



Foliage Type and Deprivation Alters the Movement Behavior of Late Instar European Gypsy Moth *Lymantria dispar* (Lepidoptera: Erebidae)

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Abstract The movement behavior of insects characterizes their ability to disperse, establish, compete, forage, seek mates, and ultimately reproduce. Understanding the movement of invasive insects is particularly important for developing management policies. We conducted laboratory experiments in Minnesota, USA to determine how host type and food deprivation affected the movement of late instars of the European gypsy moth *Lymantria dispar* (Lepidoptera: Erebidae), an invasive forest insect in North America. Gypsy moth larvae can feed on over 300 species of trees and shrubs. During outbreaks food availability to conspecifics can become severely restricted as developing instars consume increasing amounts of foliage. Larvae were raised on one of five foods: *Quercus macrocarpa*, *Larix laricina*, *Acer platanoides*, *Acer saccharinum*, or artificial diet. Subsets of fifth and sixth instar larvae were also deprived of food for zero, 24, or 48 h. After the food deprivation period, late instar larvae were placed on a servosphere and their movement paths were recorded. Larvae raised on *Q. macrocarpa*, a preferred host, were unlikely to move unless starved. They moved farther the longer they were starved. In contrast, when larvae were raised on less preferred hosts, they were more likely to move without prior starvation. These results suggest that feeding on optimal hosts provides gypsy moth larvae

with the energy and nutritional requirements to move more quickly to more food when there is none immediately available.

Keywords Servosphere · *Lymantria dispar* · Foliage · Movement behavior · Locomotion compensator

Introduction

Understanding the movement behavior of insects is critical to understanding their ecology (Bowler and Benton 2005). It is especially important to understand the movement behavior of invasive insects, as their movement determines their ability to disperse and establish across long distances (Jones et al. 1980; Conradt et al. 2003; Fahrner and Aukema 2018) and affects their competitive success in procuring food or shelter at smaller spatial scales (Campbell et al. 1975; Bengtsson et al. 2004). The physiological internal state of an insect and its capacity for movement may be affected by external factors such as nutrition (Srygley et al. 2009) and food availability (Defagó et al. 2016). The range of host plants on which oligophagous or polyphagous insects can feed determines the nutrition and energy available to herbivorous insects. Not all host plant choices are optimal or preferred, and sub-optimal hosts may affect dispersal (Lance and Barbosa 1982; Erelli and Elkinton 2000). Feeding on suboptimal hosts by immature sweet potato whiteflies *Bemisia tabaci* (Hemiptera Aleyrodidae), for example, caused the adult insects to leave their hosts earlier in life (Blackmer and Byrne

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1993). Similarly, *Phthorimaea operculella* (Lepidoptera: Gelechiidae) are more likely to fly as adults when raised on lower quality hosts (Coll and Yuval 2004). Feeding on suboptimal hosts may also affect how insects respond during periods when food becomes unavailable, either spatially or temporally. Food deprivation may cause insects to adjust their locomotory activity (Nakamuta 1987), with variations ranging from increases in activity (Edney 1937; Brady 1972; Verhoef 1984), decreases (Reynierse et al. 1972), or simply no perceived change.

To date, there have been few studies that have examined how suboptimal hosts, food deprivation, and the interaction of these two affect movement capacity or propensity to move. Such factors may be important in the context of the spread of polyphagous invasive species such as the European gypsy moth *Lymantria dispar dispar* (Lepidoptera: Erebididae) into new areas. The European gypsy moth is an invasive forest pest in North America (Fernald and Forbush 1896). The insect is able to feed on over 300 species of trees and shrubs (Lance 1983) but exhibits a preference for trees in the genera *Quercus* and *Populus* (Barbosa 1978). Larvae of gypsy moth are typically active in the late spring and early summer, beginning with post-eclosion dispersal (Mason and McManus 1981; Sharov and Liebhold 1998; Erelli and Elkinton 2000). Post eclosion, first-instar larvae spin down from trees on strands of silk and balloon into the air on gusts of wind (Leonard 1981). First-instar larvae are more likely to balloon if they encounter food that is not preferred (Capinera and Barbosa 1976; Erelli and Elkinton 2000).

To our knowledge, no research has explored how feeding on suboptimal or nonpreferred hosts interacts with food deprivation to influence movement capacity and behavior, especially of late instar larvae. Gypsy moth progress through five (males) or six (females) instars (Fernald and Forbush 1896). Late instar larvae are able to crawl more than 100 m across open fields and use the incident angle of polarized light to orient their movement (Doane and Leonard 1975), frequently moving toward dark colored bark and objects that subtend the largest angles in their field of vision when searching for hosts (Roden et al. 1992; Smitley et al. 1993). Food deprivation, especially of preferred food sources, frequently occurs following outbreaks of gypsy moth as larvae defoliate large tracts of forest (Hough and Pimentel 1976). Preferred hosts are generally considered more suitable than many of the other host plants on

which larvae can feed (Lance 1983), while feeding on non-preferred hosts, such as maple (*Acer*), can increase developmental time, decrease frass production and pupal weight (Barbosa and Capinera 1977), and may reduce survival (Hough and Pimentel 1976). Understanding the movement of late instar larvae feeding on various hosts is of particular importance, as federal quarantine regulations in the USA specify methods to restrict the movement of late instar larvae in forested ecosystems (USDA-APHIS-PPQ 2010).

In this study, we examined the movement capacity of fifth and sixth instar larvae of European gypsy moth after feeding on hosts of different putative suitabilities with different periods of food deprivation under no-choice conditions. We used a servosphere (i.e. a locomotory compensator) to evaluate their movement capacity. Servospheres have been used in the study of insect movement in response to different visual or olfactory stimuli (Bell and Kramer 1980; Hammock et al. 2007; Otálora-Luna and Dickens 2011; Otálora-Luna et al. 2013) and provide a useful platform for performing individual based movement studies. We hypothesized that larvae that were fed on suboptimal hosts would not move as far as those feeding on optimal hosts, and that movement would decrease as food deprivation period increased.

Materials and Methods

Experimental Insects

Egg masses from the 76th generation of the New Jersey Standard Strain (NJSS; a laboratory colony of gypsy moth) were received from the U.S. Department of Agriculture (USDA) – Animal and Plant Health Inspection Service (APHIS) Otis Laboratory, Buzzards Bay, MA (APHIS Permit P526P-15-02469) to the University of Minnesota, Minnesota Agricultural Experiment Station (MAES)/Minnesota Department of Agriculture (MDA) Containment facility. Eggs masses of gypsy moth were held at 4 °C prior to use. Larvae were reared from eggs in an incubator at 25 °C, 67% humidity, and a 16 L:8D photoperiod. Larvae were raised in 2.2 L plastic containers (Ziploc) with mesh lids and approximately 50 larvae in each container.

We were interested in the effect of food type or host species on the movement behavior and capability of larvae, so we selected foliage from hosts that are

qualitatively considered susceptible, somewhat susceptible, and not susceptible to gypsy moth defoliation (Liebhold et al. 1995). Accordingly, we fed larvae one of five food types: foliage from bur oak (*Quercus macrocarpa*) (“susceptible”), eastern larch (*Larix laricina*) (“susceptible”), Norway maple (*Acer platanoides*) (“somewhat susceptible”), silver maple (*Acer saccharinum*) (“not susceptible”), or artificial diet (USDA Gypsy Moth Diet, Hamden Formula). Larvae that were raised on foliage received fresh foliage every 2 days during rearing. Larvae raised on artificial diet received new diet if they consumed all available diet, the diet became moldy, or if the diet became desiccated, which occurred every three to 5 days. Foliage was collected from trees on the University of Minnesota’s Saint Paul campus.

In addition to different food treatments, all larvae were assigned to one of three different food deprivation periods: 0 (“satiated” control), 24, or 48 h. To deprive larvae of food, they were removed from the containers with food and placed individually into 160 mL plastic cups (GP PRO Dixie Plastic Portion Cup) and held in their incubator until needed for trials. Larvae were randomly assigned to a food and starvation treatment.

Servosphere Trials

Larvae were placed one at a time on a 77 cm diameter servosphere that recorded the movement of each untethered insect as it walked on the apex of the sphere (Syntech LC-770, TrackSphere 3.1 software, Hilversum, Netherlands). As the insect walks in any direction at the apex of the sphere, a CMOS camera placed above the sphere detects movement of the insect. The software integrates this information to direct the movement of motors at the base which rotate the sphere to keep the insect on top. Movement was recorded every 10 ms at 0.1 mm resolution. Our servosphere was surrounded with a box constructed of plywood and painted black. The box was open on the top but blocked vision of the room from the gypsy moth larvae, providing a uniform visual environment and hiding objects and/or people in the room.

Larvae were randomly selected from all available insects/treatments on a given day and weighed using an electronic scale to the nearest 0.01 g before being placed on the servosphere. Once on the servosphere, larvae were provided 5 min to acclimate and move. The 5 min acclimation period was selected based on

preliminary trials that showed that larvae needed approximately 5 min to adjust to the environment before they began to move. If larvae did not move at all in these 5 min, they were removed from the servosphere and returned to their feeding container. If larvae moved during the 5 min acclimation period, larvae were given an additional 10 min to move. The movement of the larvae was recorded during the entire 15 min period but only the last 10 min were analyzed (see below). We ran trials for each of the 12 experimental combinations (i.e. 4 diets × 3 feeding statuses) until we had at least 20 trials for each treatment combination where larvae moved more than 10 cm (see Results). This procedure was chosen to ensure that we could assess if there were any differences in moving behavior (i.e. not just the propensity to move); it was not uncommon for larvae to move a small amount during the acclimation period but then cease during the trial period. The servosphere was cleaned with an 80% ethanol solution in between each trial to remove any silk that larvae may have laid, although silk-laying behavior was never observed.

All trials with gypsy moth raised on artificial diet were performed from March 21, 2018 – April 13, 2018. Foliage trials were conducted from June 4, 2018 – June 21, 2018. All trials were done in the same room in the BSL-2 containment facility, which averaged 22.7 °C and 63% humidity. Trials were performed between 0900 h and 1900 h. Lighting in the room was provided by Sylvania Premium 32 W T8 5000k fluorescent bulbs.

Track Analysis

When analyzing searching behavior, we discarded the 5 min acclimation period from all trials and analyzed only the 10 min trial period. Each path recording provides the change in time between position recordings (10 ms) as well as the change in position of the insect in the x and y directions. We condensed raw movement recordings from 10 ms intervals to 1 s intervals. This procedure allowed us to more efficiently analyze track data and reduced noise in the position recordings created by side-to-side movement of the insect or sudden direction changes. From these data, we calculated the distance between each recording position. We aggregated these variables over each path to calculate total distance moved, net displacement, tortuosity, mean number of stops, and mean length of stops. Net displacement was calculated as the change in distance between the start

location of larvae and their ending location. Tortuosity is a unitless ratio of net displacement to total distance moved and is used as a measure of path straightness: a value of one indicates a perfectly straight path. As tortuosity decreases towards zero the path becomes more “meandering” (Benhamou 2004). Velocities lower than 0.1 cm/s were caused by minor movements of the insect as it looked around or moved appendages and were considered “stops”. Paths were classified qualitatively into two categories: lines and loops. Loops were defined as a path where the larvae made at least two clear, consecutive circular paths while moving. Lines were defined as paths devoid of loops (Fig. 1). All track analysis and variable calculations were carried out in R (R Core Team 2016) using the package “servosphereR” (Wittman and Aukema 2019).

Statistical Analysis

The primary goals of our analyses were to determine if there were differences in the movement of larvae feeding on different foods and if food type affected their behavior as they were starved (i.e. the interaction of food type and starvation time). To assess any differences among food treatments, we conducted one-way ANOVAs restricting data to only the larvae in the satiated groups. Our response variables were total distance moved, net displacement, mean length of stops. These ANOVAs were fit using generalized linear models (GLM) with a gamma distribution and identity link to normalize residuals and ensure equal variances in residuals. We used logistic regression to model the probability of moving (i.e. a GLM with a binomial distribution and logit link). Similarly, we used a GLM with a negative binomial distribution and log link to meet model assumptions and account for overdispersion when analyzing the number of stops. For an ANOVA examining the effect of food type on tortuosity, the response variable was arcsin square-root transformed to normalize residuals and meet assumptions of homoscedasticity. Tukey’s Honest Significant Difference (HSD) was used for post-hoc means comparisons.

To determine how starvation time affected movement, we fit GLMs with food type as a factor, time since last feeding as a covariate, and the interaction of those two terms. These models were fit using the same distributions or response variable transformations as described above. We used likelihood ratio or F -tests to determine if the interaction between food and starvation

time was significant ($\alpha = 0.05$). If there was a significant effect of the interaction between food type and starvation time, we report the factor-adjusted slopes for the relationship between the food type and starvation time.

After analyses, we added a term for the time of day that trials occurred to each model to see whether accounting for the time of day affected inferences regarding effect of food type and deprivation times on any movement parameter. In the field, gypsy moth larvae can exhibit different patterns of behavior depending on the time of day (Doane and Leonard 1975; Leonard 1981). The time of day at which the trial was conducted did not affect movement of gypsy moth larvae for any parameter measured ($P > 0.05$ for all results), so it is not further reported. All statistical analyses and graphs were done in R (R Core Team 2016). All means are reported \pm one standard error.

Results

Overall, 358 gypsy moth larvae were placed on the servosphere, with 244 individuals moving more than 10 cm during the 10 min trial window. Thirty-seven out of these 244 larvae moved in a looping path (Fig. 1). There were no significant differences among food treatments or an effect of starvation time on the probability of looping.

Larval Development

Larvae developed from egg to fifth instar in approximately 33 days, with variation depending on diet type. Larvae raised on bur oak reached fifth instar approximately 1 week before larvae raised on larch or Norway maple. Larvae restricted to leaves of silver maple did not develop well; most larvae died by the end of the third week. Those larvae that survived reached the third instar by the time cohorts on alternate diets were at least in their fifth instar. No larvae feeding on silver maple reached the fifth instar so no silver maple-fed larvae were used in movement trials. Larvae raised on artificial diet developed at a similar rate to those fed larch and Norway maple. There was no significant effect of food type on final larval mass, so mass was not included in any analyses.

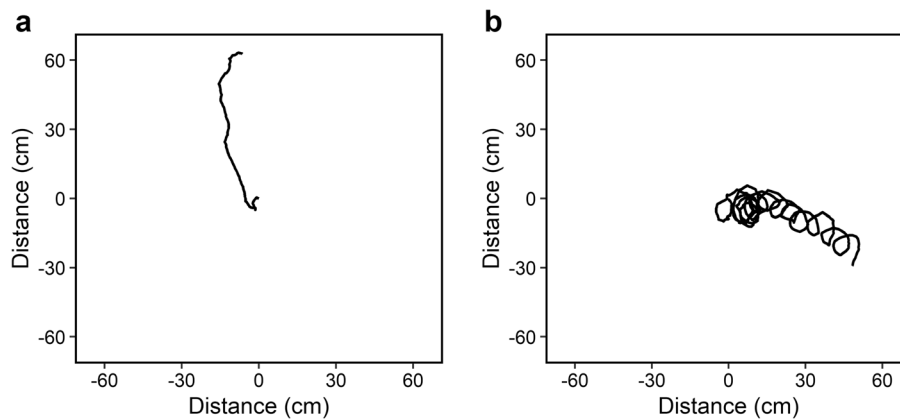


Fig. 1 Two examples of movement paths taken by late instar larvae of European gypsy moth on a servosphere in laboratory settings from March 21, 2018 – June 21, 2018 in Minnesota, USA. These plots represent (a) the most common type of path seen in these experiments consisting of mostly straight-line movements

with a few direction changes and (b) an obvious “looping” path, defined as more than two consecutive loops in a single path. Approximately 10% of larvae placed on the servosphere walked in a “looping” path

Probability of Moving

Among satiated larvae, there was a significant effect of food type on the probability of moving ($\chi^2 = 36.591$, $df = 3$, $p < 0.0001$). Overall, larch-fed larvae were the most likely to move as 20 out of 22 larch-fed larvae moved more than 10 cm during the trial recording period, followed by larvae that fed on artificial diet (20 out of 33). These movement probabilities were not significantly different from one another; however, the probability that the larch cohort would move was significantly greater than the probabilities of moving in both the Norway maple and bur oak cohorts (Table 1). Two-fifths of larvae reared on Norway maple moved more than 10 cm when placed on the servosphere, or 20 out of 50 insects, while approximately a quarter of insects that had fed on bur oak exhibited movement sufficient to record (21 out of 76) (Table 1). The time of day that trials occurred did not affect the probability that larvae would move ($\chi^2 = 3.039$, $df = 1$, $p = 0.081$).

Across all food treatments, as starvation time increased, the probability an insect would move more than 10 cm increased ($\chi^2 = 82.049$, $df = 1$, $p < 0.0001$) (Fig. 2). The interaction of starvation and food was also significant ($\chi^2 = 8.890$, $df = 3$, $p = 0.0308$), indicating that the likelihood an insect would move with different lengths of food deprivation varied among diets on which the insects had previously been feeding. Cohorts that had fed upon artificial diet ($p = 0.0156$), bur oak ($p < 0.0001$), and Norway maple ($p = 0.0002$) were more likely to move the longer they were deprived of

food. There was no significant association between the probability of movement and starvation for the larch cohort ($p = 0.2081$) (Fig. 2c). The time of day that trials occurred did not affect the probability that an insect would move.

Distances Moved

The food upon which larvae were raised had a significant effect on the total distance they moved ($\chi^2 = 36.904$, $df = 3$, $p < 0.0001$) (Fig. 3a). Artificial diet-fed satiated larvae moved 82.8 ± 40.2 cm, which was significantly less than the other three cohorts ($p < 0.0001$ for all three comparisons). There was no significant difference among the total distances moved by larvae feeding on foliage of bur oak (259.4 ± 38.2 cm), larch

Table 1 Probabilities that satiated (i.e. unstarved) larvae of European gypsy moth raised on a particular food will move more than 10 cm when placed on a servosphere. Lab trials were conducted from March 21, 2018 – June 21, 2018 in Minnesota, USA. Probabilities sharing a letter are not significantly different from one another

Food	Probability of moving	95% CI	<i>n</i>
Bur oak	0.276 c	0.187–0.387	122
Artificial diet	0.606 ab	0.433–0.756	81
Larch	0.909 a	0.700–0.977	63
Norway maple	0.400 bc	0.275–0.540	92

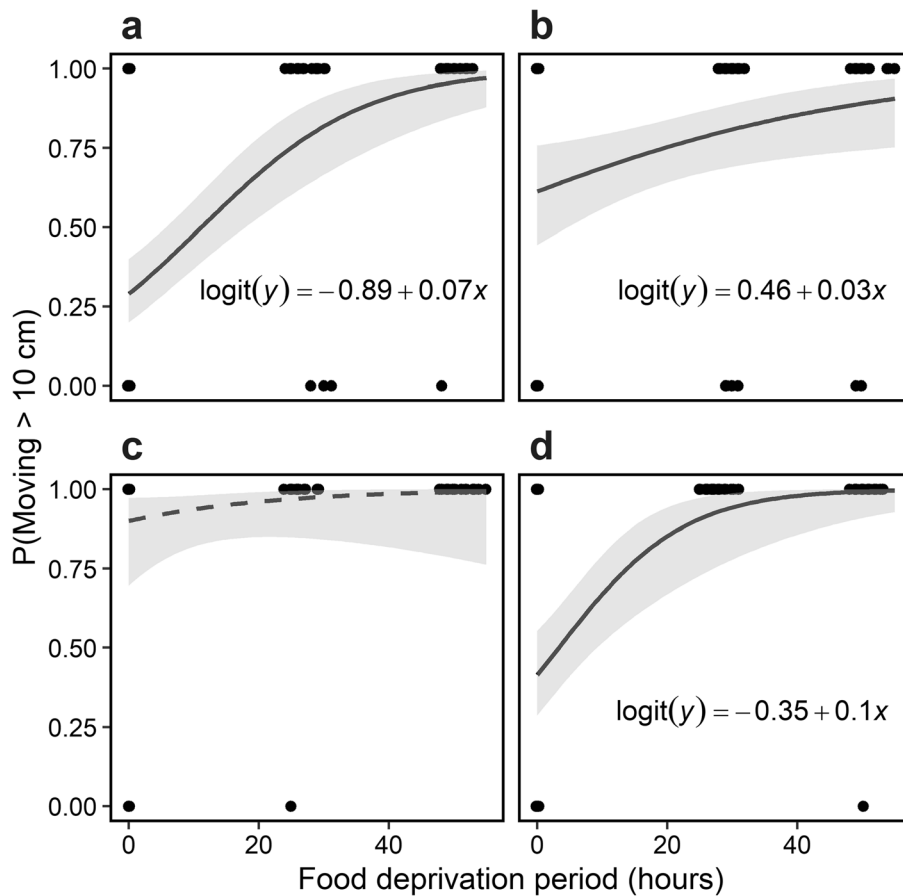


Fig. 2 Relationship between the probability larvae of European gypsy moth ($n = 358$) will move greater than 10 cm on a servosphere and how long larvae have been deprived food for (a) bur oak, (b) artificial diet, (c) larch, (d) Norway maple. Lab

trials were conducted from March 21, 2018 – June 21, 2018 in Minnesota, USA. Grey bands represent 95% confidence interval for model predictions. Solid lines represent statistically significant trend lines; in such cases equations are reported

(281.7 ± 57.1 cm), or Norway maple (223.0 ± 50.9 cm). We did note, however, that distances traveled varied depending on length of food deprivation time ($\chi^2 = 9.789$, $df = 1$, $p = 0.0018$), and the slope of these relationships varied with previous diet type (food and starvation time interaction) ($\chi^2 = 11.983$, $df = 3$, $p = 0.0074$) (Fig. 4). Larvae that ate bur oak and larvae that ate diet moved farther the longer they were starved (bur oak = 3.2 ± 1.4 cm/h starved, $p = 0.0188$; artificial diet = 1.7 ± 0.5 cm/h starved, $p = 0.0002$) (Fig. 4a & b). Such relationships disappeared for insects feeding on larch and Norway maple; insects appeared to move less the longer they were starved (slope estimate = -1.2 ± 0.9 cm/h starved for larch; Fig. 4c and -0.1 ± 1.1 cm/h starved for Norway maple; Fig. 4d). These latter slopes were not significantly different from zero, however ($p > 0.05$) (Fig. 4c).

Similar to total distanced traveled, net displacement varied significantly among the satiated larvae depending on their food type ($\chi^2 = 14.545$, $df = 3$, $p = 0.0020$) (Fig. 3b). Larvae raised on larch had the highest net displacement (252.0 cm \pm 69.5 cm); displacement among the larch-fed larvae was significantly higher than that of the artificial diet-fed larvae (60.1 ± 32.2 cm). The bur oak cohort had an average net displacement of 115.4 ± 28.4 cm, which was similar to net displacement for the larvae raised on Norway maple (112.0 ± 32.2 cm). Neither the cohorts feeding on bur oak nor Norway maple exhibited net displacements that were significantly different from the artificial diet or larch cohorts.

Displacement was also significantly affected by time spent starved ($\chi^2 = 4.319$, $df = 1$, $p = 0.0378$) and its interaction with the food treatments ($\chi^2 = 10.599$, $p = 0.0141$) (Fig. 5). Displacement increased as larvae went

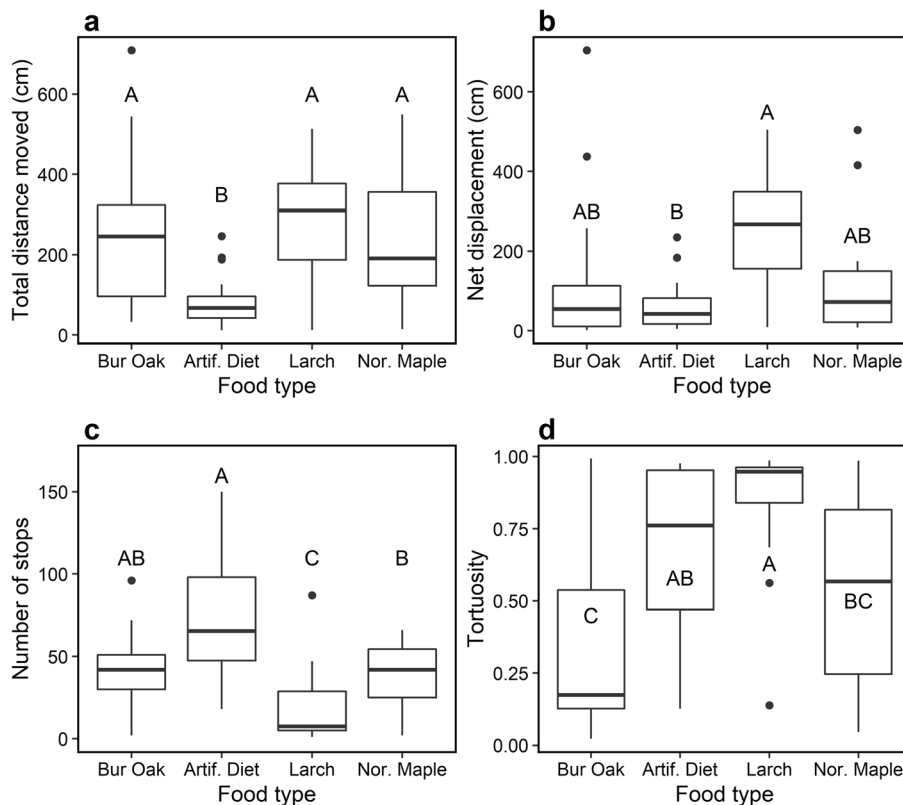


Fig. 3 Boxplots comparing the movement of satiated late instar larvae of European gypsy moth raised on different foods, on a servosphere ($n = 244$). Lab trials were conducted from March 21, 2018 – June 21, 2018 in Minnesota, USA. The thick line in the middle of the box represents the median observation for that group. The top and the bottom of the box represent the first and third quartiles, respectively. The “whiskers” extending from the box extend no farther than 1.5 times the interquartile range (the

distance between the third and first quartiles). Dots represent values larger or smaller than 1.5 times the interquartile range. Uppercase letters represent post-hoc means comparisons with Tukey’s HSD test; groups with different letters are significantly different from one another ($p < 0.05$). **a** Total distance moved. **b** Net displacement. **c** Number of stops during a 10 min trial. **d** Tortuosity (the ratio of net displacement to total distance moved) of larval movement paths

longer without food for both the bur oak (2.8 ± 1.1 cm/h starved, $p = 0.0117$) and Norway maple-fed larvae (2.5 ± 1.2 cm/h starved, $p = 0.0348$) (Fig. 5a & d). The larch-fed cohort tended to stay closer to their point of origination with increasing starvation time, but again this relationship was not significant (1.7 ± 1.1 cm/h starved, $p = 0.1265$) (Fig. 5b). There was a small positive relationship between displacement and starvation time in the larvae raised on diet, but this relationship was also not statistically significant (0.8 ± 0.4 cm/h starved, $p = 0.0834$) (Fig. 5c).

Behavior

The number of times that satiated larvae stopped moving on the servosphere varied with their diet ($\chi^2 = 38.81$, $df = 3$, $p < 0.0001$) (Fig. 3c). The satiated diet cohort also stopped significantly more (72.20 ± 11.17 stops)

than the larch (17.25 ± 2.79 stops) and Norway maple cohorts (39.50 ± 6.18 stops), but not the bur oak cohort (42.62 ± 6.50 stops). Bur oak and Norway maple-raised larvae stopped significantly more times than the larch cohort. There was no significant effect of food type or food deprivation on the length of stops that larvae took but there was a statistically significant effect of the interaction of time since last feeding and food type on the number of stops exhibited during the trial ($\chi^2 = 14.846$, $df = 3$, $p = 0.0020$) (Fig. 7). The cohorts feeding on artificial diet and bur oak foliage stopped less the longer they went without food (artificial diet $p = 0.0214$; bur oak $p = 0.0303$), while the larch cohort stopped more as starvation time increased ($p = 0.0297$). There was no significant relationship between the number of stops and time without food for Norway maple-fed larvae ($p = 0.4894$) (Fig. 6d).

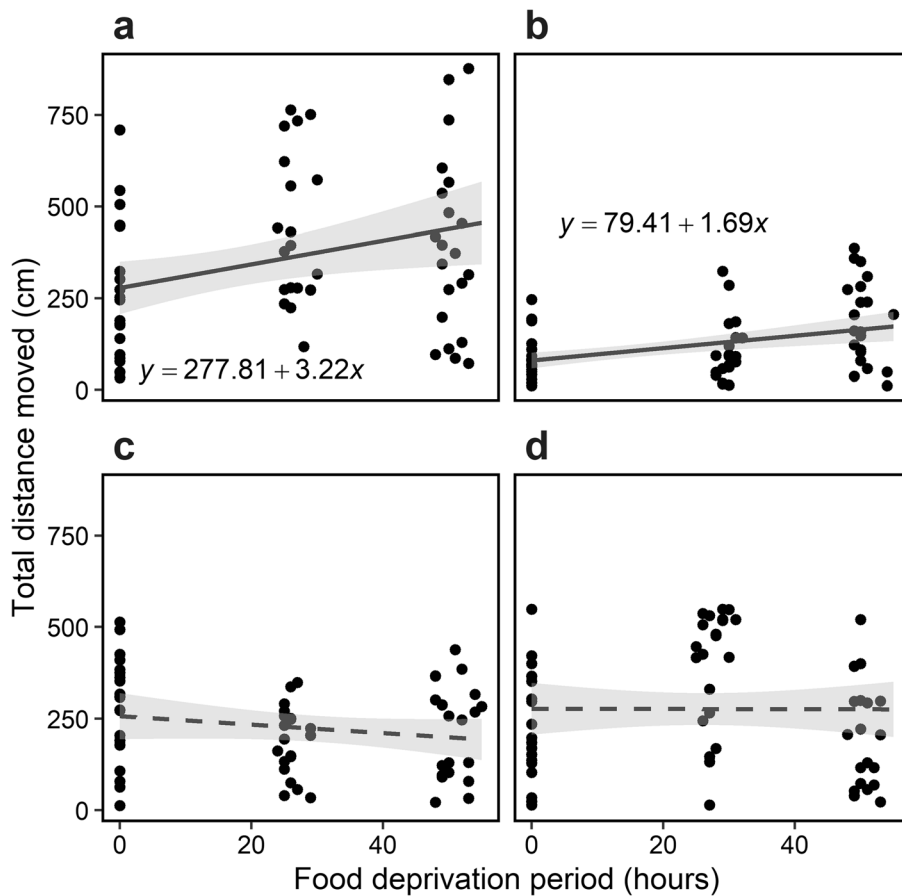


Fig. 4 Total distance moved by late instar larvae of European gypsy moth on a servosphere as time without food increased ($n = 244$). Larvae were raised on one of four foods: **a** bur oak, **(b)** artificial diet, **(c)** larch, **(d)** Norway maple. Lab trials were

conducted from March 21, 2018 – June 21, 2018 in Minnesota, USA. Grey bands represent 95% confidence interval for model predictions. Solid lines represent statistically significant trend lines; in such cases equations are reported

Among satiated larvae, food type had a significant effect on the tortuosity of movement paths ($F_{3,77} = 4.25$, $p < 0.0001$) (Fig. 3d). The larch-fed cohort had the straightest paths with tortuosity 0.86 ± 0.19 , which was significantly straighter than the Norway maple (0.54 ± 0.12) and the bur oak cohorts (0.35 ± 0.07). Additionally, the artificial diet-fed larvae had a mean tortuosity of 0.68 ± 0.15 , which was approximately double that of the bur oak-fed larvae. Starvation time alone did not account for variation in tortuosity but the interaction of starvation time and food source did ($F_{3, 236} = 3.77$, $p = 0.0113$) (Fig. 7). The Norway maple-fed cohort moved in straighter paths the longer they were starved (0.005 ± 0.002 units/h starved, $p = 0.0159$) (Fig. 7d); the tortuosity of the other cohorts did not change with starvation time.

Discussion

Both food type and food availability alter the movement behavior and capabilities of gypsy moth larvae and responses are not uniform across all food types for this polyphagous invasive defoliator. Larvae that fed on foliage (as opposed to the artificial diet) had the same initial capacity for movement in terms of total distance moved but only larvae that fed on bur oak, a preferred food source (Barbosa and Greenblatt 1979; Liebhold et al. 1995), moved farther as they went longer without food (Figs. 3a & 4). In contrast, larvae that fed on larch and Norway maple traversed approximately the same distance no matter how long they went without food (Figs. 4 cd). These disparate responses are likely due to the differences in the suitability of host foliage.

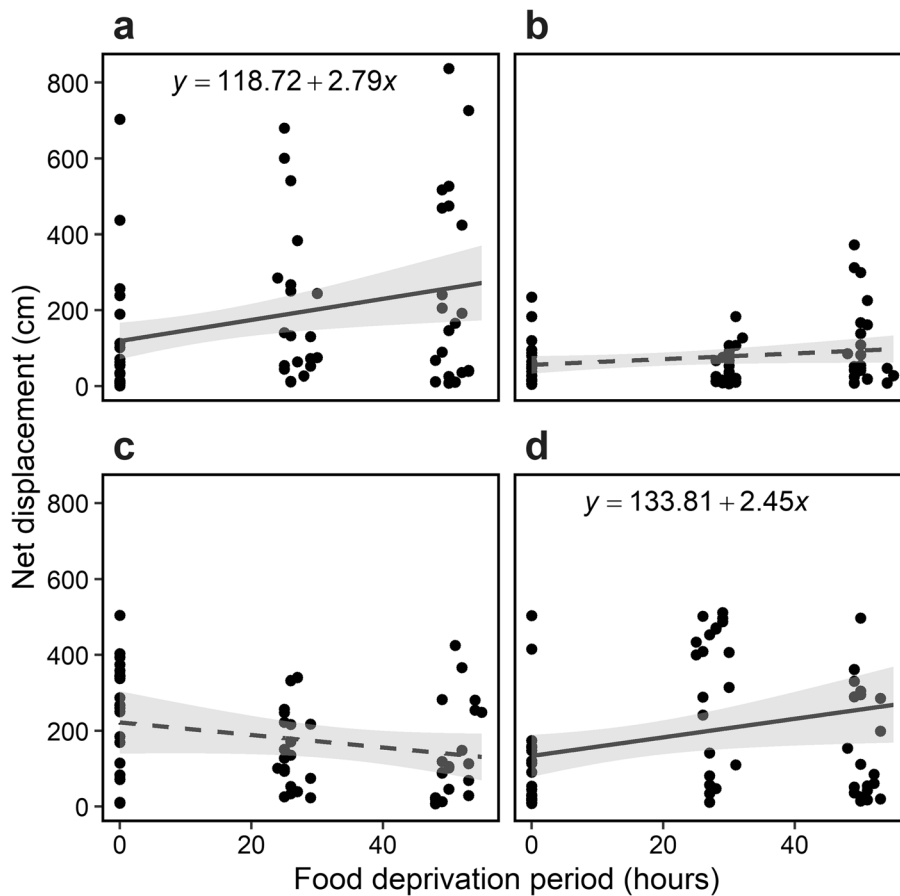


Fig. 5 Net displacement of late instar larvae of European gypsy moth on a servosphere as time without food increased ($n = 244$). Larvae were raised on one of four foods: **a** bur oak, **(b)** artificial diet, **(c)** larch, **(d)** Norway maple. Lab trials were conducted from

March 21, 2018 – June 21, 2018 in Minnesota, USA. Grey bands represent 95% confidence interval for model predictions. Solid lines represent statistically significant trend lines; in such cases equations are reported

Suitability is a qualitative metric that takes into consideration propensity to feed on foliage, survival, and/or life history characteristics such as developmental rate, the weight of different life stages, and fecundity (Hough and Pimentel 1976; Liebhold et al. 1995). The differences in these characteristics are often due to the quality and quantity of nutrients available in the host foliage (Lindroth et al. 1991, 1997). When herbivores feed on non-preferred or less preferred hosts, they often have to increase consumption rates to obtain the necessary amounts of macro and micronutrients (Lindroth et al. 1991; Couture et al. 2016) or make up energy deficits mediated by host plant defenses (Yu 1983). While few studies have examined the effects of specific nutritional deficiencies or sub-optimal hosts on movement, it seems plausible that the costs incurred by feeding on sub-optimal hosts would also impact the movement behavior of herbivores.

Bell (1990a, b) discusses, for example, how the internal physiological state of the insect influences searching behavior. The behavior chosen by an insect is based on the magnitude of the nutrient or energy deficit and the rate at which the behavior can be performed, which is itself a function of the magnitude of the deficit. In other words, it is reasonable to conclude that when a gypsy moth has been feeding on an optimal host such as *Quercus* or *Populus* spp. and is suddenly deprived of food, it would have the necessary stores of energy and nutrients to move farther and more quickly in search of food relative to a gypsy moth that has been feeding on a sub-optimal host such as *Acer* spp. We may have seen more exaggerated responses if we had held food-deprived larvae in laboratory containers larger than 160 mL. Larger containers would have permitted increased potential movement during the starvation period, which may have decreased insects' use of energy stores before being placed on the servosphere.

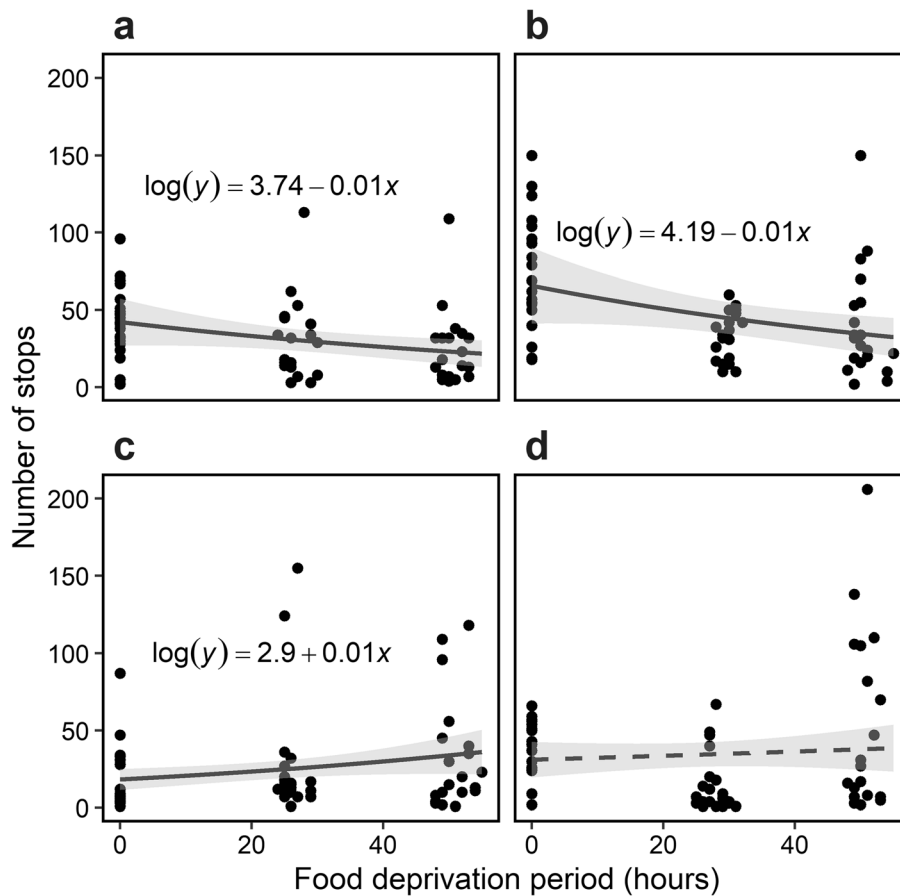


Fig. 6 Number of stops taken by late instar larvae of European gypsy moth while moving on a servosphere as time without food increased ($n = 244$). Larvae were raised on one of four foods: **a** bur oak, **(b)** artificial diet, **(c)** larch, **(d)** Norway maple. Lab trials were

conducted from March 21, 2018 – June 21, 2018 in Minnesota, USA. Grey bands represent 95% confidence interval for model predictions. Solid lines represent statistically significant trend lines; in such cases equations are reported

Even though European gypsy moths are polyphagous, larval gypsy moths are better suited to feed on species from their native range, such as *Quercus* spp., than North American *Acer* spp. or eastern larch, which is also native to North America (Barbosa and Capinera 1977). Eastern larch is a deciduous conifer and is one of the only conifers listed as suitable for gypsy moths (Campbell and Sloan 1977; Liebhold et al. 1995). Needles of conifers are generally not chosen over foliage of other, more preferred hardwood species (Leonard 1981) but may be consumed when population levels are high (Leonard 1981; Liebhold et al. 1994). Our finding that “satiated” larvae that fed on larch were the most likely to move (Fig. 2c), suggests that they were not truly “satiated”. Moreover these insects did not increase their total distances when deprived of food but instead became more likely to

stop while walking. The opposite responses were seen from the bur oak and artificial diet-fed cohorts, suggesting that such metrics may be functions of host suitability, and that early spring-flushing needles of eastern larch are only marginally suitable for this insect. More research is needed to determine if the frequency of stops is related to nutrition or energy requirements. Norway maple is native to Europe but listed as marginally suitable (Liebhold et al. 1995), similar to most *Acer* species (Barbosa and Capinera 1977). Yet, the responses of satiated insects feeding on Norway maple leaves were not statistically different from those of the highly suitable host bur oak (Fig. 3) and insects feeding on Norway maple were less likely to move than those that fed on marginally suitable eastern larch from North America (Fig. 2).

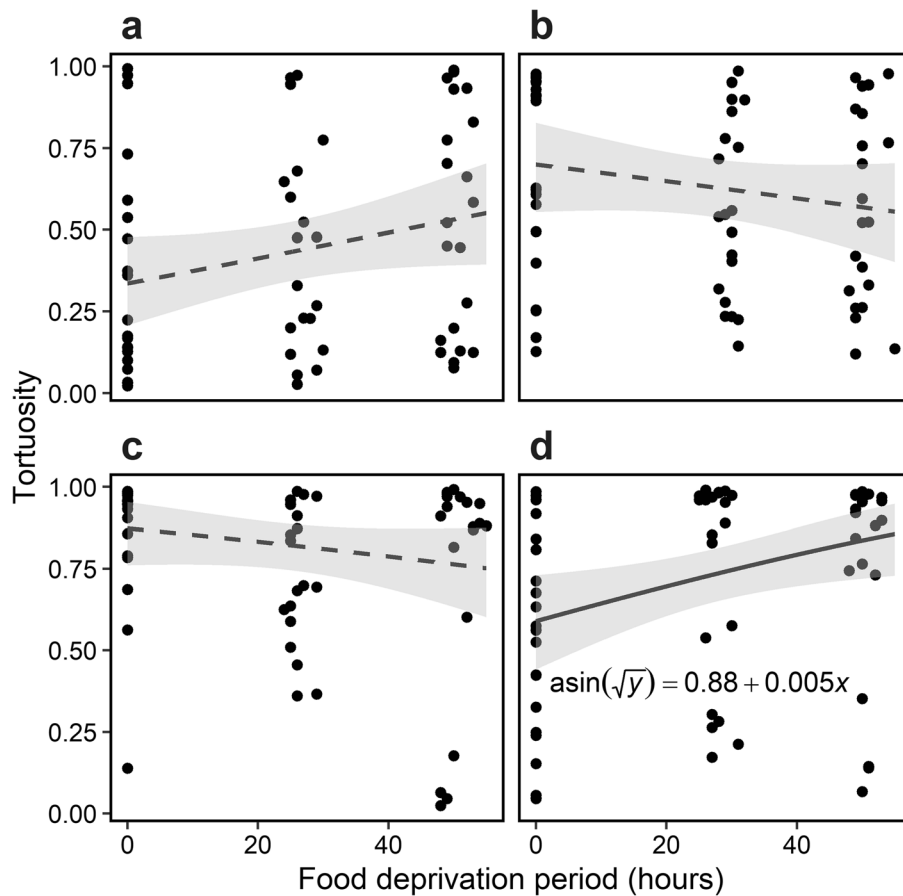


Fig. 7 Tortuosity (the ratio of net displacement to total path distance) of late instar larvae of European gypsy moth on a servosphere as time without food increased ($n = 244$). Larvae were raised on one of four foods: **a** bur oak, **(b)** artificial diet, **(c)** larch,

(d) Norway maple. Lab trials were conducted from March 21, 2018 – June 21, 2018 in Minnesota, USA. Grey bands represent 95% confidence interval for model predictions. Solid lines represent statistically significant trend

The gypsy moth larvae showed a variety of movement paths while on the servosphere with no consistent, discernible patterns. The efficiency of a search pattern depends on the spatial and temporal distribution of hosts (Jones 1977; Jones et al. 1980; Conrath et al. 2000) but in the absence of information a straight line of movement with periodic direction changes may be most efficient (Bell 1990a). Based on a qualitative assessment of the paths, most of the larvae moved in straight lines with occasional directional changes. Approximately 10% moved in spiral paths, which may be more efficient if the patches of available food are relatively small (Bell 1990a). The Norway maple cohort did show an increase in path straightness as food deprivation time increased, increasing displacement without increasing total distance moved (Figs. 4d & 5d). As the Norway maple cohort's nutritional or energy deficits increased, it is

possible that the internal physiological and/or neurological states induced a more efficient search pattern. We saw large variation, however, in the tortuosity of movement paths of larvae across the other foliage treatments so interpret such results with caution.

Larvae raised on artificial diet exhibited the same trends as the bur oak group, albeit at a lower magnitude, in total distance moved and net displacement (Figs. 4ab & 5ab). It is unclear why the larvae raised on artificial diet would show responses lower in magnitude than those raised on bur oak, but there appeared to be less variability in the response of the artificial diet-fed larvae in total distance moved and displacement. These results suggest that the artificial diet composition exhibits less nutritional variation than leaves of bur oak, which may be expected from an artificial diet that is designed to be consistent. Grayson et al. (2015) noted decreased

performance in life history characteristics for the laboratory strain of larvae raised on foliage compared to artificial diet.

Previous research has demonstrated that gypsy moth larvae exhibit distinct movement patterns based on the time of day, with larvae being more active during crepuscular periods and night (Doane and Leonard 1975; Leonard 1981). When population densities are high though, larvae will increase activity during the day as well (Leonard 1981). The time of day we performed our trials did not affect the movement behavior of our larvae likely because our experiments took place during the 16 h photophase. Population density was not explicitly incorporated into our experiments; however, because larvae were consuming all or most of the foliage available to them before it was replaced, our rearing conditions likely better approximated high-density vs. low-density populations. Such conditions may explain why we saw relatively high activity levels during photophase when gypsy moth larvae are otherwise less likely to move.

These results advance our understanding of gypsy moth movement ecology and may have implications for practice in managing gypsy moth as an invasive forest pest in North America. A significant amount of effort and resources are spent on limiting the spread of the gypsy moth outside the quarantine zone, for example. Preventing gypsy moth larvae from pupating in or on products that may later be moved out of quarantine, such as logs, is done by placing these objects in a 100 ft radius buffer zone area devoid of any host vegetation. This buffer zone is thought to be effective in keeping larvae from pupating in the shelter provided by the logs, emerging as adults, and laying eggs that then may be inadvertently transported long distances by humans (Bigsby et al. 2011). There are several aspects of immature dispersal that are still not well understood, however, such as cues affecting the propensity for larval ballooning (Diss et al. 1996) and the timing and drivers of pupation site selection (Mauffette and Lechowicz 1984). Our results suggest that actively-feeding larvae on preferred hosts might be less likely to enter a buffer zone at moderate population densities. This hypothesis is consistent with a recent field study that demonstrated that late-instar larvae are less likely to move when preferred hosts are present on the edge of the buffer zone (Wittman et al. 2019). Larvae might be more likely to move into buffer zones, however, during outbreaks where larvae strip all available foliage (Leonard 1981).

Outbreaks are more likely in forest stands that are composed of greater than 20% preferred host species (Herrick and Gansner 1986; Liebhold et al. 1997). Larvae may also move into buffer zones as they cease feeding and search for pupation sites. Past work has shown, for example, that pupal abundance may be negatively correlated with preference of larval hosts (Barbosa 1978; Mauffette and Lechowicz 1984). Future work may aid our understanding of prepupal movement and contribute to informing management decisions for this invasive defoliator in field situations.

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