

Comparison between a native and exotic adelgid as hosts for *Laricobius rubidus* (Coleoptera: Derodontidae)

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Abstract

Laricobius rubidus LeConte, native to eastern North America whose primary host is *Pineus strobi* Hartig, has been observed in association with *Adelges tsugae*, an exotic and lethal pest of hemlock trees in eastern North America. *Pineus strobi* and *A. tsugae* were used to evaluate host preference and suitability of this predatory beetle to determine in part its suitability as a potential biological control agent of *A. tsugae*. In a paired-choice oviposition test, *L. rubidus* preferred to oviposit in *P. strobi* ovisacs over those of *A. tsugae*. However, in the no-choice oviposition test, there was no significant difference in the mean number of eggs laid by *L. rubidus* females in *P. strobi* or *A. tsugae* ovisacs. There were no significant differences in larval developmental time or survivorship for *L. rubidus* reared on a diet of either *P. strobi* or *A. tsugae*. *Laricobius rubidus* completed development to the adult stage on *A. tsugae* indicating that it is a suitable host and therefore has the potential to contribute to biological control of *A. tsugae* in the eastern United States. The introduction of a congener, *Laricobius nigrinus* to eastern North America may result in competition with *L. rubidus* on *A. tsugae*, but the inability of *L. nigrinus* to complete development on *P. strobi* suggests that the two congeners will not compete on the primary host of *L. rubidus*.

Keywords: *Laricobius rubidus*, *Derodontidae*, *Pineus strobi*, *Adelges tsugae*, *Adelgidae*, host suitability, biological control

Introduction

The genus *Laricobius* Rosenhauer is one of four genera comprising the family Derodontidae (Lawrence & Hlavac 1979). Three genera *Peltastica* Mannerheim, *Derodontus* LeConte, and *Nothoderodontus* Crowson feed solely on fungi or fungal metabolites, while *Laricobius* has evolved a predatory habit (Leschen 2000). Members of this genus prey on members in the family Adelgidae (Hemiptera) (Lawrence & Hlavac 1979). There are four species of *Laricobius* present in North America (Lawrence 1989). *Laricobius erichsonii* Rosenhauer was imported from

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Europe to North America in the 1950s for biological control of balsam woolly adelgid, *Adelges piceae* Ratzeburg (Clark & Brown 1960; Brown & Clark 1962). *Laricobius nigrinus* Fender is native to the Pacific Northwest (Fender 1945). It was recently found in association with the hemlock woolly adelgid, *Adelges tsugae* Annand (Zilahi-Balogh et al. 2003a). This close association has prompted examination of this beetle as a potential biological control agent of *A. tsugae* in the eastern United States (Zilahi-Balogh et al. 2002; Zilahi-Balogh et al. 2003b), where *A. tsugae* is exotic and lethal to eastern hemlock, *Tsuga canadensis* (L.) Carrière, and Carolina hemlock, *Tsuga caroliniana* Engelmann. *Laricobius laticollis* Fall has been collected from Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco in the Pacific Northwest (Lawrence & Hlavac 1979), but no prey associations have been reported to date.

Laricobius rubidus LeConte is the only *Laricobius* sp. native to eastern North America with a distribution extending from the District of Columbia north to New Brunswick and west to Michigan (Brown 1944; Lawrence 1989) and more recently as far south as North Carolina (Wallace & Hain 2000). The primary host of *L. rubidus* is the pine bark adelgid, *Pineus strobi* Hartig (Hemiptera: Adelgidae), but *A. piceae* (Clark & Brown 1960) has also been reported as a host. *Laricobius rubidus* adults have also been infrequently collected from *A. tsugae* infested eastern hemlock in Connecticut (Montgomery & Lyon 1996) and northern North Carolina and southern Virginia (Wallace & Hain 2000), but larvae have not been reared on *A. tsugae* to determine if it is a suitable host.

The life history of *L. rubidus* was studied in New Brunswick (Clark & Brown 1960). It is similar to that reported for *L. erichsonii* (Clark & Brown 1958; Franz 1958; Brown & Clark 1962). Adults are active between late March and early June with peak activity between mid-April to mid-May in eastern Canada (Clark & Brown 1960), Connecticut (Montgomery & Lyon 1996) and Virginia (G. Zilahi-Balogh, pers. observ.) Eggs are laid within the woolly ovisacs of *P. strobi*. Four larval instars are present in the field for a period of 2 months (Clark & Brown 1960), disappearing from the branches by late June (G. Zilahi-Balogh, pers. observ.). Mature larvae migrate to the soil to pupate (G. Zilahi-Balogh, pers. observ.). Adults undergo an aestival diapause and become briefly active in October to early November in Connecticut (Montgomery & Lyon 1996) and Virginia (G. Zilahi-Balogh, pers. observ.), after which the adults disappear from the branches. In Virginia, *L. rubidus* adults have been recovered from duff samples from a mixed hemlock – white pine stand in late January (C.D. Broeckling, unpublished data). Our observations reveal that *L. rubidus* adults behave similarly to *L. erichsonii* by migrating from the branches to the duff where they undergo a hibernal diapause. Clark & Brown (1960) reported that the life cycle is fairly well synchronized with that of *P. strobi*.

Our interest in studying *L. rubidus* coincides with our on-going research in evaluating a congener, *L. nigrinus* for potential biological control of *A. tsugae* in the eastern United States. With the recently observed association of *L. rubidus* on *A. tsugae*-infested eastern hemlock several questions are considered: Is *A. tsugae* a suitable host for *L. rubidus*? What is the preferred host of *L. rubidus*? Can *L. rubidus* contribute to biological control of *A. tsugae* in the eastern United States? Can we expect any intra-guild interactions between *L. rubidus* and *L. nigrinus*, a congener currently being evaluated for classical biological control of *A. tsugae* in the eastern United States? The objective of this study was to determine host acceptance, host suitability and development of *L. rubidus* on two adelgid hosts – *Pineus strobi* and

A. tsugae. In addition, we compare developmental times of *L. rubidus* and *L. nigrinus* reared on *A. tsugae*.

Materials and methods

Laricobius rubidus adults used in this study were field collected between 6 and 30 April 2001 using a beating tray from *A. tsugae* infested eastern hemlocks. The trees were located in a 0.2-ha research plot at the Virginia Polytechnic Institute and State University, Prices Fork Research Facility, Blacksburg, VA. The plot was comprised of ~78% eastern hemlock (dbh 2.5 to 6.3 cm), ~20% Fraser fir, *Abies fraseri* (Pursh) Poir (dbh 1.3–7.5 cm), and ~2% eastern white pine, *Pinus strobus* L. (dbh 7.5 to 10 cm). Infestation levels of *A. tsugae* on eastern hemlock ranged from low to high (<1 to >4 *A. tsugae* per cm twig). Eastern white pine was lightly infested with *P. strobi* (<1 adelgid per cm² of bark surface). Fraser fir had gouting on branches which is evidence of *A. piceae* attack, but no live adelgids were observed.

Insects were maintained on field collected *A. tsugae* infested eastern hemlock twig cuttings in an environmental chamber at 18°C, 12:12 (L:D) h, and 70–87% RH before tests as no artificial diet is available for this species. Adults were separated by sex and females were used in oviposition preference experiments.

Adelgid infested eastern hemlock used in lab studies was field collected from various sites within the Blacksburg Ranger District of the Jefferson and Washington National Forests, Giles Co., VA. *Pineus strobi* collected from ornamental eastern white pine trees in Blacksburg was used to infest potted saplings of eastern white pine (0.5–1 m height). These saplings were moved to a greenhouse (~24°C) in spring 2001 to accelerate development and egg laying by over-wintering *P. strobi* before being used in tests. Eggs of adelgids are typically laid in a mass by a sessile female and surrounded by flocculence (waxy/woolly filaments). This life-stage was selected to test for host acceptance and suitability because we observed *L. rubidus* females laying eggs within the woolly ovisacs of *A. tsugae*.

No-choice (single-prey) and paired-choice oviposition experiments were conducted 21 to 29 April 2001 to evaluate the effect of prey type on acceptance and preference by *L. rubidus* females for oviposition. A no-choice test was conducted with either *Adelges tsugae* ($n=5$) or *Pineus strobi* ($n=5$) in 150 × 15 mm polystyrene petri dishes (Lab-tek™, Nalge Nunc International, Rochester, NY). In the paired-choice test, *P. strobi* was paired with *A. tsugae* in petri dishes ($n=8$) lined with a single layer of filter paper (Thomas Scientific, Swedesboro, NJ) at the base containing a 1-cm length cotton wick moistened with water as a drinking source for adults. Individual *L. rubidus* females were randomly assigned to petri dishes that contained either a single bouquet of associated host plant infested with associated adelgid species (no-choice) or two adjacent bouquets of host plant with associated adelgid species (paired-choice). A bouquet was made up of two to four terminal tip branches (10–12 cm length) of adelgid-infested hemlock or white pine held together by wrapping the cut end with parafilm to prevent the twigs from drying out. The same numbers of adelgids (50–60 adelgid ovisacs per bouquet) were used in each test. Excess adelgids were removed from the twigs using fine forceps. Duration of each experiment was 3 days. At the end of each test, the number of *L. rubidus* eggs deposited within adelgid ovisacs on each host plant bouquet was tallied by using a dissecting microscope and prying open each ovisac with fine probes. Experiments were conducted at 18°C, 12:12

(L:D) h and 75–87% RH in an environmental chamber using a completely randomized experimental design.

Development time of *L. rubidus* on either *A. tsugae* or *P. strobi* as prey was followed from the egg to pre-pupal stage at 18°C; 12:12 (L:D) h in an environmental chamber. Eggs from oviposition tests (~3 days old) were used for this study. A single egg, attached to a section (2–4 cm) of adelgid-infested host twig was followed for hatch and larval development in a 50 × 9-mm tight fitting polystyrene petri dish (Falcon®, BD Biosciences, Franklin States, NJ). Petri dishes contained a 2-cm diameter ventilation hole made in the lid that was covered with PeCap® polyester mesh (0.14 mm²) (Sefar America Inc., Kansas City, MO). 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. Life stages were observed using a dissecting microscope. Eggs were checked daily for hatch. Larvae were inspected daily for survival and molt by presence of an exuvium. Larvae were provided fresh prey (ovisacs of host prey on twig cuttings) at each inspection. When mature larvae left the food source in search for a suitable pupation site, they were recorded as pre-pupae. Pre-pupal and pupal development was not followed as these stages occur in the soil.

In a second experiment, egg, larval, pre-pupal and pupal development of *L. rubidus* were determined at 15°C, 12:12 (L:D), and egg development at 18°C 12:12 (L:D) in environmental chambers using *A. tsugae* as the host to enable direct comparisons with *L. nigrinus* development time at those temperatures (Zilahi-Balogh et al. 2003b). *Laricobius rubidus* eggs (≤24 h old) were retrieved from oviposition containers described in Zilahi-Balogh et al. (2003b) held at either 15 or 18°C and each egg was assigned to a 50 × 9-mm petri dish described above. In the 15°C chamber, once the pre-pupal stage was reached, sterilized peat (Premier Horticulture Inc., Quakertown, PA) moistened with methyl paraben solution was placed at the base of each petri dish and acted as a pupation medium. Daily inspections continued until adult eclosion.

Statistical analyses

Data for all studies were tested for heterogeneity of variance and/or non-normal sample distributions (Zar 1984). No-choice and larval development tests were analyzed using a *t*-test. In the paired-choice test, a paired *t*-test was used to determine prey preference for oviposition. Larval survivorship on the two adelgid diets was analyzed using the chi square statistic for contingency tables. Developmental time data were analyzed using descriptive statistics. All statistical tests were carried out using SAS® (SAS 1989). In all cases $\alpha = 0.05$.

Results and discussion

In the 3-day no-choice oviposition test, there was no significant difference in the mean number of eggs laid by *L. rubidus* females in *P. strobi* or *A. tsugae* ovisacs (*t*-statistic = -1.79; DF = 8; *P* = 0.11). The mean (±SE) number of eggs laid in *P. strobi* and *A. tsugae* ovisacs were 15.0 ± 2.65 and 9.0 ± 2.07 eggs, respectively. In contrast, in the 3-day paired choice test, *L. rubidus* females laid significantly more eggs in *P. strobi* ovisacs than in *A. tsugae* ovisacs (*t*-statistic = 2.54; df = 1, 7; *P* = 0.04). The mean (±SE) number of eggs laid in *P. strobi* and *A. tsugae* ovisacs were 9.13 ± 2.68 and

1.50 ± 0.50 eggs, respectively. In the paired-choice test, oviposition was more than six times greater on *P. strobi* than on *A. tsugae*, indicating an ovipositional preference for *P. strobi*.

There were no significant differences in larval development times for *L. rubidus* reared on a diet of *P. strobi* or *A. tsugae* at 18°C (*t*-statistic = -0.34; DF = 32; *P* = 0.74). In addition, survival of *L. rubidus* was found to be independent of diet ($\chi^2_{0.05, 1} = 1.47$; *P* = 0.23). Percent survivorship and mean duration on the two diets are provided in Table I. Because *L. rubidus* completed development to the adult stage on a diet of *A. tsugae*, it is considered to be a physiologically suitable host.

Mean development time from egg to adult for *L. rubidus* (56.4 days) fed a diet of *A. tsugae* at 15°C is shorter than what had been reported for *L. nigrinus* (64.8 days) at 15°C (Zilahi-Balogh et al. 2003b). However, egg development of *L. rubidus* (5.9 days) at 18°C was longer than egg development time of *L. nigrinus* (4.3 days) at the same temperature (Zilahi-Balogh et al. 2003b). Mean developmental times of various life stages of *L. rubidus* fed on *A. tsugae* at 15°C are shown in Table II. Comparisons between developmental times between *L. rubidus* and *L. nigrinus* suggest that thermal requirements of the two species are similar.

Laricobius rubidus develops as well on *A. tsugae* as it does on its primary host, *P. strobi*. The extent that *A. tsugae* is an ecological host is less clear as *L. rubidus* readily oviposited in *A. tsugae* ovisacs, and developed to the adult stage on *A. tsugae*. However, it showed a preference for *P. strobi* in paired-choice oviposition tests. This is in contrast to *L. nigrinus* which prefers to oviposit in *A. tsugae* ovisacs over other adelgids (including *P. strobi*) in both the no-choice and choice situation (Zilahi-Balogh et al. 2002). In addition, *L. nigrinus* only completes development to the adult stage on *A. tsugae* (Zilahi-Balogh et al. 2002). Observations of adult *L. rubidus* by others (Montgomery & Lyon 1996; Wallace & Hain 2000) suggest that *A. tsugae* is used by

Table I. Survivorship and duration (days) of *Laricobius rubidus* larvae feeding on either *Pineus strobi* or *Adelges tsugae* from egg hatch to mature larval stage, at 18°C, 12:12 (L:D) h photoperiod, and 75–87% RH

Target	Initial no. individuals	Survivorship to next stage (%) ^a	Duration (days) mean ± SE ^b
<i>Pineus strobi</i>	35	42.9a	13.6 ± 0.51a
<i>Adelges tsugae</i>	33	57.6a	13.4 ± 0.47a

^aMeans followed by the same letter in the same column are not significantly different at $\alpha = 0.05$, Chi-square test. ^bMeans followed by the same letter in the same column are not significantly different at *P* = 0.05, *t*-test.

Table II. *Laricobius rubidus* developmental time (days) (mean ± SD) for each life stage at 15°C and egg development at 18°C, 12:12 (L:D) h on a diet of *A. tsugae*

Temperature	15°C		18°C	
	<i>n</i>	Mean ± SD (days)	<i>n</i>	Mean ± SD (days)
Egg	54	8.3 ± 0.7	32	5.9 ± 0.5
Larva	43	16.0 ± 1.6	–	–
Pre-pupa	28	14.7 ± 2.0	–	–
Pupa	19	18.5 ± 1.0	–	–
Egg to adult	18	56.4 ± 2.0	–	–

L. rubidus as an alternative host. While the introduction of *L. nigrinus* in the eastern North America may result in competition with *L. rubidus* on *A. tsugae* infested hemlock, the inability of *L. nigrinus* to complete development on *P. strobi* (Zilahi-Balogh et al. 2002) suggests that the two congeners will not compete on the primary host of *L. rubidus*.

Adelges tsugae is a suitable host for *L. rubidus*. However, based on our observations and surveys conducted by others (Montgomery & Lyon 1996, Wallace & Hain 2000), *L. rubidus* densities in the natural forest setting are currently too low to significantly impact populations of *A. tsugae*. Unless augmentative releases are made with *L. rubidus*, this native predator is not likely to contribute much to reducing *A. tsugae* populations in the natural forest setting.

Further research on the phenology of *L. rubidus* in relation to that of *A. tsugae*, and investigations on intra-guild interactions between *L. rubidus* and other beetle predators currently being evaluated as potential biological control agents of *A. tsugae* in the eastern United States is needed.

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