A PREDATOR CASE HISTORY: *LARICOBIUS NIGRINUS*, A DERODONTID BEETLE INTRODUCED AGAINST THE HEMLOCK WOOLLY ADELGID

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ABSTRACT

The hemlock woolly adelgid, *Adelges tsugae* Annand (Homoptera: Adelgidae) is an invasive alien pest of eastern North American hemlocks (*Tsuga* spp.) and is the target of a classical biological control program in the eastern United States. Host range testing conducted under quarantine in Blacksburg, Virginia determined the suitability of *Laricobius nigrinus* Fender (Coleoptera: Derodontidae) a predatory beetle, as a biological control agent of this pest. Members of the genus *Laricobius* are known to feed on adelgids. *Laricobius nigrinus*, native to western North America, was tested on three other adelgid and three non-adelgid species of Homoptera in three families. Host acceptance and host suitability tests were conducted on test prey. In paired-choice and no-choice oviposition tests, *L. nigrinus* females preferred to oviposit in HWA ovisacs over the other test species. Feeding tests showed that *L. 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. These results suggest that *L. nigrinus* has a narrow host range and that it has potential for biological control of HWA. *Laricobius nigrinus* was cleared for field release by USDA APHIS in 2000 based on these findings and NAPPO Guidelines for 'Petition for Release of Exotic Entomophagous Agents for the Biological Control of Pests'. Test design will be discussed in a retrospective analysis in relation to the practical realities of host range testing in this system and compared with what might be the ideal.

INTRODUCTION

HEMLOCK WOOLLY ADELGID

The hemlock woolly adelgid (HWA), *Adelges tsugae* Annand is an invasive alien pest of native hemlocks (*Tsuga* sp.) in eastern North America (McClure 1996). This insect was first observed in North America in the Pacific Northwest in the early 1920's where it was described from specimens collected on western hemlock, *T. heterophylla* (Raf.) Sargent (Annand 1924). 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 13 states on the eastern sea board (USDA FS 2004).

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 within four years after initial attack (McClure 1991). HWA 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, HWA has little impact on Asian and western North American species of hemlock. Tree resistance and natural enemies have been reported as playing a role in maintaining HWA below injurious levels in these regions (Cheah and McClure 1996; Montgomery and Lyon 1996).

LARICOBIUS NIGRINUS

Members of the genus *Laricobius* are predacious on woolly adelgids (Homoptera: Adelgidae) (Lawrence and Hlavac 1979; Lawrence 1989). *Laricobius nigrinus* Fender is native to western North America (Fender 1945; Hatch 1962; Lawrence 1989). It was found in close association with HWA on western hemlock in British Columbia, Canada (Zilahi-Balogh *et al.* 2003) where HWA is not considered a forest pest. We hypothesized that *L. nigrinus* may play a role in regulating HWA abundance in the Pacific Northwest and therefore warranted investigation as a candidate biological control agent of HWA in the eastern United States.

We studied the life history of *L. nigrinus* over two years in British Columbia (Zilahi-Balogh *et al.* 2003). This beetle is univoltine. Females lay eggs singly within the woolly ovisacs of HWA from January to May. Onset of oviposition by *L. nigrinus* coincides with oviposition by the over-wintering (sistens) generation of HWA. After hatching, larvae feed preferentially on the eggs of HWA. On completion of feeding, mature larvae migrate to the soil to pupate. After eclosion, adults remain in the soil in an aestival diapause resuming activity in late September to early October at about the same time that aestivating first instar HWA sistens resume development (Zilahi-Balogh *et al.* 2003). Adult feeding by *L. nigrinus* in the winter contributes significantly to adelgid mortality (Lamb *et al.* 2005a). The phenology of *L. nigrinus* in Virginia (Lamb *et al.* 2005a) is similar to that in British Columbia (Zilahi-Balogh *et al.* 2003).

A summary of host specificity tests on *L. nigrinus* followed by a retrospective analysis of host range testing procedures addressing issues presented in this symposium are discussed. The issues are: 1) test design (Withers and Mansfield 2005), 2) statistical design (Hoffmeister 2005), and 3) genetics: relation of local populations to whole species (Hopper *et al.* 2005).

MATERIALS AND METHODS

Laricobius nigrinus adults used in this study were field collected from HWA infested western hemlock from coastal British Columbia, and imported to Virginia for quarantine evaluation (Zilahi-Balogh *et al.* 2002). Field collection and testing of adults coincided with the ovipositional period (peak oviposition is early to mid-March) of *L. nigrinus* (Zilahi-Balogh *et al.* 2003). Immature stages tested were progeny of field collected adults. Insects were main-

tained on field collected HWA infested eastern hemlock twig cuttings in environmental chambers at 15°C, 12:12 (L:D) h, and 75-87% RH.

Six species of test prey in the order Homoptera in three families (Adelgidae, Aphididae, Diaspididae) were used in host specificity tests. They were selected based on taxonomic or ecological similarity to HWA as well as availability. Test prey species are listed in Table 1. With the exception of *M. persicae*, all test prey could be encountered by *L. nigrinus* in the natural forest setting in southeast United States.

Table 1. Test prey on associated host plants used in host range tests conducted between February and April 2000 (from Zilahi-Balogh *et al.* 2002).

Test Prey	Distribution	Host Plant
	Family Adelgidae	
Adelges tsugae Annand (HWA)	Asia, North America[a] (Target insect)	*Tsuga canadensis* (L.) Carrière
Adelges piceae (Ratzeburg)	Europe, North Americaa	*Abies fraseri* (Pursh) Poir
Adelges abietis (L.)	Europe, North America, North Africa, India[a]	*Picea abies* (L.) Karst.
Pineus strobi (Hartig)	North America, Europe[a]	*Pinus strobus* L.
	Family Aphididae	
Cinara pilicornis (Hartig)	Europe, Australia, New Zealand, North and South America[a]	*Picea abies* (L.)Karst.
Myzus persicae (Sulzer)	World wide[b]	*Capsicum frutescens* L. var. *grossum* Bailey
	Family Diaspidae	
Chionaspis pinifoliae (Fitch)	North America[c]	*Pinus cembra* L.

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

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 (Zilahi-Balogh *et al.* 2003). *Chionaspis pinifoliae* (Diaspididae) over-winters in the egg stage underneath the female scale. In May, these hatch into crawlers which move over the needles for a few days and then settle down to feed (Kosztarab 1996). Host plant material infested with *C. pinifoliae* were field collected in the early spring and held at 4°C until used in tests. HWA differs from the other adelgids tested 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 spring when buds begin to break (April or May) (Arthur and Hain 1984; Craighead 1950; Friend and Wilford 1933; Gambrell 1931; Johnson and Lyon 1991; USDA 1985). The challenge was synchronizing development of the various adelgid species with that of HWA. This was achieved by moving adelgid infested potted saplings (Table 1) from an outdoor nursery

into a greenhouse (~ 24°C) beginning in January to accelerate development before being used in tests. Test prey in the family Adelgidae and Diaspididae remain attached to their host plant once crawlers settle. Excess individuals were removed from the host plant with fine forceps when numbers exceeded those required for a particular test. Test prey in the family Aphididae were tested at the early instar nymphal stage as adult females exhibited vivipary. Individuals within the family Aphididae were transferred onto or removed from their respective host plant with a fine brush to attain the appropriate number on the host plant cutting.

Host specificity tests (Zilahi-Balogh *et al.* 2002) 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 (Browne and Withers 2002; Kok *et al.* 1992). Host suitability tests therefore are more crucial in determining potential host range.

HOST ACCEPTANCE

Oviposition tests. Both no-choice (single-prey) and paired-choice oviposition tests were conducted to evaluate the effect of prey type on acceptance and preference by *L. nigrinus* females for oviposition. All tests were conducted in 14 x 2.5 cm plastic petri dishes. One male-female pair was placed in a petri dish with either one bouquet of associated host plant twigs housing test prey (no-choice test) or two adjacent bouquets of host plant with associated prey (paired-choice test). A bouquet was made up of two to four terminal tip branches (10-12 cm length) of prey infested host plant held together by wrapping the cut end with parafilm to prevent the twigs from drying out. In the paired-choice tests, HWA was paired with each of the six test prey. The same numbers of prey (~60 individuals per bouquet) were used in each test. Duration of each test was three days. The number of *L. nigrinus* eggs deposited on each plant bouquet was counted at the end of each test (Zilahi-Balogh *et al.* 2002). A 3-day test was selected based on preliminary trials that showed that three days was a long enough interval to get a treatment effect without resulting in host plant desiccation or having to add additional prey.

Adult feeding test. Prey acceptance by adult *L. nigrinus* was examined in a single-prey feeding experiment using eggs of the four adelgid species, HWA, *A. abietis*, *A. piceae*, and *Pineus strobi*. Even though *L. nigrinus* adults preferentially feed on nymphs and adult stages of adelgids, eggs were selected to test because they are uniform in size within and between adelgid species. Adult *L. nigrinus* starved for 12 h, were placed individually in 50 x 9 mm petri dishes containing one of four prey types attached to sections (< 5 cm) of host plant. Egg numbers of test prey were estimated before introduction of the predator. After 3 d, adult beetles were removed and the number of eggs that remained were counted (see Zilahi-Balogh *et al.* 2002 for details).

Host Suitability. Development and survivorship of *L. nigrinus* were followed from the egg to adult stage on all test prey except *M. persicae*. We did not evaluate *M. persicae* because it was the only test prey that *L. nigrinus* females did not oviposit on during the oviposition tests. *Laricobius nigrinus* eggs (d•24 h old) were transferred individually onto test prey in petri dishes 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. Egg hatch was followed daily. Other stages were examined daily or every other day for survivorship until adult emergence. Fresh prey was added each time an individual larva was examined. Larval molt was determined by recording the presence of an exuvium. Once the pre-pupal stage was reached, moistened sterilized peat 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 that mature larvae left the twig with abundant prey and appeared to be actively searching for a suitable pupation site (Zilahi-Balogh *et al.* 2002).

RESULTS AND DISCUSSION

HOST ACCEPTANCE

Oviposition tests. In both the no-choice and paired-choice oviposition tests, *L. nigrinus* females laid significantly more eggs in HWA ovisacs ($P < 0.0001$ to 0.02) over the other test prey (Zilahi-Balogh *et al.* 2002). In the paired-choice test, no eggs were laid on host plants housing non-adelgid prey (*C. pilicornis, C. pinifoliae,* and *M. persicae*). Oviposition was more than five times greater on HWA than on adelgid test prey (*A. piceae, A. abietis, Pineus strobi*) in the paired-choice tests. These differences indicate an ovipositional preference for HWA over these other adelgids (Zilahi-Balogh *et al.* 2002). In no-choice tests, no eggs were laid on sweet pepper housing *M. persicae,* and very few eggs (mean: $d \leq 0.2$ eggs) were laid on host plants housing the other non-adelgid Homoptera (*C. pilicornis* and *C. pinifoliae*). In no-choice tests, *L. nigrinus* laid ~ 2 to 12 times more eggs in HWA ovisacs over the other adelgid non-target prey.

Adult feeding test. In this no-choice feeding test, eggs of all the test adelgids were fed on by adult *L. nigrinus.* Significantly more eggs of HWA were consumed than eggs of the *A. piceae* and *Pineus strobi,* but not *A. abietis.* Though not statistically significant, *L. nigrinus* adults consumed on average 2x more eggs of HWA (48.4) than *A. abietis* (24.7) (Zilahi-Balogh *et al.* 2002).

HOST SUITABILITY

Laricobius nigrinus only completed development to the adult stage on a diet of HWA. *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 (see Zilahi-Balogh *et al.* 2002 for details).

RETROSPECTIVE ANALYSIS

TEST DESIGN

Host specificity tests are designed to determine host acceptance and host suitability (defined earlier) (Kok *et al.* 1992). No-choice and choice tests have been used widely to evaluate host

ranges for both weed and arthropod biological control (Sands and Van Driesche 2003; Van Driesche and Hoddle 1997; Van Driesche and Murray 2004a).

No-choice tests combine the biological control agent with a single test species for a set period of time (Van Driesche and Murray 2004a; Withers and Mansfield 2005). Sequential no-choice tests involve the presentation of target and non-target hosts in a sequence. Choice tests utilize two or more test species with the biological control agent simultaneously (Withers and Mansfield 2005). The paired-choice test includes two treatments (i.e., hosts or prey) being offered simultaneously to the biological control agent. In our tests, the target prey (HWA) was always paired with a non-target prey. We used both no-choice and paired-choice for ovipositional preference and no-choice tests for adult feeding and larval development. Both no-choice and choice tests contribute to information on possible ecological host range of the biological control agent and ideally both should be used in combination (Withers and Mansfield 2005).

Estimation of physiological host range examines the suitability of a candidate biological control agent to survive and complete development on a test host/prey. No-choice larval development tests are able to determine physiological host range and may be more restrictive than no-choice oviposition tests. Physiological host range testing can be challenging when assessing endoparasitoids as it requires observing whether the parasitoid develops and emerges from a test species that has been previously accepted by a female in an oviposition test (Van Driesche and Murray 2004a; Withers and Mansfield 2005). However with a predator, eggs can be transferred easily onto test prey and assessed for feeding and development (Zilahi-Balogh *et al.* 2003). We were able to assess host suitability for larval development to the adult stage. In our case, even though *L. nigrinus* developed to the fourth instar on several non-target hosts, it was only on HWA that this predator developed to the adult stage.

No-choice tests are important in host range testing because negative results can provide good evidence that a test species is not likely to be a field host. Host acceptance in a no-choice test can identify low ranked hosts missed in choice tests. Choice tests are useful in ranking order of preference within a list of possible hosts (Van Driesche and Murray 2004a). With choice tests, we expect a bigger difference in predation or oviposition between target and non-target (lower ranked hosts) (Withers and Mansfield 2005). In our oviposition tests, *L. nigrinus* accepted more non-target hosts than in the paired-choice tests. In the paired choice tests, none of the non-adelgid test prey were accepted as hosts for oviposition. This is consistent with what we expect.

Physiological and behavioral factors can influence the outcome of host range lab assays whether they are choice or no-choice (Withers and Mansfield 2005). Several relevant to our study system are discussed.

Prior experience. A confounding factor in interpretation of results from no-choice and choice tests is prior experience to host or prey (Withers and Mansfield 2005). Studies on both parasitoids and predators have shown there is an enhanced responsiveness in foraging behavior with prior experience to that host (prey) or volatile (Van Driesche and Murray 2004a; Withers *et al.* 2000; Withers and Browne 2004; Withers and Mansfield 2005).

A weakness in our test design is prior experience of adult *L. nigrinus* to HWA prior to tests. *Laricobius nigrinus* adults used in host specificity tests were field collected and therefore were preconditioned to the target prey. This has introduced bias in favor of the target prey (HWA). Though not ideal because of preconditioning of *L. nigrinus* to HWA, it was a practical reality in our system. *Laricobius nigrinus* is a difficult species to rear in the laboratory because of the obligatory aestival diapause exhibited by adults. We initially experienced high mortality in aestivating adults in laboratory culture. A mass rearing protocol has subsequently been developed for *L. nigrinus* (Lamb *et al.* 2005b), but it can only be kept in culture if reared on HWA. No artificial diet has been developed for this species yet. Withers and Browne (2004) suggested that predators and parasitoids should be reared and maintained on species other than the target host (prey) or on artificial diet if possible in order to minimize any experience-induced bias in favor of the target species, especially in the context of choice tests. The use of artificial diet to rear insects can creates some inherent problems because such diets are seldom optimal for development.

Time dependent effects. The period of food or oviposition site deprivation can have major effects on the acceptance threshold of a biological control agent to host cues (Browne and Withers 2002). The consequence of host deprivation is that deprived insects may accept a wider range of hosts than non-deprived individuals (Browne and Withers 2002; Withers and Mansfield 2005). In our studies, beetles were deprived of prey for 12 h prior to feeding tests, but were not deprived prior to oviposition tests. Had females been deprived of host prior to oviposition, would the outcome of the tests be different? We do not think so because of the longevity of *L. nigrinus.* Long-lived species are more likely to resorb eggs in the absence of suitable oviposition sites, as is typical of synovigenic species.

Physiological state of test insects. An important consideration in all bioassays with insects is ensuring that all test insects are of a similar physiological age and have been exposed to the same conditions. When doing oviposition bioassays, it is important to have an understanding of the life history and reproductive biology of the biological control agent. In our case, we were dealing with a predator that is univoltine, and undergoes an obligatory aestival diapause for ~ 4 months of the year (Zilahi-Balogh *et al.* 2003). As mentioned earlier, practical considerations necessitated the use of field collected beetles. Beetles were collected in February, within the ovipositional period of *L. nigrinus* (Zilahi-Balogh *et al.* 2003).

Negative controls. Though not discussed by Withers and Mansfield (2005), the use of negative controls (arenas with no predators) in no-choice feeding tests and controls (with no prey) in oviposition tests are useful for interpretation of results (Van Driesche and Murray 2004b). Negative controls in a feeding test account for any mortality in prey not attributed by the biological control agent, while a no-prey control in an oviposition test can account for the potential of prey dumping in the absence of prey-related cues (Van Driesche and Murray 2004b). We did not include negative controls in our feeding tests or a no-prey control in our oviposition tests. In retrospect, we should have considered these controls, but do not think that it would change our findings. Had we used a no-prey control, and oviposition in this treatment was not significantly different from non-adelgid homopteran hosts, we might have been able to conclude that oviposition on these non-target hosts may be due to egg dumping

rather than host acceptance. In our feeding tests, we were not assessing mortality. We assessed the difference between the number of test prey eggs present before predator introduction and number of test prey eggs present after the predator was removed three days later.

STATISTICAL DESIGN

Hoffmeister (2005) argued that the problem in host range testing is assigning a probability of accepting the null hypothesis of no effect, i.e. that the biological control agent does not include a given non-target host into its host range. This may be impossible to prove with certainty, but what is required is utilizing an experimental design that aims at achieving accuracy and precision from the sample population that is tested. This requires a robust experimental design and decision by researchers on the magnitude of an effect that is desirable to be detected, appropriate sample size to use, and knowledge of the power of the statistics used (Hoffmeister 2005).

Statistical power. The power of a statistical test, defined as 1-β is the probability of rejecting the null hypothesis when the null hypothesis is false and should be rejected (Zar 1984). Power is dependent on the α-level, variance, sample size (n) and effect size (Quinn and Keough 2002). Power analysis can be done *a priori*, for a given level of variability, sample size and power (0.80 is common) to determine how big the change (i.e., effect size) is needed before it would be detected as significant (Hoffmeister 2005; Quinn and Keough 2002).

In our study, preliminary no-choice and paired-choice oviposition tests were done to determine an appropriate length of time to use for a bioassay that allowed for adequate oviposition to occur without host plant material desiccating or having to add additional host material. The number of replicates used for these preliminary tests were n=12 and n=20. Using the variance from the preliminary tests, we could have conducted power analysis to calculate the minimum detectible effect size for a given level of power, or calculate sample size to decide on how much replication is necessary given a level of power, variability, effect size and α (α=0.05 is standard) (Quinn and Keough 2002). Instead, after appropriate analyses of the preliminary tests, we determined that n=12 was a reasonable sample size to get a significant treatment effect. Sample sizes in oviposition tests ranged between n=11 and n=20. We used n=7 in the no-choice feeding test. The limited sample size is this case was due to the limited availability of test predators. Even with this limited number of replications, when we compared number of eggs eaten by *L. nigrinus* when adult predators were presented with eggs of either target prey (HWA) or non-target prey, the predator consumed significantly more HWA eggs than two of the three non-target prey. Though not statistically significant, *L. nigrinus* adults consumed ~ 51% fewer non-target *A. abietis* eggs than target HWA eggs (Zilahi-Balogh *et al.* 2002). A larger sample size might have shown a significant difference in predator consumption between HWA and *A. abietis* eggs.

Statistical analysis. Both paired-choice and no-choice tests were used in our study. The response variable in these tests is quantitative (i.e., number of eggs laid, number of prey consumed). Therefore ANOVA and paired-t tests are an appropriate choice as long as data are normally distributed and there is homogeneity of variance (Horton 1995; Zar 1984). Prior to analysis, data were examined for normality using the Shapiro-Wilk W Test and for homogeneity of variance using Levene's test for Equality of Variance (SAS 1989). The Shapiro-Wilk

test was done on the difference between paired observations in the paired-choice tests. Transformation of data using log (x+1) prior to analysis was done as necessary to correct for heterogeneity of variance and/or non-normal sample distributions. Parametric tests on transformed data were selected over non-parametric tests as they are more powerful than non-parametric tests.

Pseudoreplication. Pseudoreplication is defined as the use of inferential statistics to test for treatment effects with data from experiments where either treatments are not replicated (though samples may be) or replicates are not statistically independent (Hurlbert 1984). If treatments are spatially or temporally segregated, if replicates of a treatment are interconnected somehow, or if replicates are only samples from a single experimental unit, then replicates are not independent (Hurlbert 1984). It is important to determine the experimental unit. Steel and Torrie (1980) define the experimental unit as the unit to which one application of a treatment is applied. The treatment is the procedure whose effect is to be measured and compared with other treatments (Steel and Torrie 1980). For all experiments in this study, the experimental unit was an individual *L. nigrinus* adult, male-female pair, or egg (larva) in a petri dish. The treatment was the host/prey material (host plant with associated homopteran prey) in which the predator was exposed. Pseudoreplication did not apply to our study.

GENETICS: RELATION OF LOCAL POPULATIONS TO WHOLE SPECIES – IMPLICATIONS FOR HOST RANGE TESTS

In classical biological control it has been common practice to introduce natural enemies from many geographic locations (Unruh and Woolley 1999). However, it has been well documented that different populations have shown differences in host affinities and behavior (Hopper *et al.* 2005, and references within). The term biotype has commonly been used for populations that display differences in some biological attributes (Unruh and Woolley 1999). Diehl and Bush (1984) categorized insect biotypes by their genetic polymorphisms, non-genetic polyphenisms, geographic variation and host races. Molecular genetics provides tools to unraveling this variation. Hopper *et al.* (2005) discussed the implications of using distinct populations in host range testing.

All collections of *L. nigrinus* evaluated under quarantine were collected from the same site in a HWA infested western hemlock seed orchard near Victoria, British Columbia, Canada and thus would be considered the same 'local' population. Although this may not represent all existing populations of the species, it allowed for the elimination of inter-population variations.

CONCLUSIONS

A summary and interpretation of our test results is shown in Table 2. Although adult feeding tests indicated feeding acceptance on other adelgid species in addition to HWA, no-choice larval development tests showed that *L. nigrinus* only completed development to the adult stage on HWA. Based on the larval development tests, we concluded that these adelgid species are not suitable hosts for completion of larval development of HWA. If we solely based our conclusions on the paired-choice and no-choice oviposition and no-choice adult feeding

test, our interpretation would be that the other test adelgids would be inside the host range of *L. nigrinus* (see Table 4, Sands and Van Driesche 2000). Oviposition and feeding tests are concordant with larval development tests. We consistently see HWA ranked as the most preferred host. Non-host adelgids rank second, while non-adelgid hosts rank at the bottom.

Table 2. Summary of results of acceptance and suitability tests of Homoptera prey screened as hosts of *Laricobius nigrinus* (from Zilahi-Balogh *et al.* 2002).

	Acceptance[a]		Suitability[a]	
Test species	**Oviposition**	**Adult feeding**	**Larval development**	**Final host status[b]**
Adelgidae				
Adelges tsugae Annand	+	+	+	Yes
Adelges piceae (Ratzeburg)	+	+	-	No
Adelges abietis (L.)	+	+	-	No
Pineus strobe (Hartig)	+	+	-	No
Aphididae				
Cinara pilicornis (Hartig)	+	x	-	No
Myzus persicae (Sulzer)	-	x	x	No
Diaspididae				
Chionaspis pinifoliae (Fitch)	+	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.

Laboratory host range tests are further strengthened by the synchrony between ovipositional period of *L. nigrinus* and presence of suitable oviposition sites (i.e., HWA ovisacs) in the field (Zilahi-Balogh *et al.* 2003). There is poor synchrony between ovipositional period of *L. nigrinus* and availability of suitable oviposition sites with the non-target adelgids tested (Arthur and Hain 1984; Craighead 1950; Friend and Wilford 1933; Gambrell 1931; Johnson and Lyon 1991; USDA 1985). When this information is combined with the larval development tests, we predict that these adelgids are outside of the ecological host range of *L. nigrinus*. We conclude that adult feeding by *L. nigrinus* may occur under natural field conditions on the other test adelgids, but that these hosts are phenologically and/or physiologically unsuitable for larval development.

Though not without flaws, we believe our host specificity tests provide a consistent pattern in regards to the predicted ecological host range of *L. nigrinus*.

ACKNOWLEDGEMENTS

We thank Ashley Hillyer and Canadian co-operators at Natural Resources Canada Canadian Forest Service, Pacific Forestry Centre for technical assistance. This research was supported

by grants from the USDA Forest Service, Forest Health Technology Enterprise Team and Special Technology Development Program.

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