Biology of *Laricobius nigrinus* Fender (Coleoptera: Derodontidae) and its Potential as a Biological Control Agent of the Hemlock Woolly Adelgid, *Adelges tsugae* Annand (Homoptera: Adelgidae) in the eastern United States

by

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Abstract

The biology of *Laricobius nigrinus* Fender (Coleoptera: Derodontidae) and its potential for control of hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Homoptera: Adelgidae) in the eastern United States were investigated. *Laricobius nigrinus* completed development on HWA in laboratory studies. There are four larval instars. Mean larval consumption was 225.9 and 252.3 HWA eggs at 12 and 18 °C, respectively. Post-aestivation activity period was 36.6 and 30.8 weeks for males and females at 13 °C, respectively. Mean lifetime fecundity was 100.8 eggs over a mean ovipositional period of 13.2 weeks.

Laricobius nigrinus is host specific. In paired-choice and no-choice oviposition tests, *L. nigrinus* laid more eggs in HWA ovisacs than other test species. *Laricobius nigrinus* consumed more eggs of HWA than eggs of *Adelges piceae* (Ratzeburg) and *Pineus strobi* (Hartig) but not of *Adelges abietis* (L.). In larval development tests, *L. nigrinus* only completed development on HWA.

A two-year field study conducted in British Columbia showed that: 1) the life cycle of HWA in British Columbia is similar to that previously reported in Virginia and Connecticut; 2) *L. nigrinus* adults undergo an aestival diapause that coincides with the diapause of the first instar HWA sistens. Adult activity beginning in autumn coincides with resumption of development of the sistens generation; 3) oviposition and subsequent larval development of *L. nigrinus* coincide with oviposition by HWA sistens adults.

Temperature-dependent development was determined for egg, larval, pre-pupal and pupal stages of *L. nigrinus* at five constant temperatures (9, 12, 15, 18 and 21 °C). Development time was inversely proportional to temperature between 9 and 18 °C. *Laricobius nigrinus* did not complete development at 21 °C. Minimum developmental temperatures for eggs (6.5 °C), larvae (5.1 °C), pre-pupae (3.8 °C), and pupae (3.1 °C) were determined by extrapolation of linear regression equations to the *x*-intercept. Degree-days calculated for eggs, larvae, pre-pupae, pupae, and egg to adult were 54.6, 161.3, 196.1, 212.8, and 666.7, respectively. The laboratory derived degree-day model for predicting egg hatch was validated with field data collected in British Columbia. Observed median egg hatch at three of four sites over two years was within 15% of the predicted DD value (54.6).

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Chapter 1

Introduction and Literature Review

Hemlock woolly adelgid in the eastern United States

The hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, is a serious threat to hemlock landscape and forest stands in the eastern United States (McClure 1996). Eastern hemlock (*Tsuga canadensis* (L.) Carrière) and Carolina hemlock (*T. caroliniana* Engelmann) are very susceptible to HWA attack and infested trees have died in as little as four years (McClure 1991). Hemlock woolly adelgid is exotic to eastern North America (McClure 1987). First reported in the eastern United States in Virginia in 1952 in an ornamental setting (Souto et al. 1996), it has spread to forests where it occurs along the eastern seaboard from North Carolina to southern New England (Salom et al. in press). The main front of the HWA infestation is advancing at approximately 25 km per year (McClure 2001).

Hemlock woolly adelgid was first observed in North America in the Pacific Northwest in the early 1920's where Annand (1924) described it from specimens collected on western hemlock*, T. heterophylla* Raf. Sargent in Oregon. An earlier description in 1922 identified the species as *Chermes funitectus* Dreyfus, also from western hemlock in Vancouver, British Columbia (Annand 1928). Annand (1928) reported that the two species were the same.

Species of hemlock in Asia (McClure and Cheah 1999, Montgomery et al. 2000, Yu et al. 2000) and western North America (Furniss and Carolin 1977) are attacked by

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HWA but are seldom damaged. In the Pacific Northwest, HWA is not considered a forestry pest but it can weaken and kill ornamental trees (Furniss and Carolin 1977). Tree resistance and natural enemies likely play a role in maintaining HWA below injurious levels in these two regions (Cheah and McClure 1996, Montgomery and Lyon 1996). In contrast, hemlock species in eastern North American have little or no tolerance to HWA attack. Infested trees exhibit poor crown condition and reduced terminal branch growth, which often results in mortality when trees are predisposed by other stresses (McClure et al. 1996, Souto et al. 1996). Hemlock woolly adelgid feeds on parenchyma cells of the xylem rays which are cells that transfer and store nutrients (Shields et al. 1996). This type of feeding depletes nutrients in storage cells, may introduce toxic saliva and likely interferes with the movement of water and nutrients through the xylem. These stressors in combination with others (e.g. drought) likely contribute to tree death (Shields et al. 1996).

Hemlocks have considerable ecological importance in the predominantly hardwood eastern North American forest ecosystem. Hemlocks are long-lived, late successional, shallow rooted conifers with dense evergreen crowns (Quimby 1996). They are the most shade and least drought tolerant of any conifer and are restricted to areas with cool humid climates (Farjon 1990). Hemlock stands provide important habitats for a number of bird, wildlife, small mammal, amphibian and fish species (Evans et al. 1996, Quimby 1996). In addition, hemlock is economically important in several areas of the eastern United States as a landscape tree and as source of timber wood (Rhea 1995). Thus, HWA has significant ecological and economic impacts which will become more severe as its distribution expands.

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Management of HWA is important for preservation of eastern hemlock as a forest and ornamental species. Hemlock woolly adelgid can be controlled effectively with a number of insecticides, insecticidal soaps, and horticultural oils in landscape and nursery settings (McClure 1987, 1992). Because eastern hemlock ecosystems are patchy in distribution (Quimby 1996), grow slowly and therefore are usually subdominant in a stand for centuries (Burns and Honkala 1990, Quimby 1996), and are often associated with riparian habitats because of moist soil requirements (Williams and Moriarity 2000), chemical control in forested areas is impractical if not impossible to obtain (McClure 1987). Since HWA populations in the eastern United States are not regulated by effective natural enemies (McClure 1987, Montgomery and Lyon 1996, Wallace and Hain 2000), classical biological control has become the most promising management option. Since the early 1990's research on promising predators has been underway (Cheah and McClure 1996, 1998, McClure et al. 2000, Montgomery et al. 2000, Lu and Montgomery 2001).

Biology of Hemlock Woolly Adelgid

Life histories within the family Adelgidae are complicated and involve a succession of morphologically different forms and life cycles (Blackman and Eastop 1994). Host alternation and cyclic parthenogenesis are two life history characteristics that are represented within this group (Moran 1988, 1992, Blackman and Eastop 1994). The genus *Picea* is the primary host in holocyclic species of both *Adelges* and *Pineus*, the two genera within the family Adelgidae. The secondary hosts of *Adelges* spp. include the genera *Abies, Larix, Pseudotsuga* and *Tsuga*. The secondary hosts of *Pineus* spp. are *Pinus* spp. (Blackman and Eastop 1994). Hemlock woolly adelgid is known to be

holocyclic in Japan, alternating between *Picea polita* (Siebold & Zucc.) Carrière and *Tsuga sieboldii* Carrière, while in North America and China it is apparently anholocyclic (Blackman and Eastop 1994).

The life history of HWA was first studied in Connecticut after its establishment in 1985 (McClure 1987). Subsequent studies in Virginia by Gray and Salom (1996) reported the life histories to be similar in the two locations, with possible differences in a faster rate of development of the over-wintering sistens generation in Virginia.

Hemlock woolly adelgid completes two asexual generations on hemlock, represented by sistens, present from July through April, and progrediens present from April through June (McClure 1989b, McClure 1996). Sistens of the over-wintering generation mature in February and deposit eggs into white, woolly ovisacs containing as many as 300 eggs (McClure 1987, 1996). A portion of the eggs develop into progrediens (wingless) with the rest developing into sexuparae, a winged migratory form (McClure 1987). The proportion of eggs that become sexuparae increases with adelgid density in response to reduced host quality (McClure 1991). In April, eggs of both types hatch into crawlers (first instar nymphs). Individual crawlers, which are active for only one or two days, can be passively dispersed by birds, deer and wind (McClure 1987, 1989a, 1990). Crawlers search for a suitable site to settle and insert their stylets at the base of hemlock needles on young branches. Feeding in late winter and early spring impedes new growth in the spring and causes existing needles to desiccate and drop from the branches (McClure 1987). Nymphs develop through four instars and mature in June (McClure 1987, 1996).

Progrediens remain on hemlock and produce ovisacs in June and July. Sistens crawlers hatch from these eggs, settle on new growth on hemlock branches and shortly

thereafter enter into summer diapause (aestivation). Nymphs resume development in October. They feed during the warmer periods of late autumn and winter and mature by February (McClure 1987).

The development of a winged form of HWA suggests that spruce (*Picea* spp.) plays a role in the life cycle of HWA. Studies by McClure (1987) showed no survivorship of HWA nymphs (sexuales) produced by sexuparae that oviposited on 12 species of spruce, not even on *Picea polita*, the recorded primary host in Japan. Sexuales have not been observed to date to develop successfully on any spruce species in North America (McClure et al. 1996).

Hemlock woolly adelgid is regulated by density dependent factors (McClure 1991). Studies in Connecticut showed that HWA at densities as low as four individuals per 20 mm^2 of branch, inhibits the production of new growth by hemlocks the following year. Adelgids are then forced to feed on less preferred older growth where survival and fecundity are reduced. In addition, the proportion of sexuparae produced increases with decreased host quality. This generation disperses and probably dies without reproducing because no primary host (spruce) is available. This reduces the population further (McClure 1991).

The genus *Laricobius*

Laricobius is one of four genera in the family Derodontidae that inhabits relatively humid forests in temperate regions (Lawrence and Hlavac 1979). The genus *Laricobius* is holarctic in distribution (Lawrence 1989), and is the only genus in this family that feeds on woolly adelgids (Adelgidae). In the remaining three genera, both larvae and adults feed on various kinds of fungi or on the by-products of fungal

metabolism (Lawrence and Hlavac 1979, Bright 1991). The genus *Laricobius* is poorly studied. The small size (< 3 mm) and cryptic habits of larvae and adults, coupled with the occurrence of adult activity in the cooler months of the year, make this genus rare in collections. Table 1.1 lists 11 species of *Laricobius* described with host associations if known. There are four species of *Laricobius* present in North America. They are *L. erichsonii* Rosenhauer, *L. laticollis* Fall, *L. nigrinus* Fender and *L. rubidus* LeConte. *Laricobius erichsonii* is native to Europe and was introduced into North America in the 1950's and 1960's as a biological control agent of *Adelges piceae* Ratzeburg, while the three remaining species are native to North America (Hatch 1962, Clausen 1978, Lawrence 1989, Bright 1991). Only the biology and ecology of *L. erichsonii* has been reported in any detail (Franz 1958a).

Franz (1958a, 1958b) studied distribution, life history, ecology and external morphology of *L. erichsonii* in Europe. Clark and Brown (1958) investigated its establishment, spread, life history, and effectiveness as a predator of *A. piceae* in Canada. The life histories of *L. erichsonii* in Europe and Canada are similar (Franz 1958a, Clark and Brown 1958). Clark and Brown (1960) studied the life cycle of *L. rubidus* in New Brunswick. The life cycle of *L. rubidus* appears to be similar to that of *L. erichsonii*. *Laricobius laticollis* was described from specimens found on Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco from Washington and has been collected in British Columbia (Brown 1944, Bright 1991). It has been little studied and there are no published reports on its biology or host preferences. *Laricobius nigrinus* was recently found in association with HWA on western hemlock in British Columbia (L.M. Humble, Research Scientist, Canadian Forest Service-Pacific, unpublished data). Prior to our investigations there were no published reports on the biology and ecology of *L. nigrinus*, or its relationship

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with HWA. This recent association and the need to evaluate potential natural enemies for HWA in the eastern United States provided the impetus for this research.

Biological Control of Adelgidae

Biological control has been well documented in forestry (Pschorn-Walcher 1977, Dahlsten and Mills 1999, and references therein). The order Homoptera, especially Aphidoidea, as a group of pests has also been targeted for biological control (Clausen 1978).

Several biological control programs have been carried out on adelgid pests. Previous attempts to control adelgids using natural enemies have been more successful for the genus *Pineus* (Culliney et al. 1988, Zondag and Nuttall 1989, Mills 1990) than for *Adelges* (Mitchell and Wright 1967, Amman and Speers 1971, Clark et al. 1971, Clausen 1978, Harris and Dawson 1979, Schooley et al. 1984). Table 1.2 summarizes predators that have been released to control various adelgid pests. There are no known parasitoids that attack any member of the family Adelgidae (Montgomery and Lyon 1996).

The classical biological control program for the balsam woolly adelgid, *Adelges piceae* in North America is well documented (Smith and Coppel 1957, Mitchell and Wright 1967, Amman and Speers 1971, Clark et al. 1971, Harris and Dawson 1979, Schooley et al. 1984). More than 25 species of predators released over approximately 35 years resulted in seven European species becoming established, including *Laricobius erichsonii* (Table 1.2). Despite establishment, these introduced predators failed to provide significant control of *A. piceae* (Clark et al. 1971, Clausen 1978, Schooley et al. 1984). Poor synchronization between various predators and *A. piceae*, inadequate searching ability of predators, inability to adapt to harsh winter climates in eastern

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Canada, poor over-wintering conditions in the soil, and inability of host trees to withstand even low populations *of A. piceae*, have been suggested as reasons why adequate biological control was not achieved (Mitchell and Wright 1967, Clark et al. 1971, Clausen 1978, Harris and Dawson 1979, Schooley et al. 1984). The biological control of *Pineus laevis* Maskell in Australia failed because of the lack of establishment of the five predators released (Clausen 1978). However, successful control of *P. laevis* was achieved in both Chile and New Zealand with the establishment of the European chamaemyiids *Leucopis (Neoleucopis) obscura* Haliday (Mills 1990) and *L. (N.) tapiae* Blanchard (Zondag and Nuttall 1989), respectively. Similarly, in Hawaii, *Pineus pini* (Macquart) was successfully controlled with the introduction and establishment of *L. (N.) tapiae* (Culliney et al. 1988, Greathead 1995) and *Leucopis nigraluna* McAlpine from Pakistan (Mills 1990). In Kenya, *Tetraphleps raoi* Ghauri (Hemiptera: Anthocoridae) was successfully introduced into Kenya from Pakistan for biological control of *P. pini* and established in pine plantations (Mailu et al. 1980, Aloo and Karanja 1986). The establishment of *T. raoi* was followed by a decline in field populations of *P. pini*, but successful biological control of *P. pini* has not yet been confirmed (Aloo and Karanja 1986).

Research on biological control of HWA began in the early 1990's. Field surveys of native and established natural enemies of HWA in eastern United States were conducted by McClure (1987) and Montgomery and Lyon (1996) in Connecticut and Wallace and Hain (2000) in North Carolina and Virginia. In both regions, natural enemies were found at densities too low to significantly reduce populations of HWA. In addition, most natural enemies sampled were found to be generalist predators (Montgomery and Lyon 1996, Wallace and Hain 2000). Foreign exploration in Japan

(Cheah and McClure 1996) and China (Montgomery et al. 2000, Yu et al. 2000) for candidate natural enemies of HWA was initiated in 1992 and 1996, respectively. Two species, *Diapterobates humeralis* (Hermann) (Oribatida: Ceratozetidae) and *Pseudoscymnus tsugae* Sasaji & McClure collected in Japan, were selected as the most promising candidates for introduction into North America (Cheah and McClure 1996). Observations on foraging behavior of *D. humeralis* revealed that these mites did not feed on adelgid eggs and nymphs, but consumed the woolly filaments that surrounded HWA eggs. This feeding behavior dislodged eggs (McClure 1995a). Although *D. humeralis* resulted in approximately 65 percent mortality of HWA eggs in Japan (McClure 1995a), its low fecundity, difficulty of lab culture, as well as its distribution throughout temperate regions of the Northern Hemisphere, made it an unsuitable candidate for release in North America (Cheah and McClure 1996). In Japan, *P. tsugae* was found to occur in over 30 percent of forest and ornamental sites sampled, where adelgid mortality was observed to be greater than 85 percent (McClure 1995b). *Pseudoscymnus tsugae* was imported into eastern United States and is currently undergoing laboratory and field evaluations as a potential biological control agent of HWA (Cheah and McClure 1996, 1998). Quarantine studies in Connecticut revealed that *P. tsugae* possesses many attributes of a successful biological control agent (Cheah and McClure 1996, 1998). In addition, it is amenable to mass culture in the laboratory (Cheah and McClure 1998, McClure and Cheah 1999). Since 1995, more than 160,000 adults of *P. tsugae* have been released in forests of Connecticut, New Jersey and Virginia (McClure 2001, Salom et al., in press).

In China, the following families with number of species in brackets were collected from hemlocks: Coccinellidae (54), Anthocoridae (4), Miridae (3), Syrphidae (2) and Cecidomyiidae (1) (Yao and Hongbin 1998, Montgomery et al. 2000, Yu et al.

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2000).   Three species, *Scymnus (Neopullus) sinuanodulus* Yu & Yao, *S. (N.) camptodromus* Yu & Liu, and *S. (N.) ningshanensis* Yu & Yao, in the family Coccinellidae (subfamily: Scymninae) were imported into eastern United States for further study under quarantine (Yao and Hongbin 1998).   Lu and Montgomery (2000) determined that all three species are univoltine and feed on HWA, preferring eggs over other stages.   However*, S. (N.) camptodromus* eggs undergo a diapause (Lu and Montgomery 2000), making it difficult to culture and therefore unsuitable as a potential candidate for release (M.E. Montgomery, Research Scientist, USDA FS, Hamden, CT, pers. commun., 2001). Field evaluation of *S. (N.) sinuanodulus* and *S. (N.) ningshanensis* using sleeve cages, began in 1999 and 2001, respectively (Salom et al. in press, M.E. Montgomery, pers. commun., 2001). Although these lady beetles reduced the population of HWA significantly, this reduction may not be sufficient to prevent HWA from causing damage (M.E. Montgomery, pers. commun., 2001).

To date, *P. tsugae* is the only predator released in the field for control of HWA in eastern United States. Results have been encouraging where *P. tsugae* has been released (McClure and Cheah 1999, Cheah and McClure 2000, McClure 2001). Compared with control sites, HWA densities were reduced 47 to 87 percent (McClure 2001). However, it remains to be determined if adequate control can be achieved by *P. tsugae* alone. Most likely, adequate control of HWA will be achieved by a complex of predators (McClure 2001, Salom et al. in press).

Objectives

The overall objective of this research was to investigate the biology of *Laricobius nigrinus* and determine its suitability as a potential biological control agent of the hemlock woolly adelgid, *Adelges tsugae* in the eastern United States. Specific objectives were to determine:

1) Life cycle, development, and reproductive biology of *L. nigrinus*;

2) Host specificity of *L. nigrinus* for potential release in the eastern United States as a biological control agent of HWA;

3) Seasonal abundance of *L. nigrinus* and synchrony with HWA in British Columbia;

4) The influence of temperature on the development of life stages of *L. nigrinus*; and

   (a) Estimate lower developmental threshold temperatures for each life stage and thermal sums necessary to complete development;

   (b) Validate the degree-day model derived from constant temperature experiments with field data collected on *L. nigrinus* in British Columbia;

Table 1.1. World distribution and host range or habitat of described species of *Laricobius*

Species	Origin and Distribution[a]	Host and/or Habitat	References[b]
Laricobius nigrinus Fender	Nearctic - BC, WA, OR, ID	*Adelges tsugae* on *Tsuga heterophylla* (3, 4)	1, 2, 3, 4
L. laticollis Fall	Nearctic - BC, WA, OR, ID	on *Pseudotsuga menziesii* (*A. cooleyi* Gillette is the assumed host because of reported host tree) (5)	1, 2, 5
L. rubidus LeConte	Nearctic - ON, QB, NB, MI, NY, PA, DC, ME, NH, MA, CT, VA	*Pineus strobi* Hartig on *Pinus* (2, 6); HWA on *T. canadensis* (4, 8, 9); *A. piceae* on *Abies* (6)	2, 4, 6, 7, 8, 9
L. erichsonii Rosenhauer	Palaearctic - Italian Alps to southern Denmark; introduced to North America BC, NB, NS, NF, WA, OR, NH, VT, ME, NC	*A. piceae*, *A. nüsslini* Borner on *Abies*; *P. pineoides* (Cholodkovsky) on *Picea* (10, 11); *P. strobi* on *Pinus* (10, 11); *A. cooleyi* on *Pseudotsuga* (10, 11)	2, 7, 10, 11
L. caucasicus Rost	Palaearctic - Caucasus Mountains	host or habitat not reported	10
L. sahlbergi Reitter	Palaearctic - Siberia	Adelgidae	12
L. minutus Nikitsky	Palaearctic - Siberia	Adelgidae	12
L. kovalevi Nikitsky	Palaearctic - Siberia	Adelgidae	12
L. mirabilis Háva & Jelínek	Palaearctic - China (Asia)	host or habitat not reported	13
L. schawalleri Háva & Jelínek	Palaearctic - Nepal (Himalayas)	*Tsuga-Quercus-Rhododendron* habitat	14
L. loebli Jelínek & Háva	Palaearctic - Nepal (Himalayas)	host or habitat not reported	15

[a] BC British Columbia, CT Connecticut, DC District of Columbia, ID Idaho, MA Massachusetts, ME Maine, MI Michigan, NB New Brunswick, NC North Carolina, NF Newfoundland, NH New Hampshire, NS Nova Scotia, ON Ontario, OR Oregon, PA Pennsylvania, QB Quebec, VA Virginia, VT Vermont, WA Washington

[b] 1. Hatch 1962; 2. Lawrence 1989; 3. L.M. Humble (unpublished data); 4. G.Z.-B. (unpublished data); 5. Brown 1944; 6. Brown and Clark 1962; 7. Bright 1991; 8. Montgomery and Lyon 1996; 9. Wallace and Hain 2000; 10. Franz 1958a; 11. Lawrence and Hlavac 1979; 12. Nikitsky and Lafer 1992; 13. Háva and Jelínek 1999; 14. Háva and Jelínek 2000; 15. Jelínek & Háva 2000

Table 1.2. Summary of predator releases against the family Adelgidae

Target Pest	Target Region	Year of Release	Origin of Species Released	Species Released	Agents Established	References[g]
Adelges piceae	Canada/USA[a]	1933-1969		**Coleoptera: Coccinellidae**		
(Ratzeburg)			Europe	*Aphidecta obliterata* (L.)[b]	Yes[b]	5, 7, 9
				Exochomus quadripustulatus (L.)[d]	No[e]	1, 5
				Scymnus (Pullus) impexus (Mulsant)[b]	Yes[b]	1, 2, 5, 6
			India/Pakistan	*Coccinella (Adalia) luteopicta* Mulsant[b]	No	2, 4, 5, 7
				Adalia tetraspilota (Hope)[b]	No	2, 5, 7
				Coccinella septempunctata L.[e]	No[e]	7
				Exochomus lituratus Gorham[b]	No	2, 5, 7
				Exochomus uropygialis Mulsant[b]	No	2, 5, 7
				Harmonia breiti Mader[b]	No	2, 3, 5, 7
				Harmonia (Leis) dimidiata (Fabricius)[c]	No[e]	2, 5
				Harmonia (Ballia) eucharis [=*dianae*] Mulsant[b]	No	2, 5, 7
				Oenopia (Synharmonia) conglobata (L.)[c]	No	2, 5
				Oenopia sauzeti Mulsant[c]	No	2
			Japan	*Adalia ronina* (Lewis) [= *A. conglomerata* L.][d]	Yes[d]	5, 8
				Chilocorus kuwanae Silvestri[c]	No[e]	2, 5
			Australia	*Diomus (=Scymnus) pumilio* (Weise)[b]	No	2, 5, 7
				Coleoptera: Derodontidae		
			Europe	*Laricobius erichsonii* Rosenhauer[b]	Yes[b]	1, 2, 4, 5, 6, 8

Table 1.2 continued

Target Pest	Target Region	Year of Release	Origin of Species Released	Species Released	Agents Established	References[g]
Adelges piceae	Canada/USA			**Diptera: Cecidomyiidae**		
(Ratzeburg)			Europe	*Aphidoletes thompsoni* Möhn[b]	Yes[b]	1, 2, 4, 5, 6, 7
				Diptera: Chamaemyiidae		
			Europe	*Cremifania nigrocellulata* Czerney[b]	Yes[b]	1, 4, 5, 6, 7, 9
				Leucopis (Neoleucopis) atratula Ratz.[d]	Yes[c]	7, 9
				Leucopis hennigrata McAlpine [= *L.* sp.nr. *melanopus* Tanasijtshuk][b]	Yes[d]	7
			Europe	*Leucopis (Neoleucopis) obscura* Haliday[b]	Yes[b]	1, 2, 7
				Lipoleucopis praecox de Meij[d]	No	5
				Diptera: Syrphidae		
			Europe	*Cnemodon* spp.[d]	No	1
				Hemiptera: Anthocoridae		
			India/Pakistan	*Tetraphleps abdulghani* Ghauri[d]	No	7
				Tetraphleps raoi Ghauri[d]	No	7
				Neuroptera: Hemerobiidae		
			Europe	*Hemerobius nitidulus* Fabricius[d]	No	1, 5
				Hemerobius stigma Stephens[d]	No	1, 5
				Neuroptera: Chrysopidae		
			India/Pakistan	*Chrysopa* spp.[c]	No	2, 5

Table 1.2 continued

Target Pest	Target Region	Year of Release	Origin of Species Released	Species Released	Agents Established	References[g]
Adelges tsugae Annand	Eastern USA	1995 to present	Japan	**Coleoptera: Coccinellidae**		
				Pseudoscymnus tsugae Sasaji & McClure	Yes	10, 11
Pineus laevis (Maskell) [= *P. boerneri* Annand]				**Neuroptera: Hemerobiidae**		
			UK	*Westmaelius concinnus* Stephens	No	5
				Diptera: Chamaemyiidae		
			UK	*Leucopis (Neoleucopis) obscura* Haliday	No	5
				Lipoleucopis praecox de Meij	No	5
			California	*Leucopis atrifacies* Ald.	No	5
	Chile	1945	Europe	*Leucopis (Neoleucopis) obscura* Haliday	Yes	12
	New Zealand	1926-1934	Australia	**Coleoptera: Coccinellidae**		
				Diomus (=Scymnus) pumilio (Weise)	No	13
			UK	**Diptera: Chamaemyiidae**		
				Leucopis(Neoleucopis) tapiae Blanchard	Yes	13
			UK	Neuroptera: Hemerobiidae		
				Hemerobius stigma Stephens	No	13
	Chile	1945	Europe	*Leucopis (Neoleucopis) obscura* Haliday	Yes	12

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Table 1.2 continued

Target Pest	Target Region	Year of Release	Origin of Species Released	Species Released	Agents Established	References[g]
			India/Pakistan	**Acari: Erythraeidae**		
				Balaustium spp. [d]	No	7
	New Zealand	1926-1934	Australia	**Coleoptera: Coccinellidae**		
				Diomus (=Scymnus) pumilio (Weise)	No	13
Pineus laevis	New Zealand	1926-1934	UK	**Diptera: Chamaemyiidae**		
(Maskell)				*Leucopis(Neoleucopis) tapiae* Blanchard	Yes	13
			UK	Neuroptera: Hemerobiidae		
				Hemerobius stigma Stephens	No	13
Pineus pini				**Diptera: Chamaemyiidae**		
(Macquart)	Hawaii	1972, 1977	Pakistan	*Leucopis nigraluna* McAlpine	Yes	14
		1976-1979	Europe	*Leucopis (Neoleucopis) tapiae* Blanchard[f]	Yes	14, 15
	Kenya	1975	Pakistan	**Hemiptera: Anthocoridae**		
				Tetraphleps raoi Ghauri	Yes	12, 16, 17

[a] Canada 1933-1969, USA 1955-1965;[b]east and west; [c]west only; [d]east only; [e]did not establish on *A. piceae*, but established elsewhere (8);

[f] previous reported as *L. obscura*, but subsequently determined to be *L. tapiae* (15).

[g] 1. Smith and Coppel 1957; 2. Mitchell and Wright 1967; 3. Amman and Speers 1971; 4. Clark et al. 1971; 5. Clausen 1978; 6. Harris and Dawson 1979; 7. Schooley et al. 1984; 8. Gordon 1985; 9. Humble 1994; 10. McClure and Cheah 1999; 11. Cheah and McClure 2000; 12. Mills 1990; 13. Zondag and Nuttall 1989; 14. Culliney et al. 1988; 15. Greathead 1995; 16. Mailu et al. 1980; 17. Aloo and Karanja 1986

Chapter 2

Development and Reproductive Biology of *Laricobius nigrinus*

Introduction

The hemlock woolly adelgid, *Adelges tsugae* Annand is an exotic insect pest of eastern (*Tsuga canadensis* (L.) Carrière) and Carolina (*T. caroliniana* Engelmann) hemlocks (McClure 1996). Hemlock species in eastern North America are very susceptible to HWA attack and infested trees have been reported to die in as little as four years (McClure 1991). In contrast, species of hemlock in Asia and western North America are attacked, but seldom severely damaged by HWA. Tree resistance and natural enemies likely play a role in maintaining HWA below injurious levels in these regions (Cheah and McClure 1996, Montgomery and Lyon 1996).

In 1993, the Hemlock Woolly Adelgid Working Group was established in the eastern United States to coordinate research efforts to reduce the impact of HWA and slow its spread in eastern hemlock forest ecosystems (Reardon and Bullard 1996). Biological control was identified as an area of emphasis for management of HWA in a forest setting under the USDA Forest Service Technology Enterprise Team (Onken 1996, Reardon and Bullard 1996). Studies by McClure (1987) and Montgomery and Lyon (1996) in Connecticut, and Wallace and Hain (2000) in North Carolina and Virginia, documented a number of native or established predators associated with HWA, but they were generally found at densities too low to significantly impact populations of HWA. No parasitoids that attack any member of the family Adelgidae are known. Since the

mid-1990's several predators have been investigated for potential biological control of HWA in the eastern United States (Cheah and McClure 1998, McClure et al. 2000, Montgomery et al. 2000, Zilahi-Balogh et al. 2000, Lu and Montgomery 2001).

Laricobius nigrinus Fender (Coleoptera: Derodontidae) has a known distribution in British Columbia, Washington, Oregon and northern Idaho (Fender 1945, Hatch 1962, Lawrence 1989). Members of the genus *Laricobius* are predacious on woolly adelgids (Homoptera: Adelgidae). The other three genera in this family of four genera feed on fungi or the by-products of fungal metabolism (Lawrence and Hlavac 1979, Lawrence 1989, Bright 1991). Derodontids inhabit relatively humid forests in cooler temperate regions (Lawrence 1989). This family is not commonly encountered in the field, possibly due to its small size (< 3 mm).

Laricobius nigrinus has been found in close association with HWA on western hemlock, *Tsuga heterophylla* (Raf.) Sargent in British Columbia (Humble, L.M., Natural Resources Canada, Canadian Forest Service-Pacific, unpublished data), where HWA is not considered a forestry pest. Since 1997, it has been imported annually into Virginia from British Columbia for quarantine evaluation as a potential biological control agent of HWA in the eastern United States.

With the exception of a few studies on taxonomy and distribution (Fender 1945, Hatch 1962, Lawrence and Hlavac 1979, Lawrence 1989 Bright 1991), there are no published reports on the biology of *L. nigrinus* or its association with HWA. I report the results of laboratory studies on the development and reproductive biology of this predator as part of our on-going investigations in evaluating it as a potential biological control agent of HWA in the eastern United States.

Materials and Methods

Insect collection

   Laricobius nigrinus adults were collected using a beating tray from HWA infested western hemlock, *Tsuga heterophylla* (Raf.) Sargent near Victoria BC, Canada (48.65° N, 123.43° W) in March 1998 and February 1999, 2000. They were shipped to a USDA approved quarantine facility at Virginia Polytechnic Institute and State University, Blacksburg, VA. A colony of *L. nigrinus* was established on twig cuttings of field collected HWA on eastern hemlock.

Life cycle

   Observations on the life cycle of *L. nigrinus* were made while rearing it in the laboratory. *Laricobius nigrinus* is active in the cooler months, therefore it was reared under spring-like conditions in environmental chambers at 15 and 18°C, 75-90 % R.H., and 12:12 (L:D) h. Rearing and oviposition cages consisted of 950 ml clear polystyrene containers with ventilation provided by a 10 cm diameter hole cut out of the lid and covered with PeCap® polyester mesh (0.14 mm^2) (Sefar America Inc., Kansas City, MO). Since no morphological characteristics reliably distinguish adult males from females, 10 to 15 adults were placed into each oviposition cage. Each week, twigs with predator eggs were placed in rearing containers and provided with fresh HWA infested twigs. Twigs were held in a block of moistened floral foam (Oasis®) to prevent them from drying out. The base of these cages was lined with two layers of filter paper (Whatman No. 1; 100 mm diam.), followed by approximately 1 cm thickness of sterilized peat (Premier Horticulture Inc.) moistened with methyl paraben solution (0.42 g/250 ml de-ionized water) that acted as a fungal inhibitor. Peat was pre-sifted using hardware cloth (3 X 3

mm mesh size) and served as a pupation medium. After four to five weeks, the peat was sifted weekly to collect pupae. Pupae were separated by sex using external genitalic characters (Figure 2.1) and placed in 50 mm petri dishes (Falcon) containing sterilized peat lightly moistened with methyl paraben solution and checked weekly for adult emergence. Adult male beetles were marked on one of the elytra using a water-soluble marker (Pentel® K106 white, milky gel roller). Determining sex of adults is impossible without dissection because genitalia retract into the body when adults eclose (Figure 2.2).

Egg and larval development

Observations on development of *L. nigrinus* life stages were made on individuals reared from egg hatch to adult with HWA as the host. Egg and larval development were determined at 12, 15 and 18 °C and 12:12 (L:D) h. *Laricobius nigrinus* eggs (24 h old), attached to sections (2 to 4 cm) of HWA infested hemlock twigs, were placed individually in 50 mm polystyrene petri dishes (Falcon®). Petri dishes were considered as experimental units with 70, 45 and 60 replicates at 12, 15 and 18 °C, respectively. Petri dishes contained a 2 cm ventilation hole made in the lid that was covered with polyester mesh (PeCap®). The base of each petri dish was lined with two layers of filter paper (Whatman No. 1) moistened with methyl paraben solution. Life stages were observed using a dissecting microscope. Eggs were checked daily for hatch. Other developmental stages were inspected daily or every other day until adult emergence. Larval molts were determined by presence of exuvia. Larvae were provided fresh prey (HWA ovisacs on twig cuttings) at each inspection. Once the pre-pupal stage was reached, sterilized peat moistened with methyl paraben solution was placed at the base of each petri dish. The

pre-pupal stage was determined to be the stage when a mature larva leaves the food source in search of a suitable pupation site (Figure 2.3).

Dead larvae collected from development studies and the colony were placed in 70% ethanol. Head capsule widths were measured using a dissecting microscope equipped with a calibrated ocular scale.

Larval feeding

Consumption by *L. nigrinus* larvae on eggs of HWA was determined at 12 and 18 °C. Cumulative larval consumption for each instar to the end of the larval period was determined at 12 °C. Cumulative total larval consumption was also determined at 18 °C. Eggs (24 h old) attached to sections of HWA infested hemlock twigs were placed in petri dishes as described above for the development study. Petri dishes containing an individual *L. nigrinus* egg were considered as replicates. After egg hatch, larvae were inspected daily (24 h intervals) and provided with fresh prey. Each larval molt was determined by presence of an exuvium. At 12 °C, within 24 h of a larval molt to the next stadium, larvae from each instar (L2, n = 10; L3, n = 8; L4, n = 11; Pre-pupa, n = 10) were collected and placed individually in vials containing 70% ethanol together with all host material that was provided as prey. At 18 °C, larvae (n = 10) and host material were placed in ethanol within 24 h of the beginning pre-pupal stage. Body length measurements of larvae were later made using a dissecting microscope equipped with a calibrated ocular scale to determine the relationship between consumption and body size. Larvae feed on HWA eggs by sucking out the contents. Eggs of HWA fed on by *L. nigrinus* larvae are collapsed and translucent in color, in contrast to the intact purple-

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colored eggs that were not fed on. Twigs were examined using a dissecting microscope to determine the number of HWA eggs fed on by larvae.

Activity, oviposition period and fecundity

Weekly observations on activity, oviposition, and fecundity were made on male-female pairs of *L. nigrinus* adults between August 1999 and October 2000. Activity period was measured instead of longevity. Longevity was not measured because date of eclosion of individual beetles from the colony used in this study, was not recorded. Adults were determined to be active (i.e. not in aestivation) once they were no longer found in the peat and exhibited positive phototaxis. Total activity period was defined as the time period from first activity until death. Pre-ovipositional period was the period from first activity to first oviposition and post-ovipositional period was the period following last oviposition until death. Adults used in this study were lab reared and eclosed during April and May 1999. They were kept at 13 °C, 12:12 (L:D) h in an environmental chamber. On 17 August, aestivating adults previously sexed in the pupal stage were paired (n = 45) and placed in 50 mm polystyrene petri dishes lined with two layers of filter paper moistened with methyl paraben solution and covered with sterilized peat. Each petri dish (without lid) was then set inside a 950 ml clear polystyrene rearing container with HWA infested hemlock twigs, held in moistened floral foam and maintained at 13 °C. A temperature of 13 °C was selected based on a previous study (Chapter 4) that determined that peak egg laying in the field (Victoria, Canada) was in early March which corresponded to maximum daily temperatures between 8 and 15 °C. Females that died before initiating oviposition or beetles that were lost or died of unnatural causes, were not included in the data analysis. Individuals were followed until

death. Body length of intact dead females was measured using a dissecting scope equipped with a calibrated ocular scale to determine the relationship between fecundity and body size.

Data Analysis

The t-test procedure, for normal data with equal variance, was used to test for differences in the number of HWA eggs consumed by *L. nigrinus* larvae at 12 and 18 °C. Regression analysis was used to determine whether HWA consumption by *L. nigrinus* larvae was related to their body length. Numbers of HWA eggs consumed were transformed using a $\log_{10}$ transformation (Zar 1984). The Wilcoxon Ranks Sums Test for non-normal data with equal variance was used to test for differences in total activity period between males and females. Linear regressions were carried out to determine whether fecundity of *L. nigrinus* was related to oviposition period and/or female body length. The F value of analysis of variance (ANOVA) was used to determine the significance of the regression. All statistical tests were done using SAS® (SAS Institute 1989), at $P < 0.05$ significance level.

Results

Life cycle

Female *Laricobius nigrinus* lay eggs singly in the woolly ovisacs of HWA. Eggs are bright yellow, oval in shape with a shiny chorion and appear to be deposited with a sticky secretion on the surface to aid in adhering to the wool. Egg color changes to pale greenish-yellow with age. Just before hatch, dark-colored stemmata can be seen through

the chorion. Four instars were determined from head capsule measurements (Table 2.1). The growth ratio between larval instars is consistent with Dyar's constant (Dyar 1890). Both *L. nigrinus* adults and larvae fed on HWA. Adults were observed to feed primarily on nymphs and adults of HWA, while larvae fed predominantly on eggs. Larvae were also observed to occasionally feed on crawlers and nymphs. Larvae used their mandibles to grasp prey and suck out liquid contents from the egg or body of prey. Mature larvae migrated to the soil to pupate. Eclosed adults remained in the soil in aestival diapause, resuming activity in the fall.

Egg and larval development

Developmental time of *L. nigrinus* was found to be inversely proportional to temperature (Table 2.2). Total development time (± SD) from egg to adult was 88.8 ± 2.2, 64.8 ± 2.6 and 46.6 ± 2.01 d at 12, 15 and 18 °C, respectively. Egg development time decreased by over 60 percent with an increase of 6 °C in temperature (Table 2.2).

Larval feeding

There was no significant difference in the cumulative number of HWA eggs consumed by larvae reared at 12 and 18 °C ($F_{9, 9} = 1.02$, $P = 0.98$). The number of HWA eggs consumed (mean ± SE) during the entire larval period at 12 and 18 °C was 225.9 ± 18.1 and 252.3 ± 17.9 eggs, respectively. The $\log_{10}$ [number of eggs consumed] related positively to larval body length at 12 °C (Figure 2.4).

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Activity, oviposition and fecundity

Males broke aestival diapause earlier than females. Male activity was first observed on 1 September 1999 with 6.7 % of males active (n = 30). Female activity was first observed on 19 September with 10.7 % females active (n = 28). By 6 October, 70 % males and 46.4% females were active. All males and females were active and feeding by 8 November. The presence of purple colored liquid excrement on the needles was evidence of feeding activity. The purple color of the excrement reflects the body color of HWA. There was no significant difference in total activity period between males and females (Wilcoxon Rank Sums Test, z = -1.81, $P > 0.05$). Total activity period of males and females (mean ± SE) was 36.6 ± 2.4 and 30.8 ± 2.2 wk, respectively.

Males were observed to lie lateral to females during copulation. This position was also described by Franz (1958a) and Clark and Brown (1958) for *Laricobius erichsonii* Rosenhauer. Multiple mating between male-female pairs was observed. Mean (± SD) pre-ovipositional, ovipositional and post-ovipositional periods at 13 °C were 14.1 ± 2.01, 13.2 ± 8.2 and 3.6 ± 7.0 wk, respectively. Oviposition began in week 9 (27 December) with the active egg laying period between weeks 10 and 32 (7 January and 13 June, respectively) (Figure 2.5). Peak egg laying occurred week in 18 (7 March) (Figure 2.5).

Lifetime fecundity (mean ± SD) was 100.8 ± 89.6 (range, 2 - 396) eggs. Fecundity of *L. nigrinus* was positively related to length of oviposition period ($F_{1, 26}$ = 11.55, $P < 0.01$) (Figure 2.6) and female body length ($F_{1, 14}$ = 21.5; $P < 0.001$) (Figure 2.7). However, an over-sized female (body length = 3.01 mm) appeared to give leverage to the positive relationship. When it was omitted, the regression of fecundity on length of female was not significant (y = 206.5x – 407.1, r^2 = 0.20; $F_{1, 13}$ = 3.33, P = NS).

Discussion

The number of larval instars determined for *L. nigrinus* is consistent with what has been reported for *L. erichsonii* (Clark and Brown 1958, Franz, 1958a) and *L. rubidus* (Clark and Brown 1960). *Laricobius nigrinus* is univoltine. Our laboratory observations of the life cycle of *L. nigrinus* is similar to that described for *L. erichsonii* (Franz 1958a) and *L. rubidus* (Clark and Brown 1960). However, Franz (1958a) reported that emergent *L. erichsonii* adults feed before undergoing aestivation from August to October. This behavior was not observed for *L. nigrinus*.

Mean developmental time from egg to adult for *L. nigrinus* (46.6 d) at 18 °C is comparable to that determined for *Pseudoscymnus tsugae* Sasaji & McClure (Coleoptera: Coccinellidae) (Cheah and McClure 1998) and *Scymnus sinuanodulus* Yu & Yao (Coleoptera: Coccinellidae) (Lu and Montgomery 2001), predators also being evaluated for biological control of HWA. Developmental time from egg to adult for *Pseudoscymnus tsugae* was ~ 40.1 d at 20°C, 16:8 (L:D) h, and ~ 41.9 d for *Scymnus sinuanodulus* at 20°C, 12:12 (L:D) h. Mean (± SE) egg development at 20 °C was 10.3 ± 0.22 d and 10.21 ± 0.06 d for *P. tsugae* and *S. sinuanodulus*, respectively (Cheah and McClure 1998, Lu and Montgomery 2001). Egg developmental time of these two coccinellids was more than two times greater than that of *L. nigrinus* (4.3 ± 0.06 d) at 18 °C. This fact suggests that *L. nigrinus* is active earlier and may be better adapted to cooler temperatures than *P. tsugae* and *S. sinuanodulus*. In field studies (Chapter 4), oviposition and subsequent larval development of *L. nigrinus* coincided with egg laying by the sistens (over-wintering) generation. These findings indicate good synchrony of *L. nigrinus* with suitable prey stages of HWA. Development times for other *Laricobius*

species or other members of the family Derodontidae have not been previously reported in the literature.

Laricobius nigrinus adults became active after a 3 to 4 month aestival diapause in the soil. Although males became active earlier than females, there were no significant differences in the total activity period of males and females. Mean (± SD) ovipositional period of *L. nigrinus* (13.2 ± 8.2 wk) is comparable to *P. tsugae* (13.0 ± 3.8 wk) (Cheah and McClure 1998).

Fecundity was highly variable, ranging from 2 to 396 eggs per female. Mean lifetime fecundity of *L. nigrinus* was comparable to that for *S. sinuanodulus* F1 generations (116.5 eggs) (Lu and Montgomery 2001), but less than half that of *P. tsugae* (247.8 eggs) at 20 °C (Cheah and McClure 1998). Fecundity was positively related with duration of ovipositional period and female body length (Figures 2.3 and 2.4). Thus, ovipositional period and female body length have an influence on fecundity as has been reported with a number of insect species (Ernsting and Huyer 1984, Heinz and Parella 1990, Zeng et al. 1993). Small sample size (n = 15) may have accounted for the poor relationship between fecundity and female body length when a large female was omitted from the analysis.

Observations on the life cycle of *L. nigrinus* in the laboratory are consistent with our field studies (Chapter 4). The aestival diapause exhibited by *L. nigrinus* corresponds with that of the sistens generation of HWA (McClure 1987, 1996, Gray and Salom 1996). Aestivation by *L. nigrinus* may be an adaptation to more closely synchronize its feeding activity with the availability of suitable prey stages.

Conclusions

This study provides previously unreported information on the life cycle, development and reproductive biology of *L. nigrinus*. *Laricobius nigrinus* oviposited and completed development on a diet of HWA indicating that HWA is a suitable host for this predator. In addition, *L. nigrinus* appears to be well synchronized with HWA development, has a long oviposition period, and high fecundity. Eggs are laid only in sites which are suitable for development of progeny (i.e. within the woolly ovisacs of HWA), so that larvae do not have to search for prey. These findings contribute to our on going investigations in evaluating the suitability of *L. nigrinus* as a potential biological control agent of HWA in the eastern United States.

Table 2.1. Head capsule width measurements of *L. nigrinus* larvae

| Instar | n | Head capsule width (mm) | | Dyar's ratio |
		Mean ± SD	Range	
I	31	0.20 ± 0.01	0.17 – 0.22	-
II	60	0.27 ± 0.01	0.24 – 0.30	1.35
III	126	0.36 ± 0.02	0.31 – 0.40	1.33
IV	161	0.48 ± 0.03	0.41 – 0.55	1.33

Table 2.2. *Laricobius nigrinus* developmental time (d) (mean ± SD) for each life stage and from egg to adult at

12, 15 and 18°C, 12:12 (L:D) h

Temperature	12° C		15° C		18° C	
Stage	n	Mean ± SD (d)	n	Mean ± SD (d)	n	Mean ± SD (d)
Egg	69	11.8 ± 1.12	29	9.1 ± 0.99	59	4.3 ± 0.48
Larva	39	26.7 ± 3.13	41	19.0 ± 1.29	35	13.9 ± 1.09
Pre-Pupa	9	27.4 ± 3.61	32	19.4 ± 2.25	26	14.1 ± 1.90
Pupa	4	26.3 ± 2.22	25	17.8 ± 1.04	21	14.6 ± 1.47
Egg to Adult	4	88.8 ± 2.22	25	64.8 ± 2.58	21	46.6 ± 2.01
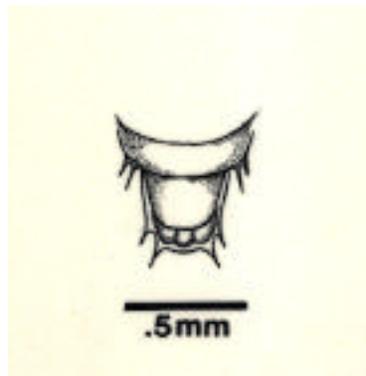
Figure 2.1. *Laricobius nigrinus* pupa (ventral) with genitalic characters of
female (top) and male (bottom) shown at the terminal abdominal
segments.
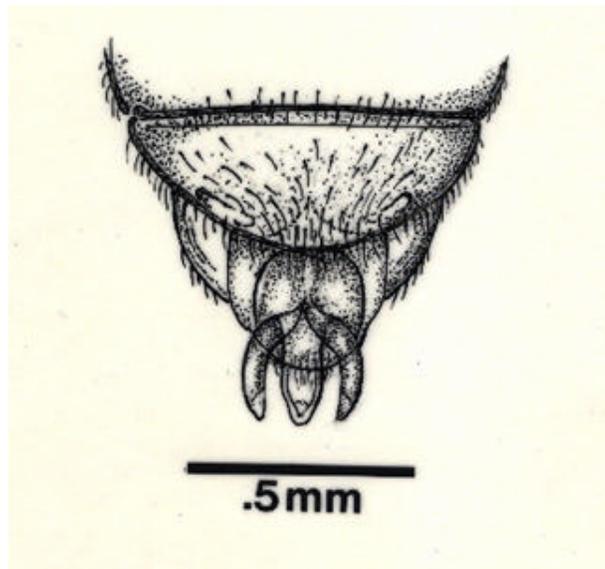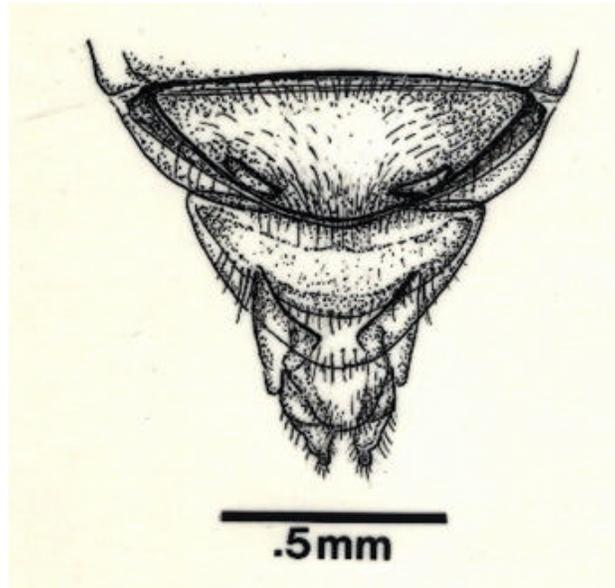
Figure 2.2. Protracted genitalic characters of adult *Laricobius nigrinus* (ventral view)
of female (top) and male (bottom).
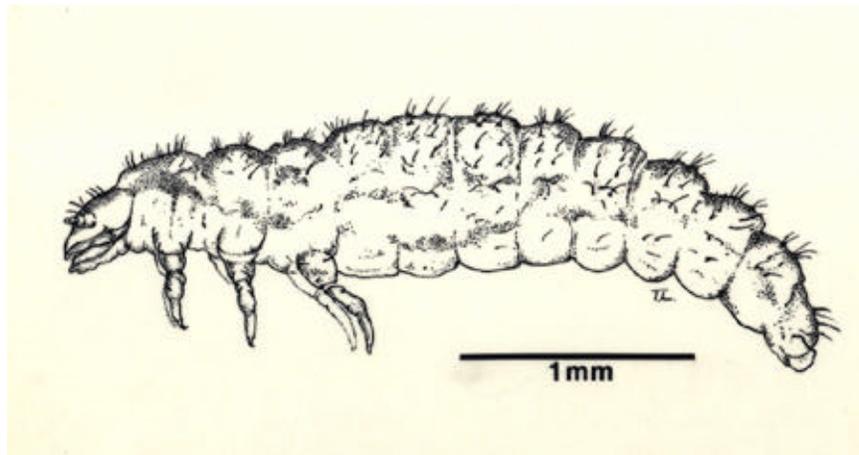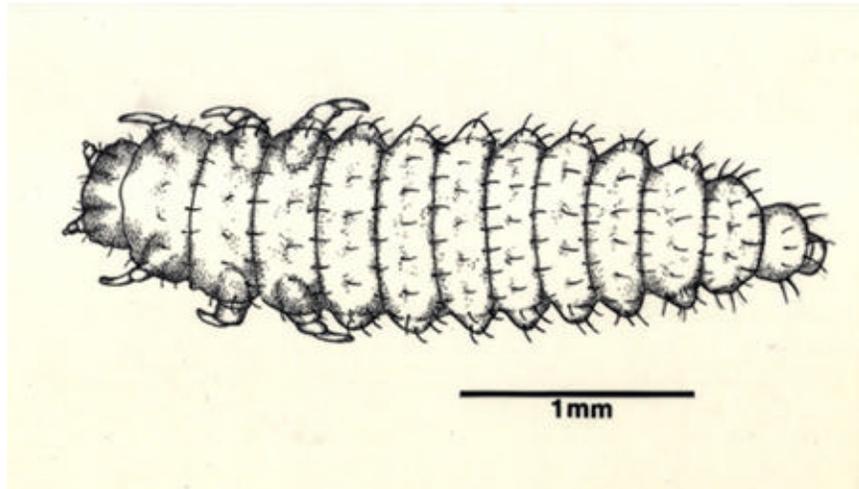
Figure 2.3. Mature larvae of *Laricobius nigrinus*; dorsal view (top), lateral view
(bottom).
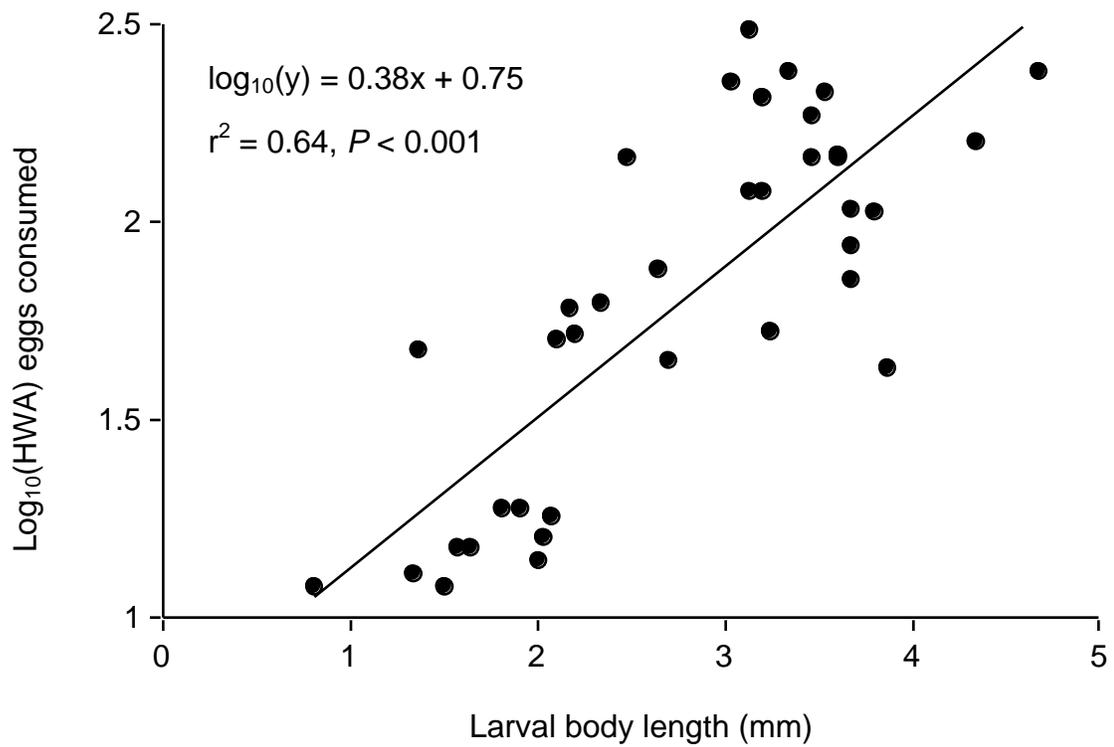
Figure 2.4. Relationship between $\log_{10}$ (*A. tsugae* eggs consumed) and body length (mm) of *Laricobius nigrinus* larvae (n = 37) at 12 °C.
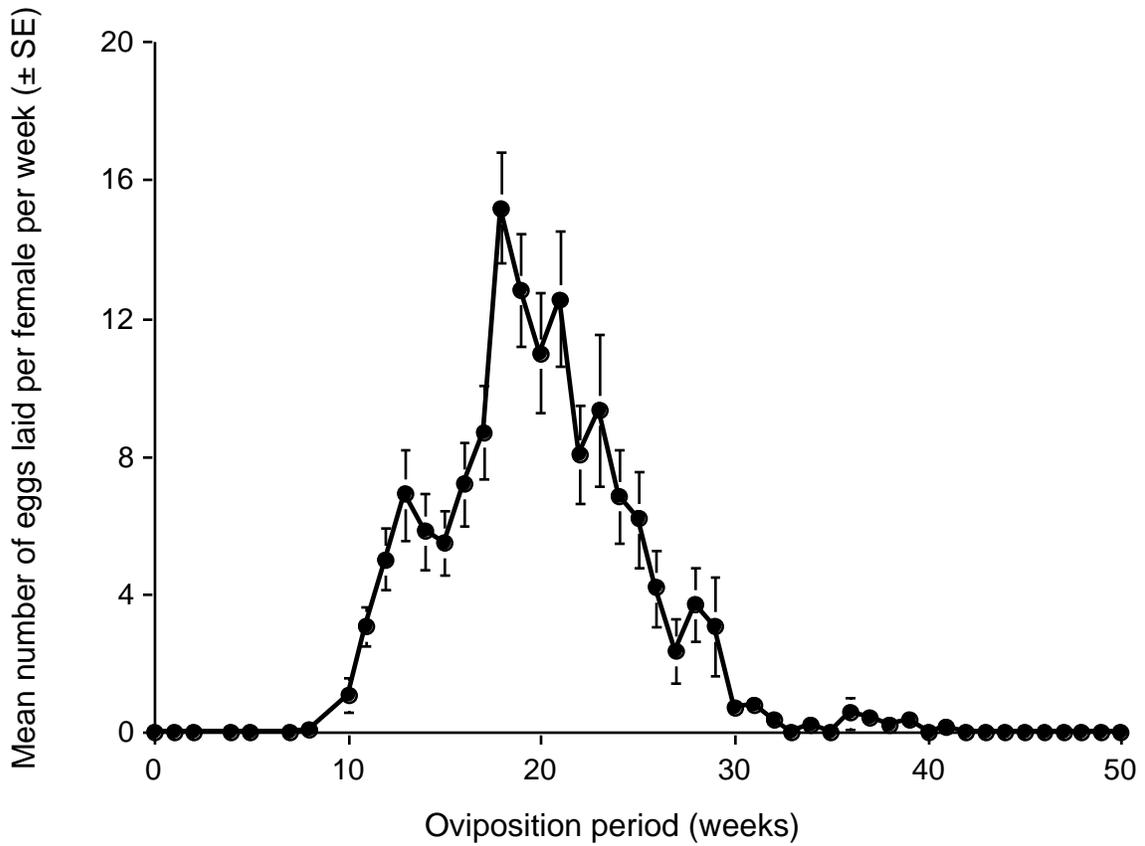
Figure 2.5. Mean number of eggs laid by *Laricobius nigrinus* females per week. Oviposition period (wk) is shown on the x-axis beginning 8 November 1999 (week 1), when 100 percent of beetles were observed to be active and ending when last beetle died (17 October 2000).
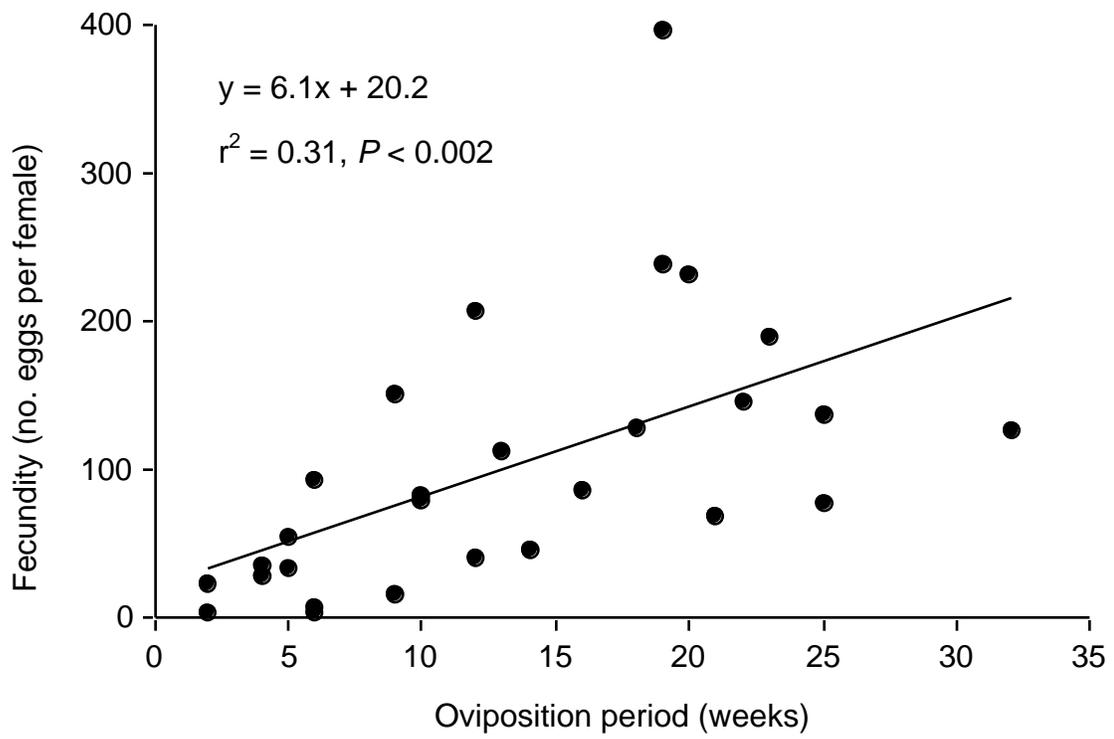
Figure 2.6. Relationship between fecundity (number eggs per female) and
oviposition period (wk) of *Laricobius nigrinus* females (n = 28).

$y = 384x - 832.3$

$r^2 = 0.61$, $P < 0.001$
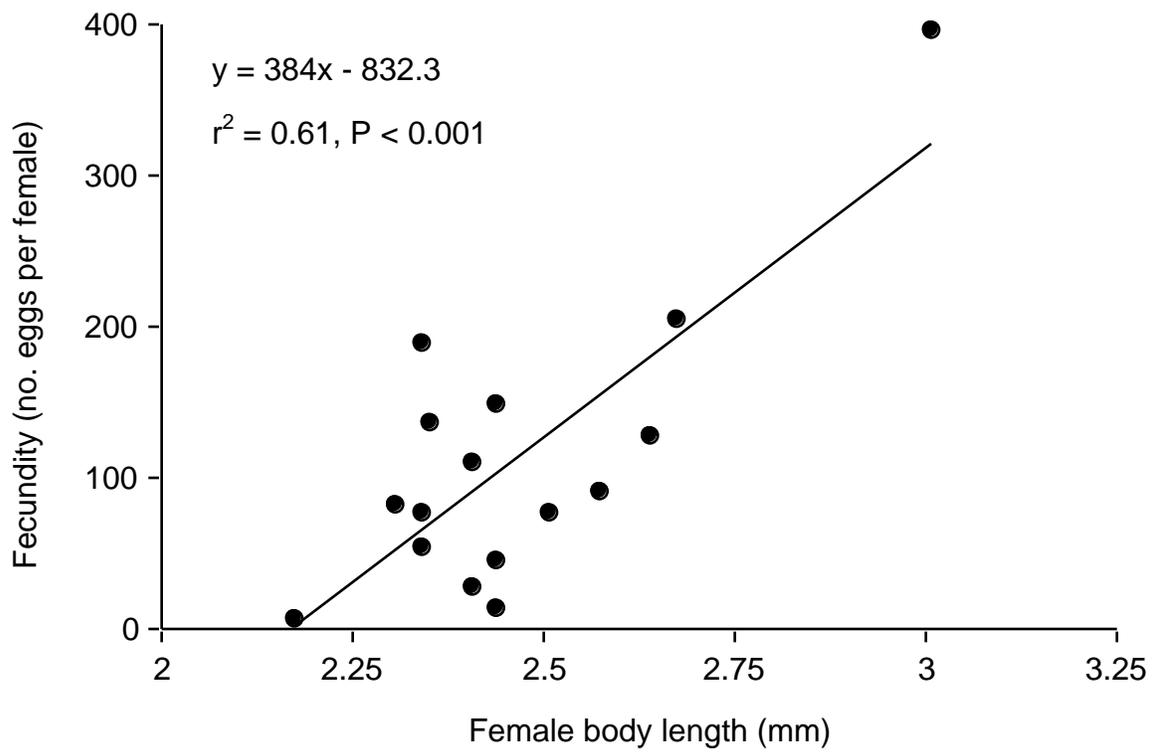
Figure 2.7. Relationship between fecundity (number eggs per female) and body
length (mm) of *Laricobius nigrinus* females (n = 16).

Chapter 3

Host Specificity of *Laricobius nigrinus*

Introduction

The hemlock woolly adelgid, *Adelges tsugae* Annand, thought to be of Asian origin (McClure 1987), is a serious threat to eastern (*Tsuga canadensis* (L.) Carrière) and Carolina (*T. caroliniana* Engelmann) hemlocks (McClure 1996). This insect was first observed in North America in the Pacific Northwest in the early 1920's where Annand (1924) described it from specimens collected on western hemlock, *T. heterophylla* (Raf.) Sargent. Since its introduction into the eastern United States in the early 1950's (Souto et al. 1996), HWA has spread along the eastern seaboard in parts of eleven states from Massachusetts to North Carolina and currently infests approximately 25% of the 1.3 million ha of eastern hemlock ecosystem (Rhea, J.R., USDA Forest Service, N.C., pers. comm.).

Eastern hemlock is an important ornamental and forest tree that is very susceptible to HWA attack. Infested trees exhibit poor crown condition, reduced terminal branch growth and needle loss, and have been reported to die in as little as four years (McClure 1991). Hemlock woolly adelgid populations in the eastern United States are not regulated by effective natural enemies (McClure 1987, Montgomery and Lyon 1996, Wallace and Hain 2000). In contrast, species of hemlock in Asia and western North America are attacked but seldom damaged by HWA. Tree resistance and natural enemies likely play a role in maintaining HWA below injurious levels in these regions (Cheah and

McClure 1996, Montgomery and Lyon 1996). Even though eastern hemlocks are not considered a major timber species, they are important components of forest and landscape settings in the eastern United States. They are ecologically important in riparian areas and provide unique habitats for a number of bird and wildlife species within the predominantly deciduous eastern forests (Evans et al. 1996, Quimby 1996). A conservative projection of the benefit-cost ratio for biological control of HWA in the eastern United States was estimated at 26.2 in 1998 (Rhea, J.R., USDA Forest Service, N.C., pers. comm.).

Five species of insects, four in the family Coccinellidae (Tribe: Scymnini) and one in the family Derodontidae, are being evaluated for potential biological control of HWA in the eastern United States (Cheah and McClure 1998, McClure et al. 2000, Montgomery et al. 2000, Zilahi-Balogh et al. 2000, Lu and Montgomery 2001). *Pseudoscymnus tsugae* Sasaji & McClure (Coccinellidae) is currently undergoing field evaluation as a potential biological control agent (Cheah and McClure 1996, 1998, McClure et al. 2000).

Members of the genus *Laricobius* are predacious on woolly adelgids (Homoptera: Adelgidae) (Lawrence and Hlavac 1979, Lawrence 1989). *Laricobius nigrinus* is native to western North America (Fender 1945, Hatch 1962, Lawrence 1989). It has been found in close association with HWA on western hemlock in British Columbia (Humble, L.M., Natural Resources Canada, Canadian Forest Service-Pacific, unpublished data) where HWA is not considered a forestry pest, and was imported into Virginia from British Columbia, Canada for quarantine evaluation as a potential biological agent of HWA in the eastern United States. Adult *L. nigrinus* are black (< 3 mm) with oval punctures on the pronotum and the elytral striae, and covered by fine dense pubescence (Figure 3.1).

In British Columbia, oviposition of over-wintered females occurs from January to May (Chapter 4). Onset of egg laying by *L. nigrinus* coincides with egg laying by the over-wintering sistens generation of HWA. Eggs are laid singly in the woolly ovisacs of HWA. After hatching, larvae feed on the eggs of HWA. On completion of feeding, mature larvae migrate to the soil to pupate. Emergent adults remain in the soil in aestival diapause over the summer and resume activity in the fall at about the same time that aestivating nymphs of HWA sistens resume development. Our observations indicate that it has one generation a year (Zilahi-Balogh et al. 2000). Little is known about *L. nigrinus* as there are no reports on its biology or its association with HWA.

Results of host specificity tests on *L. nigrinus* are reported here. The objective of these host specificity tests was to determine the suitability of *L. nigrinu*s for release in the eastern United States for biological control HWA.

Materials and Methods

Laricobius nigrinus adults used in this study were collected using a beating tray from HWA infested western hemlock, *Tsuga heterophylla* (Raf.) Sargent near Victoria BC, Canada (48.65° N, 123.43° W) in February 2000 and shipped to a USDA approved quarantine facility at Virginia Tech, Blacksburg, VA. Insects were maintained on field collected HWA infested eastern hemlock cuttings in environmental chambers at 15 °C, 12:12 (L:D) h, 70-90% R.H. Adult insects were allowed to acclimate to the new surroundings for three weeks before being used in tests and were maintained on HWA infested eastern hemlock for at least one week between tests.

Test Prey

Six species of prey used in host specificity tests were selected based on taxonomic or ecological similarity to HWA. Selected test prey were species of Homoptera comprising five genera in three families (Blackman and Eastop 1984, 1994). They are listed with their associated host plants (Bailey 1924, Farjon 1984, 1990) in Table 3.1. With the exception of *Adelges piceae* (Ratzeburg), all test prey were collected from ornamental trees in Blacksburg, Virginia or the Jefferson National Forest in Montgomery and Giles Co., Virginia. *Adelges piceae* was obtained from a Fraser fir, *Abies fraseri* (Pursh) Christmas tree plantation in Avery County, North Carolina. Ten *A. fraseri* trees (1- 2 m high) infested with *A. piceae* were dug up in November, 1999 and brought back to Virginia Tech, potted, and held in an outdoor nursery. Saplings (0.5 - 1 m high) of *Pinus strobus* L. and *Picea abies* (L.) Karst., infested the previous year (spring 1999) with *Pineus strobi* (Hartig) and *Cinara pilicornis* (Hartig), respectively, were potted. Potted trees were held in an outdoor nursery. *Chionaspis pinifoliae* (Fitch), HWA and *A. abietis* (L.) were field collected from their associated tree at the appropriate stage (Table 3.1) before tests.

The egg stage was used in all tests for members in the family Adelgidae and Diaspididae. Eggs of adelgids are typically laid in a mass by a sessile female and surrounded by flocculence (waxy/woolly filaments). This stage was selected because we found *L. nigrinus* females laying eggs in the woolly ovisacs of HWA. Hemlock woolly adelgid differs from the other three adelgids tested (Table 3.1) in that it breaks aestival diapause in late September/October, develops throughout the winter and begins to lay progrediens and sexuparae eggs in February (McClure 1987). In contrast, *A. piceae*, *A. abietis* and *P. strobi* over-winter as early instar nymphs and begin to lay eggs in the

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spring when buds begin to break (April or May) (Gambrell 1931, Friend and Wilford 1933, Craighead 1950, Arthur and Hain 1984, Johnson and Lyon 1991, USDA 1985). Potted host trees (Table 3.1) infested with *A. piceae* and *P. strobi* were brought into the greenhouse (~ 24 °C) in January 2000 to accelerate adelgid development and oviposition before being used in tests. Egg masses of *A. abietis* were field collected in early April from cuttings made from an infested *P. abies* tree. Female *C. pinifoliae* (Diaspididae) lay over-wintering eggs in the fall under the protective scale and subsequently die. Twig cuttings of a *Pinus cembra* L. tree infested with *C. pinifoliae* were made in March 2000 (before egg hatch) and held at 4 °C in moistened floral foam (Oasis) until used in tests. *Myzus persicae* (Sulzer) (Aphididae) and *Cinara pilicornis* (Aphididae) were tested at the early instar nymphal stage. Potted saplings of *Picea abies* containing over-wintering eggs of *C. pilicornis* were brought into the greenhouse in January 2000 to accelerate egg hatch, development, and subsequent viviparous reproduction. *Myzus persicae* was found to naturally infest potted sweet pepper plants (*Capsicum frutescens* L. var. *grossum* cv. California Wonder) in the greenhouse and were maintained on them. Because test prey in the family Adelgidae and Diaspididae remain attached to their host plant once crawlers settle, these test prey were left intact on the plant. Excess individuals were removed from the host plant with fine forceps when numbers exceeded those required for a particular test. Individuals of the two aphid species used were transferred onto or removed from their respective host plant with a fine 00 brush to attain the appropriate number on the host plant cutting. With the exception of *M. persicae*, the test insect prey used occur naturally in a forest setting on conifers in Virginia, and would likely be encountered by *L. nigrinus* when it is released.

Host Acceptance

Oviposition tests

No-choice (single-prey) and paired-choice oviposition tests were conducted between February and April 2000 to evaluate the effect of prey type (Table 3.1) on acceptance and preference by *L. nigrinus* females for oviposition. In three no-choice test experiments, treatments included HWA, *A. piceae*, *Pineus strobi* and *Cinara pilicornis* (Exp. 1); HWA, *Chionaspis pinifoliae* and *Myzus persicae* (Exp. 2) and HWA and *A. abietis* (Exp. 3). In six paired-choice tests, HWA was paired with each of the six test prey. The order of all tests and combination of treatments in no-choice tests was based on availability of test prey at the appropriate stage. Tests were conducted in 14-cm (Labtek®) plastic petri dishes lined with a single layer of filter paper (Thomas Scientific) at the base and a 1 cm water moistened cotton wick was used as drinking source for adults. Male-female pairs were randomly assigned to petri dishes that contained either a single bunch of associated host plant cuttings housing test prey (no-choice test) or two adjacent bunches of host plant with associated prey (paired-choice test). The number of petri dishes (replicates) per treatment with 1 pair of beetles per petri dish was between 11 and 20 in both no-choice and paired-choice tests. The same number of prey on each bunch was used in each test. Duration of each test was three days. The number of *L. nigrinus* eggs deposited on each plant bunch with test prey was counted at the end of each test. Tests were conducted at 18°C, 12:12 (L:D) in an environmental chamber using a completely randomized experimental design.

Adult feeding test

Prey acceptance by adult *L. nigrinus* was examined in a single-prey feeding experiment using eggs of each test prey. Eggs of the test adelgid hosts were of similar size. Adult *L. nigrinus*, starved for 12 h to control for satiation, were randomly assigned to 50 mm polystyrene petri dishes (Falcon) containing one of four prey (treatments) on sections (< 5 cm) of host plant. Petri dishes contained a 2 cm diameter ventilation hole made in the lid that was covered with PeCap® polyester mesh. Two layers of filter paper (Whatman No. 1) were placed at the base of the petri dish and moistened with methyl paraben (0.42 g/250 ml de-ionized water) that acted as a fungal inhibitor. The four treatments were HWA, *A. piceae, A. abietis* and *Pineus strobi*. Each treatment was replicated seven times. Egg numbers of test prey were counted before exposure to *L. nigrinus* for *A. piceae, A. abietis* and *P. strobi*. Eggs laid by females of these test prey are loosely surrounded by waxy wool filaments, enabling the eggs to be easily counted without destroying the integrity of the egg mass. In contrast, HWA females lay eggs within tight woolly ovisacs, making it difficult to count the eggs without destroying the integrity of the ovisac. To determine a relationship between the number of eggs within an ovisac and ovisac area (mm^2), the length and width of 22 HWA ovisacs were measured using a dissecting scope with a calibrated ocular scale. All eggs within each ovisac were subsequently counted. The number of eggs (dependent variable) was regressed against ovisac area (independent variable). A significant positive linear regression (y = 19.41x – 27.95) was found ($r^2 = 0.73$, $F_{1, 21} = 53.45$, *P* = 0.0001). Therefore, before exposure to *L. nigrinus*, the area of HWA ovisacs was determined and the number of eggs within was estimated from the above equation. After three days, adult beetles were removed and the remaining eggs were counted. Tests were conducted

at 18°C, 12:12 (L:D) in an environmental chamber in a completely randomized experimental design.

Host Suitability

Development

Development and survivorship of *L. nigrinus* were followed from the egg to adult stage on all test prey (Table 3.1) except *Myzus persicae* because no eggs were laid in this host in the no-choice test (Table 3.2). *Laricobius nigrinus* eggs (24 h old) were transferred individually with a fine 00 brush onto test prey in 50 mm polystyrene petri dishes (Falcon) as described above in the adult single-prey feeding test. The stage of test prey used was similar to that described for the oviposition tests. Eggs of *L. nigrinus* were examined daily for hatch. Other development stages were examined daily or every other day for survivorship until adult emergence. Fresh prey were added each time an individual larva was examined. Larval molt was determined by recording the presence of exuviae. Once the pre-pupal stage was reached, sterilized peat moistened with methyl paraben solution, was placed at the base of each petri dish and acted as a pupation medium. The pre-pupal stage was determined to be the stage when mature larvae left the twig with abundant prey and appeared to be actively searching for a suitable pupation site.

Data Analysis

Statistical tests were transformed where necessary using a $\log_{10}$ (x+1) transformation to correct for heterogeneity of variance and/or non-normal sample distributions (Zar 1984). Exp. 1 and 2 were analyzed using a one-way analysis of

variance (ANOVA) and the Tukey-Kramer HSD test for determination of significant differences between treatments. Exp. 3 was analyzed using a t-test, to determine the effect of prey type on ovipositional acceptance. In paired-choice tests, paired t-tests were used to determine prey preference for oviposition. The adult feeding test was analyzed using a one-way ANOVA to compare the effect of prey type on adult acceptance of prey. Larval development was summarized by percent survivorship at each stage for all test prey. All statistical tests were carried out using SAS® (SAS Institute, 1989), at a $P < 0.05$ significance level.

Results and Discussion

<u>*Host Acceptance*</u>

Oviposition tests

In three-day no-choice oviposition tests, *L. nigrinus* females laid significantly more eggs in HWA ovisacs over other adelgid and non-adelgid species of Homoptera tested (Table 3.2). No eggs were laid on sweet pepper leaves housing *Myzus persicae* (Table 3.2, Exp. 2). Similarly, in three-day paired-choice oviposition tests, *L. nigrinus* females laid significantly more eggs in HWA ovisacs over the three adelgid test prey (Table 3.3). No eggs were laid on host plant cuttings housing the non-adelgid test prey - *Chionaspis pinifoliae* (Diaspididae), *Cinara pilicornis* (Aphididae) and *Myzus persicae* (Aphididae). In paired-choice tests, oviposition was more than five times greater on HWA than on *A. piceae*, *A. abietis* or *Pineus strobi* (Table 3.3). These differences indicate an ovipositional preference for HWA over these test prey.

Adult feeding test

Eggs of all test prey were fed on by adults of *L. nigrinus*. Significantly more eggs of HWA were eaten than eggs of the *A. piceae* and *Pineus strobi*, but not *A. abietis* (Table 3.4).

<u>*Host Suitability*</u>

Larval Development

Laricobius nigrinus only completed development to the adult stage on a diet of HWA (Table 3.5). *Adelges piceae* and *P. strobi* supported larval development to the fourth instar, providing evidence of larval feeding, but did not support further development. Larvae provided with *A. abietis*, *C. pilicornis* or *C. pinifoliae* did not survive beyond the first instar (Table 3.5).

A summary of test results on oviposition, feeding and larval development indicate that *L. nigrinus* has a narrow host range (Table 3.6). Host specificity tests conducted under quarantine were of two types – host acceptance and host suitability. Host acceptance tests determine whether a candidate biological control agent will feed and/or oviposit on a host. Host suitability tests determine whether the agent is able to complete development to the adult stage and produce viable offspring on a particular host (Kok et al. 1992). Host suitability tests therefore are more crucial in determining potential host range. Although adult feeding tests indicated feeding acceptance on other adelgid species in addition to HWA, larval development tests showed that *L. nigrinus* only completed development to the adult stage on HWA. Therefore, these other adelgid species are not suitable hosts.

Conclusions

In field studies (Chapter 4), oviposition and subsequent larval development of *L. nigrinus* coincided with egg laying by the sistens (over-wintering) generation of HWA. These findings indicate good synchrony of *L. nigrinus* with suitable prey stages of HWA. Since other adelgid species begin egg laying two to three months later than HWA, poor synchrony of *L. nigrinus* with suitable prey stages of these species is evident (Gambrell 1931, Friend and Wilford 1933, Craighead 1950, Arthur and Hain 1984, Johnson and Lyon 1991, USDA 1985).

Previous records describing members of the genus *Laricobius* as adelgid specialists (Lawrence and Hlavac 1979, Lawrence 1989) support our data. Based on our overall results, *L. nigrinus* is host specific on the family Adelgidae and prefers HWA over the other adelgids tested. The possibility that a few non-target species of Adelgidae may be attacked should be balanced with the potential benefit that comes with control of HWA. In September 2000, the Animal and Plant Health Inspection Service, United States Department of Agriculture approved the field release of *Laricobius nigrinus* in the eastern United States.

Table 3.1. Test prey on associated host plants used in host specificity tests conducted between February and April 2000.

Test Prey (Common name)	Distribution	Host Plant (Common name)
Family Adelgidae		
Adelges tsugae Annand	Asia, North America[a]	*Tsuga canadensis* (L.) Carrière
(Hemlock woolly adelgid)	Target insect	(Eastern hemlock)
Adelges piceae (Ratzeburg)	Europe, North America[a]	*Abies fraseri* (Pursh) Poir
(Balsam woolly adelgid)		(Fraser fir)
Adelges abietis (L.)	Europe, North America,	*Picea abies* (L.) Karst.
(Eastern spruce gall adelgid)	North Africa, India[a]	(Norway spruce)
Pineus strobi (Hartig)	North America, Europe[a]	*Pinus strobus* L.
(Pine bark adelgid)		(Eastern white pine)
Family Aphididae		
Cinara pilicornis (Hartig)	Europe, Australia, New Zealand,	*Picea abies* (L.)Karst.
(Giant conifer aphid)	North and South America[a]	(Norway spruce)
Myzus persicae (Sulzer)	World wide[b]	*Capsicum frutescens* L. var. *grossum* Bailey,
(Green peach aphid)		(Sweet pepper *cv*. California wonder)
Family Diaspididae		
Chionaspis pinifoliae (Fitch)	North America[c]	*Pinus cembra* L.
(Pine needle scale)		Swiss stone pine

[a]Blackman and Eastop 1994; [b]Blackman and Eastop 1984; [c]Kosztarab 1996

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Table 3.2. Mean number of eggs laid by *Laricobius nigrinus* females in separate 3-day no-choice (single-prey) tests at 18 °C and 12:12 (L:D) h photoperiod in March and April, 2000.

Exp.	Date	Prey	n	Mean # eggs laid ± SE[a]
1	8 – 11 March	*Adelges tsugae*	11	17.1 ± 2.52a
		Pineus strobi	11	7.9 ± 1.45b
		Adelges piceae	12	3.1 ± 1.81bc
		Cinara pilicornis	11	0.2 ± 0.11c
2	20 – 23 March	*Adelges tsugae*	12	10.9 ± 2.07a
		Chionaspis pinifoliae	12	0.1± 0.08b
		Myzus persicae	12	0b
3	8 – 11 April	*Adelges tsugae*	12	8.6 ± 1.59***[b]
		Adelges abietis	12	0.7 ± 0.31

[a]Means followed by the same letter in the same column for Exp. 1 and 2 are not significantly different at $P = 0.05$ using Tukey-Kramer HSD test; [b] ***t - test $P < 0.001$.

Table 3.3. Mean number of eggs laid by *Laricobius nigrinus* females on *Adelges tsugae* (control) and on other test prey

(treatments) in paired-choice tests conducted between February and April 2000 at 18°C, 12:12 (L:D) h.

| | | Mean number of eggs laid | | | | | |
| | | Control | | | | | |
Treatment	n	(HWA) ± SE	Treatment ± SE	Difference ± SE	*t* - statistic	*df*	*P* - value
Adelges piceae	12	10.1 ± 2.40	1.8 ± 1.04	8.3 ± 3.05	2.71	1, 10	0.0200
Adelges abietis	11	7.6 ± 2.43	0.4 ± 0.20	7.2 ± 2.40	2.99	1, 9	0.0140
Pineus strobi	20	12.3 ± 1.42	2.3 ± 0.40	10.0 ± 1.48	6.76	1, 18	0.0010
Chionaspis pinifoliae	12	17.5 ± 2.02	0	17.5 ± 2.02	8.68	1, 10	0.0001
Cinara pilicornis	12	12.4 ± 2.24	0	12.4 ± 2.24	5.54	1, 10	0.0002
Myzus persicae	12	9.8 ± 1.92	0	9.8 ± 1.92	5.13	1, 10	0.0003

Table 3.4. Mean number of eggs (± SE) consumed by adult *Laricobius nigrinus* in

a 3-day single-prey test.

Host	n	Mean no. eggs consumed ± SE
Adelges tsugae	7	48.4 ± 8.21a
Adelges abietis	7	24.7 ± 7.25ab
Pineus strobi	7	21.0 ± 4.04b
Adelges piceae	7	6.9 ± 3.40b

Test conducted at 18°C and 12:12 (L:D) photoperiod, 12–15 April 2000. PROC ANOVA, $F_{3,24} = 6.65$, $P = 0.002$. Means followed by the same letter in the same column are not significantly different at $P = 0.05$, Tukey-Kramer HSD test.

Table 3.5. Percent survivorship of *Laricobius nigrinus* from egg to adult on various adelgid and non-adelgid hosts.

Life stage	*Adelges tsugae* %	n[e]	*Adelges piceae* %	n[e]	*Pineus strobi* %	n[e]	*Adelges abietis* %	n[e]	*Cinara pilicornis* %	n[e]	*Chionaspis pinifoliae* %	n[e]
Egg	100.0	69	100.0	172	100.0	149	100	42	100.0	35	100.0	50
Instar 1	87.0	60	85.5	147	68.5	102	100	42	68.6	24	78.0	39
Instar 2	79.7	55	22.7	39	24.2	36	0	0	0.0	-	0.0	0
Instar 3	69.6	48	15.1	26	11.4	17	-	-	-	-	-	-
Instar 4	58.0	40	11.1	19	6.7	10	-	-	-	-	-	-
Pre-pupa	42.0	29	0.0	0	0.0	-	-	-	-	-	-	-
Pupa	18.8	13	-	-	-	-	-	-	-	-	-	-
Adult	17.4	12	-	-	-	-	-	-	-	-	-	-

[e]number beginning given life stage

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Table 3.6. Summary of results of acceptance and suitability tests of Homoptera prey screened as hosts of *Laricobius nigrinus.*

Family: Test species	Acceptance[a]		Suitability[a]	
	Oviposition	Adult feeding	Larval development	Final host status[b]
Adelgidae				
Adelges tsugae	+	+	+	Yes
Adelges piceae	+	+	-	No
Adelges abietis	+	+	-	No
Pineus strobi	+	+	-	No
Aphididae				
Cinara pilicornis	+	x	-	No
Myzus persicae	-	x	x	No
Diaspididae				
Chionaspis pinifoliae	+	x	-	No

[a] +, positive response on test prey; -, negative response on test prey; x, test not conducted; [b] Whether the species could serve as a host to *L. nigrinus*.
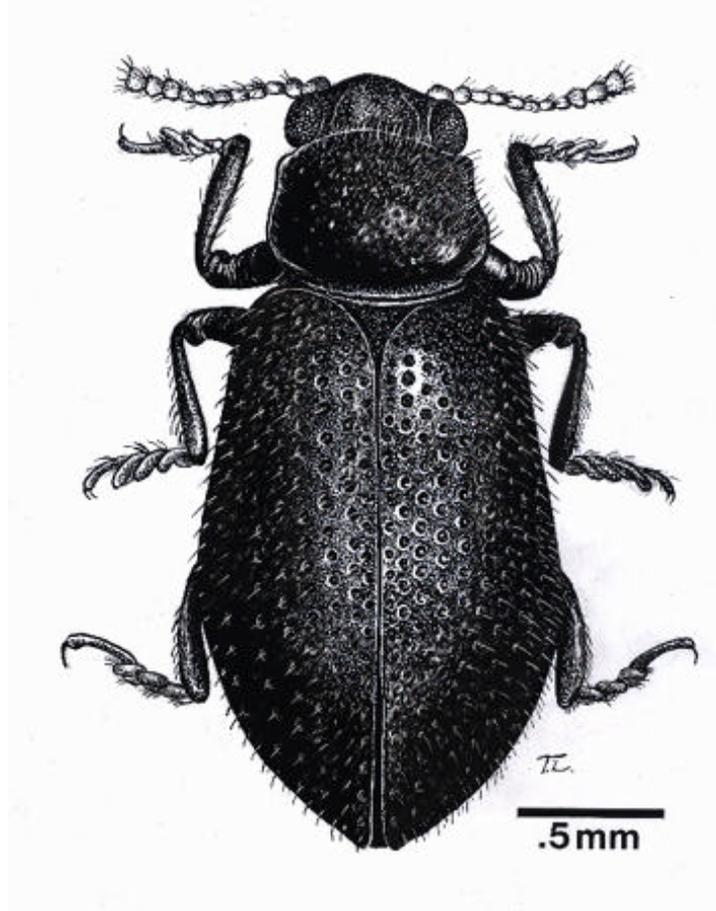
Figure 3.1. *Laricobius nigrinus* adult

Chapter 4

Seasonal Abundance of *Laricobius nigrinus* and synchrony with

Adelges tsugae in British Columbia

Introduction

    Laricobius nigrinus, native to western North America (Fender 1945, Hatch 1962, Lawrence 1989), has recently been found in close association with the hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Homoptera: Adelgidae) on western hemlock (*Tsuga heterophylla* Raf. Sargent) in British Columbia, Canada (Humble, L.M., Natural Resources Canada, Canadian Forest Service-Pacific, unpublished data). Members of the genus *Laricobius* are predacious on woolly adelgids (Homoptera: Adelgidae) (Lawrence and Hlavac 1979, Lawrence 1989).

    There is no published literature on any aspect of the biology of *L. nigrinus* or its association with HWA. The few published accounts report on taxonomy (Fender 1945, Lawrence and Hlavac 1979) and distribution (Hatch 1962, Lawrence 1989, Bright 1991). The lack of attention paid to HWA in western North America, along with the life habits and small size of *L. nigrinus* may account for this lack of biological data. This beetle is active in the cooler months of the year and is < 3 mm in size, making it rare in collections.

    The emphasis on biological control of HWA in the eastern United States and the recent association found between *L. nigrinus* and HWA in British Columbia (L.M. Humble, unpublished data), provided the impetus to study the interaction between these two species. Results of a 2-year field study (1998-2000) on the seasonal abundance and

life history of *L. nigrinus* and synchrony with HWA in BC are presented here. This study is the first to detail the seasonal abundance and interaction between *L. nigrinus* and HWA, and provides information that is useful in assessing the potential of *L. nigrinus* as a biological control agent of HWA.

Materials and Methods

The study area was located in the Lost Lake Seed Orchard, operated by Western Forest Products Ltd., Victoria, British Columbia, Canada (48°65' N, 123°43' W). These seed orchards are even-aged plantations consisting of a single species represented by genetic families or clones artificially selected for commercially desirable traits. They are isolated to avoid or reduce pollination from outside sources and managed to produce genetically superior seed for reforestation on managed forest lands (Zobel and Talbert 1984). Trees are planted in regular and widely spaced rows. Practices such as fertilization, hormonal treatments, girdling, moisture stress, or top and root pruning may be used several times during the life of the orchard to produce frequent and abundant seed crops (Zobel and Talbert 1984, Turgeon et al. 1994).

Western hemlocks are attacked by HWA but seldom at damaging levels in a natural forest setting (Furniss and Carolin 1977). However, in a seed orchard setting HWA can reach damaging levels and kill trees (pers. obs.). Several factors likely contribute to high levels of HWA in seed orchards. First, hemlock trees are shallow rooted and are particularly susceptible to drought stress (Farjon 1990). Second, trees in a seed orchard are purposely stressed to induce seed production. A seed orchard was selected over the natural forest setting for this study to ensure that infested branches were

accessible for sampling and that adequate levels of HWA would be present to allow sampling for the predator, *L. nigrinus*.

Study Area

The Lost Lake Seed Orchard contains four western hemlock orchards, two western red cedar (*Thuja plicata* Donn ex D. Don.) orchards and two Sitka spruce (*Picea sitchensis* (Bong.) Carrière.) orchards (Figure 4.1). Two western hemlock orchards (Sites 1 and 2) separated by a 1.5 ha western red cedar orchard, were selected for sampling. Site 1 was 2.3 ha with 999 trees representing 44 clones, while Site 2 was 2.4 ha with 1137 trees representing 38 clones. Orchard maps provided clone identity and identification of each individual tree. Clones appeared to be randomly distributed within each orchard. Site 2 had no aspect, while Site 1 had a slight eastern aspect. The ground cover in both orchards was grass that was regularly grazed by sheep. No irrigation was used in 1999 resulting in radical moisture stress to trees and a large cone crop was harvested. Adequate moisture levels were applied in 1998 and 2000. In May 1998 and 2000, 42 and 58 trees, respectively were treated with gibberellic acid to induce a cone crop for breeding purposes (Cook, C., Senior Forestry Technician, Tree Improvement, Western Forest Products Ltd., Saanichton, BC, Canada, pers. commun.)

Seasonal abundance and phenology

To relate seasonal abundance of *L. nigrinus* with phenology of HWA, Sites 1 and 2 were sampled within the Lost Lake Orchard over a two-year period from 1998 to 2000. In August 1998, before initiating sampling, all trees in the two sites were assigned a level of HWA infestation by visually inspecting the underside of branches for presence of

HWA. The categories used were: none, low, moderate and high which represented 0, 1 to 10, 11 to 20 and 21 settled HWA sistens per 10 cm terminal twig, respectively. Twenty-five trees (5 replicates of 5 clones) with moderate to high levels of infestation were randomly selected as sample trees in each site. Sample trees were marked. Bi-weekly sampling began in September until January and continued weekly thereafter. Sampling was conducted between September and June in year 1 (1998-1999) and September and August in year 2 (1999-2000). In year 2, sampling was missed on 6 February, 22 June and 3 August. The same trees were sampled in both years where possible. Dead or declining trees were replaced by other sample trees of the same clone in year 2.

On each sample tree, a terminal branch tip (1 to 6 cm in length) was removed from each of the four cardinal directions at a height of 0.9 - 1.5 m. Branch tips from the same sample tree were pooled and placed into a 20 ml plastic scintillation vial containing 70% alcohol. All life stages of HWA and egg and larval stages of *L. nigrinus* were subsequently recorded using a dissecting scope calibrated with an optical scale. Life stages of HWA were determined from body length measurements and shape reported by McClure (1989, 1996) and by counting the number of cast skins associated with each individual. Larval stages of *L. nigrinus* were determined from head capsule measurements as described in Chapter 2. Density counts of HWA and *L. nigrinus* were determined from the number counted on a twig sample divided by the length of the twig to obtain a count per cm twig. On the same sample tree, adult beetles were sampled using a beating tray (42 X 48 cm), with a single beat made on a randomly selected branch (0.9 - 1.5 m height). All adults collected in the beating tray were recorded.

Temperature (1998-1999) and rainfall data (1998-2000) were obtained from an Environment Canada, Meteorological Service of Canada weather station located at the University of Victoria campus ~ 4 km SE of the seed orchard. In 1999-2000, temperature data loggers (Hobo®, Onset, Pocasset, MA) were placed on the bole of the tree (~ 1.4 m height) nearest to the center of each site.

Data Analysis

The densities of HWA and *L. nigrinus* eggs and larvae were computed on sub-samples (n = 4) to get a mean density per sample tree. Mean densities for each sample date were then computed from sample tree means (n = 25). Data were tested within each year for differences in mean densities between the 2 sites using PROC GLM, where 'Site' was the whole plot and 'Time' the subplot (SAS 1989). Data were pooled where there was no Site*Time interaction (Steel and Torrie 1980). Median proportion of individuals was approximated by the logistic equation, $Y = [1 + \exp(-K(X-C)^{-1})]$ (Régnière 1984) using least squares estimation and the solver function in MS Excel®, where Y is cumulative frequency and X is sampling period. Parameter K determines the steepness of the sigmoidal curve. Parameter C determines the midpoint of Y (median). The distribution of adult *L. nigrinus* from beat samples was evaluated using Taylor's power law (Taylor 1961) by regressing log s^2 (variance) on log m (mean) for each site in both years. The constants α and β were estimated from the regression model $\log(s^2) = \log \alpha + \beta \log(m)$, where α and β are the intercept and slope, respectively. Values of Taylor's regression slopes $\beta > 1$, $\beta = 1$ $\beta = 0$ indicate a clumped, random and regular distribution, respectively (Taylor 1961). A Student's t-test (Zar 1984), was performed to test if β was significantly different from 1. The chi-squared goodness of fit test (Zar

1984) was conducted to determine if the distribution of eggs laid by *L. nigrinus* within HWA ovisacs (n = 104) differed from a Poisson distribution. All statistical tests were done at a $P < 0.05$ significance level.

Results

Seasonal abundance and phenology

HWA

Sampling began on 26 and 27 September in 1998 and 1999, respectively. Occurrence of the various HWA life-stages was similar between sites and years. However, abundance of life-stages varied between sites within a year (Figures 4.2 and 4.3). In year 2, populations of all life stages of the sistens and progrediens generation were significantly lower in Site 2 than Site 1 (range, $F_{1, 36} = 5.60 - 9.96$; $P < 0.0001$, for all life stages) (Figure 4.3). After undergoing summer dormancy, sistens resumed development by the first sampling period as shown by the small number of second instar (post-aestival) nymphs recorded (Figures 4.2 and 4.3). In year 1, first sistens adults appeared on 25 October and 8 November at Site 1 and 2, respectively and were present until early June at both sites (Figure 4.2). Median proportion of sistens adults present was estimated at 25 March 1999 for Sites 1 and 2 (Figure 4.2). In year 2, sistens adults appeared at both sites on 21 November. They also occurred at both sites until early June (Figure 4.3). The median proportion of sistens adults present was estimated at 22 and 9 March for Sites 1 and 2, respectively (Figure 4.3). The earliest progrediens eggs laid by sistens adults were observed at the end of January - early February and were present until late May or early June in both years (Figure 4.2 and 4.3). In year 1, the median proportion of progrediens eggs present was estimated at 30 and 31 March for Sites 1 and

2, respectively (Figure 4.2). In year 2, the median proportion of progrediens eggs present was estimated at 27 and 13 March for Sites 1 and 2, respectively (Figure 4.3). Because sampling ended in early June in year 1, the progrediens generation was not sampled. The progrediens generation was sampled in year 2 (1999-2000) only. Progrediens adults and sistens eggs (laid by progrediens adults) first appeared 15 and 29 June at Sites 1 and 2, respectively and were present until 20 July (Figure 4.3). Settled first instar sistens were first recorded 20 July (Figure 4.3).

Laricobius nigrinus

Abundance of *L. nigrinus* adults was not significantly different between sites in both year 1 ($F_{1, 27} = 0.70$; $P = 0.87$) and year 2 ($F_{1, 36} = 1.28$; $P = 0.12$) and therefore data were pooled. Adult *L. nigrinus* activity was first recorded on 11 and 10 October in 1998 and 1999, respectively (Figure 4.4). The appearance of *L. nigrinus* adults on hemlock in the fall (October) coincided with the resumption of development of the HWA sistens generation (Figures 4.2 - 4.4). Adult activity patterns were similar between years and fluctuated from fall through spring (Figure 4.4). Adults were not recorded on branches on 20 December 1998, when the mean daily temperature was below 0 °C (Figure 4.4). Between tree distribution of adult *L. nigrinus* on hemlock was determined to be clumped ($\beta > 1$) at both sites over 2 years (Table 4.1).

The earliest eggs laid by *L. nigrinus* females were observed mid-January in both 1999 and 2000 (Figures 4.5 - 4.8). In year 1, the median proportion of *L. nigrinus* eggs oviposited was estimated to occur on 8 and 7 March (1999) for Sites 1 and 2, respectively (Figures 4.5 and 4.7). In year 2, median proportion of *L. nigrinus* eggs oviposited was

estimated to occur on 12 and 6 March (2000) for Sites 1 and 2, respectively (Figures 4.6 and 4.8).

Female *L. nigrinus* preferred to oviposit eggs singly within the woolly ovisacs of HWA rather than multiple eggs per ovisac (x^2 = 89.2; df = 2; P = 0.001) (Table 4.2). Onset of oviposition by *L. nigrinus* females coincided with the beginning of egg laying by sistens adults (Figures 4.5 and 4.6). Four larval instars were observed between 14 March and 7 June and 12 March and 18 June in 1999 and 2000, respectively (Figures 4.7 and 4.8). Larval stages of *L. nigrinus* exhibited a large temporal overlap (Figures 4.7 and 4.8). The larval period also coincided with the presence of HWA ovisacs (progrediens eggs) laid by the sistens (over-wintering) generation (Figures 4.5 and 4.6). When twigs were examined under the dissecting microscope, the majority of the larvae were found within the woolly ovisacs of HWA. This observation supports laboratory studies (Chapter 2) that revealed *L. nigrinus* larvae feeding predominantly on the eggs of HWA. Pupae were not found on the branches and support laboratory findings that mature fourth instars migrate to the soil to pupate (Chapter 2).

Discussion

HWA

The life cycle of HWA sistens and progrediens in British Columbia (Figures 4.2 and 4.3) is consistent with that found in Virginia (Gray and Salom 1996) and Connecticut (McClure 1987, 1989b). However no sexuparae (winged migratory morph) were ever observed. In addition, maturation of the sistens generation in British Columbia was 2 to 3 months earlier than what had been reported in Virginia (Gray and Salom 1996) and Connecticut (McClure 1989b). Earliest sistens matured between January and early

February in Virginia (Gray and Salom 1996) and February in Connecticut (McClure 1989b). The appearance of progrediens eggs in British Columbia (end of January) (Figures 4.2 and 4.3) is comparable to that in Virginia and Connecticut. In Virginia, the earliest progrediens eggs appeared between early February and mid-March (Gray and Salom 1996), while in Connecticut, they appeared in February (McClure 1989b). The occurrence of the progrediens generation and presence of sistens eggs in British Columbia is similar to findings reported by McClure (1989b), but later than that reported by Gray and Salom (1996). Adult progrediens reached maturity ~ 1 month earlier in Virginia than in British Columbia and Connecticut (McClure 1989b, Gray and Salom 1996). In Virginia, progrediens adults and sistens eggs first appeared late May - early June and were present until late June (Gray and Salom 1996), while in British Columbia they first appeared mid to late June (Figure 4.3). McClure (1989b) indicated that progrediens adults were present during June and July in Connecticut.

Temperature differences between years likely account for observed differences in the phenology of the progrediens generation of HWA between the three regions. Regional differences in temperature in the winter appear to account for the earlier maturation of the sistens generation in Victoria, British Columbia than in Virginia or Connecticut (Table 4.3).

Laricobius nigrinus

The life cycle of *L. nigrinus* is similar to that described for *L. erichsonii* Rosenhauer (Franz 1958a) and *L. rubidus* LeConte (Clark and Brown 1960). However, Franz (1958a) reported that *L. erichsonii* adults, recently emerged from pupae in the soil, fed briefly on *A. piceae* on the bole of *Abies* spp., before returning to the soil by August

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where they remained until the following spring. This behavior was not observed for *L. nigrinus*. The fact that *L. nigrinus* adults were not collected on branches from the end of May to August, suggest that adults remained in the soil in aestival diapause until early October (Figure 4.4). Observations in the laboratory (Chapter 2) support this explanation. Both *L. erichsonii* and *L rubidus* were reported to over-winter in the soil and resume activity at the end of March – early April (Franz 1958a, Clark and Brown 1960). *Laricobius nigrinus* adults were found to be active on hemlock branches all winter at temperatures above 0 °C (Figure 4.4). At this time it is not known whether they migrate to the soil or the bole (hiding in cracks in the bark) during periods when temperatures are below 0 °C. Over-wintering survival of *L. nigrinus* in eastern United States has not yet been determined.

This field study and biological studies in the laboratory (Chapter 2), have revealed that *L. nigrinus* is univoltine. This finding is consistent with *L. erichsonii* (Franz 1958a) but not with *L. rubidus* (Clark and Brown 1960). Clark and Brown (1960) reported collecting a few *L. rubidus* second and third instars in early August in New Brunswick, Canada, suggesting a partial second generation in New Brunswick. Preliminary sampling in Virginia found no adult or larval *L. rubidus* on HWA infested *T. canadensis* after early June (G.Z.-B., unpublished data).

Laricobius nigrinus appears to be well synchronized with HWA. It undergoes an aestival diapause (Chapter 2) that coincides with the first instar sistens nymphs of HWA (McClure 1987, 1989b). Beginning of adult activity coincides with resumption of development of the sistens generation. In addition, oviposition and subsequent larval development of *L. nigrinus* coincide with oviposition by the sistens adults. These findings indicate good synchrony with suitable prey stages of HWA.

Table 4.1. Taylor's power law regression indices (and standard errors) for the

distribution of adult *Laricobius nigrinus* on *Adelges tsugae* infested western

hemlock in Victoria, BC (1998 – 2000). Values of $\beta > 1$ indicate a clumped

distribution.

Year	Site	n	Log(α)	β	r^2
1998 - 1999	1	25	- 0.03 (0.03)	2.08 (0.12)**	0.93
	2	25	- 0.02 (0.03)	1.84 (0.12)**	0.90
1999 – 2000	1	25	- 0.01 (0.02)	1.59 (0.09)**	0.91
	2	25	- 0.01 (0.02)	1.79 (0.12)**	0.87

Slopes tested for significant difference from 1 by using Student's t test, ** $P < 0.001$

Table 4.2. Proportion of eggs laid by *Laricobius nigrinus* females in *Adelges tsugae*
ovisacs (n = 104) in 1998

L. nigrinus eggs laid per HWA ovisac	n	Proportion
1	79	0.76
2	21	0.20
3	3	0.04

Chi-squared test for equal proportions. $x^2 = 89.21$; df = 2 $P = 0.001$

Table 4.3. Mean monthly temperatures in Victoria, British Columbia (BC) (1994-2000), Blacksburg, VA (1990-2000), and Hartford, CT (1990-2000).

	Temperature °C								
	Victoria, BC[a]			Blacksburg, VA[b]			Hartford, CT[c]		
Month	Max	Min	Mean	Max	Min	Mean	Max	Min	Mean
January	8.3	1.7	5.0	6.3	-4.7	0.8	2.1	-6.9	-2.4
February	9.6	1.6	5.6	8.7	-3.3	2.7	3.9	-6.0	-1.1
March	11.4	2.6	7.0	11.5	-0.9	5.3	8.9	-2.0	3.4
April	14.5	4.2	9.4	17.3	3.8	10.5	15.5	3.5	9.5
May	17.7	6.9	12.3	22.1	8.7	15.4	21.9	8.8	15.3
June	20.3	9.5	14.9	26.0	13.7	19.8	26.9	14.1	20.5
July	23.2	11.3	17.2	28.4	16.2	22.4	29.1	17.0	23.0
August	23.1	11.1	17.1	27.2	14.9	21.1	27.9	16.0	22.0
September	20.8	9.1	14.9	24.3	11.1	17.7	23.4	11.5	17.4
October	14.8	5.6	10.2	19.2	4.2	11.7	17.5	5.2	11.4
November	10.7	3.4	7.1	12.2	-0.6	5.8	10.3	0.4	5.4
December	8.0	1.8	4.9	7.0	-4.3	1.3	4.8	-4.3	0.3

[Source: National Climatic Data Center, National Oceanic and Atmospheric Administration website http://www.ncdc.noaa.gov/]

[a]Victoria International Airport station, 48°39'N/ 123°26'W; 19 m asl; [b]Blacksburg Nwso station, 37°12'N/ 80°25'W; 640.1 m asl.

[c]Hartford Bradley International Airport station, 41°56'N/ 72°41W; 48.8 m asl.
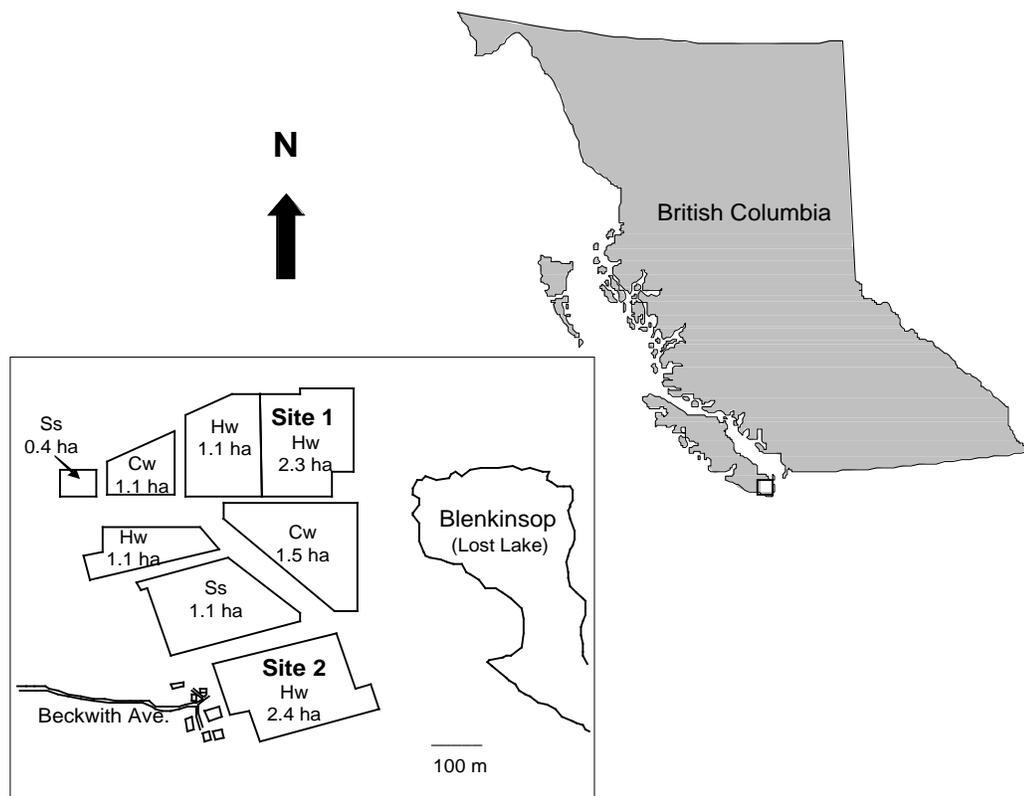
Figure 4.1. Map of British Columbia, Canada showing study site, a commercial seed orchard, on Vancouver Island near Victoria (square) and in detail (inset). Hw = western hemlock; Ss = Sitka spruce; Cw = western red cedar. Sampling occurred in Sites 1 and 2.
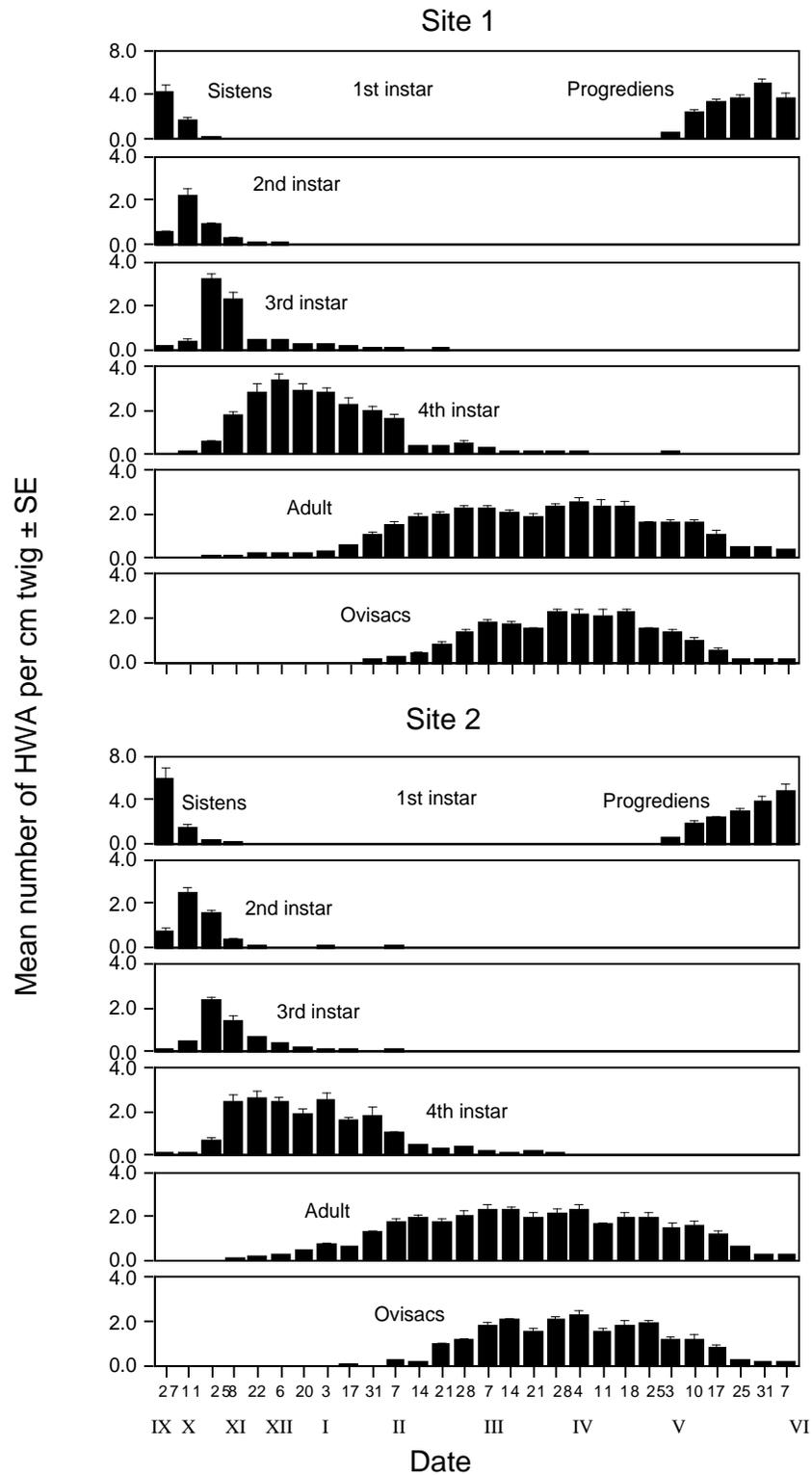
Figure 4.2. Seasonal abundance of over-wintering sistens and beginning of progrediens generations of HWA at Site 1 (top) and Site 2 (bottom) in Victoria, British Columbia from 27 September 1998 to 7 June 1999.

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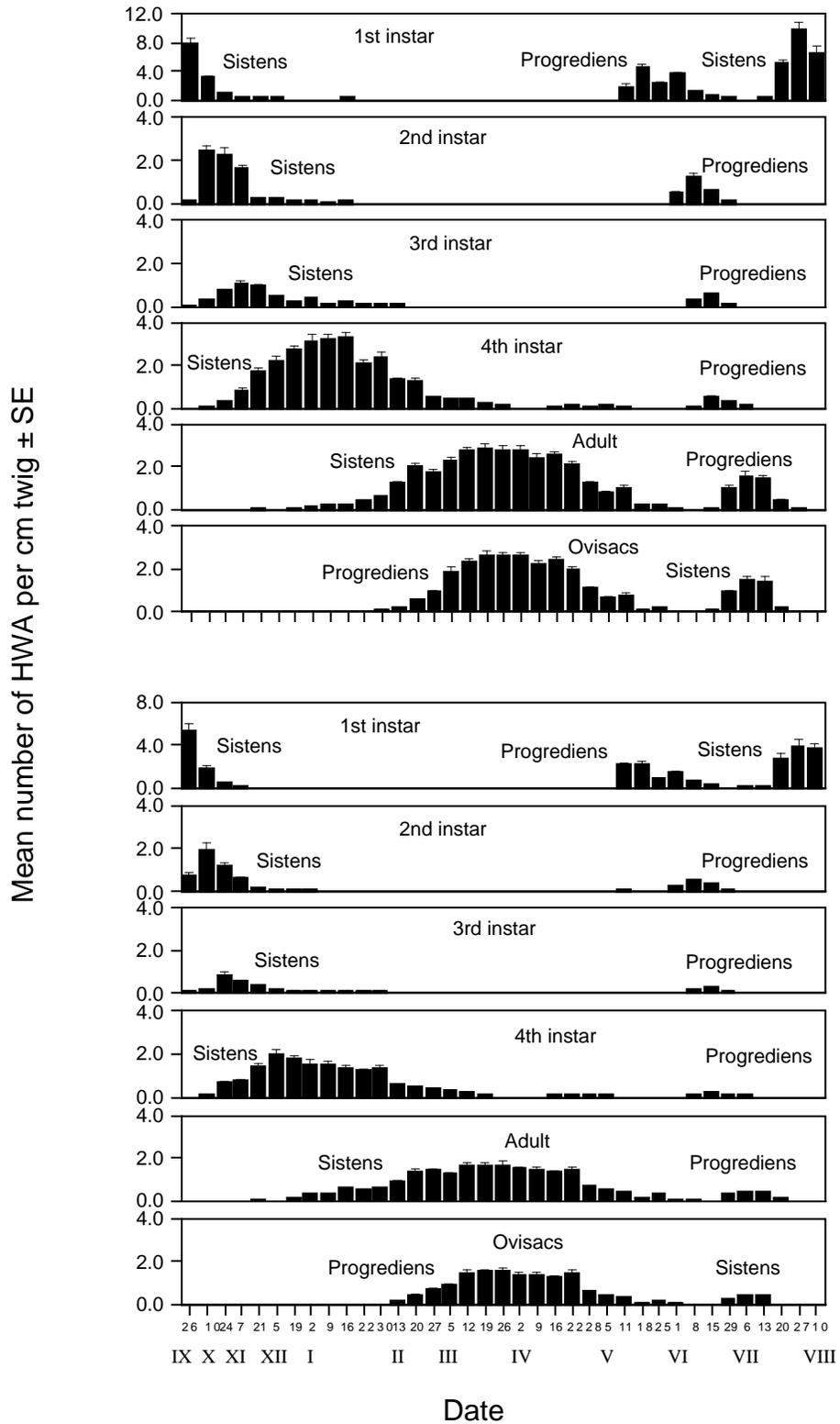
Figure 4.3. Seasonal abundance of over-wintering sistens (1st), progrediens and aestivating sistens (2nd) generations of HWA at Site 1 (top) and Site 2 (bottom) in Victoria, British Columbia from 26 September 1999 to 10 August 2000.

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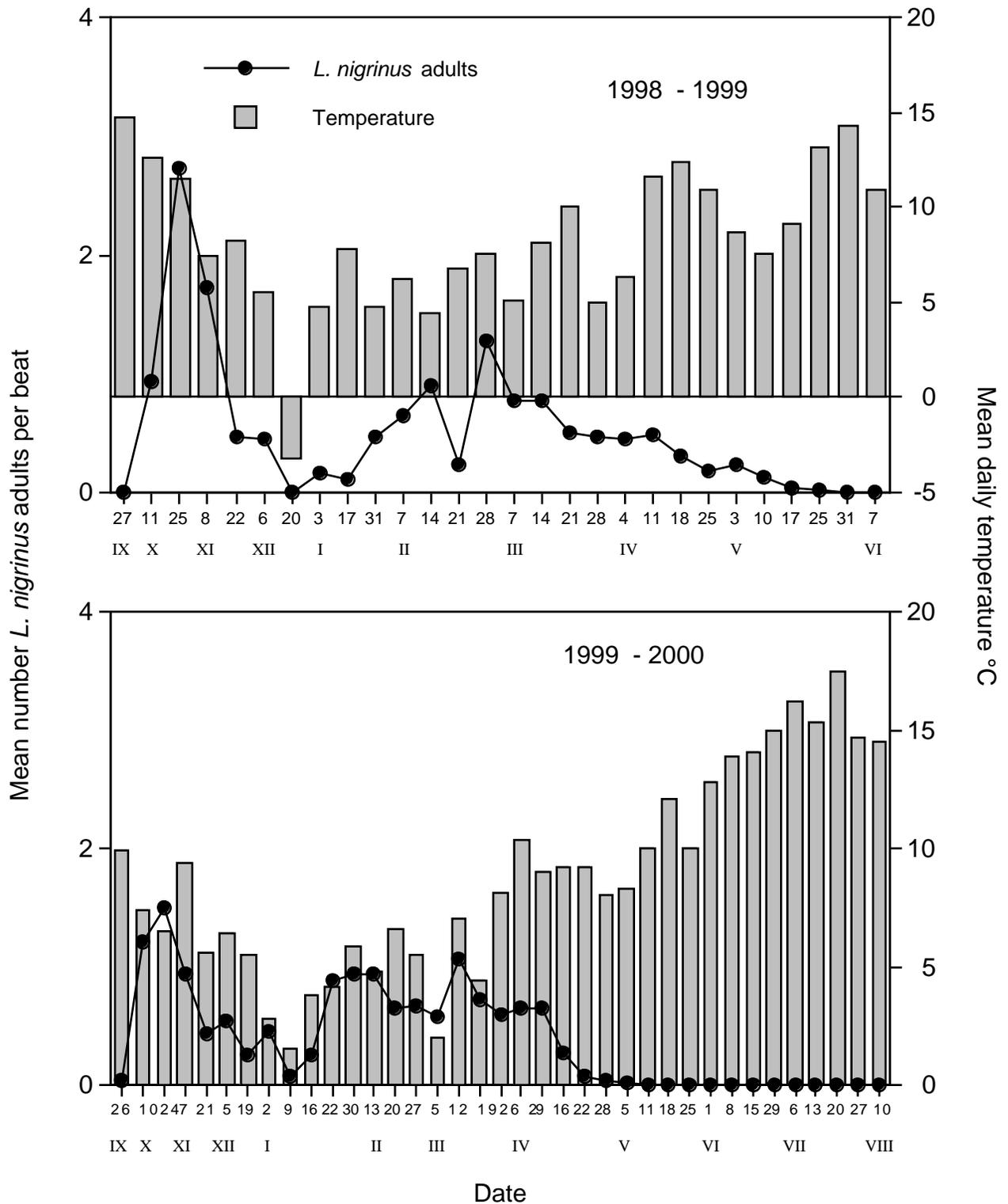
Figure 4.4. Seasonal pattern and abundance of *Laricobius nigrinus* adults in Victoria, BC and mean temperature (°C) on sampling dates in 1998-1999 and 1999-2000. Adults in Sites 1 and 2 were pooled in both years as there were no significant differences between the two sites.
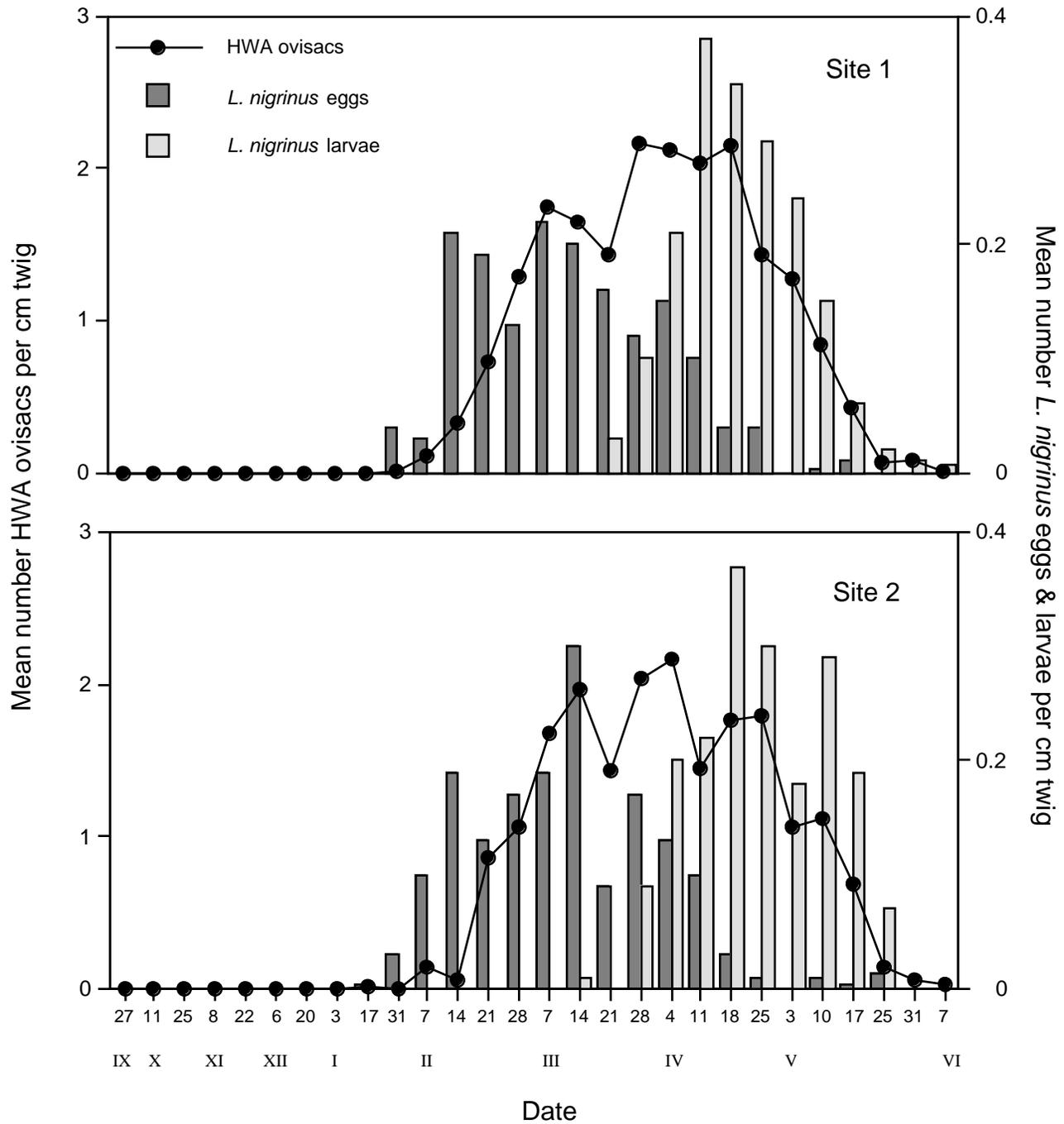
Figure 4.5. Seasonal pattern and abundance of HWA ovisacs and *Laricobius nigrinus* eggs and pooled larvae (instars 1 - 4) at Site 1 (top) and Site 2 (bottom) in Victoria, British Columbia from 27 September 1998 to 7 June 1999.

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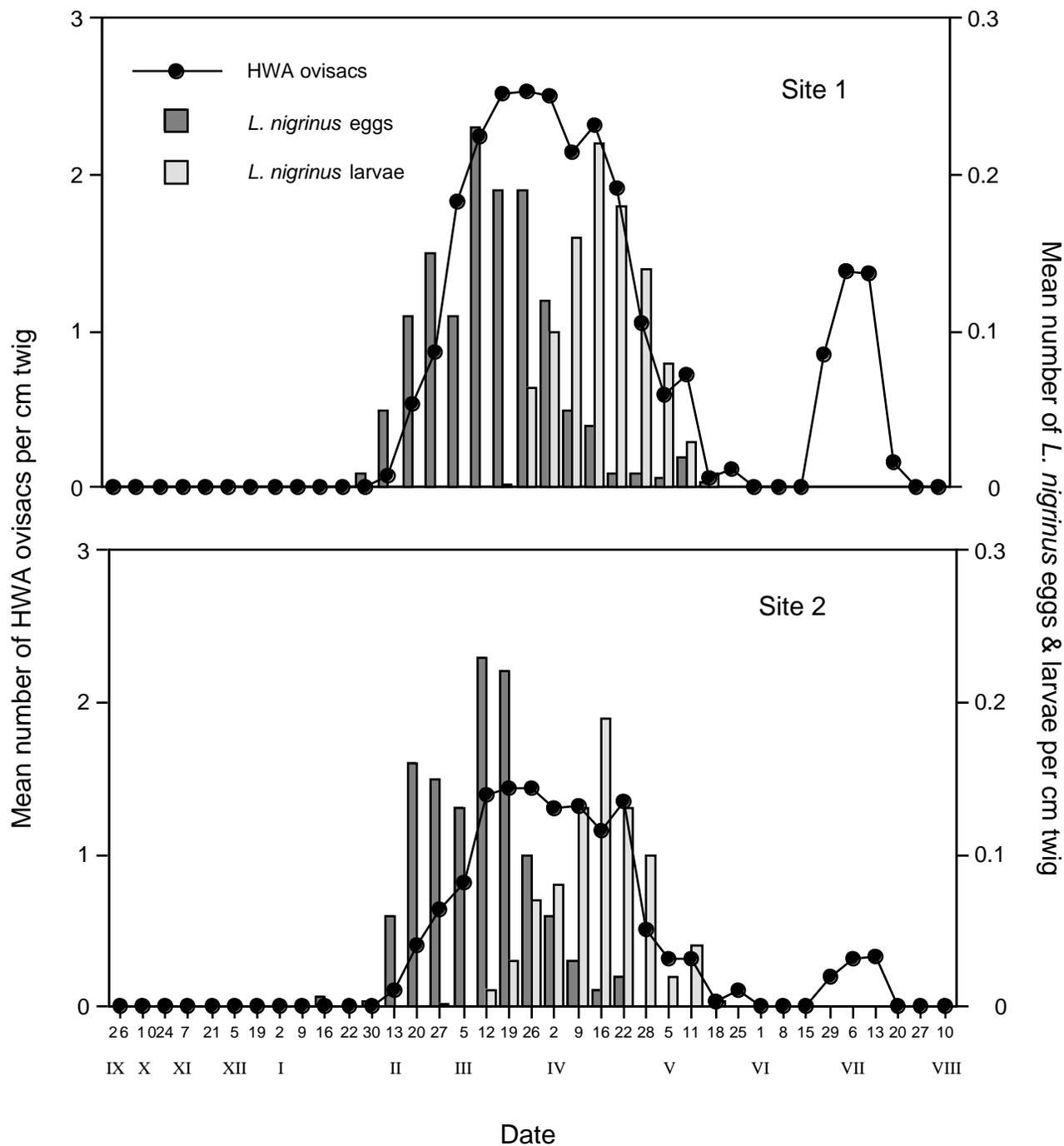
Figure 4.6. Seasonal pattern and abundance of HWA ovisacs and *Laricobius nigrinus* eggs and pooled larvae (instars 1 - 4) at Site 1 (top) and Site 2 (bottom) in Victoria, British Columbia from 26 September 1999 to 10 August 2000.

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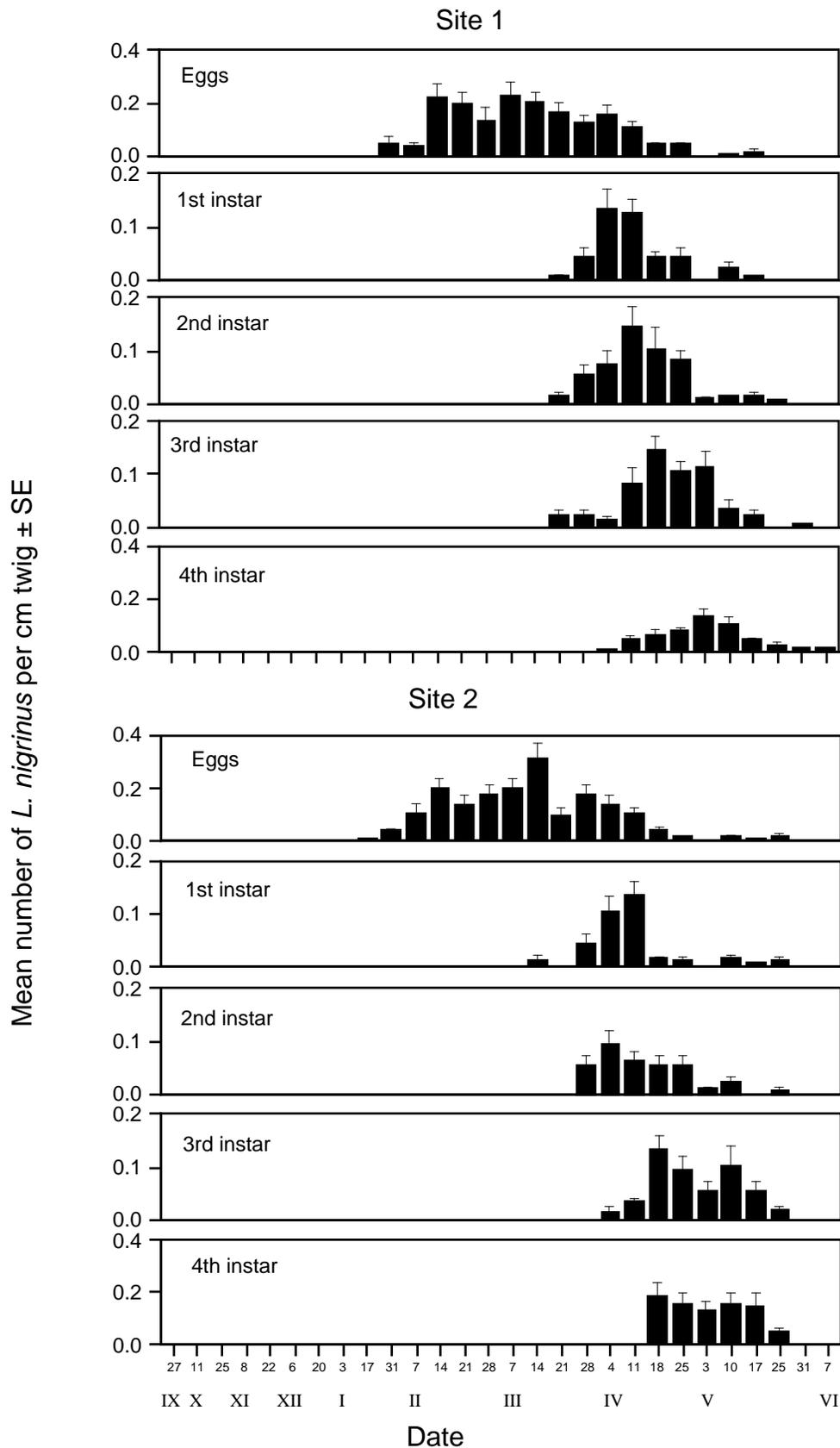
Figure 4.7. Seasonal abundance of *Laricobius nigrinus* eggs and larvae at Site 1 (top) and Site 2 (bottom) in Victoria, British Columbia from 27 September 1998 to 7 June 1999.

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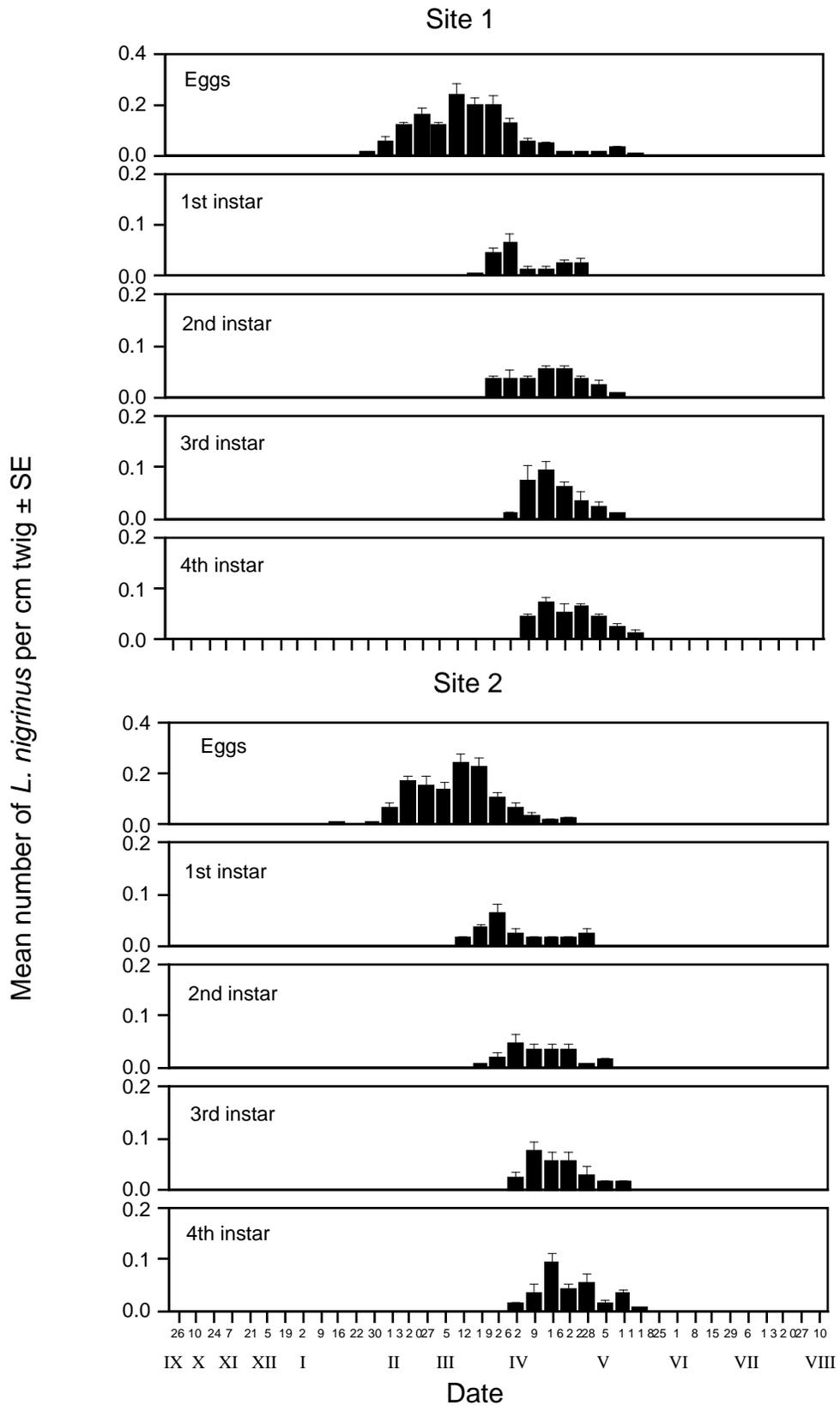
Figure 4.8. Seasonal abundance of *Laricobius nigrinus* eggs and larvae at Site 1 (top) and Site 2 (bottom) in Victoria, British Columbia from 26 September 1999 to 10 August 2000.

Chapter 5

# Temperature-dependent development of *Laricobius nigrinus*

on *Adelges tsugae*

Introduction

Insects, like other ectothermic organisms, depend on temperature for development (Taylor 1981, 1982). The amount of heat required over time for an insect to complete some aspect of its development is a thermal constant (Campbell et al. 1974). Calculation of a thermal constant requires knowledge of the developmental threshold temperature of a particular stage of development of the insect as well as its rate of development in relation to temperature (Dent 1997).

Studies on temperature-dependent development are important in understanding the dynamics of predator-prey relationships, including biological control (Miller and Paustian 1992, Rodriguez-Saona and Miller 1999, Cheah and McClure 2000). In addition, they provide information on the biology and distribution of a species (Frazer and McGregor 1992), contribute knowledge that enhances the efficiency of mass rearing (Rodriguez-Saona and Miller 1999), are useful in predicting development and activity in the field (Fan et al. 1992, Judd et al. 1993, Judd et al. 1994, Davis et al. 1996), and are used in models to estimate insect growth, development and reproduction (Roltsch et al. 1990, Petitt et al. 1991, Paine 1992, Allen et al. 1995). Differences in developmental rates among progeny of individual females (Rodriguez-Saona and Miller 1999), among populations (Miller 1992, Lamana and Miller 1998), among species (Frazer and

McGregor 1992) as well as species reared on different prey or hosts (Campbell et al. 1974, Michels and Behle 1991), may influence population dynamics of natural enemies when imported into novel climates for biological control of pests (Miller and Paustian 1992).

The life cycle and seasonal abundance of *L. nigrinus* described in Chapters 2 and 4, respectively, are the first contributions to the biology of this insect. Knowledge of thermal development requirements will provide baseline phenological information on the biology of *L. nigrinus* and how it relates to the phenology of its host. Comparison of thermal development requirements with HWA in Virginia will help determine whether *L. nigrinus* is synchronized with suitable prey stages of HWA. Synchrony of phenologies between predator and prey can be used to assess in part the suitability of *L. nigrinus* as an effective biological control agent of HWA in the eastern United States.

The objectives of this study were to: 1) determine the influence of temperature on the development of life stages of *L. nigrinus*; 2) estimate lower developmental threshold temperatures for each life stage and thermal sums necessary to complete development; and 3) validate the degree-day model derived from constant temperature experiments with field data collected on *L. nigrinus* in British Columbia.

Materials and Methods

Constant Temperature Experiments

Individuals used in constant temperature experiments were obtained from adult *L. nigrinus* collected from HWA infested western hemlocks (*Tsuga heterophylla* Raf. Sargent) from a commercial seed orchard (Chapter 4) in Victoria, British Columbia, Canada and imported to a USDA approved quarantine facility at Virginia Polytechnic

Institute and State University, Blacksburg, VA. Adults were maintained on HWA from eastern hemlock (*Tsuga canadensis* (L.) Carrière) twig cuttings that were field collected from Giles and Montgomery Co., VA.   Experiments were conducted during the ovipositional period of *L. nigrinus* (February to May).

This beetle is active in the cooler months of the year (Chapter 4), therefore, development was studied at temperatures ranging from 9 to 21 °C. Five constant temperatures were used: 9, 12, 15, 18 and 21 °C (± 1°C) at a photoperiod of 12:12 (L:D) h and RH of 75 – 87%. Eggs used were collected every day (24 h old) from adults held at each constant temperature in oviposition containers, described in Chapter 2. Because *L. nigrinus* females each oviposit a single egg in a woolly ovisac of HWA, rather than dissect out the egg, sections of twig (2 to 4 cm) bearing a single HWA ovisac containing a single *L. nigrinus* egg were cut and placed individually in 50 mm polystyrene petri dishes (Falcon®). Petri dishes contained a 2 cm ventilation hole made in the lid that was covered with polyester mesh (0.14 mm^2) (PeCap®, Sefar America Inc., Kansas City, MO). The base of each petri dish was lined with two layers of filter paper (Whatman No. 1) moistened with methyl paraben solution (0.42 g/250 ml de-ionized water), that acted as a fungal inhibitor. Eggs (n = 45 - 100) were randomly assigned to different constant temperature environmental chambers (Percival®, Boone, Iowa; models: E-30B, I-30BLL, I-36LL). Hobo (Onset Computer Corp., Pocasset, MA). Temperature and RH data loggers were used in each chamber with readings made at 0.5 h intervals. Constant temperature experiments at 15 and 18 °C were conducted in 1999, while experiments at 9, 12 and 21 °C were conducted in 2000. A second experiment at 12 °C was repeated in 2001 as survivorship to the adult stage in 2000 was poor. Data from the two replicates at 12 °C were pooled as there were no significant differences in development times. Life

stages were examined using a dissecting microscope. Eggs were examined daily (24 h) for hatch. Subsequent developmental stages were inspected daily or every other day to adult emergence. Survivorship at each stage per treatment temperature determined the number of initial individuals used in observations for subsequent development stages, except for egg development at 15 °C. In this case, the temperature in the environmental chamber deviated from the experimental temperature by > 1°C, and eggs retrieved from adult females were not used to measure egg development. Egg development was followed on a subsequent cohort of eggs. Larval molts were determined by presence of exuvia. Larvae were provided fresh prey (HWA ovisacs on twig cuttings) at each inspection. Once the pre-pupal stage was reached, sterilized peat moistened with methyl paraben solution (0.42 g/250 ml de-ionized water) was placed at the base of each petri dish. The pre-pupal stage was considered to begin when a mature larva left the food source in search of a suitable pupation site.

Model Development

Cumulative frequency distributions of developmental times at each constant temperature were determined for eggs, larvae, pre-pupae, and pupae. Median developmental time for each life stage at each constant temperature was approximated by the logistic equation, $Y = [1 + \exp(-K(X - C)^{-1})]$ (Régnière 1984) using least squares estimation and the solver function in MS Excel®, where Y is cumulative frequency and X is developmental time. Parameter K determines the steepness of the sigmoidal curve. Parameter C determines the midpoint of Y (median development time). Median developmental times were converted to developmental rates by taking their reciprocals (1/days) for eggs, larvae, pre-pupae and pupae, respectively. Median developmental rates

for each life stage (dependent variable) were then regressed against temperature (independent variable) using least-squares linear regression analysis in SAS$^{®}$ (PROC REG) (SAS 1989). Developmental threshold (D_{th}) temperature was determined by $-a/b$ where a is the y-intercept and b is the slope of the linear equation relating temperature to developmental rate. The degree-day (DD) requirements were determined as the inverse of the slope ($1/b$) of the linear equation.

Model Validation

The degree-day model derived from constant temperature development studies for the egg stage was compared with field observations of *L. nigrinus* in British Columbia at two sites in 1999 and 2000 (see Chapter 4). Cumulative frequency distributions of mean numbers of individuals on each sample date were generated for the egg and larval stages. The logistic equation, as described above, was used to estimate when 50% of the population (median) entered the next stage. The sine-wave method (Allen 1976) was used to generate degree-day accumulations, using D_{th} estimated from the regression equation, described above for the egg stage. Degree-day summations were made from the time that 50% of eggs were laid to 50% egg hatch. Degree-day summation at 50% egg hatch in the field were compared with the DD value predicted from the linear model. Degree-days were not summed for larval development because pre-pupae were not sampled and therefore 50% of the population reaching the pre-pupal stage as a cut-off date for larval degree-day accumulation could not be estimated. In 1999, daily maximum and minimum temperatures were obtained from an Environment Canada, Meteorological Service of Canada weather station located on the University of Victoria campus ~ 4 km SE of the Lost Lake Seed Orchard (see Chapter 4). In 2000, temperature data loggers

(Hobo®, Onset, Pocasset, MA) were placed on the bole of the tree (~ 1.4 m height) nearest to the center of each orchard being sampled. Daily max-min temperatures were summarized from readings taken at 15 minute intervals on the data loggers.

Data Analysis

Data from British Columbia were tested within each year for differences in mean densities between the 2 sites using PROC GLM, where 'Site' was the whole plot and 'Time' the subplot (SAS 1989). Data were pooled where there was no Site*Time interaction (Steel and Torrie 1980). Hemlock woolly adelgid progrediens ovisac data for each site was pooled in both years.

Results

Constant Temperature Experiments

Developmental times for egg, larval, pre-pupal, and pupal stages are given in Table 5.1. Developmental time of eggs decreased as temperature increased, up to 18 °C. Egg developmental time increased slightly at 21 °C (Table 5.1). Larval developmental time was inversely proportional to temperature between 9 and 21 °C. However, larval mortality was two to three times higher at 21 °C than between 9 and 18 °C (Table 5.1). *Laricobius nigrinus* did not complete development at 21 °C. Pre-pupal mortality was > 75% at 9 and 12 °C (Table 5.1). Developmental times for pre-pupae and pupae were similar. Median developmental times from egg to adult were 118.2, 86.7, 64.2 and 45.7 days at 9, 12, 15 and 18 °C, respectively (Table 5.1).

Relationships between temperature and developmental rates of all life stages were described by linear regressions (Figure 5.1). Estimated regression parameters are given

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in Table 5.2. Significant positive linear relationships ($P < 0.05$) were observed between developmental rate and temperature for all life stages (Table 5.2). Degree-day requirements for completion of development at each life stage were different and increased with each successive life stage. Median developmental times of eggs, larvae, pre-pupae, and pupae required 54.6, 161.3, 196.1, and 212.8 degree-days above the minimum developmental temperatures, respectively (Table 5.2). The estimated lower developmental thresholds for eggs, larvae, pre-pupae, and pupae were 6.5, 5.1, 3.8, and 3.1 °C, respectively (Table 5.2). When life stages were pooled for individuals that completed development to the adult stage, minimum temperature for development from oviposition to adult eclosion was 3.7 °C, requiring 666.7 DD to complete development (Table 5.2).

Model Validation

Using the logistic equation described above on cumulative frequency distributions of egg and first instar larval stages, median occurrence of eggs were estimated on 8 and 7 March for Site 1 and 2, respectively in 1999, while in 2000, on 13 and 7 March for Sites 1 and 2, respectively. Median egg hatch in 1999 occurred at 64.2 and 64.3 DD (6 April), at Sites 1 and 2, respectively, while in 2000, at 48.0 (2 April) and 33.7 DD (27 March), at Sites 1 and 2, respectively. The mean ($\pm$ SE) of these four observed values is 52.6 $\pm$ 7.4 DD, which is close to the DD value (54.6) predicted by the reciprocal of the slope of the linear development model for the egg stage (Table 5.2). In 1999 and 2000, median occurrence of HWA progrediens ovisacs was estimated on 30 March and 27 March, respectively, which was within 4 or 5 days of median egg hatch of *L. nigrinus* (see Figures 4.5 and 4.6, Chapter 4). It is evident that oviposition by *L. nigrinus* is

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synchronized with availability of HWA progrediens ovisacs, as shown by the temporal overlap between egg and larval stages of *L. nigrinus* and suitable stages of HWA.

Discussion

High larval mortality at 21 °C as well as the fact that *L. nigrinus* did not complete development at 21 °C suggests that development is probably maximal between 18 and 21 °C. High pre-pupal mortality (> 75%) compared with pupal mortality (26.7 – 50%) at 9 and 12 °C, suggest that the pre-pupal stage is particularly sensitive to low soil temperatures (Table 5.1).

Data on developmental thresholds and thermal constants are important to matching natural enemies to new climates for biological control of pests. The lower developmental threshold from egg to adult for *L. nigrinus* (3.7 °C) is similar to that for the HWA progrediens generation from second instar to adult (3.9 °C) (Salom et al. 2001), indicating that *L. nigrinus* and HWA are adapted to similar climatic regimes. Egg developmental threshold of *L. nigrinus* (6.5 °C) was higher than the HWA progrediens threshold. This delay in natural enemy development ensures that suitable stages of the host or prey population can become established before the natural enemy (Campbell et al. 1974).

Deviations of observed degree-day summations of median egg hatch in British Columbia were 15.0 and 15.1 % at Sites 1 and 2, respectively, in 1999 and 12.1 and 38.3 % at Sites 1 and 2, respectively, in 2000, from the predicted value of 54.6 DD. Three of the four observed DD values came within the 15% acceptable range suggested by Ratte (1985). It is unclear why Site 2 underestimated egg hatch by such a wide margin (38.3%) since Site 2 is less than 500 m from Site 1. In addition, there were no significant

differences in average temperatures between sites at each sampling period. In 2000, at Site 2, *L. nigrinus* first instars were observed one sample period (= 1 week) earlier than at Site 1. This may explain why estimated median egg hatch at Site 2 was ~ 1 week earlier than at Site 1.

When observed degree-days in both years were averaged (52.6), the value was close to the predicted degree-day value (54.6) derived from taking the reciprocal of the slope of the regression equation. In three of four sites over two years, the observed degree-days approximated the predicted value. It can be concluded that this regression model is a useful predictor of egg hatch in Victoria, British Columbia. This model still needs to be validated for Virginia to determine if oviposition by *L. nigrinus* is synchronized with oviposition by HWA sistens females.

Both *L. nigrinus* and HWA are cool adapted insects. HWA has been reported to survive extremely low (- 30°C) winter temperatures (Parker et al. 1998). Lawrence (1989) reported that the family Derodontidae occupies cool temperate regions of the world. There are no published reports on any member of the family Derodontidae for comparisons with *L. nigrinus* on temperature-dependent development. The genus *Tsuga* is similarly adapted to climatic conditions as the insects associated with it (Burns and Honkala 1990, Farjon 1990).

Comparison of thermal development requirements of other natural enemies targeting the same pest species provides information on temporal overlap that is useful when examining possible interactions among biological control agents. The developmental threshold for *Pseudoscymnus tsugae* Sasaji & McClure (Coleoptera: Coccinellidae), another predator being evaluated for biological control of HWA, was 9.5 °C with 405 DD required to complete development from egg to adult (Cheah and

McClure 2000). This information suggests that *P. tsugae* becomes active later in the spring. Cheah and McClure (2000) reported that over-wintering adults begin to lay eggs late April or May in Connecticut with adults emerging mid-June or July. These adults begin to lay eggs of the second generation beginning mid-July. Based on results from British Columbia (Chapter 4*)*, *L. nigrinus* eggs and larvae overlap completely with the HWA progrediens ovisac stage. In Connecticut, this stage extends from February to June (McClure 1987). In the event that *L. nigrinus* and *P. tsugae* become established in the same region, it is expected that there will be some temporal overlap between the two species since *P. tsugae* adults begin to lay eggs in the latter part of the progrediens/sexuparae ovisac stage (Cheah and McClure 2000). Whether these two species of predators will provide increased HWA suppression by an additive effect or eventually compete for resources and reduce the overall effect of HWA suppression, needs to be determined.

Table 5.1. Survivorship and duration (days) of *Laricobius nigrinus* eggs, larvae, pre-pupae and pupae reared on

Adelges tsugae at 4 or 5 constant temperatures

Life stage	Temp. (°C)	Initial no. Individuals	No. surviving to next stage	% survivorship	Mean ± SD	Median	Median rate (1/d)
Egg	9	100	87	87.0	16.3 ± 0.86	15.7	0.0635
	12	165	105	63.6	11.5 ± 1.05	11.0	0.0906
	15	45	29	64.4	9.1 ± 0.99	8.5	0.1175
	18	70	59	84.3	4.3 ± 0.48	3.9	0.2545
	21	105	80	76.2	4.4 ± 0.54	3.9	0.2564
Larva	9	87	64	73.6	32.8 ± 2.92	32.1	0.0311
	12	105	74	70.5	25.1 ± 2.77	24.2	0.0413
	15	65	41	63.1	19.0 ± 1.29	18.3	0.0546
	18	59	35	59.3	13.9 ± 1.10	13.4	0.0745
	21	80	22	27.5	9.6 ± 1.00	9.3	0.1079
Pre-pupa	9	64	15	23.4	36.2 ± 4.40	35.3	0.0283
	12	74	18	24.3	26.0 ± 3.34	25.4	0.0394
	15	41	32	78.1	19.4 ± 2.25	18.7	0.0535
	18	35	26	74.3	14.1 ± 1.90	13.5	0.0744
	21	22	0	0.0	-	-	-
Pupa	9	15	11	73.3	33.9 ± 1.38	33.5	0.0299
	12	18	9	50.0	26.6 ± 2.19	25.7	0.0389
	15	32	25	78.1	17.8 ± 1.04	17.3	0.0577

Table 5.2. Estimates for parameters in regression equations, developmental threshold (D_{th}), and degree-day (DD) requirements for *Laricobius nigrinus* from constant temperature experiments

Life Stage	Temperature range (°C)	Regression equations[a]	P	r^2	D_{th} (°C)	DD (1/b)
Egg	9.0 – 21.0	$y = -0.1183 + 0.0183x$	0.017	0.89	6.5	54.6
Larva	9.0 – 21.0	$y = -0.0315 + 0.0062x$	0.006	0.94	5.1	161.3
Pre-pupa	9.0 – 18.0	$y = -0.0196 + 0.0051x$	0.01	0.98	3.8	196.1
Pupa	9.0 – 18.0	$y = -0.0144 + 0.0047x$	0.008	0.98	3.1	212.8
Egg to Adult	9.0 – 18.0	$y = -0.0056 + 0.0015x$	0.013	0.97	3.7	666.7

[a]Regression model is $y = a + bx$, where y is development rate (1/days), x is temperature.
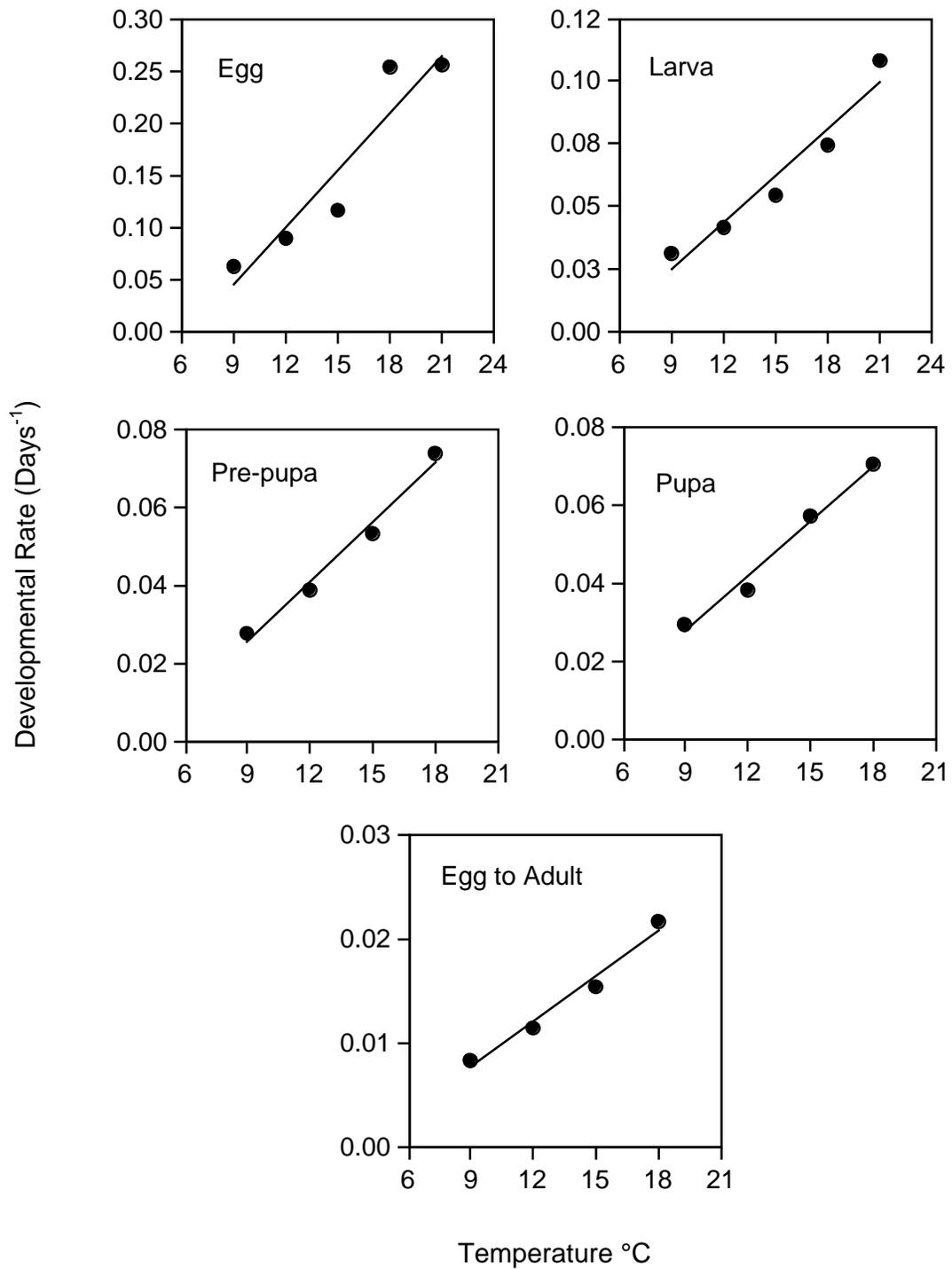
Figure 5.1. Linear relationship between median developmental rate (Days⁻¹) and temperature (° C) for each life stage and egg-adult. See Table 5.2 for regression parameters.

Chapter 6

Summary

The hemlock woolly adelgid is an exotic pest to eastern North America (McClure 1987). Eastern hemlocks are very susceptible to attack by HWA and can be killed in as little as four years if trees are predisposed to other stresses (Shields et al. 1996). Hemlocks in eastern North America have significant ecological and economic importance. Management of HWA is important for preservation of eastern hemlocks as a forest and ornamental species. Because of the silvics of eastern hemlocks, chemical control in a forest setting is an impractical option (McClure 1987, Burns and Honkala 1990, Quimby 1996, Williams and Moriarity 2000). Since HWA populations in the eastern United States are not regulated by effective natural enemies (McClure 1987, Montgomery and Lyon 1996, Wallace and Hain 2000), classical biological control has become the most promising option in a natural forest setting.

The overall objective of this research was to investigate the biology *Laricobius nigrinus* and determine its suitability as a potential biological control agent of the hemlock woolly adelgid, *Adelges tsugae* in the eastern United States.

In Chapter 2, the life cycle, development and reproductive biology of *L. nigrinus* were determined. *Laricobius nigrinus* completed development on a diet of HWA. It is univoltine, has four larval stages and undergoes an aestival diapause after adult eclosion. Females are long-lived (> 30 weeks) and have a high fecundity (> 100 eggs).

In Chapter 3, host specificity was determined. *Laricobius nigrinus* is host specific. In paired-choice and no-choice oviposition tests, *L. nigrinus* laid more eggs in HWA ovisacs than other test species. *Laricobius nigrinus* only completed development on HWA.

In Chapter 4, the seasonal life history of both *L. nigrinus* and HWA in British Columbia revealed that the life cycle of HWA in British Columbia is similar to that previously reported in Virginia and Connecticut. In addition, *L. nigrinus* adults undergo an aestival diapause that coincides with the first instar sistens nymphs of HWA. Adult activity beginning in autumn coincides with resumption of development of the sistens generation. Finally, oviposition and subsequent larval development of *L. nigrinus* coincide with oviposition by HWA sistens adults.

In Chapter 5, the influence of temperature on development of *L. nigrinus* were determined. Development time was inversely proportional to temperature between 9 and 18 °C. *Laricobius nigrinus* did not complete development at 21 °C. The minimum temperature for development of *L. nigrinus* eggs to adult (3.7 °C) was determined to be similar to HWA progrediens second instar to adult (3.9 °C), suggesting that these two species are adapted to similar climatic regimes.

Based on research findings, it can be concluded that *L. nigrinus* has several attributes that make it a good candidate for biological control of HWA in the eastern United States:

    a) It is highly host specific, feeding specifically on adelgids and preferring HWA over other adelgid species;

    b) Its life cycle and feeding stages are highly synchronized with that of HWA;

    c) Females are long-lived;

d) Females restrict oviposition to sites suitable for development of progeny;

e) It is adapted to similar climatic regimes as HWA;

Based on this research, *L. nigrinus* was removed from quarantined status by APHIS-PPQ in September 2000. However, because *L. nigrinus* is univoltine and undergoes an aestival diapause, it is less amenable to mass culturing.

This research is the first contribution to studies on the biology and ecology of *L. nigrinus* and interactions with its prey, HWA. More research is required on various aspects of *Laricobius nigrinus* biology and interactions with HWA that will provide more information on its potential efficacy as a biological control agent in Virginia. Recommendations for further research include:

1) Validate the degree-day model developed for egg development in Virginia to determine if oviposition by *L. nigrinus* is synchronized with oviposition by HWA sistens.

2) Investigate the genetic variation between populations of HWA in western North America, eastern North America and various locations in Asia. Results of these studies will shed light on where HWA in eastern North America was introduced from and whether HWA in western North America and eastern North America are the same species.

3) The type of diapause exhibited by *L. nigrinus* adults should be examined. Is diapause facultative or obligatory? If diapause is facultative, what environment cues or their combinations influence diapause initiation and termination? This information will be useful for mass culturing *L. nigrinus*.

4) How adult *L. nigrinus* select a habitat and patch in search of prey, or how a female selects a suitable patch for oviposition will provide information on searching ability.

5) Interactions between *L. nigrinus* and other predators being evaluated for biological control of HWA need to be examined to determine possible effects (i.e. complementary or detrimental).

6) Possible non-target impacts (i.e. displacement, competition) on native predators resulting from release of biological control agents should be examined.

7) Hemlock resistance to HWA attack should be investigated in *Tsuga canadensis* as it appears to play a role both in Asian and western North American hemlock species.

8) There needs to be a greater understanding of what drives HWA populations in the eastern United States. What mortality factors are involved in the population dynamics of HWA? How much mortality is caused by native and introduced predators? What type of impact are each of these natural enemies having on the dynamics of this system? These studies will reveal what factors have the most impact on HWA population regulation and what role introduced natural enemies play.

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