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Source: Journal of Insect Science, 15(1) : 1-7

Published By: Entomological Society of America

URL: <https://doi.org/10.1093/jisesa/ieu038>

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RESEARCH

Postgression Feeding Enhances Growth, Survival, and Nutrient Acquisition in the Endoparasitoid *Toxoneuron nigriceps* (Hymenoptera: Braconidae)

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Subject Editor: Paul Ode

J. Insect Sci. (2015) 15(1): 51; DOI: 10.1093/jisesa/ieu038

ABSTRACT. *Toxoneuron nigriceps* Viereck (Hymenoptera: Braconidae), a koinobiont endoparasitoid of the tobacco budworm, *Heliothis virescens* F. (Lepidoptera: Noctuidae), derives nutrition from the host hemolymph during the internal portion of its larval development but feeds destructively on host tissues externally after egression. To investigate the importance of this tissue-feeding phase, and to evaluate the behaviors associated with postgression feeding, *T. nigriceps* larvae were subjected to one of four treatments: 1) allowed to carry out normal tissue feeding, 2) deprived of tissue feeding, 3) presented with tissues scraped away from the host remains, and 4) fed tissues scraped from an unparasitized *H. virescens* larva. Additionally, total carbohydrates, lipids, and proteins were quantified from pre and posttissue feeding *T. nigriceps* larvae to examine the effect of postgression feeding on parasitoid nutritional physiology. Parasitoids that received no tissues after egression, or that received tissue from an unparasitized *H. virescens* larva, had significantly smaller body masses at all stages than those allowed to feed naturally or fed tissues scraped from a parasitized host. Parasitoids that underwent normal host feeding after egression also reached larger masses than those fed scraped host tissue. Parasitoids that received no tissue after egression survived to adulthood significantly less often than those that were presented with any *H. virescens* tissue. This suggests that postgression tissue feeding is a vital developmental step for *T. nigriceps*, and that *T. nigriceps* will not only feed when normal postgression behavior is disrupted, but will also feed on unparasitized tissue. The quantification of macronutrients in the tissues of pre and posttissue feeding *T. nigriceps* larvae showed significantly elevated proportions of proteins, lipids, and carbohydrates in the tissues of larvae that had completed feeding, with the greatest difference being in total lipids.

Key Words: parasitoid, insect rearing, Hymenoptera, feeding behavior, nutrition

Parasitoids are specialized parasites that utilize a single host during immature development, but are free-living as adults (Godfray 1994). Immature parasitoids often kill their host during development, and those hosts that are left alive by the parasitoid do not survive long enough to reproduce. Thus, many parasitoids have great potential for use as biological control agents. Biological control is the practice of using natural enemies, rather than chemicals, to control populations of pest species. This practice can reduce the need for pesticides, which not only reduces the exposure of consumers, agricultural workers, and the environment to potentially harmful chemicals, but also can be more economically efficient. Although monetary costs have been difficult to measure precisely, in some cases biological control has been estimated to save hundreds of thousands of dollars in chemical control and prevent millions of dollars' worth of damages (Gutierrez et al. 1999). Although parasitoids and predators have both been used for the biological control of insect pests, hymenopteran parasitoid species account for the majority of cases, mostly due to their higher degree of host specificity and, thus, decreased risk of nontarget effects (Gordh et al. 1999, van Lenteren 2012).

One obstacle to the use of parasitoids as biological control agents, however, is the inefficiency of mass rearing many species due to the need to rear not only the parasitoid but also its host. To increase the efficiency of mass rearing, many attempts have been made to develop artificial diets for parasitoids, with varying degrees of success. As of 2011, 61 hymenopteran parasitoid species have been reported as having been reared partially or completely in vitro (Cônsoi and Grenier 2010). Most parasitoids that have been reared through their entire life cycle on artificial diets are ectoparasitoids, which develop externally of their hosts, or egg parasitoids. None is koinobionts, which develop internally as endoparasitoids of active, developing hosts (Thompson 1999, Grenier 2009). Some koinobionts, however, have been reared partially

in vitro, from egg to larva or through several larval instars. One such parasitoid, *Toxoneuron nigriceps* Viereck, has been reared in artificial media from egg to second-larval instar (Pennacchio et al. 1992) and from second to late third instar, the final-larval instar of *T. nigriceps* (Kuriachan et al. 2006). Although the third-larval instar marks the end of *T. nigriceps* larval development, parasitoids reared to this stage failed to survive to pupation. One factor that may have contributed to this failure to develop beyond the larval stage was the lack of an equivalent to the tissue-feeding phase normally carried late third instar *T. nigriceps* larvae after it exits the host, a process usually referred to as 'egression' in parasitoids. Highly host specific, *T. nigriceps*, develops only in the tobacco budworm, *Heliothis virescens*, a serious pest that damages buds in a number of crops including tobacco, cotton, soybean, and tomato (Fitt 1989). Historically *T. nigriceps* offered a high degree of natural control of *H. virescens*, with one estimate putting field parasitism rates at 50 to nearly 100% (Chamberlin and Tenhet 1926). Later field study in Georgia estimated parasitism rates of 96% 1 year and 76% the following year (Lewis et al. 1972).

Endoparasitoids, which develop within the body cavity of their hosts, are often divided into two informal groups, 'hemolymph feeders' and 'tissue feeders', based on their feeding habits during larval development. Hemolymph feeders are those endoparasitoids that feed only nondestructively by filtering nutrients from host hemolymph as larvae, though some may consume varying amounts of the fat body before egression, and then pupate outside of the host (Harvey et al. 2008). This nondestructive feeding strategy generally leaves the host alive, though moribund, at the time of larval egression. The hemolymph feeding strategy appears to be prevalent in the braconid subfamilies Microgastrinae, Cheloniinae, and Cardiochilinae (Gauld and Bolton 1988, Harvey et al. 2000).

Tissue feeders begin their development in a fashion similar to that of hemolymph feeders, feeding nondestructively on hemolymph;

however, late in their development they will make a behavioral switch and begin a destructive tissue-feeding phase. The timing of the switch from hemolymph to tissue feeding can be critical. If the switch occurs too early, the host will be killed before it is large enough to provide sufficient resources for maximal parasitoid growth. Letting the host grow too large, however, can also be fatal to the parasitoid, as many tissue feeders pupate within the host cuticle, and excess tissue can impede pupation (Hemerik and Harvey 1999). Tissue feeding is the more common strategy by far, occurring in most ichneumonid endoparasitoids as well as many braconids (Gauld and Bolton 1988), and appears to be an ancestral trait (Harvey et al. 2008).

Toxoneuron nigriceps, a member of the braconid subfamily Cardiochilinae, begins its development much like a hemolymph feeding parasitoid would. From the first until the third-larval instar *T. nigriceps* feeds nondestructively on the hemolymph of its host, *H. virescens*, and egresses without having consumed vital host tissues. During its late third instar, however, it begins a destructive tissue-feeding phase. *Toxoneuron nigriceps* is somewhat unusual in this respect, as destructive feeding occurs not within the host, as it does among most tissue feeders, but externally, after parasitoid egression. As the mature larva exits the host, it does not separate completely from the host but leaves its posterior end anchored within the hole in the cuticle through which it egressed. The larva then curves around to form a second hole in the host cuticle through which it feeds on the remaining host tissues, which by this point have been liquefied into a viscous pulp. External feeding can last for several hours, during which time the parasitoid alters drastically in appearance, changing from the bright green of its host's hemolymph to a dull whitish color and nearly doubling in length and girth.

As many members of Cardiochilinae are strictly hemolymph feeders, and *T. nigriceps*, until egression, develops in a manner very similar to that of hemolymph feeders, it appears that in this case the tissue-feeding strategy may have arisen secondarily from an ancestrally hemolymph feeding group. The tissue-feeding phase of *T. nigriceps* is likely an important component of larval development, and deprivation of such feeding could have significant developmental consequences. Such deprivation may have been relevant in the failure of *T. nigriceps* to develop past the third-larval instar when reared by Kuriachan et al. (2006) on an artificial diet. Third instar parasitoid larvae reared in vitro by Kuriachan et al. (2006) had an opaque, fragile appearance, unlike in vivo-reared counterparts, perhaps owing to a lack of some tissue components that are normally taken up immediately after egression from the host.

In this study, newly egressed *T. nigriceps* larvae were subjected to four different postegression feeding treatments in order to investigate the importance of postegression tissue feeding as well as some of the behavior associated with postegression feeding. To evaluate the effects of this feeding phase, some larvae were allowed to feed naturally on their hosts while others were prevented from any postegression feeding. To determine whether or not *T. nigriceps* will commence feeding after having their normal postegression behavior disrupted and having tissue presented to them in an unfamiliar form, *T. nigriceps* larvae were fed experimentally with tissue scraped out of the host after parasitoid egression. To investigate whether or not *T. nigriceps* will feed on unparasitized *H. virescens* tissue and, if so, whether the parasitoid can benefit from this unparasitized tissue, other *T. nigriceps* larvae were fed tissues scraped from healthy *H. virescens* prepupae. The nutritional benefits of postegression feeding were also evaluated by extracting and quantifying total proteins, lipids, and carbohydrates from tissue of both newly egressed *T. nigriceps* larvae and from larvae that had completed postegression feeding. Comparison of the basic nutritional states of *T. nigriceps* larvae before and after tissue feeding may elucidate what macronutritional benefit larvae gain from this step of feeding. This information could be valuable for formulating an artificial postegression diet, if it appears that *T. nigriceps* will accept a substitute for the tissue of its own parasitized host.

Materials and Methods

Rearing Practices. *Heliothis virescens* larvae were reared on artificial corn earworm diet (BioServ Inc., Frenchtown, NJ) under controlled conditions (a temperature of $29 \pm 1^\circ\text{C}$; $60 \pm 10\%$ relative humidity and a photoperiod of 14:10 [L:D] h) (Vanderzant et al. 1962). *Toxoneuron nigriceps* was reared in a laboratory culture according to Vinson et al. (1973). Hosts were parasitized by placing a late fourth instar *H. virescens* larva with a single mated female wasp under the lid of a 35 by 5-mm plastic petri dish. The wasp and host larva were observed until the wasp oviposited once, at which time the larva was removed and placed in a rearing vial with corn earworm diet. Hosts were reared one per vial to prevent cannibalism. Parasitized hosts were removed from the diet ~ 10 d after parasitization and placed individually in 16 by 100-mm glass test tubes for continuation of parasitoid growth and to facilitate observation of parasitoid egression, which occurs after the host molts to its fifth-larval instar.

Postegression Feeding Treatments. As mature *T. nigriceps* larvae egressed from their fifth instar hosts, they were subjected to one of four feeding treatments, with 30 larvae being subjected to each treatment. In the first treatment, as a control, larvae were allowed to feed normally on host tissues for 3 h before being removed from the host remains, weighed, and placed individually in 0.5 ml gelatin capsules (Electron Microscopy Sciences, Fort Washington, PA). Gelatin capsules served as artificial pupation chambers for parasitoid larvae, aiding in the formation of cocoons and subsequent pupation (Henderson et al. 2011). The second treatment served as a negative control, in which larvae were removed from the host remains immediately upon egression, weighed, and placed in gelatin capsules with no postegression feeding having occurred. In the third treatment, parasitoid larvae were removed from the host remains, which were then opened by cutting down the dorsal midline. The remaining internal tissues were scraped out of the cuticle using the spatula end of a Spoonula (Thermo Fisher Scientific, Waltham, MA) and placed on filter paper in a 35 by 10-mm plastic petri dish. The *T. nigriceps* larva was placed on the filter paper with its mouthparts and posterior end just touching the collected host remains. The petri dish was then closed and placed in the rearing incubator, where the larva was allowed to feed on the tissues for 3 h before being weighed and placed in a capsule. Although normal tissue feeding by *T. nigriceps* can last up to 4 h, the scraped host tissue became dry and hardened after 3 h and was thus unsuitable for feeding by the parasitoid. Larvae in the fourth treatment were handled similarly to those in the previous treatment, but were each presented with tissue scraped from an unparasitized *H. virescens* larva at the prepupal stage. This stage was chosen because the tissue was somewhat homogenized and viscous, and was more similar to the liquefied tissue in parasitized larval remains than the tissue of other larval stages. Again, *T. nigriceps* larvae were allowed to feed on tissue for 3 h before being removed from any remaining tissue, weighed, and placed in a gelatin capsule. After the feeding treatment, each larva in its gelatin capsule was placed individually in a labeled glass test tube for observation. These test tubes were held upright in a test tube rack in the rearing incubator and observed daily for cocoon formation and emergence of adult wasps.

Data Analysis for Feeding Experiments. Larvae and pupae (within cocoons) were measured in wet mass, with only adult size measured as dry mass. The masses of larvae (postfeeding treatment), cocoons, and adults from each treatment were logarithm transformed to normalize data, then compared using a repeated measures MANOVA (JMP 7, SAS Institute Inc., Cary, NC, 2007) followed by Bonferroni corrected pairwise comparisons. To look for evidence of larval feeding on scraped host tissues and scraped unparasitized tissues, initial larval mass was subtracted from final larval mass to calculate the mass gained through feeding in each of these treatments. One sample *t*-tests were used to determine if mass gain was significantly greater than zero in the scraped, parasitized and scraped, unparasitized tissue treatments (SPSS 16.0, SPSS Inc., Chicago, IL). Mass gain was compared with these two treatments using an independent samples *t*-test (SPSS 16.0, SPSS

Inc.). The proportions of parasitoids in each treatment that went on to form cocoons and to emerge as adult wasps were compared using *G*-tests, followed by a Tukey-type multiple comparison among proportions (Zar 2010) in cases where a significant difference was found.

Quantification of Proteins, Lipids, and Carbohydrates. Mature *T. nigriceps* larvae were collected from hosts that had been removed from diet containers ~10 d after parasitization—at which point the hosts no longer feed—and placed individually in 16 by 100-mm glass test tubes for observation. The wasp larvae were collected either immediately after they finished egression, before the initiation of tissue feeding, or immediately after they had completed tissue feeding and removed their head from the host remains. Larvae from both treatments were then weighed, placed individually in microcentrifuge tubes, and flash frozen in liquid nitrogen. The frozen larvae were stored in a freezer at -80°C until the extractions were carried out, at which time larvae were crushed and homogenized within the tubes using a glass rod coated in clean parafilm.

Total carbohydrates in larval samples were measured following a procedure modified from van Handel (1985a) using anthrone reagent, which changes from yellow to blue-green in the presence of carbohydrates. Anthrone reagent was prepared by measuring 150 ml of deionized water into a 1-l Erlenmeyer flask, placing the flask in an ice bath, and adding 380 ml concentrated (97%) sulfuric acid. Approximately 750 mg anthrone (J. T. Baker, Phillipsburg, NJ) was then added to this solution and mixed until it dissolved completely. The complete anthrone reagent was then stored in a refrigerator to prolong its shelf life.

A small sample of homogenized tissue (1–5 mg) was taken from each larva and placed in a 12 by 75-mm glass culture tube, with the mass of each sample recorded. To measure the total carbohydrate content of each sample, 5 ml of anthrone reagent was added directly to the culture tube. Tubes were vortexed, and then placed in a test tube heater and held at $90\text{--}92^{\circ}\text{C}$ for 17 min. After cooling to room temperature, the absorbance of each sample was read at 625 nm in a USB4000 Fiber Optic Spectrometer (Ocean Optics, Inc., Dunedin, FL). Carbohydrate content was calculated from absorbance using a standard curve generated by measuring three sets of 25, 50, 100, 150, and 200 μl portions of 1 mg/ml solution of sucrose in 25% ethanol.

Total lipids in larval samples were measured following a procedure modified from van Handel (1985b) using a vanillin-phosphoric acid reagent, which changes from clear to pink when it reacts with lipids. The vanillin-phosphoric acid reagent was prepared by adding 600 mg vanillin ReagentPlus (Sigma-Aldrich Co., St. Louis, MO) to 100 ml hot deionized water then mixing until the vanillin dissolved. This solution was then cooled to room temperature before 400 ml concentrated (85%) phosphoric acid was added. The reagent was stored at room temperature in a sealed glass bottle covered in aluminum foil, to keep the light-sensitive reagent from being degraded.

Small samples of larval tissue (1–10 mg) were measured into 12 by 75-mm glass culture tubes, to which 0.5 ml of 1:1 (v:v) chloroform-methanol solution was added and mixed to extract lipids. Tubes were then heated until the solvent evaporated completely. Next, 0.2 ml of concentrated (97%) sulfuric acid was added, and the samples were placed back in the heating block and held at $\sim 95^{\circ}\text{C}$ for 10 min. Samples were then removed from the heating block and cooled to room temperature before vanillin-phosphoric acid reagent was added up to a previously determined 5 ml mark on each culture tube. Samples were carefully poured back and forth between two test tubes four times to mix thoroughly. Absorbance of each sample was measured at a wavelength of 525 nm, or at 490 nm for samples with particularly high lipid concentrations. Standard curves at 525 and 490 nm were generated using three sets of 50, 100, 200, and 400 μl samples of a standard solution of 1 mg/ml vegetable oil in chloroform.

Total protein in larval samples was measured using a Pierce bicinchoninic acid (BCA) protein assay kit (Thermo Scientific, Rockford, IL). With this kit, protein assays are carried out using a

solution in which Cu^{+2} is reduced to Cu^{+1} by proteins, causing a color change in a bicinchoninic acid reagent. The procedure was carried out following kit instructions. For each sample, a small amount of homogenized *T. nigriceps* larval tissue (between 1 and 10 mg) was measured into a microcentrifuge tube. Following the procedure of Wheeler and Buck (1996), 1 ml of 1 M NaOH was added to each sample to extract nonwater soluble proteins. Samples were agitated on a vortex machine for one minute to thoroughly mix the solution. A 0.1 ml aliquot of each sample was added to a 12 by 75-mm glass culture tube, to which 2 ml of the BCA reagent was then added. Test tubes were briefly vortexed again, to mix the sample and reagent, and then incubated in a heating block for 30 min at 37°C . After cooling to room temperature, samples were measured in the spectrometer at a wavelength of 562 nm. A protein-standard curve was generated using three sets of 2.5, 12.5, 25, 50, 75, 100, 150, and 200 μg samples of bovine serum albumin standard.

Data Analysis for Macronutrient Quantification. The proportions of larval tissue composed of protein, lipid, and carbohydrate in the tissues of pre and posttissue feeding-larvae was calculated by dividing the estimated mass of each macronutrient in a sample by the total mass of the sample it was taken from. These proportions of carbohydrate, lipid, and protein—measured as mg macronutrient per mg sample (wet mass)—were compared with pre and posttissue-feeding larvae. Because all three assays were carried out using samples from the same 60 larvae, comparisons were made using a MANOVA (JMP 7, SAS Institute Inc., 2007).

Results

Postgression Feeding Treatments. Feeding treatment had a significant effect on parasitoid size (Fig. 1) ($F = 30.217$, $P < 0.001$), with parasitoids from the control treatment being significantly larger than those fed scraped, parasitized tissue ($F = 21.654$, $N = 30$, Bonferroni corrected $P = 7.55 \times 10^{-5}$), fed unparasitized *H. virescens* ($F = 75.544$, $N = 30$, Bonferroni corrected $P = 1.94 \times 10^{-12}$), or not fed at all ($F = 48.889$, $N = 30$, Bonferroni corrected $P = 4.19 \times 10^{-9}$). Parasitoids fed scraped host tissue were also significantly larger than those fed unparasitized *H. virescens* tissue ($F = 15.366$, $N = 30$, Bonferroni corrected $P = 0.0011$) and those that were not fed ($F = 9.036$, $N = 30$, Bonferroni corrected $P = 0.0211$). The masses of larvae fed unparasitized *H. virescens* tissue and those not fed after egression did not differ significantly ($F = 0.106$, $N = 30$, Bonferroni corrected $P > 1.0$). The mean masses of parasitoids in each treatment are summarized in Table 1.

Larvae presented with scraped, parasitized host tissue gained significant mass during the 3-h feeding period ($t = 10.0878$, $N = 30$, $\text{df} = 29$, $P < 0.001$), as did larvae presented with tissues scraped from unparasitized *H. virescens* prepupae ($t = 6.6581$, $N = 30$, $\text{df} = 29$, $P < 0.001$). This indicated that larvae in both treatments consumed the tissues presented to them (Fig. 2). However, larvae fed parasitized tissue gained significantly more mass than those fed unparasitized tissue ($t = 5.7705$, $N = 30$, $\text{df} = 58$, $P < 0.001$), with those from the scraped parasitized tissue treatment gaining 15.4 ± 1.5 mg (mean $\pm$ SE) and those from the scraped unparasitized tissue treatment gaining 5.4 ± 0.8 mg.

Feeding treatment did not have a significant effect on the proportion of *T. nigriceps* larvae in each treatment that formed cocoons ($G = 3.527$, $N = 30$, $P = 0.317$) (Fig. 3). However, feeding treatment significantly affected the proportion of parasitoids that emerged as adult wasps (Fig. 4). Parasitoids that were not fed after egression survived to adulthood in 50% of cases, a significantly smaller proportion than in the control treatment ($q = 5.5097$, $N = 30$, $P < 0.005$), the scraped, parasitized tissue treatment ($q = 4.3635$, $N = 30$, $P < 0.025$), and the scraped, unparasitized tissue treatment ($q = 4.3635$, $N = 30$, $P < 0.025$). The proportions of parasitoids that emerged as adults in the control treatment and in the two scraped tissue treatments did not differ significantly.

Quantification of Proteins, Lipids, and Carbohydrates. Concentrations of proteins, lipids, and carbohydrates were significantly

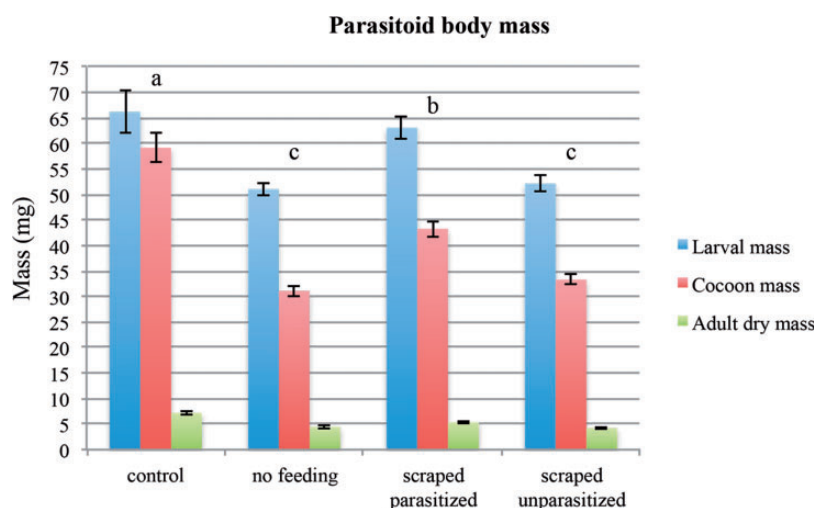


Fig. 1. The mean masses of parasitoids as larvae, pupae (inside cocoons), and adults (dry mass) in each feeding treatment. Error bars represent SE. All three measures of mass were affected similarly by the treatments. Different letters above columns indicate a statistical difference between treatments ($P < 0.05$; MANOVA).

Table 1. Mean masses ($\pm$ SE) of larvae, cocoons, and adult parasitoids (in mg)

Treatment	Final larval mass	Cocoon mass	Adult dry mass
Control	66.2 (4.2)	59.2 (2.8)	7.2 (0.3)
No feeding	51.0 (1.3)	31.1 (1.1)	4.5 (0.3)
Scraped, parasitized	63.1 (2.2)	43.3 (1.6)	5.4 (0.3)
Scraped, unparasitized	52.1 (1.6)	33.3 (1.0)	4.3 (0.2)

Larvae and cocoons were measured in fresh mass, and adults in dry mass.

higher in the tissues of *T. nigriceps* larvae that had completed postegression tissue feeding than in the tissues of those that had not experienced tissue feeding (Fig. 5) ($F = 52.147$, $N = 30$, $P = 3.2 \times 10^{-16}$). Concentrations of proteins, lipids, and carbohydrates are displayed in Table 2. The increase in tissue concentration of carbohydrates and proteins were similar, with an ~ 1.5 -fold increase in both. The difference in lipid concentration was much greater, with the tissues of postfeeding larvae containing nearly threefold greater concentration.

Discussion

Postegression tissue feeding is clearly a vital step in the development of *T. nigriceps*. Deprivation of host tissues after parasitoid egression did not noticeably affect the ability of mature *T. nigriceps* larvae to produce silk and spin cocoons, but only half of the parasitoids that were tissue deprived as larvae developed to adulthood. This is a significantly smaller proportion than seen among parasitoids that had fed naturally (93.3%) or had been provided with scraped tissues (86.7%). A similar effect was demonstrated in a recent study by Kuriachan et al. (2011), in which larvae prevented from tissue feeding after egression went on to form significantly fewer cocoons than those that had been allowed to carry out normal tissue feeding, and only 58% of larvae prevented from tissue feeding emerged from cocoons as adult wasps, compared with the 100% emergence rate of larvae allowed to tissue feed. Additionally, many adults that emerged from the nonfeeding treatment were deformed, had incomplete wing development, and generally suffered from a reduction in longevity compared with those that had fed on tissue.

In the current study, *T. nigriceps* larvae that were disrupted from their normal postegression-feeding behavior but were later provided with *H. virescens* tissue survived to adulthood in proportions similar to those that had undergone natural-feeding behavior, indicating that lack of tissue, rather than disruption and manipulation of parasitoid larvae,

was the likely cause of the reduced rate of survival seen among parasitoids that received no *H. virescens* tissues. It also appeared that only a comparatively small amount of *H. virescens* tissue was needed to ensure development of *T. nigriceps* to adulthood, as parasitoids fed scraped tissues were smaller than those that had fed naturally, but developed to adulthood in similar proportions. There may, however, have been undocumented fitness consequences for these smaller parasitoids, as body size is often linked to greater longevity and fecundity among parasitoids, with larger individuals presumed to have greater fitness (Godfray 1994).

Idiobiont parasitoids, which develop on permanently paralyzed hosts or quiescent host stages, utilize a predetermined and relatively static nutritional source during their larval development. In contrast, koinobionts, which utilize active, developing hosts, may encounter a highly dynamic nutritional environment. Several koinobiont species have been shown to actively alter the nutritional physiology of their hosts, likely to suit the changing nutritional requirements of the parasitoid (Dahlman and Vinson 1975, 1976; Thompson 2001; Salvador and C onsoli 2008). As a koinobiont, *T. nigriceps* experiences variation in its host's nutritional physiology during development, with concentration of proteins in the hemolymph increasing as the host reaches its fifth and final-larval instar (Pennacchio et al. 1993).

Toxoneuron nigriceps also alters the nutritional physiology of *H. virescens* during internal development, but the postegression switch from feeding on hemolymph to feeding on host tissues marks a greater nutritional alteration. Although hemolymph can contain relatively high concentrations of proteins, both carbohydrates (in the form of trehalose) and lipids (bound in lipophorins) are found in lower concentrations (Wyatt 1961). This change in nutrition is reflected in the difference in macronutritional makeup of homogenized *T. nigriceps* larvae before and after tissue feeding. Larger proportions of proteins, carbohydrates, and lipids in postfeeding *T. nigriceps* larvae are to be expected, as these larvae are consuming a large amount of matter, but what draws attention is the especially large increase in lipid concentration in the homogenized tissues of postfeeding larvae, which was 2.7 times greater than in prefeeding larvae. Larvae consuming host tissues are feeding, in part, on the liquefied remains of the fat body, where the majority of the host's lipid supply is stored in the form of triglycerides. This is likely to be an important nutritional source for developing *T. nigriceps*, especially because a number of parasitoid wasps (including several members of Braconidae and Ichneumonidae) are not capable of lipogenesis and must rely on stores of lipids acquired from the host during larval development (Visser and Ellers 2008).

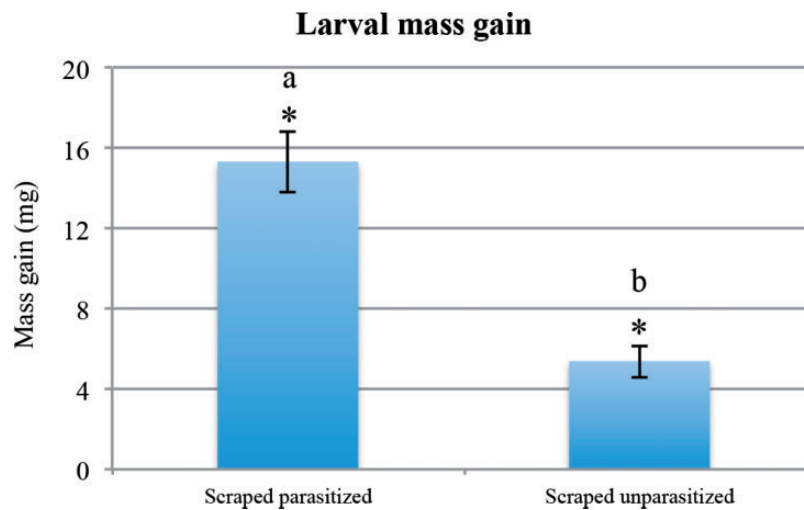


Fig. 2. Larval mass gain (mg). Error bars represent SE. An asterisk indicates a gain significantly different from zero ($P < 0.001$; one sample t -test). Different letters above columns indicate a statistical difference between treatments ($P < 0.001$; independent samples t -test).

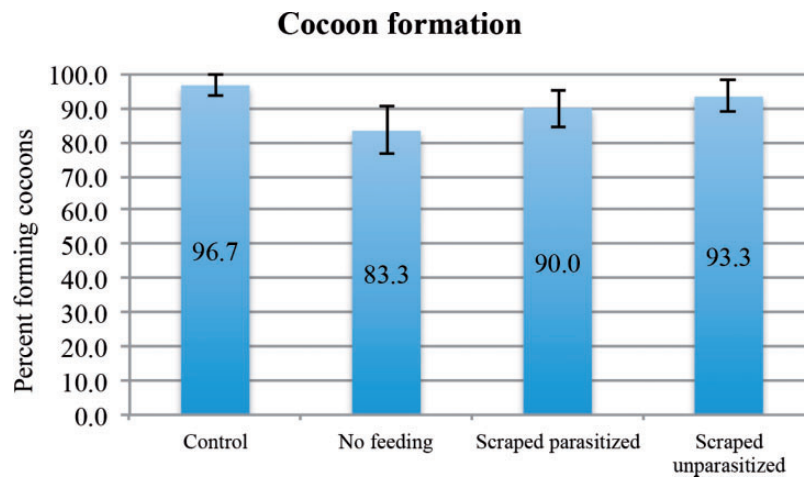


Fig. 3. The percent of parasitoids in each treatment that formed complete cocoons. Error bars represent SE. No significant differences were found ($G = 3.527$, $N = 30$, $P = 0.317$; G -test).

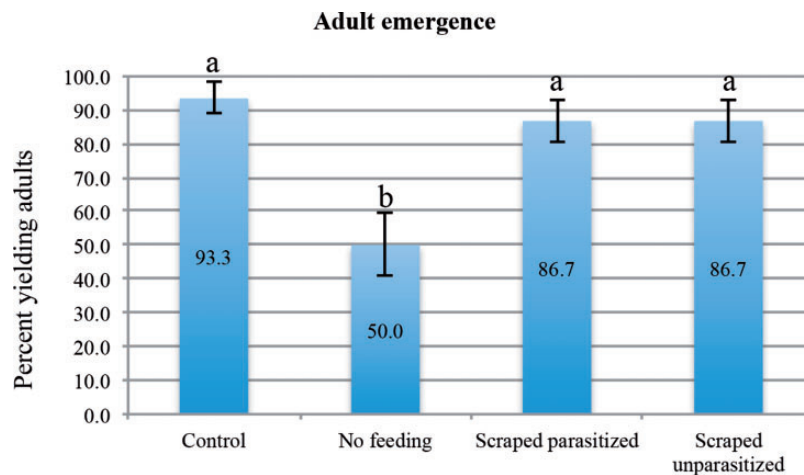


Fig. 4. The percent of parasitoids in each treatment that emerged as live adult wasps. Error bars represent SE. Different letters above columns indicate a statistical difference between treatments ($N = 30$, $P < 0.05$; Tukey-type multiple comparison among proportions).

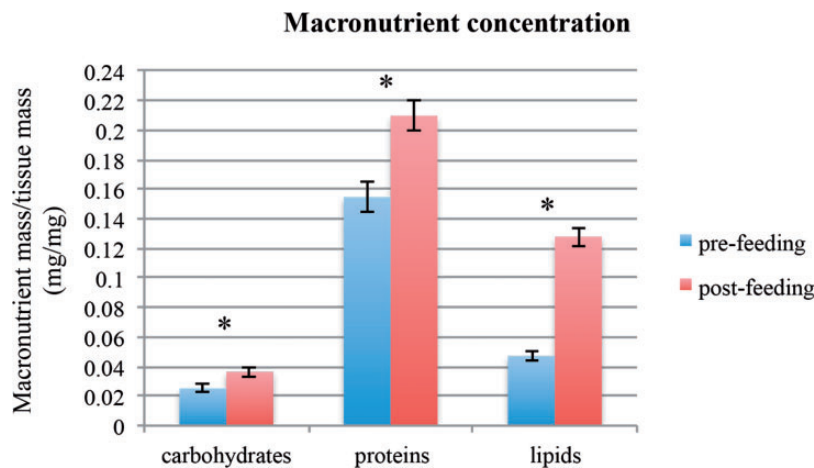


Fig. 5. Mean concentration (mg macronutrient/mg tissue sample [wet mass]) of carbohydrates, proteins, and lipids in the tissues of pre- and posttissue feeding *T. nigriceps* larvae. Different letters above columns indicate a statistical difference between treatments ($N = 30$, $P < 0.05$; MANOVA).

Table 2. The mean macronutrient concentration (mg macronutrient/mg sample wet mass) of, and difference (%) between, homogenized tissue from pre and posttissue-feeding <i>T. nigriceps</i> larvae			
Treatment	Protein concentration (±SE)	Lipid concentration (±SE)	Carbohydrate concentration (±SE)
Prefeeding	0.1547 (0.0102)	0.0471 (0.003)	0.0253 (0.0024)
Postfeeding	0.2097 (0.0103)	0.1279 (0.006)	0.0365 (0.0035)
Difference (%)	35.56	171.51	44.27%

The switch from hemolymph feeding to tissue feeding, clearly vital to the development of *T. nigriceps*, may have been what was lacking in the in vitro rearing of this parasitoid by Kuriachan et al. (2006). Although 100% of second instar *T. nigriceps* larvae fed certain artificial diets molted to their third and final larval instar, none of these larvae formed cocoons or pupated. However, as demonstrated here, late third instar *T. nigriceps* larvae that have their normal postegression behavior disrupted will initiate feeding on tissue that is presented to them in a perhaps unfamiliar form. Even more encouraging is the fact that larvae will feed on tissue from unparasitized *H. virescens* larvae and do benefit from it, suggesting that there is not a parasitism-specific nutrient that is required by *T. nigriceps* during postegression feeding. If *T. nigriceps* can be induced to feed on an artificial postegression diet, it may survive past the larval stage when reared in vitro. This diet should reflect the difference in macronutrient concentration exhibited by pre and posttissue-feeding larvae. The diet would need to contain carbohydrates, but would likely need to have a proportionally greater protein and, perhaps most importantly, a proper lipid composition and concentration. Another vital factor will be a source of sterols as *T. nigriceps*, like other insects, is incapable of synthesizing these de novo (Clark and Bloch 1959).

Another factor to consider, apart from nutrition, is the consistency of a potential diet. By the time mature larvae egress from their hosts, the remaining tissues inside have been partially liquefied, turning them into a viscous, pulpy substance. This is likely important for mature *T. nigriceps* larvae, which do not have mandibles that are well suited for chewing solid foods. A diet with too liquid a consistency, however, would pose the problem of larvae drowning in it, rather than being able to feed. Kuriachan et al. (2006) successfully reared *T. nigriceps* on an artificial diet from second to third-larval instar, and a previous in vitro rearing study by Pennacchio et al. (1992) succeeded in rearing *T. nigriceps* from egg to second-larval instar. If *T. nigriceps* larvae previously reared on artificial media can be induced to feed on an artificial postegression diet, perhaps this parasitoid can some day be reared

completely in vitro from egg to adult. This would be a first among koinobiont parasitoids (Thompson 199, Grenier 2009).

Funding

Funding for this research was provided by the Texas A&M University Department of Entomology.

Acknowledgments

Thank you to Dr. Julio Bernal, of the Texas A&M Department of Entomology, and Dr. Thomas DeWitt, of the Department of Wildlife and Fisheries Sciences, for assistance with statistical techniques.

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Received 19 February 2013; accepted 12 June 2013.