugae.*

PSEUDOSCYMNUS (COLEOPTERA: COCCINELLIDAE)

Development. *Pseudoscymnus* n. sp. belongs to the Tribe Scymnini, which are characteristically small coccinellids (< 3mm) that specifically prey on homopteran pests such as aphids, mealybugs, scales, and adelgids (Pang and Gordon 1986) and thus present great potential for biological control. No information is hitherto known for this previously undescribed species; our studies to date have concentrated on understanding the basic biology and life cycle, with evaluation of this species for biological control of *A. tsugae.* Developmental stages include the egg, four larval instars and a pupa. Newly-hatched first instar larvae are reddish brown but soon darken to a medium gray after feeding. The four larval stages last about 17 - 20 days, and the late fourth instar forms a woolly prepupa which lasts two to three days. The pupa is reddish brown and lasts 8 - 9 days. Newly emerged coccinellid adults are golden brown but soon harden off to a jet black within a day. Total development in the laboratory from egg hatch to adult emergence is between 28 - 32 days at 20 °C (Table 2; Cheah and McClure in prep.).

Predation. Both larvae and adult *Pseudoscymnus* n. sp. actively prey on all stages of *A. tsugae* from egg or nymphs to adults, feeding on whatever stage of adelgid is available at the time. Larvae search actively along hemlock branches in search of their prey, then feed by alternate suction and regurgitation of the fluid contents of the adelgid. Drained shells of the adelgid stage consumed remain as evidence of predation. Larval predation impact is heaviest during the third and fourth larval instars when more than 70% of the total adelgid consumption occurs. Studies have shown that a single larva can consume about 500 eggs or 50 - 100 adelgid nymphs (in this experiment, nymphal instars 2-4) for completion of development to adult emergence (Table 3). The total number of adelgids consumed per larva is dependent on the stage and relative size of the adelgid available. Adult beetles are strong fliers and actively move on hemlock branches in search of adelgids. They consume the smaller stages of *A. tsugae* whole, usually leaving no trace of the eggs, crawlers, or settled first instar nymphs eaten. Adult predation is not as easily quantifiable, but experiments have shown that an adult can consume an average of 48 $\pm$ 20 second and third instar nymphs a week, although this predation is likely to be modified by oviposition trends (Cheah and McClure in prep.).

Life cycle. The life cycle of *Pseudoscymnus* n. sp. has been determined to be very well synchronized with that of its prey. Spring egg hatch for the coccinellid in May is timed to coincide with peak adelgid egg hatch, and adults continue to oviposit through the spring and mid-summer months. All stages of the coccinellid are present through June. Most importantly, a second generation of the coccinellid is produced when adults of the first spring generation begin oviposition with the start of egg production of the summer generation of the adelgid. Adult coccinellids are able to survive the summer aestivation period (ca. July 1 - Oct.15) of the adelgid by feeding on the settled first instar nymphs. In the laboratory, it has been observed that as adelgid dormancy is broken in October, oviposition is resumed by the two generations of adult beetles produced in the spring and summer. Such larvae produced in the fall are able to complete development by feeding on adelgid nymphs (Cheah and McClure in prep.). The occurrence of this fall generation in the field is currently being studied.

Longevity and fecundity. The maximum adult lifespan recorded was 12 months for a female. However, adults more commonly live an average of 4 months during the active oviposition and mating season. It appears that fecundity influences the total lifespan in female *Pseudoscymnus* n.sp., as females that have yet to deposit their full complement of eggs in a season apparently survive to the next active adelgid period. Oviposition occurs over several generations of adelgids; some females survive to oviposit on two successive spring generations of the adelgid. Oviposition behaviour appears to be related to the availability of developing adelgids, as egg production is sharply curtailed in late summer when the adelgid enters a period of summer aestivation. Fecundity is maintained by repeated matings and is variable. In a study which followed the egg production of individual female coccinellids for 10 months, the average lifetime fecundity was 224 $\pm$ 71 eggs (n=18). Adult females generally deposit their eggs in concealed locales such as curled bud scales, within male cones, on or under old or new adelgid wool, and under bark flaps. Between 1 - 4 eggs are laid at a time in such protected sites. Adult beetles have been monitored to produce up to 50 eggs in a week but an average of 20 eggs are laid per week, during a season (Cheah and McClure in prep.).

Field Studies. Efforts since January 1995 have concentrated on the mass rearing of *Pseudoscymnus* n. sp. for spring and summer field experiments and release. A spring shipment of natural enemies from Japan in 1995 has yielded more coccinellid eggs, larvae, and adults to augment genetic diversity in the mass rearing procedure. Field experiments have included the gradual release of 2025 adults from April to June 1995 on a select group of adelgid-infested hemlock with predator exclusion cages in a natural forest environment in Windsor, Connecticut. Studies are planned for next spring to monitor for dispersal and predation impact at this site. An additional 100 adults were released at another forest site in Cheshire, Connecticut. Sleeve cage experiments were also conducted at Lockwood Farm, Connecticut, to determine the impact of adult and larval predation on adelgid populations. Further studies are planned to investigate density responsiveness and overwintering ability of *Pseudoscymnus* n. sp.

SUMMARY AND CONCLUSIONS

The attributes of a successful biological control agent are generally agreed to include the following: host/prey specificity, synchrony of life cycle with that of the pest, density responsiveness, adaptability to varying physical conditions, high searching efficiency and dispersal ability, high rate of population increase with respect to the pest, and survival during pest-free periods. *Pseudoscymnus* n.sp., with its targeted attack of its prey, the hemlock woolly adelgid, has clearly evolved as a specialized predator-prey system in Japan and appears to be the most promising biological control agent for study. Our experience in Connecticut has shown that the coccinellid is amenable to mass culturing on live *A. tsugae* collected from the field. Preliminary laboratory indications are that the coccinellid may feed on other adelgids it encounters, such as *Adelges cooleyi* and *Pinius strobi*, but preference tests still need to be conducted. The ability to feed on other adelgids may prove to be an adaptive strategy in times of *A. tsugae* scarcity, yet may also reduce the efficiency of the beetle to concentrate on *A. tsugae*. The life cycle of *Pseudoscymnus* n. sp. is extremely well synchronized with that of its prey, a fact that is enhanced by the production of a second generation in the summer for maximum impact. That adults and larvae attack all stages of *A. tsugae* available is an added advantage over other predators which target only the eggs. Dormant first instar *A. tsugae* nymphs are also consumed during the summer by adults and larvae, a behavior that increases and sustains the impact of predation on adelgid populations. Fecundity is substantial and prolonged by repeated matings and a relatively long lifespan. Although much remains to be determined as to whether the coccinellid will be effective in the field and be able to survive the winter conditions, all indications are that *Pseudoscymnus* n. sp. is a favorable candidate for biological control of *A. tsugae*.

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TABLE 1. Occurrence and impact of natural enemies on hemlock woolly adelgid in Japan (adapted from McClure 1995b, 1996)

Natural Enemy	Number of Infested Sites		% Mortality of Adelgids *	
	Forest (37)	Ornamental (37)	Forest	Ornamental
Coccinellidae *Pseudoscymnus* n.sp.	13	11	99	86
Chrysopidae nr.*Mallada prasina*	4	6	99	79
Cecidomyiidae *Lestodiplosis* sp.	5	3	96	69
Syrphidae unidentified sp.	1	2	97	70
Ceratozetidae *Diapterobates humeralis*	17	23	91	98
None	4	3	0	0

* based on sites where only one species of natural enemy was present

TABLE 2. **Duration (in Days) of Developmental Stages and Total Development Time from Hatch to Adult Emergence for *Pseudoscymnus* n. sp. (Spring * and Fall ** Generations) at 20 °C**

Generation	L 1 - L 4	L4(Prepupa)	Pupa	L 1 - Adult
Spring	17.1 ± 3.7	2.3 ± 0.5	7.9 ±1.0	28.2 ± 4.1
Fall	20.0 ± 3.5	2.8 ± 0.8	8.7 ± 1.1	32.5 ± 3.4

* Development of spring generation larvae measured on a diet of *A. tsugae* eggs
**Development of fall generation larvae measured on a diet of *A. tsugae* nymphal instars 1-4

TABLE 3. Mean Number and Stages of *A. tsugae* Consumed for Completion of Development from Hatch to Adult Emergence for *Pseudoscymnus* n. sp.

Time of Hatch	Total Number Consumed by Larva	Adelgid Stage(s) Consumed
June	489.7 ± 103.6	Egg
Mid October	109.0 ± 11.6	N 1 - N 4
Early November	83.7 ± 6.8	N 2 - N 4
Mid November	60.1 ± 14.7	N 2 - N 4
End November	52.0 ± 9.5	N2 - N 4