

Emergence of *Laricobius nigrinus* (Fender) (Coleoptera: Derodontidae) in the North Georgia Mountains¹

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Abstract Hemlock woolly adelgid, *Adelges tsugae* Annand, is currently found throughout most of the range of eastern hemlock, *Tsuga canadensis* (L.) Carrière. Biological control agents have been released in attempts to control this pest, but how different climates influence the efficacy and survival of these agents has not been studied. One predatory beetle of *A. tsugae*, *Laricobius nigrinus* Fender, is native to the Pacific Northwest and, therefore, experiences a much different summer climate in the north Georgia mountains. To better understand survival of this predator as it aestivates in the soil, 5 mesh cages were set up at each of 16 sites with 4 sites located at an elevation below 549 m, 4 sites between 549 m – 732 m, 5 sites between 732 m – 914 m, and 3 sites over 914 m. At each site 30 larvae were placed inside one of the cages during March, April, or May on a bouquet of adelgid infested hemlock twigs, and emergence of adults was monitored in the fall. Of the 1440 larvae placed at the 16 sites, only 4 adult beetles emerged between 06 October 2012 and 05 November 2012. The overall success rate remains unknown, and more research is needed to assess the efficacy of *L. nigrinus* as a biological control agent in Georgia.

Key Words Hemlock woolly adelgid, *Adelges tsugae*, biological control, emergence

Hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae), is an invasive, aphid-like insect that feeds on the sap of hemlock trees and is devastating eastern (Canadian) hemlock, *Tsuga canadensis* (L.) Carrière, and Carolina hemlocks, *Tsuga caroliniana* Engelmann, in the eastern United States. *Adelges tsugae* can be found as far north as Maine, south to Georgia, and west into Tennessee and Kentucky. Hemlock mortality occurs in 4 - 6 years (Mayer et al. 2002, McClure 1991) and faster in the southern range due to other factors such as drought stress (Ward et al. 2004) and mild winters that favor *A. tsugae* survival (Trotter and Shields 2009).

To save a portion of the mature hemlock trees in north Georgia, the U.S. Forest Service created 114 Hemlock Conservation Areas (HCAs) within the Chattahoochee National Forest to be treated either with biological controls and/or insecticides (Fig. 1). One of the major biological control agents reared and released in Georgia (since 2007) is *Laricobius nigrinus* (Fender) (Coleoptera: Derodontidae), a small black beetle native to the Pacific Northwest where it feeds on *A. tsugae* (Zilahi-Balogh et al. 2002). It is univoltine, with winter-active adults laying eggs when overwintering

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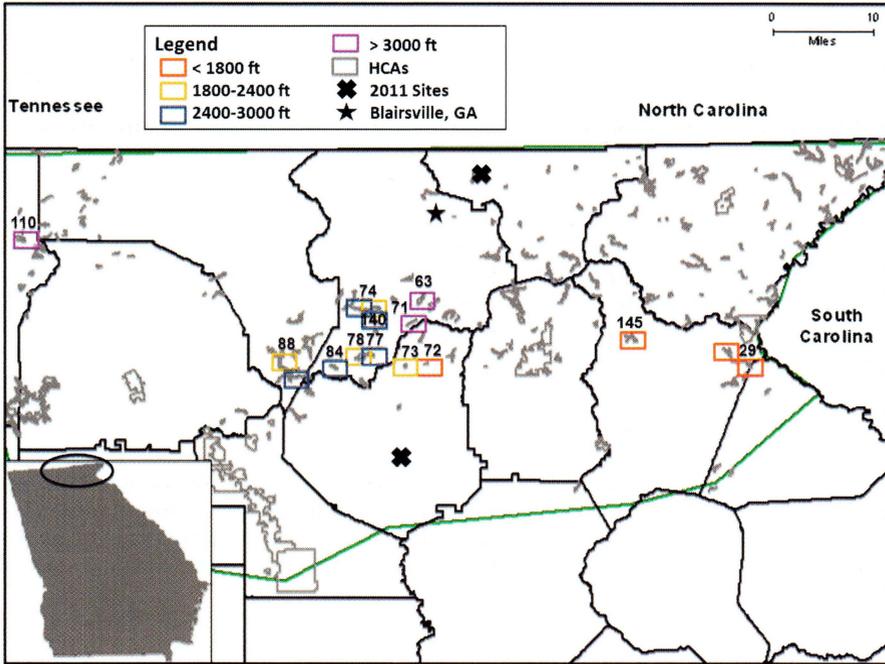


Fig. 1. A map of *L. nigrinus* soil aestivation sites in north Georgia displaying 2011 sites, 2012 sites separated by elevation bracket, hemlock conservation areas (HCAs) in Georgia, and the location of Blairsville, GA where temperature data was compared with Seattle, WA.

A. tsugae sisten egg-laying occurs. The beetle larvae feed on the adelgid eggs and, upon maturation, drop to the soil to pupate (Zilahi-Balogh et al. 2002).

Whereas *L. nigrinus* has been studied for cold tolerance (Mausel et al. 2010, 2011) and north Georgia is comparable to Seattle, WA, in terms of cold hardness zone, the effect of heat/moisture tolerance of pupae/adults in the soil has never been considered. The summer climate of Seattle (mean high of $23 \pm 3^\circ\text{C}$ in August 2012; source www.farmersalmanac.com/) is quite different from that of north Georgia (mean high of 28.3°C in Blairsville, GA [elevation 590 m] in August 2012; source www.georgiaweather.net). Soil temperatures in a Douglas fir/western hemlock stand in southwestern Washington (elev. 300 m) did not rise above 16°C throughout the year (Devine and Harrington 2007). The annual precipitation also differs with the Pacific Northwest receiving an average annual precipitation of 988 ± 139 mm and Seattle receiving 918 mm with dry summers (monthly avg. 53.2 ± 27 mm, March through October) and wet winters (monthly avg. 123.1 ± 28 mm, November through February; source www.weatherdb.com/). North Georgia receives an average annual rainfall of 1426 ± 81 mm with Blairsville annual precipitation being 1510 mm distributed equally throughout the year with a monthly average of 120.5 ± 17 mm from March through October and $136.5.8 \pm 14$ mm from November through February (source www.weatherdb.com/).

The phenology of *A. tsugae* in Georgia varies from the Pacific Northwest in that sisten adults are found 2 months later, the occurrence of progredien adults and their sisten eggs ends 1 month earlier, and the end of the sisten generation aestivation period occurs a month later in Georgia (Joseph et al. 2011, Zilahi-Balogh et al. 2003a). In a laboratory study, *L. nigrinus* prepupae were unable to complete development when temperatures were higher than 21°C (Zilahi-Balogh et al. 2003b). Based on average temperatures in Georgia, *L. nigrinus* would need to complete development by mid-April to avoid temperatures exceeding that. In Georgia, with their life cycle being synchronized with *A. tsugae*, adult *L. nigrinus* emerge when *A. tsugae* breaks its summer aestivation in October (Joseph et al. 2011). Thus, *L. nigrinus* must survive 2 months longer in Georgia than in its native range under soil moisture and temperature conditions much different from where it evolved in the Pacific Northwest.

In one study on factors influencing aestivation of *L. nigrinus*, fewer adults emerged when aestivating adult were stored at 20°C (26.7%) compared with 15°C (37.3%) and 10°C (44.4%) in a laboratory setting. Temperature had a significant influence on time spent in the soil, with those at higher temperatures remaining in the soil longer (Lamb et al. 2007). Thus, the objective of this study was to determine how timing of *L. nigrinus* pupation in the spring and soil temperatures during their soil aestivation period in Georgia affects their survival prior to adult emergence in the fall.

Materials and Methods

Preliminary study. During 2010, air and soil temperatures were monitored at 5 sites ranging in elevation from 497 m to 919 m using HOBO® Micro Station Data Loggers (H21 - 002) with a temperature/relative humidity data logger (U23 - 002) and temperature sensor (U23 - 004)(Onset, Bourne, MA). Air temperature was recorded at a height of about 3 m near the bole of the tree and soil measurements were taken from a depth of 5 cm.

A trial of soil emergence boxes was set up in Dahlenega, GA (elv. 442 m) and Young Harris, GA (elv. 586 m) in 2011. The trial consisted of 5 aluminum boxes, with no top or bottom (25.4 cm × 18 cm × 30.5 cm; L x W x H) placed in the ground 15 - 20 cm deep at each location in January, because all *Laricobius* spp. should have emerged by that time, and the boxes were covered with no-see-um netting (Mosquito Curtains, Inc., Atlanta, GA) to prevent new larvae from dropping in. HOBO® Micro Station Data Loggers with temperature sensors attached were used at each site to record soil (depth 5 cm) and air temperatures (height 2 m) from August through November.

Boxes at each site were randomly assigned one of the following treatments: (1) no larvae (control); (2) 55 prepupae during mid-to-late March; (3) 55 prepupae during early April; (4) 55 prepupae during late April/early May; or (5) 55 prepupae during late May. The prepupae were obtained from the University of North Georgia (Dahlenega, GA) rearing laboratory, where they were collected as they dropped from infested foliage using a similar funnel design as described in Salom et al. (2012). The containers were checked twice daily for prepupae which were immediately placed in a moist 1:1 peat/sand mixture and then placed in the field within 36 h. Once the larvae were placed in the soil boxes a rectangular lid made out of no-see-um netting, over 1.27 cm hardware cloth to support the netting, was attached (Fig. 2). Prior to emergence in the fall, an inner pyramid (Fig. 2) was created to direct emerging adults into a collection area.



Fig. 2. 2011 *L. nigrinus* emergence cage design with an inner pyramid to funnel beetles into the rectangular collection area.

The containers were checked once a month until October. In October they were checked twice a month, weekly in November, and then twice a month again in December before the site was dismantled.

2012 emergence study. Sixteen sites in Hemlock Conservation Areas (HCAs) were selected with 4 located at an elevation below 549 m, 4 between 549 - 732 m, 5 between 732 - 914 m, and 3 over 914 m (Fig. 1, Table 1). Areas that had previous releases were selected to compare emergence data to recovery of the larval stage from hemlock foliage in the same areas (Jones 2013). In January 2012, 4 rectangular aluminum enclosures, 30 cm × 41 cm × 10 cm (L x W x H), were inserted 5 - 8 cm into the ground at each of the 16 sites using an edger (Hound Dog "Edge Hound"®, Griffon Corp., New York, NY) to initially cut a slit into the ground without disturbing the duff layer. A hardware cloth plus no-see-um netting lid was placed over each enclosure to prevent *Laricobius* spp. in the area from dropping into the box (Fig. 3a).

Laricobius nigrinus larvae for the study were obtained from a laboratory colony at the University of North Georgia (Dahlonoga, GA) rearing facility that was started with 400 wild-captured *L. nigrinus* adults obtained from field insectaries in Boone, NC. Oviposition jars containing 20 *L. nigrinus* adults and a bouquet of 10 *A. tsugae*-infested hemlock twigs were held at 6°C, 65% R:H, and 12:12h L:D. After 1 week, the adults were transferred to a new jar, and the bouquets were collected and placed, with an additional 20 adelgid-infested twigs, in 64.4-L clear plastic totes with locking lids

Table 1. *L. nigrinus* soil aestivation monitoring sites in north Georgia with 4 sites located at an elevation below 549m, 4 sites between 549m - 732m, 5 sites between 732m - 914m, and 3 sites over 914m.

HCA #	Site	Latitude	Longitude	Altitude (m)
29	Lower Panther	34.66906	-83.36742	225
29	Upper Panther	34.69642	-83.41708	449
145	Soque River	34.71582	-83.57611	457
72	Waters Creek	34.67639	-83.94282	526
88	Noontootla A	34.69694	-84.20783	654
78	Canada Creek 1	34.68727	-84.05750	718
74	Coopers Creek A	34.74991	-84.03668	724
73	Dockery Lake	34.67544	-83.97596	731
77	Canada Creek 2	34.68952	-84.04485	739
74	Coopers Creek B	34.75446	-84.03966	757
140	Mart Helton	34.74240	-84.02956	775
88	Noontootla B	34.64995	-84.17449	841
84	Blackwell Creek	34.64338	-84.04360	856
71	Slaughter Creek	34.74132	-83.95564	974
110	Lake Conasauga	34.85547	-84.64712	992
63	Wolf Pen Gap	34.76447	-83.95298	1024

(68 cm × 45 cm × 30 cm, 292,010, Sterilite, Townsend, MA). The totes were modified by cutting 38 cm × 23 cm holes in the longer sides and lid. The holes were then covered with no-see-um netting using hot glue. These rearing totes were held at 10°C, 65% RH, and 13:11h L:D for 1 week, 14°C the second week, and 16°C for the remainder of the rearing period. After 3 weeks fresh bouquets of 20 adelgid-infested twigs (15 - 20 cm long stuck in parafilm wrapped floral foam) to which 30 *L. nigrinus* larvae were added were taken to the field and placed in the designated cages at the appropriate times (March, April, or May). The whole bouquet was placed inside the field enclosures on a stand made of hardware cloth to elevate the twigs above the ground (Fig. 3d-e) and to allow the larvae to drop naturally to the soil. Bouquets remained in the cages for 1 month.

To monitor emerging adult *L. nigrinus*, rectangular cages slightly larger than the field enclosures (31 cm × 41 cm × 25 cm, L x W x H), were constructed from 1.27-cm mesh hardware cloth with no-see-um netting lining the inside and secured with hot glue (Fig. 3b, c, d). These were placed over the field enclosure. The lids from the field enclosures were removed, flipped over, and then used to cover the top of this cage (Fig. 3f). The no-see-um netting was at least 10 cm longer at the top and bottom to secure them to the ground, with 15-cm long nails, and to seal the top to the sides of the cages by rolling the excess material from the lid with that of the sides to create a



Fig. 3. 2012 *L. nigrinus* emergence cage design. The site was covered by a lid (a) to prevent any *Laricobius* spp. from dropping in. During the treatment time a rectangular box made of hardware cloth and no-see-um netting (b) was placed on top and secured with weather-proof duct tape (c). A stand made of hardware cloth (d) was set inside to elevate the bouquet (e). The lid was then flipped over and used as the lid for the box and secured with binder clips and the site was surrounded by a fence (f). A sleeve cage was used to determine survival of developing larvae (g).

tight seam that was held together with binder clips (Fig. 3f). White Outdoor Duck Tape® (1.88 in × 30 yd, Shurtech Brands, Avon, OH) was used to seal the no-see-um netting of the cage to the aluminum enclosure (Fig. 3c, d, e). The 4 cages at each field site were encircled with wire fencing to discourage disturbance by mammals.

Enclosures at each site were randomly selected to receive *L. nigrinus* larvae in: (1) March, (2) April, (3) May, or (4) not at all (control). The introduction of *L. nigrinus* to the box occurred within the third week of each month (March-May), and the cages were checked for emerging beetles monthly until October when they were checked twice per month. On the second visit in October, bouquets of 20 *A. tsugae*-infested hemlock twigs were placed in each container to provide a substrate for any emerging beetles to climb on to (Fig. 3e). Cages were then visited weekly to collect and replace the bouquets until the second week of December. During each visit the netting on the inside of the cage was checked for beetles and the bouquets were collected by quickly placing them into 7.6-L plastic ziplock bags, returned to the laboratory, and examined for adult *L. nigrinus*. Any beetles found were placed into 95% EtOH and stored in the freezer.

A variety of site parameters were measured to determine if they might be important factors in explaining where *L. nigrinus* was most likely to be successful. Soil temperature and moisture were measured at 5 cm depth using HOBO® Micro Station Data Loggers with temperature sensor and soil moisture sensor (S-SMD-M005). Additional

HOBO Pendant® Temperature/Alarm Data Loggers (UA-001 - 64) were placed 2 m above the emergence cages on the closest branch at each site to record air temperature. Measurements were taken every 4 h.

In December 2 leaf litter samples were obtained at each site by placing a rectangular aluminum enclosure (30 cm × 41 cm × 10 cm) on the ground in the vicinity of the cages and collecting all identifiable leaf/needle material from within the enclosure. The samples were then placed into paper bags and oven-dried at 100°C for 72 h and then immediately weighed. After the leaf litter was removed in the field, a soil core (5 cm × 10 cm) was obtained using an AMS soil core sampler with hammer attachment (77455, Forestry Suppliers Inc, Jackson, MS) and the organic layer was measured. Two additional soil samples were collected into 118 ml Glad® plastic containers with snap-on lids. Upon returning to the laboratory, 5 *Galleria mellonella* L. larvae were placed inside each of the 118-ml containers and held at room temperature for 7 - 10 days to determine the prevalence of parasitic fungi and nematodes. After that time the larvae were located and assessed as living or dead and for the presence of fungi or nematodes. Subsamples were sent off for pathogen identification (W. Gardner, Univ. of Georgia, Griffin).

Cage design test. In December 2012, 20 wild-captured *L. nigrinus* adults were introduced into the control cage on an *A. tsugae*-infested bouquet of hemlock at 12 sites to test how well the cages held beetles for collection. After 7 days the sides and lid of the cages were examined, adults were aspirated into vials, and the bouquets were quickly placed into 7.6-L plastic ziplock bags and examined at the laboratory.

Statistical analysis. Emergence success of *L. nigrinus* was analyzed using logistic regression to compare the likelihood of emergence with site parameters (elevation, maximum and minimum soil moisture and temperature, leaf litter weight, and depth of organic layer). Additional analysis using Poisson regression was conducted to compare site parameters with number of beetles recovered in a previous study (Jones et al. 2014). The data were overdispersed and were corrected using the “scale=deviance” option.

Results

Preliminary study: Average daily soil temperatures in 2010 exceeded 20°C for 110 days (June through October, max 28°C) at the lowest elevation and 43 days at the highest elevation (end of July through August, max 23°C). During the week 4 - 11 November 2011, four adults were collected from both sites (8 out of 440 total) and, in each case, they were collected from cages containing prepupae placed in March and early April. Average air temperature during the week of emergence was $9.5 \pm 5^\circ\text{C}$.

***L. nigrinus* emergence 2012.** Four beetles were recovered from emergence cages between 30 October and 1 November 2012. One beetle from the March treatment was found at Waters Creek (HCA# 72) a low elevation site < 548.64 m above sea level (Table 1, Fig. 1). Two beetles were recovered from sites between 548.64 m – 731.52 m elevation, 1 from the April treatment at the Coopers Creek A site (HCA# 74) and 1 from the May treatment at Noontootla (HCA# 88). One additional beetle from the April treatment was recovered at the Blackwell Creek site (HCA# 84) located between 731.52 m – 914.4 m elevation. Elevation of the site was not a significant predictor for *L. nigrinus* emergence.

Average monthly air temperatures in 2012 at the lowest and highest elevation sites during *L. nigrinus* larval development were $15 \pm 0.4^\circ\text{C}$ (Mean \pm SD) and $14 \pm 0.1^\circ\text{C}$ in April, $19 \pm 0.2^\circ\text{C}$ and $17 \pm 0.2^\circ\text{C}$ in May, and $21 \pm 0.4^\circ\text{C}$ and $19 \pm 0.1^\circ\text{C}$ in June (Fig. 4).

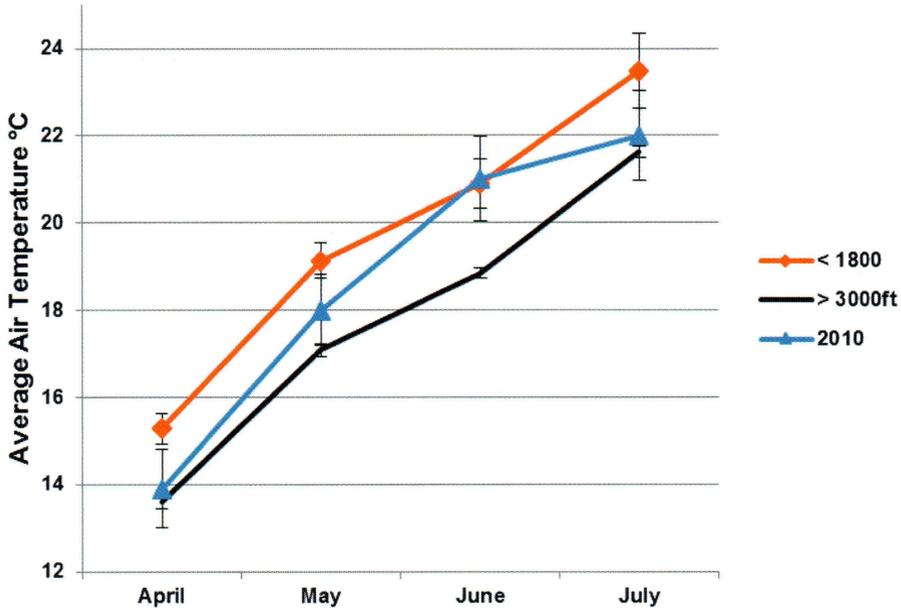


Fig. 4. Average monthly 2010 air temp. (°C) and 2012 air temp. at lowest and highest elevation sites sampled during *L. nigrinus* larval development (April- July). Error bars = St. Dev.

Air temperatures reached an average maximum of $36 \pm 0.2^{\circ}\text{C}$ at the low elevation sites and $32 \pm 0.9^{\circ}\text{C}$ at the highest elevations between 29 June 2012 and 01 July 2012 (Fig. 4).

Average 2012 monthly soil temperature at the lowest elevation sites during *L. nigrinus* pupation through adult emergence were $14.5 \pm 0.6^{\circ}\text{C}$ in April, reached the highest point in July at $22.5 \pm 0.8^{\circ}\text{C}$, and dropped to $9.3 \pm 2.1^{\circ}\text{F}$ in November (Fig. 5). Average daily temperatures exceeded 20°C starting 25 May 2012 and were $\geq 20^{\circ}\text{C}$ 67 - 110 days at the lower elevation sites. Soil temperatures were below 20°C after 22 September 2012 and remained below that level to the end of the study in December. At the highest elevation sites the average monthly soil temperatures were $12.16 \pm 0.52^{\circ}\text{C}$ in April, $19.64 \pm 0.36^{\circ}\text{C}$ in July, and $8.18 \pm 1^{\circ}\text{C}$ in November (Fig. 5). Average daily temperatures exceeded 20°C starting 29 June 2012 and reached these high temperatures 8 - 38 days at the high elevation sites from that date until 8 September 2012. Monthly averages of air and soil temperatures obtain in 2010 and 2012 were similar April through November. Minimum and maximum soil temperatures were not significant in predicting emergence of *L. nigrinus*.

Overall average soil moisture between April and December 2012 was the same ($0.13 \pm 0.02\text{m}^3/\text{m}^3$) at the low and high elevations. Likewise, average monthly soil moistures (Fig. 6) were similar at high and low elevation sites during *L. nigrinus* pupation through adult emergence (April through November) and ranged from $0.09 \pm 0.04\text{m}^3/\text{m}^3$ to $0.19 \pm 0.07\text{m}^3/\text{m}^3$ with the driest month in July. However, low elevation

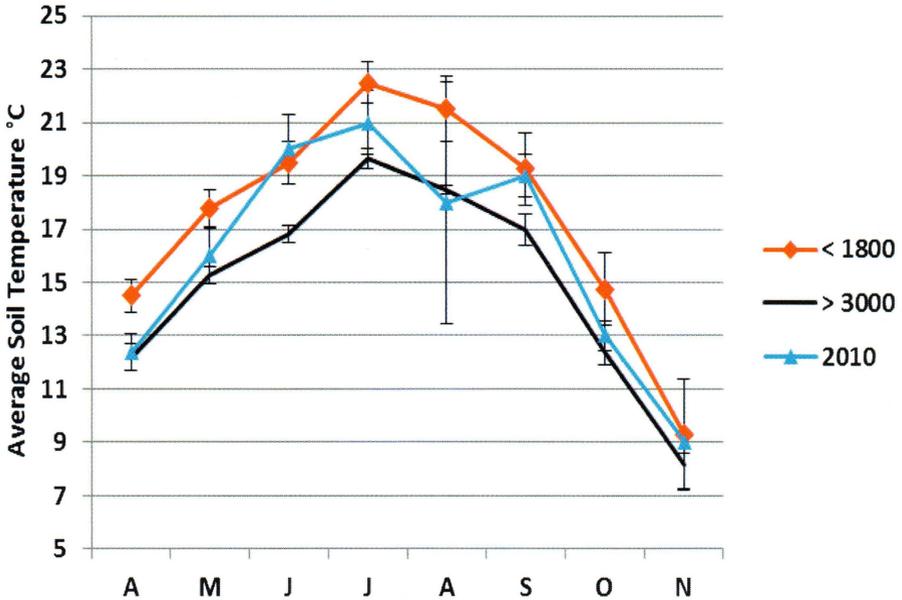


Fig. 5. Average monthly 2010 soil temp. (°C) and 2012 soil temp. at lowest and highest elevation sites sampled during *L. nigrinus* pupation to adult emergence (April–November). Error bars = St. Dev.

sites had their wettest months in April and May, whereas higher elevation sites had their wettest month in November (Fig. 6). Minimum and maximum soil moisture was not significant in predicting emergence of *L. nigrinus*.

Leaf litter samples weighed 56.7 ± 15 g, and the organic layer depth was 1.72 ± 0.77 cm. Leaf litter weight and organic layer thickness was not significant for predicting *L. nigrinus* emergence. However, analysis of the organic layer depth using Poisson regression corrected for overdispersal was significant ($P = 0.001$) in regards to recovery of *L. nigrinus* (Jones et al. 2014) suggesting that it is more likely to recover higher numbers of *L. nigrinus* with deeper organic layers.

Fungal samples from *G. mellonella* larvae were sent to Dr. Wayne A. Gardner (University of Georgia–Griffin) who identified them as *Metarhizium anisopliae* (Metchnikoff) Sorokin a common soil fungus that is saprophytic as well as pathogenic. Other *G. mellonella* larvae were found to be parasitized by nematodes.

Cage design. Of the 12 sites that had adult beetles placed into the control cage, 11 returned beetles with an average of 4.3 ± 3 beetles. The majority of the beetles recovered (78%) were recovered from the bouquet that was placed in the cage. Two of the sites also were disturbed with cages crushed or turned over by bears.

Discussion

The cage design test showed that 22% of beetles released in them were recovered a week later. The fact that only 4 of 1440 *L. nigrinus* placed in the cages as mature

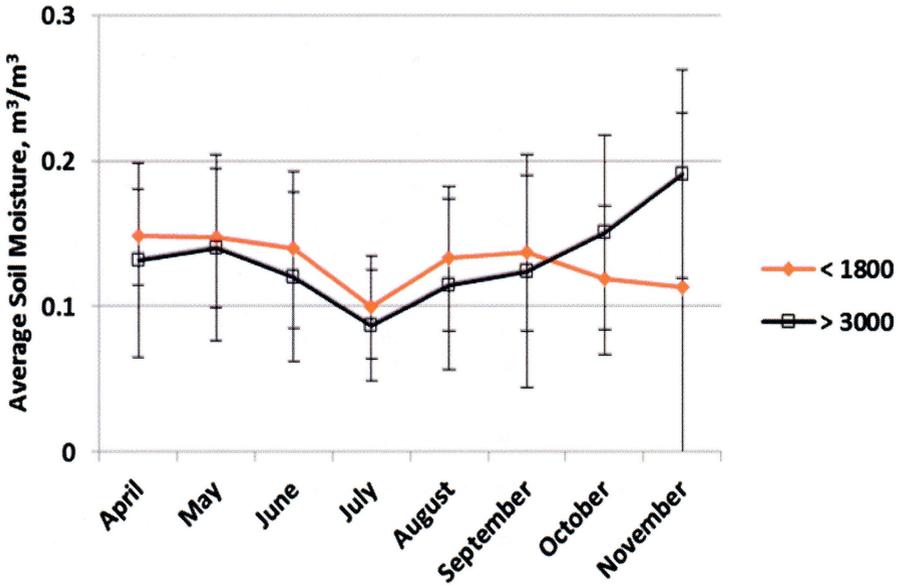


Fig. 6. Average monthly 2012 soil volumetric water content (m^3/m^3) at lowest and highest elevation sites sampled during *L. nigrinus* pupation to adult emergence (April-Novemeber). Error bars = St. Dev.

larvae in the spring were recovered as adults in the fall raises several questions. Emergence of the 4 adults occurred 163 - 221 days after the larvae were placed in cages. The emergence time was consistent with the preliminary trial conducted in 2011. A very similar study conducted at the same time in Tennessee (A. Lamb, pers. commun.) also resulted in a low emergence rate of 4% between 06 October 2012 and 05 November 2012, the same time as our beetles emerged. Likewise, an emergence study conducted in 2007 in Georgia resulted in 1.7% adult emergence in the field and 12.3% in the laboratory (T. Coleman, pers. commun.). These results suggest that the low emergence we observed was not due to cage design but to poor survival of aestivating *L. nigrinus*.

Initially, low emergence was thought to be due to the unusually warm winter and spring in 2012, but comparing air and soil temperature data obtained in 2010 showed no difference in monthly averages from April through November, and in both years, each trial resulted in very low emergence. In the laboratory, *L. nigrinus* larvae cannot complete development at temperatures above 21°C (Zilahi-Balogh et al. 2003b). It is unknown whether similar high temperatures affect aestivating pupain the soil or if the critical maximum temperature of 21°C only applies to the developing larvae.

Although it is unclear why emergence was so low in this study, we suspect the overall high temperatures in Georgia are an important factor. Because there are no data indicating survival rate of each developmental stage of *L. nigrinus* in its native range, it is impossible to estimate how well it is performing in its introduced range. Average laboratory mortality data from 2001 - 2004 indicate that there is roughly

29.5% mortality of mature larvae, 35.5% mortality of pupae, and 18.5% mortality of adults emerging from aestivation (Salom et al. 2012). This mortality occurred at optimal temperatures not experienced under field conditions in north Georgia. Using these survival rates and applying them to our study we expect that, of the 30 larvae put into an emergence cage, only 8.9 would become prepupae, 3.1 would pupate, and 0.6 adults would emerge in the fall. Because other factors could affect survival in the field, such as pathogenic microorganisms, damage during handling and placement in the field, or variable soil temperatures and moistures, our results were not totally unexpected.

In another study assessing establishment of *L. nigrinus* from 2010 - 2012 (Jones et al. 2014), *L. nigrinus* was found 1 - 3 years postrelease at various sites throughout North Georgia. Therefore, it can survive throughout the year and establish, but the annual survival rate is still unknown. More research is needed to assess the efficacy of *L. nigrinus* as a biological control agent in Georgia.

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