Effects of host type and food deprivation on the movement behavior of late-instar larvae of gypsy moth *Lymantria dispar* (Lepidoptera: Erebidae)

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Thesis Abstract

The European gypsy moth, *Lymantria dispar dispar* L. (Lepidoptera: Erebidae) is an invasive insect in North America. Gypsy moth larvae are highly polyphagous and capable of extensive defoliation during population outbreaks. The United States maintains a quarantine across the established range of the gypsy moth to help slow the spread of the moth. One component of the quarantine requires that entities that move wood products across quarantine boundaries stage those wood products within a buffer zone area devoid of any host vegetation. The purpose of the buffer zone is to reduce the likelihood that late instar gypsy moth larvae will pupate nearby, emerge as adults, mate, and lay eggs on the wood. In practice, this buffer zone is 100 feet in radius. It is difficult to evaluate the efficacy of the buffer zone practice, however, due to our limited understanding of the movement ecology of these larvae. Here, I study the movement ecology of late instar larvae of European gypsy moths. I investigate how host type and food deprivation affects the movement capacity and behavior of larvae in the laboratory using a servosphere. I then quantify the movement capacity and behavior of larvae in a field experiment simulating the buffer zone environment.

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Thesis Introduction

The gypsy moth, *Lymantria dispar*, (L.) (Lepidoptera: Erebidae) is an invasive forest insect introduced to North America in 1869 by the French naturalist Etienne Léopold Trouvelot (Fernald and Forbush 1896). The insect is classified as a pest due to its ability to reach high population levels over consecutive years, causing extensive defoliation (Leonard 1981, Elkinton and Liebhold 1990, Liebhold et al. 2000). Gypsy moths can feed on over 300 species of plants. Preferred hosts include species of oak (*Quercus*), willow (*Salix*), aspen (*Populus*), birch (*Betula*), and apple (*Malus*) (Liebhold et al. 1995). Defoliation by larvae can have negative impacts on trees, leading to ecological and economic impacts (Hattenschwiler and Schafellner 2004). Defoliation can lead to growth diameter loss and if defoliation occurs repeatedly for several years, it may cause tree death (Baker 1941, Campbell and Sloan 1977). Gypsy moth-induced mortality may lead to shifts in canopy structure from dominant tree species to sub-dominant species (Fajvan and Wood 1996). During outbreaks, numerous larvae become a public nuisance due to the large amount of frass produced, making home and recreational areas unappealing (Leonard 1981).

The gypsy moth is native to Eurasia. There are three subspecies of the gypsy moth in its native range: the European gypsy moth *L. dispar dispar* (L.), *L. dispar asiatica* Vnukovskij, and *L. dispar japonica* (Motschulsky). *Lymantria dispar asiatica* and *L. dispar japonica* are more commonly referred to as the Asian gypsy moth. Flight capability is the primary distinction between the Asian and European subspecies of gypsy moth; adult females of the Asian gypsy moth are capable of flight, whereas adult females of the European gypsy moth are not. (Schaefer et al. 1984, Reineke and Zebitz 1998, Shi et al. 2015). Since its introduction to Medford, Massachusetts, European gypsy moth has spread westward and southward in North America. Its range now extends west to Minnesota and south to North Carolina, as well as north into several Canadian provinces (Kauffman et al. 2017). Asian gypsy moths are frequently intercepted in monitoring traps along the western coast of North America but are not thought to be established currently in North America (Crook et al. 2014).

Gypsy moths are univoltine. Larvae hatch from eggs in the spring after their obligate overwintering diapause (Gray et al. 1991). Male larvae typically progress through five instars and females through six, but supernumerary instars are not uncommon, especially when larval populations are high or preferred food sources are unavailable (Hough and Pimentel 1976, Barbosa and Capinera 1977). Pupation typically occurs in late June or early July and adults will emerge a few weeks later to mate and lay eggs.

Being flightless, adult female European gypsy moths are unlikely to disperse long distances before laying eggs (Taylor and Reling 1986). First instar larvae are the life stage that is most able to disperse naturally (Mason and McManus 1981). These neonate larvae spin down from branches on silk and are carried by wind typically 1 to 3 km, but may be blown as far as 20 km (Capinera and Barbosa 1976, Mason and McManus 1981, Taylor and Reling 1986). Humans facilitate long range dispersal through inadvertent transport of egg masses, which are laid indiscriminately by adult females (Liebhold et al. 1992, Tobin et al. 2007). Larvae, for example, will look for pupation sites that are sheltered, such as bark flaps, but will use nearby man-made objects when no bark flaps are available (Campbell et al. 1976). Female moths frequently oviposit near their pupation site (Lance 1983), which may result in eggs being moved if wood, recreational vehicles, or household articles are transported from infested areas. If egg masses are moved beyond the current distribution of gypsy moth, isolated colonies may become established if individuals can overcome local Allee effects and find mates (Tobin et al. 2009). Isolated colonies, if left untreated, often eventually coalesce into larger populations along the expanding population front (Sharov and Liebhold 1998, Johnson et al. 2006).

In an attempt to reduce the rate of gypsy moth spread across the country, the "Slow the Spread" (STS) program was developed by the United States Department of Agriculture (USDA) Forest Service in 1999 (Sharov et al. 2002). STS attempts to detect and suppress satellite populations as they spread along the south- and west-ward advancing front of the infested area. A grid of pheromone-baited traps spaced evenly every 2 km in a 100 km wide strip along the length of the expanding population front is used to detect new infestations. When moths are captured, delimiting traps are placed at 0.5 km intervals in the surrounding area to determine the extent of the location of the satellite population. Once the population is delimited, the area is treated aerially with the bacterial insecticide *Bacillus thuringiensis* var. *kurstaki* to kill early instar larvae, or a sex pheromone (disparlure, mating disruption) that can suppress mating in low-density populations, enhancing Allee effects and resulting in the collapse of the population (Sharov et al. 2002, Liebhold and Bascompte 2003). The gypsy moth national management program also includes a quarantine protocol designed to address the issue of human-mediated spread and decrease the probability that egg masses will be moved by humans.

Once a county is under quarantine because gypsy moth is detected, the movement of a variety of articles, such as untreated wood products, across the quarantine zone is regulated (USDA-APHIS-PPQ 2010). Entities that move regulated articles from quarantine zones across boundaries must operate under compliance conditions designed to reduce the risk of transporting gypsy moths. One component of the compliance agreement for entities moving logs, pulpwood, and wood chips is the requirement that "Log, pulpwood and/or wood chip staging or holding areas must be set suitable distance away from standing trees" (Appendix E, USDA-APHIS-PPQ 2010). In practice, the "suitable distance" is a circle with a radius of 100 feet (MDA 2015). The intended purpose of this buffer zone area is to limit the likelihood that larvae will use the wood products as pupation sites. If the wood is used as a pupation site, it is likely that emerging females that successfully attract mates will oviposit on these products. Relatively little is known, however, about the movement ecology of late-instar larvae prior to pupal life stages, especially as it pertains to the buffer zone environment.

Movement ecology can be divided into four components that influence each other: the environment within which movement happens, the navigational capacity of the organism moving, the capacity of the organism for movement (i.e. mode of locomotion, speed, distance), and the internal physiological and neurological state of the organism (Nathan et al. 2008). Most work to date has focused on the navigational capacity of gypsy moth larvae and how they respond to their environment, specifically the cues they use to locate hosts. Larvae are most frequently found on trees that are preferred hosts, such as trees in the genus *Quercus*, but there is a slightly higher frequency of later instar larvae found on less preferred hosts (Barbosa 1978). Later instar larvae are more prone to wandering, which may explain why they are more often found on less preferred, but still suitable hosts (Barbosa and Capinera 1977, Leonard 1981). Gypsy moth larvae exhibit negative geotropism and tend to crawl up when possible. In early instars, larvae also exhibit positive phototropism, until the third instar at which point they begin to exhibit negative phototropism (i.e. movement away from light) (Wallis 1959). Larvae are also known to use the incident angle of polarized light to maintain their orientation while moving (Doane and Leonard 1975). When deciding where to move, larvae will often move towards whatever object subtends the largest angle in their field of vision. When given a choice between a light colored object and a dark colored one, larvae will move towards the darker object (Roden et al. 1992). Little is known about the movement capacities of gypsy moth larvae. One study reported larvae were capable of moving at least 125 m across a grassland field (Doane and Leonard 1975).

The goal of my thesis is to study the movement ecology and behavior of gypsy moth larvae, especially in relation to the 100 foot buffer zone requirement of the federal quarantine. At present, no scientific foundation exists with which to determine the effectiveness of the buffer zone. My thesis will address this issue through two components. In chapter 1, I measured the movement capacity and behavior of late instar gypsy moth larvae in the laboratory on a servosphere. Larvae used in this experiment were raised on different foods and subjected to different periods of food deprivation under no-choice conditions. In chapter 2, I observed the movement of late instar gypsy moth larvae in a buffer zone environment and examined how host plants staged on the edge of the buffer zone and food deprivation affected their movement. I used this field data to parameterize a Monte Carlo simulation model exploring how the probability that gypsy moth larvae would cross the buffer zone and reach wood at the center of a zone would change with different buffer zone radii. Both of these chapters contribute to our understanding of the movement capacity of gypsy moth larvae and how their external environment and physiological state influence their movement.

The chapters of this thesis were prepared for publication in peer-reviewed journals. I am the lead author, but the work of the remaining chapters is the combined results of multiple authors, hence my use of the plural voice in these sections. Chapter 1 is being prepared for submission to the *Journal of Insect Behavior*. Chapter 2 is being prepared for submission to *Environmental Entomology*. Each chapter may have some redundancy, which is included to preserve the integrity of each chapter as a stand-alone unit.

Chapter 1

Foliage type and availability alters the movement behavior of late instar European gypsy moth *Lymantria dispar* (Lepidoptera: Erebidae)

Synopsis

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 severly 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 larvae were also deprived of food for zero, 24, or 48 hours. 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 and faster 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. Implications to mitigating this invasive insect in areas of heavy defoliation on preferred hosts are discussed.

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 movement behavior of an insect is primarily determined by four factors: internal physiological and neurological state (i.e. the insects “drive” to move); knowledge (or lack thereof) about the insect’s location, orientation, and surroundings; capacity for movement; and external factors (Nathan et al. 2008). These external factors include biotic and abiotic factors present in the environment around the insect, such as habitat structure, climate, or predators. An insect’s capacity for movement is often characterized by its mode of movement (e.g. flying vs. walking), velocity, and duration or distance of movement.

Both the 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 nutrition and energy available to herbivorous insects is determined by the range of host plants on which they can feed. Not all host plant choices are optimal or preferred, and sub-optimal hosts may affect development (Barbosa and Capinera 1977), survival (Wetzel et al. 2016), fecundity (Awmack and Leather 2002), and dispersal (Lance and Barbosa 1982, Erelli and Elkinton 2000). Feeding on suboptimal hosts by the sweet potato whitefly *Bemisia tabaci* Gennadius (Hemiptera Aleyrodidae), for example, caused the insects to leave their hosts earlier in life (Blackmer and Byrne 1993). Similarly, *Phthorimaea operculella* Zeller (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 host feeding, food availability, and the interaction of the two affects 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: Erebidae) 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. The spring-hatching first instar encompasses most natural dispersal activity (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). Later 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). While preferred hosts are generally considered more suitable than many of the other host plants on which larvae can feed (Lance 1983), 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 target 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 (NJSS) were shipped 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. Egg 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 16L:8D photoperiod. Larvae were raised in 2.2 L plastic containers (Ziploc) with 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 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* Michx) ("susceptible"), eastern larch (*Larix laricina* (Du Roi) K. Koch) ("susceptible"), Norway maple (*Acer platanoides* L.) ("somewhat susceptible"), silver maple (*Acer saccharinum* L.) ("not susceptible"), or artificial diet (USDA Gypsy Moth Diet, Hamden Formula). Larvae that were raised on foliage received fresh foliage every two days during rearing, while larvae raised on artificial diet received new diet if they consumed all available diet, the diet became moldy, or if the diet became dessicated. 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 hours. To deprive larvae of food, they were removed from the containers with food and placed individually into 5.5 oz 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 of the sphere that rotate to keep the insect on top of the sphere. 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 five minutes to acclimate and move. The five minute acclimation period was selected based on preliminary trials that showed that larvae needed approximately five minutes to adjust to the environment before they began to move. If larvae did not move at all in these five minutes, they were removed from the servosphere and returned to their feeding container. If larvae moved during the five minute acclimation period, larvae were given an additional ten minutes to move. The movement of the larvae was recorded during the entire 15 minute period but only the last 10 minutes were analyzed (see below). We ran trials for each of the 12 experimental combinations (i.e. 4 diets x 3 feeding statuses) until we had at least 20 trials for each treatment combination where larvae moved more than 10 cm. 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.

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. Lighting in the room was provided by Sylvania Premium 32 Watt T8 5000k fluorescent bulbs.

Track analysis

When analyzing searching behavior, we discarded the five minute acclimation period from all trials and analyzed only the ten minute 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 and velocity between each recording position. We aggregated these variables over each path to calculate total distance moved, net displacement, tortuosity, mean velocity, 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. 1.1). All track analysis and variable calculations were carried out in R (R Core Team 2016).

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 velocity, and 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. We used the arcsin square-root transformation on tortuosity because we could not find a distribution that met the assumptions required for a GLM. 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. All statistical analyses and graphs were done in R (R Core Team 2016). All means are reported ± one mean standard error.

Results

Overall, 358 gypsy moth larvae were placed on the servosphere, with 244 individuals moving more than 10 cm during the 10 minute trial window. Thirty-seven out of these 244 larvae moved in a looping path, with the remainder moving in mainly straight lines (Fig. 1.1). 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 33 days on average, with variation depending on diet type. Larvae raised on bur oak reached fifth instar approximately one 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.

Probability of moving

Among satiated larvae, there was a significant effect of food type on the probability of moving ($\chi^2 = 34.37$, 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).

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. 1.2). 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. 1.2c).

Distances moved

Larval food type had a significant effect on the total distance they moved ($\chi^2 = 35.140$, df = 3, $p < 0.0001$) (Fig. 1.3a). 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 (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. 1.4). Larvae that ate bur oak and larvae that ate diet moved farther the longer they were starved (bur oak = 3.2 ± 1.4 cm/hour starved, $p = 0.0188$; artificial diet = 1.7 ± 0.5 cm/hour starved, $p = 0.0002$) (Fig. 1.4a & 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/hour starved for larch; Fig. 1.4c and -0.1 ± 1.1 cm/hour starved for Norway maple; Fig. 1.4d). These latter slopes were not significantly different from zero, however ($p > 0.05$) (Fig. 1.4c).

Similar to total distanced traveled, net displacement varied significantly among the satiated larvae depending on their food type ($\chi^2 = 16.577$, df = 3, $p = 0.0008$) (Fig. 1.3b). Larvae raised on larch had the highest net displacement (252.0 cm ± 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. 1.5). Displacement increased as larvae went longer without food for both the bur oak (2.8 ± 1.1 cm/hour starved, $p = 0.0117$) and Norway maple-fed larvae (2.5 ± 1.2 cm/hour starved, $p = 0.0348$) (Fig. 1.5a & 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/hour starved, $p = 0.1265$) (Fig. 1.5b). 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/hour starved, $p = 0.0834$) (Fig. 1.5c).

Behavior

Food had a significant effect on the mean velocities of larvae ($\chi^2 = 35.263$, df = 3, $p < 0.0001$) (Fig. 1.3c). Diet-raised larvae moved the slowest with a mean velocity intercept of 0.1 ± 0.1 cm/s, which was significantly lower than the velocity of the three other cohorts of larvae ($p < 0.0001$ for all three comparisons). The mean velocity for the satiated bur oak-fed larvae was 0.4 ± 0.1 cm/s, which was similar to both the velocities of both the Norway maple-fed larvae (0.4 ± 0.1 cm/s) and the larch-fed larvae (0.5 ± 0.1 cm/s). Velocities changed, however, with starvation time ($\chi^2 = 9.813$, df = 1, $p = 0.0017$), and again we noted an interaction of starvation time and food source ($\chi^2 = 12.430$, df = 3, $p = 0.0060$) (Fig. 1.6). The longer bur oak-fed larvae went without food, the faster they moved (0.0053 ± 0.0022 cm/s/hour starved, $p = 0.0180$) (Fig. 1.6a). Similarly, artificial diet-fed larvae also increased their velocity the longer they went without food (0.0028 ± 0.00074 cm/s/hour, $p = 0.0002$) (Fig. 1.6b). As before, the velocity of the larch cohort decreased slightly but non-significantly as their time since feeding increased (-0.0020 ± 0.0014 cm/s/hour starved, $p = 0.1774$) (Fig. 1.6c) and there was no significant relationship between velocity and time starved for the Norway maple-fed larvae (-4.6×10^{-5} ±, 0.0018 cm/s/hour starved, $p = 0.9795$) (Fig. 1.6d).

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. 1.3d). 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. 1.7). 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. 1.7d).

Among satiated larvae, food type had a significant effect on the tortuosity of movement paths ($F_{3,77} = 4.25$, $p < 0.0001$) (Fig. 1.3e). 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. 1.8). The Norway maple-fed cohort moved in straighter paths the longer they were starved (0.005 ± 0.002 units/hour starved, $p = 0.0159$) (Fig. 1.8d); the tortuosity of the other cohorts did not change with starvation time.

Discussion

Our results demonstrate that both food type and food availability alter the movement behavior and capabilities of gypsy moth larvae and that 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, largely due to an increase in velocity (Figs. 1.3a & 1.4). In contrast, larvae that fed on larch and Norway maple traversed approximately the same distance no matter how long they went without food and showed no changes in mean velocity (Figs. 1.4cd & 1.6cd). These disparate responses are likely due to the differences in the suitability of host foliage.

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 (1990) 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 5.5 oz. 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.

Even though European gypsy moths are polyphagous, we posit that larval gypsy moths may be better suited to feed on species from their native range than North American *Acer* spp. or eastern larch, which is also native to North America. 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. 1.2c), suggests that they were not truly "satiated", possibly because larch is not a preferred host. Moreover, these insects did not increase their total distances or mean velocities when deprived of food but instead became more likely to stop while walking. When foraging, gypsy moth will periodically stop and move their head around, seemingly to scan their environment (Doane and Leonard 1975). 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. 1.3) and insects feeding on Norway maple were less likely to move than those that fed on marginally suitable eastern larch from North America (Fig. 1.2).

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 (Turchin 1998, Conradt et al. 2000) but in the absence of information a straight line of movement with periodic direction changes may be most efficient (Bell 1991). 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 1991). The Norway maple cohort did show an increase in path straightness as food deprivation time increased, increasing displacement without increasing total distance moved (Figs. 1.4d & 1.5d). 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 appropriate caution.

Larvae raised on artificial diet exhibited the same trends as the bur oak group, albeit at a lower magnitude, in total distance moved, net displacement, and average velocity (Figs. 1.4ab, 1.5ab, & 1.6ab). 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, displacement, and average velocity. These results may be due to quality variation between food sources. Grayson et al. (2015) noted increased mortality and development times for the laboratory strain of larvae raised on foliage compared to artificial diet. Both Grayson et al. (2015) and our study used a laboratory strain of gypsy moth that has been in culture for 40 years so differences between the laboratory strain and wild-collected larvae is possible.

These results advance our understanding of science that can undergird regulatory 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. 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-foot 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). Our results indicate that the movement of gypsy moth larvae within these buffer zones likely depend on the type and quantity of hosts available in the area around the buffer zone. 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). In outbreak situations where larvae strip all available foliage, larvae will already be more prone to move due to high densities (Leonard 1981), but may become even more likely to encroach into buffer zones as they deplete foliage of preferred hosts. At the same time, our result suggest that in stands with increasing percentages of preferred hosts, larvae will be less likely to enter the buffer zone unless extensive defoliation occurs. This hypothesis is consistent with data from field studies that demonstrates that larvae are less likely to move when preferred hosts are present on the edge of the buffer zone (Chapter 2). Management decisions, then, may take into consideration the potential for insects to cause extensive defoliation on preferred hosts around a buffer zone. Increasing the size of the buffer zone when gypsy moth populations are high and few preferred hosts are available may increase the efficacy of buffer zone practices.

Table 1.1. 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 (*n* = 181). Lab trials were conducted from March 21, 2018 – June 21, 2018 in Minnesota, USA. Letters next to probabilities represent post-hoc means comparisons; probabilities sharing a letter are not significantly different from one another.

Food	Probability of moving	95% CI
Bur oak	0.276 c	0.187 – 0.387
Artificial diet	0.606 ab	0.630 - 0.905
Larch	0.909 a	0.849 - 0.992
Norway maple	0.400 bc	0.450 - 0.788

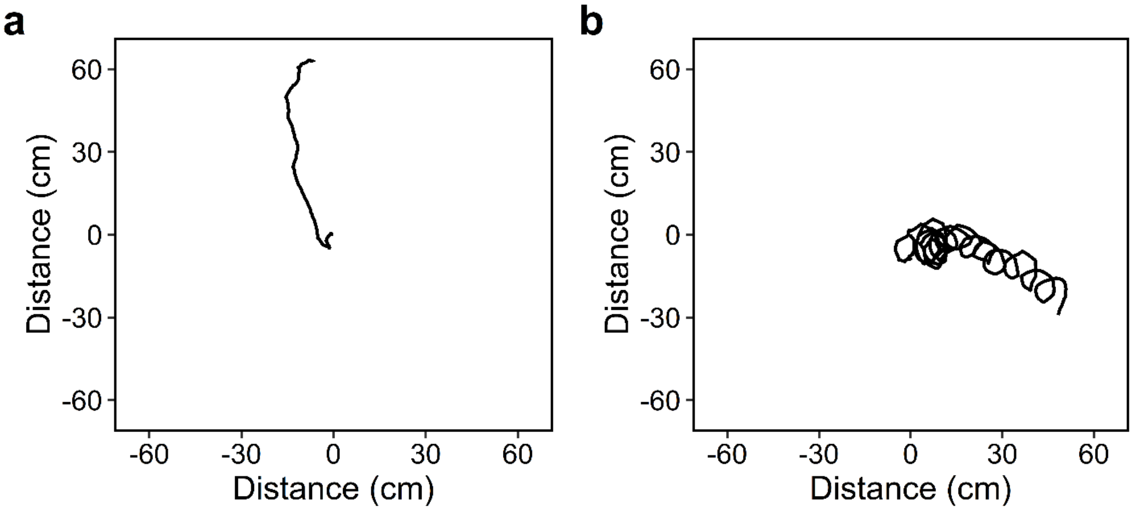

Figure 1.1. Two examples of movement paths taken by late instar larvae of European gypsy moth on a servosphere in laboratory settings from March 21, 2018 – June 21, 2018 in Minnesota, USA. These plots represent a) the most common type of path seen in these experiments consisting of mostly straight-line 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.

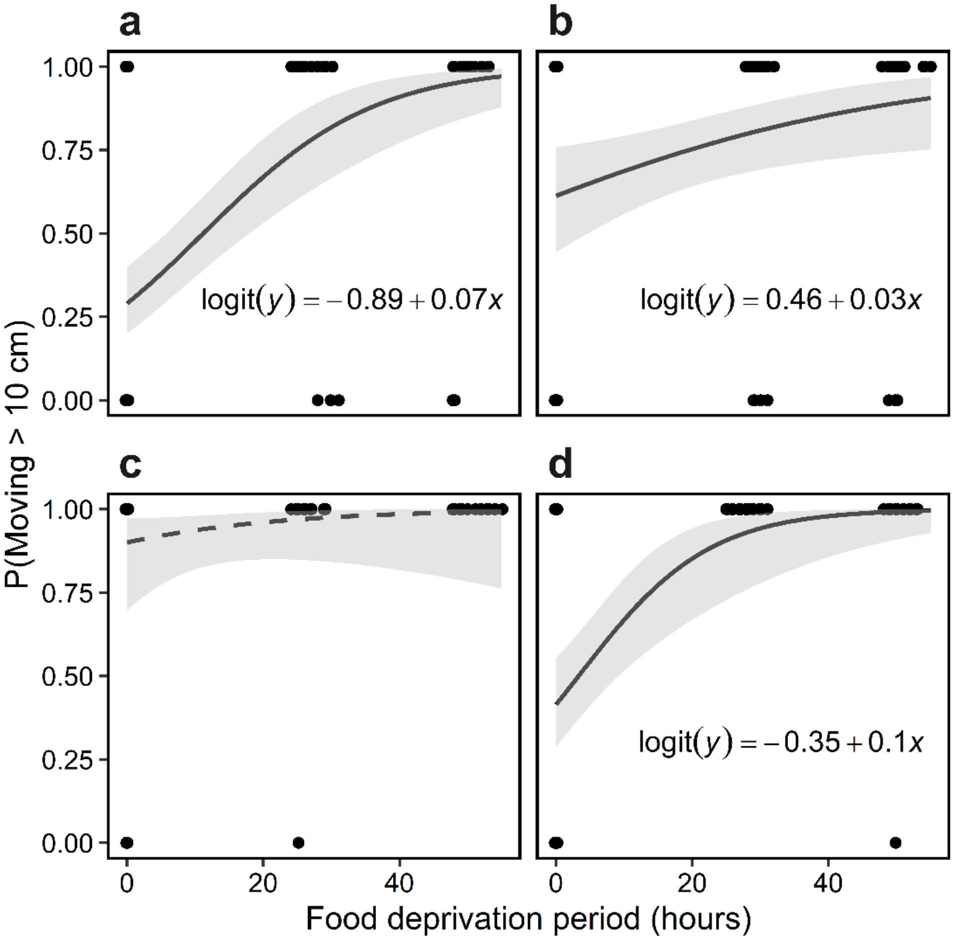

Figure 1.2. Relationship between the probability larvae of European gypsy moth (*n* = 358) will move greater than 10 cm on a servosphere and how long larvae have been deprived food for a) bur oak, b) artificial diet, c) larch, d) Norway maple. Lab trials were conducted from March 21, 2018 – June 21, 2018 in Minnesota, USA. Grey bands represent 95% confidence intervals for model predictions. Solid lines represent statistically significant trend lines; in such cases equations are reported.

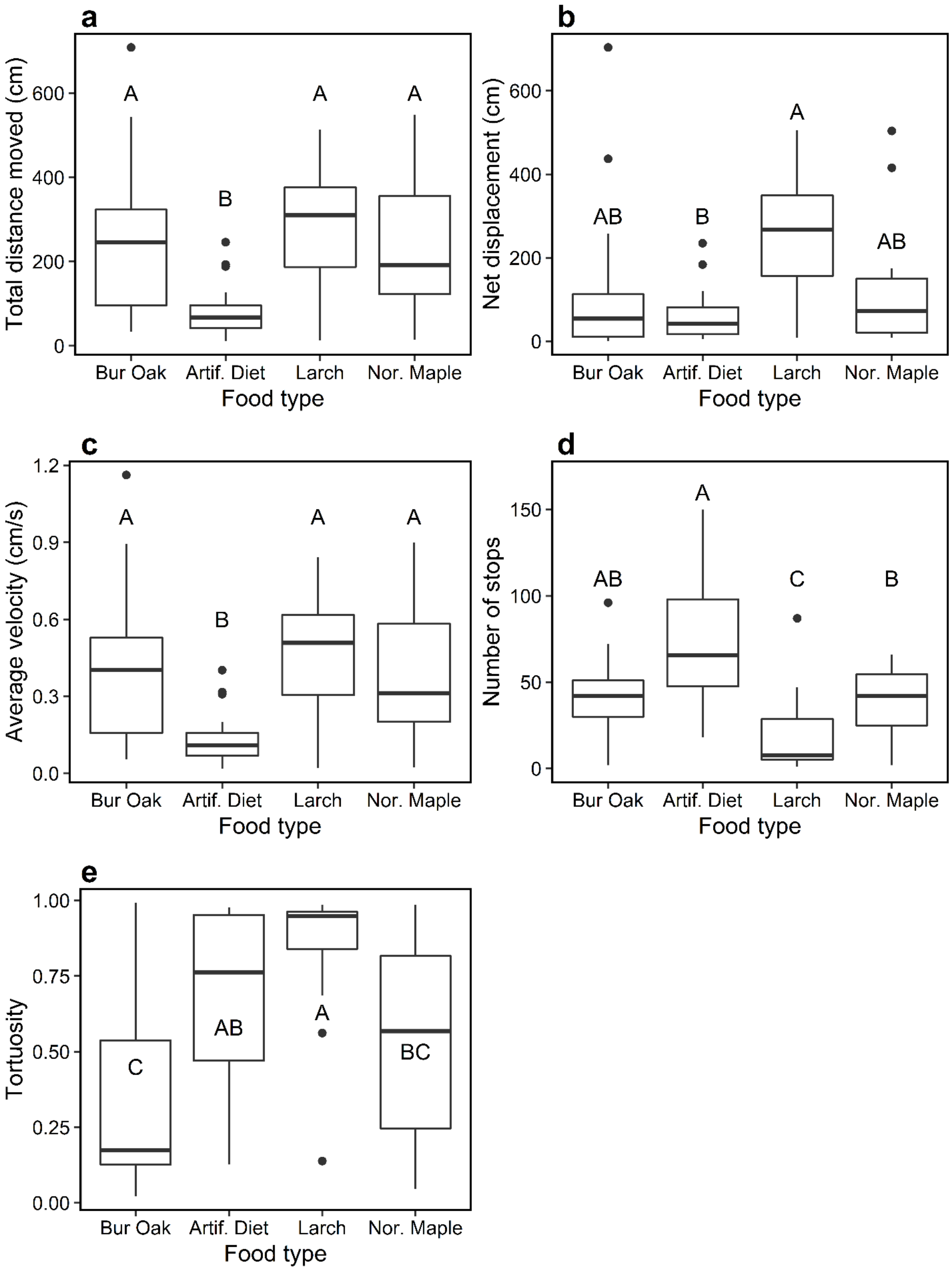

Figure 1.3. Boxplots comparing the movement of satiated late instar larvae of European gypsy moth raised on different foods, on a servosphere (*n* = 244). Lab trials were

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 quantiles, 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) Mean velocity. d) Number of stops during a 10 minute trial. e) Tortuosity (the ratio of net displacement to total distance moved) of larval movement paths.

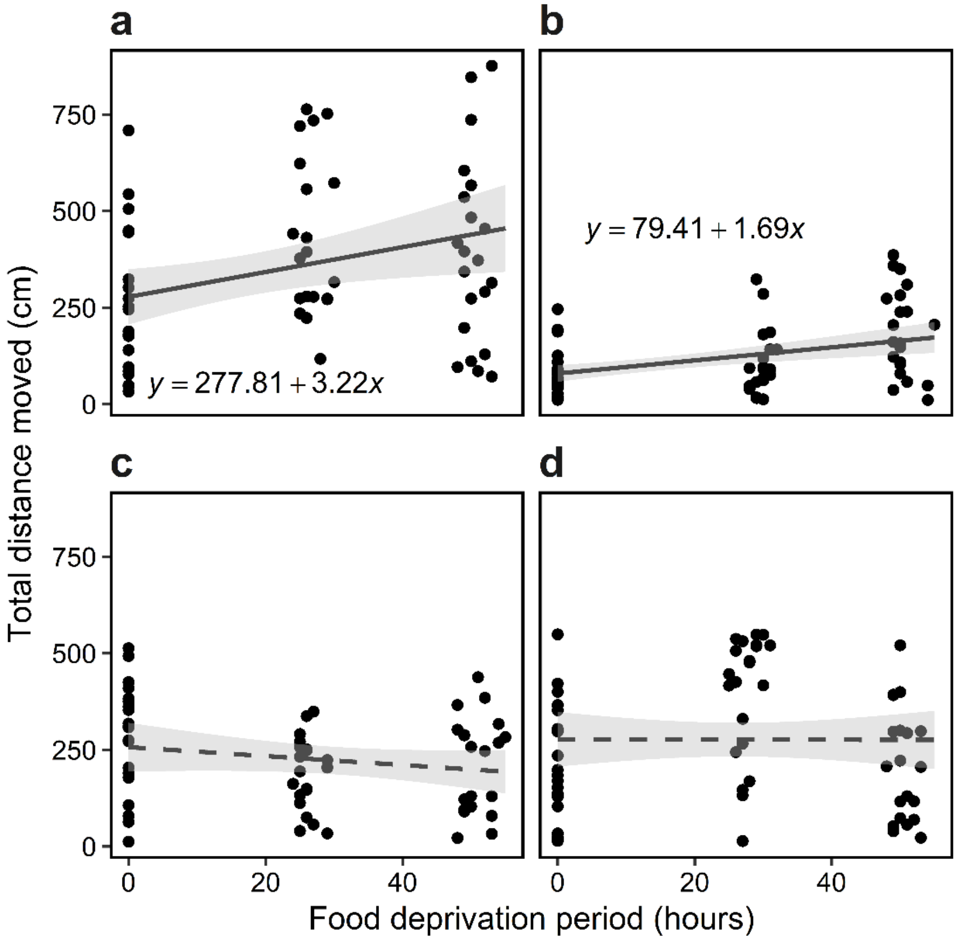

Figure 1.4. Total distance moved by late instar larvae of European gypsy moth on a servosphere as time without food increased (*n* = 244). Larvae were raised on one of four foods: a) bur oak, b) artificial diet, c) larch, d) Norway maple. Lab trials were conducted from March 21, 2018 – June 21, 2018 in Minnesota, USA. Grey bands represent 95% confidence intervals for model predictions. Solid lines represent statistically significant trend lines; in such cases equations are reported.

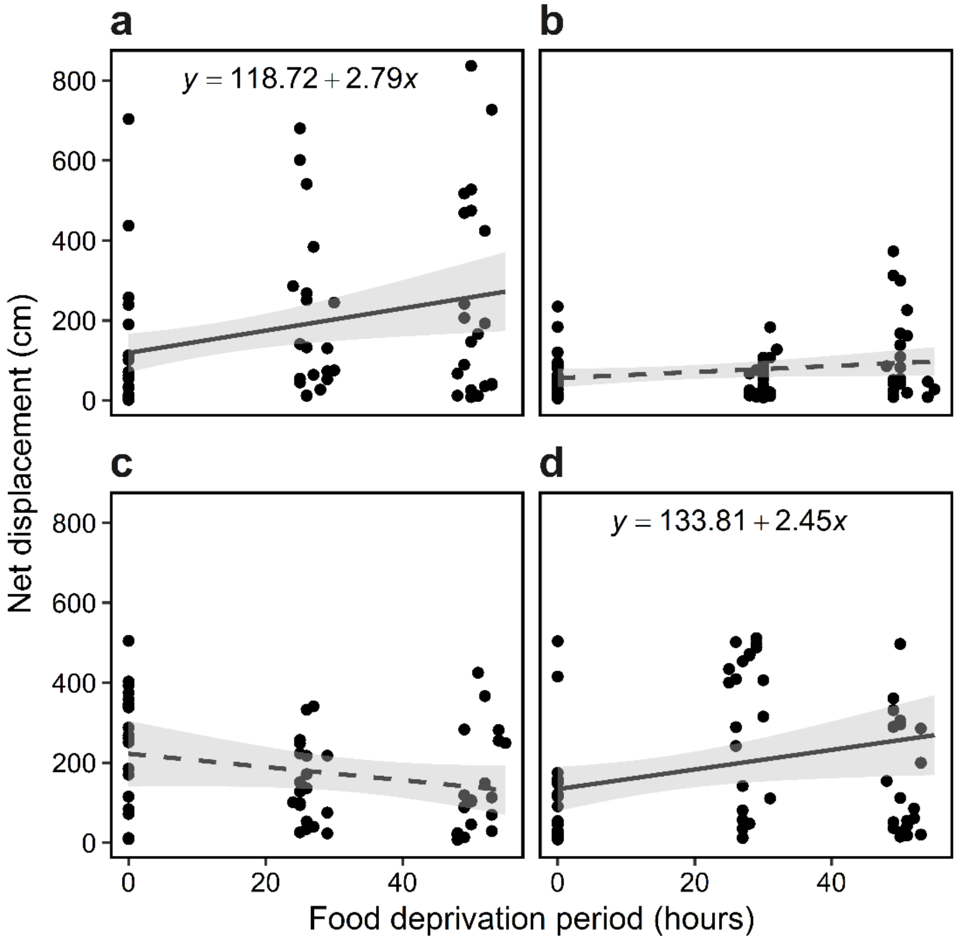

Figure 1.5. Net displacement of late instar larvae of European gypsy moth on a servosphere as time without food increased (*n* = 244). Larvae were raised on one of four foods: a) bur oak, b) artificial diet, c) larch, d) Norway maple. Lab trials were conducted from March 21, 2018 – June 21, 2018 in Minnesota, USA. Grey bands represent 95% confidence intervals for model predictions. Solid lines represent statistically significant trend lines; in such cases equations are reported.

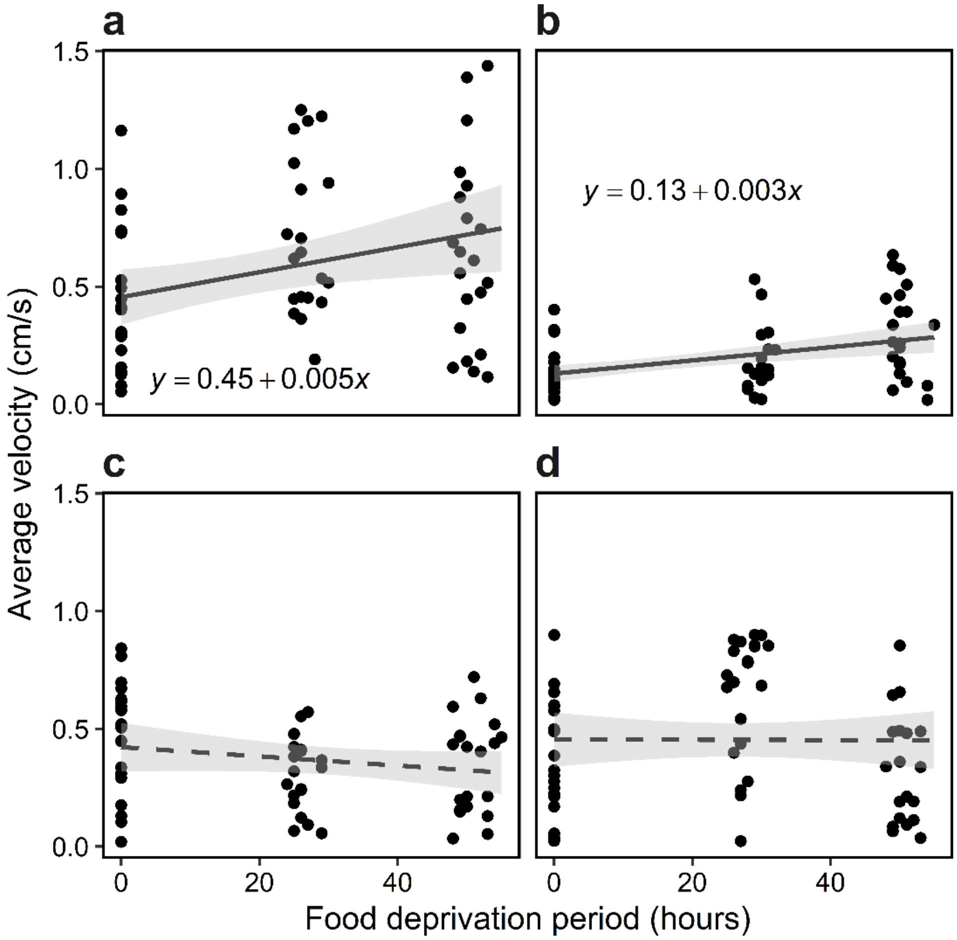

Figure 1.6. Average velocities of late instar larvae of European gypsy moth on a servosphere as time without food increased (*n* = 244). Larvae were raised on one of four foods: a) bur oak, b) artificial diet, c) larch, d) Norway maple. Lab trials were conducted from March 21, 2018 – June 21, 2018 in Minnesota, USA. Grey bands represent 95% confidence intervals for model predictions. Solid lines represent statistically significant trend lines; in such cases equations are reported.

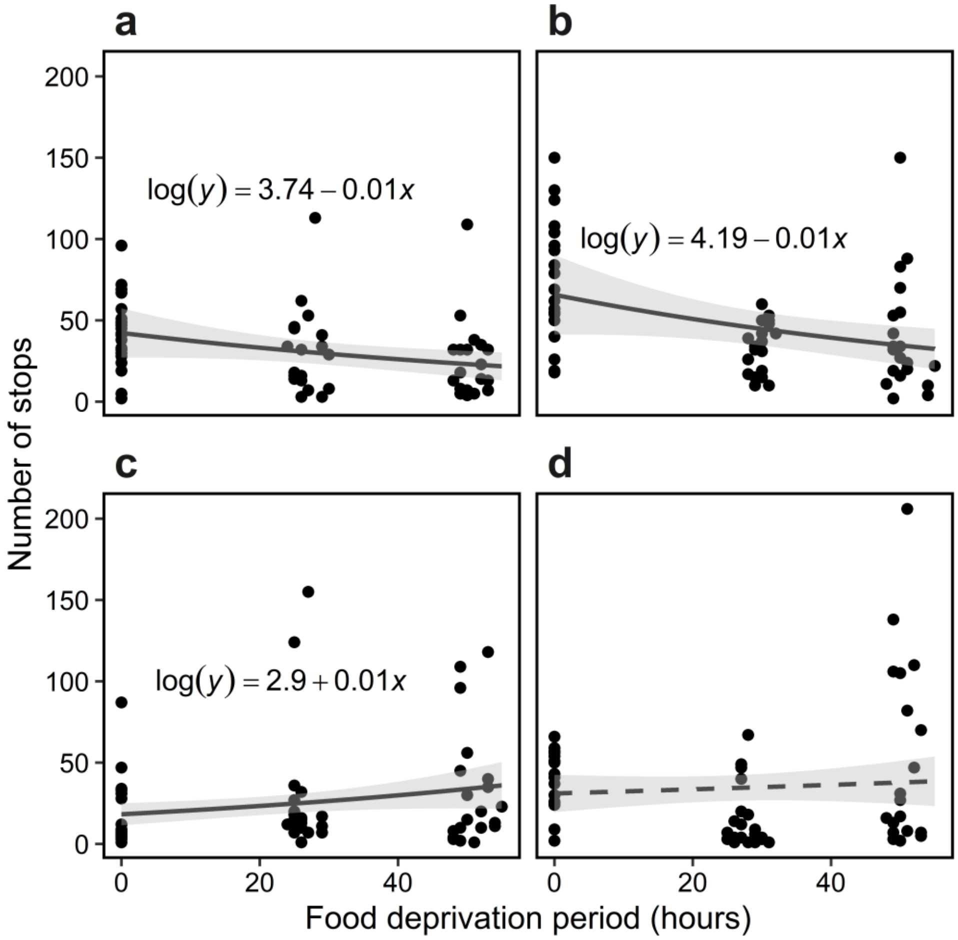

Figure 1.7. Number of stops taken by late instar larvae of European gypsy moth while moving on a servosphere as time without food increased (*n* = 244). Larvae were raised on one of four foods: a) bur oak, b) artificial diet, c) larch, d) Norway maple. Lab trials were conducted from March 21, 2018 – June 21, 2018 in Minnesota, USA. Grey bands represent 95% confidence intervals for model predictions. Solid lines represent statistically significant trend lines; in such cases equations are reported.

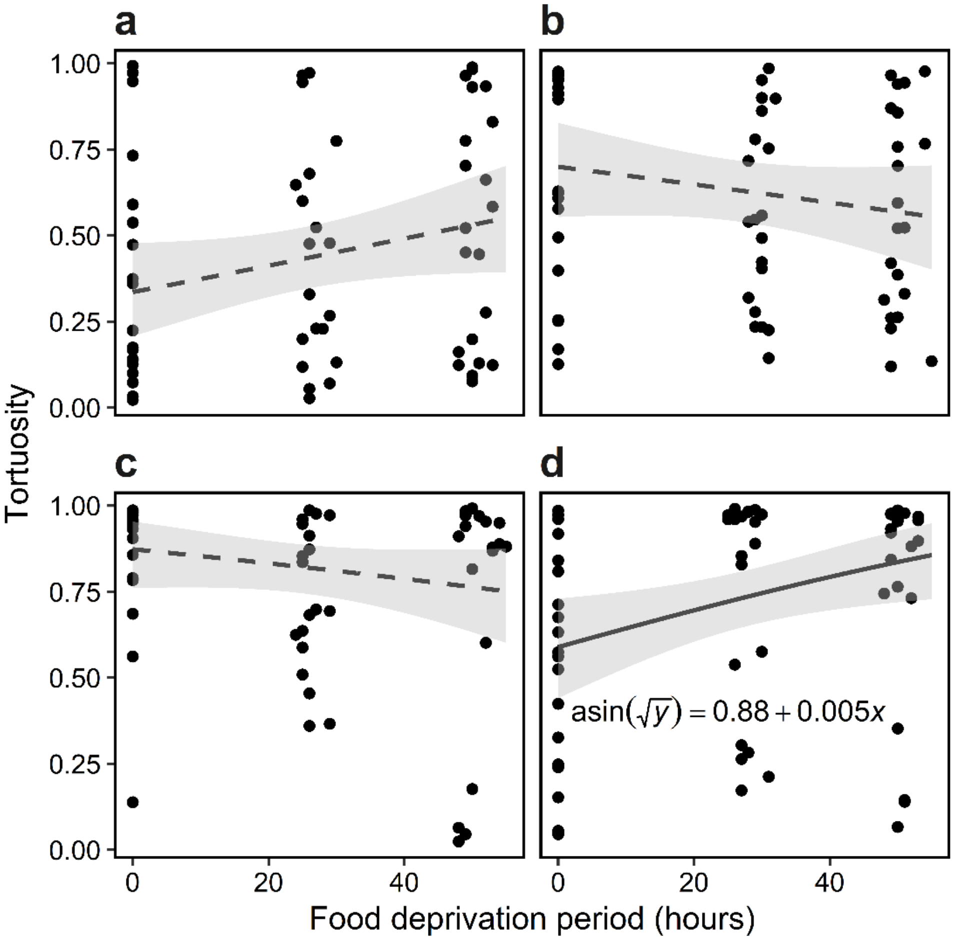

Figure 1.8. Tortuosity (the ratio of net displacement to total path distance) of late instar larvae of European gypsy moth on a servosphere as time without food increased (*n* = 244). Larvae were raised on one of four foods: a) bur oak, b) artificial diet, c) larch, d) Norway maple. Lab trials were conducted from March 21, 2018 – June 21, 2018 in Minnesota, USA. Grey bands represent 95% confidence intervals for model predictions. Solid lines represent statistically significant trend lines; in such cases equations are reported.

Chapter 2

Characterizing and simulating the movement of late instar gypsy moth (Lepidoptera: Erebidae) to evaluate the effectiveness of regulatory practices

Synopsis

The European gypsy moth, *Lymantria dispar* L. (Lepidoptera: Erebidae), is an invasive insect in North America that feeds on over 300 species of trees and shrubs and occasionally causes extensive defoliation. One regulatory practice within quarantine zones to slow the spread of this insect recommends that wood products (e.g. logs, pulpwood) originating from quarantine areas are staged within 100 foot-radius buffer zones devoid of host vegetation before transport outside the quarantine boundary. Currently there is little data underpinning the distance used. We conducted field experiments in Wisconsin, USA to assess buffer zone efficacy in reducing risk of larval gypsy moth encroachment on wood staging areas. We released late-instar gypsy moth larvae in groups around the perimeter of a 100-foot radius zone and tracked their movements for 10-hour periods using harmonic radar, and tested whether host vegetation staged around the perimeter or food availability before release altered movement patterns. Three larvae moved over 300 feet in 10 hours, but 93% of larvae moved less than 100 feet total. The presence of host vegetation reduced the likelihood of larvae entering the buffer zone by 70%. Food availability before release did not affect movement. Using these field data, we parameterized a Monte Carlo simulation model to evaluate risk of larvae crossing zones of different sizes. For zones greater than 100 feet in radius, less than 4% of larvae reached the center. This percentage decreased as zone size increased. Implications of these results for the regulatory practices of the gypsy moth quarantine are discussed.

Introduction

To control and limit the spread of invasive insects, it is important to understand how they move both locally and regionally. Insects move naturally, but also through vectors, such as humans facilitating movement (Carrasco et al. 2010). At local scales, methods to limit or prevent spread of herbivorous pests include the removal of host species, increasing the diversity of plants to reduce available hosts, and installing physical barriers that prevent movement into protected areas (Boiteau and Vernon 2001, Wratten et al. 2003, Barros and Foil 2007, Rigot et al. 2014). Implementing these strategies without understanding movement behavior, such as host-finding or dispersal, may result in wasted time, effort, and resources (Jeger 1999). Most work on insect movement behavior has focused on highly mobile species (Fahrner and Aukema 2018), while less attention has been given to less mobile, ground-crawling insects such as larval Lepidoptera (Diekötter et al. 2007).

The European gypsy moth, *Lymantria dispar dispar* L., (Lepidoptera: Erebidae) is an invasive insect in North America that is capable of extensive defoliation during outbreaks (Campbell and Sloan 1977, Elkinton and Liebhold 1990, Davidson et al. 1999). Gypsy moth overwinter in the egg stage and hatch in the early spring. Gypsy moth larvae feed until June or July. Larvae are capable of feeding on over 300 species of host plants, most of which are hardwoods (Elkinton and Liebhold 1990, Liebhold et al. 1997). After pupation, adults emerge to mate and lay eggs between mid-July through August (Leonard 1981). Females of the European gypsy moth are flightless and oviposit very close to where they emerged from their pupal case (Lance 1983).

Since the gypsy moth arrived in Massachusetts, USA in 1869, it has spread south to Virginia, west to Minnesota and Wisconsin, and north into Maine as well as the provinces of Ontario, Quebec, New Brunswick, and Nova Scotia in Canada (Fernald and Forbush 1896, Kauffman et al. 2017). Larval ballooning is the primary driver of natural dispersal, as neonate larvae in the early spring spin down from tree branches on silk and balloon on the wind (Capinera and Barbosa 1976, Weseloh 1997, Erelli and Elkinton 2000). Most ballooning larvae do not travel far from where they hatch, however, so natural dispersal accounts for only a portion of the moth's spread since its introduction (Weseloh 1985, Sharov et al. 2002). Much of the spread of the gypsy moth is thought to originate from accidental movement of egg masses by humans (McFadden and McManus 1991, Liebhold et al. 1992, Bigsby et al. 2011). If gypsy moth females choose pupation sites in or around manufactured objects (e.g. vehicles, patio furniture, and shipping containers) or natural products (e.g. firewood, logs, and nursery stock), they may oviposit on these objects. Transport of objects with cryptic egg masses can result in the transport and establishment of gypsy moth to areas beyond their current invasive range.

At present, the gypsy moth has invaded approximately one-third of at-risk areas in North America (Liebhold et al. 1997, Sharov et al. 2002). To protect areas where the gypsy moth is not established in the USA, the United States Department of Agriculture maintains a quarantine that covers the current invaded range of the moth (US Code of Federal Regulations, Title 7, Chapter III, Section 301.45). As part of the quarantine, entities that transport wood products, including logs, pulpwood, and woodchips, across the quarantine boundary must sign compliance agreements intended to limit the human-mediated movement of the gypsy moth. One practice requires that "Log, pulpwood, and/or wood chip staging or holding areas must be set suitable distance away from standing trees" (USDA-APHIS-PPQ 2010). In practice, 100 feet is often used as the "suitable distance" and the area created by this buffer zone should be vegetation free (Minnesota Department of Agriculture 2015). The goal of this practice is to reduce the likelihood that late instar larvae will ingress from surrounding vegetation and eventually pupate on the wood within the center staging area.

Little is known, however, about larval movement and behavior (Doane and Leonard 1975, Roden et al. 1992) and thus how effective the buffer zone practice may be. Doane and Leonard (1975) observed late instar gypsy moth larvae crawling across an open area surrounded by woodland and noted that the larvae tended to move in a northeast or southwest direction when they were highly active in the morning and late afternoon. Larvae moved less as in the early afternoon as the temperature increased. The movement of larvae also became more random in the afternoon, which is when polarized light from the sun is reduced, suggesting that gypsy moth larvae use polarized light to navigate. Other lepidopteran larvae are known to use polarized light to orient (Wellington et al. 1951, Sullivan and Wellington 1953, Wellington 1955). Visual cues also appeared important: when observers stood within two meters of larvae, larvae tended to move towards the observer. In the laboratory, Roden et al. (1992) performed a series of behavioral experiments examining the response of gypsy moth larvae to different visual and host stimuli. Larvae showed a preference for black objects over white objects and for darker colored bark of red oak (*Quercus rubra*) over lighter colored barks of trembling aspen (*Populus tremuloides*) and white birch (*Betula papyrifira*). Larvae also tended to move towards objects that subtended the largest angle in their field of vision, all else being equal. While these studies provide a foundation for studying the movement of gypsy moth larvae, they do not provide enough information to evaluate the effectiveness of implementing buffer zone practices in reducing the spread of this species.

In this work, we evaluate and describe the dispersal of late instar European gypsy moth in a field environment meant to simulate a buffer zone in an active tree-harvesting environment. Our objectives were to 1) characterize the movement of larvae, 2) determine the frequency with which larvae might cross a vegetation-free buffer zone, 3) evaluate factors such as host vegetation on the edge of the buffer zone and short-term starvation that might influence the likelihood that larvae would cross the buffer zone, and 4) evaluate the efficacy of the buffer zone in preventing larvae from reaching products at the center. To do this, we tracked gypsy moth larvae released at the edge of a buffer zone using harmonic radar in the summers of 2016 and 2017 in a granite quarry in central Wisconsin. We used data collected from these experiments to parameterize a Monte Carlo simulation model to investigate gypsy moth larval movement in buffer zones of varied sizes. This model helps evaluate the relative efficacy of the current regulatory practice in reducing ingress of gypsy moth larvae to access staged wood products.

Methods and Materials

Experimental site

To evaluate the movement of gypsy moth larvae, we selected a field site in Marathon County, Wisconsin that would allow us to mimic a wood product staging area with an appropriate buffer zone. The field site, at a commercial granite crushing facility (44.85 °N, 89.84 °W), consisted of a large, flat area devoid of any vegetation, with a combination of wood chips, gravel, dirt, and solid rock covering the ground. Marathon

County is within the quarantine for the gypsy moth, so we were able to release larvae. During each year of the study, we did not notice any wild populations of gypsy moth anywhere around our study site that could confound our results. We arranged an experimental plot with a rectangular hardwood log deck approximately 30 feet by 10 feet in the center of the plot area. We measured 100 feet from the log deck to establish the perimeter of our buffer zone (Fig. 2.1). (Note: The imperial system is used extensively in United States regulations and agricultural practices, thus all distances in this paper will be reported in feet.)

Test insects

Gypsy moth egg masses were obtained 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). Larvae were reared in laboratory conditions at the University of Minnesota, Minnesota Agricultural Experiment Station (MAES)/Minnesota Department of Agriculture (MDA) Containment facility, as the University of Minnesota’s St. Paul campus is outside the quarantine boundary. After receipt, egg masses were held at 4 °C until they were ready to be used. The eggs and larvae were held in a growth chamber at 25 °C, 70% humidity, and with a photoperiod of 16L:8D. Egg masses were placed in 5.5 oz plastic cups (GP PRO Dixie Plastic Portion Cup) filled approximately half-way with artificial diet (USDA Gypsy Moth Diet, Hamden Formula). After hatching, each cup held approximately 200 larvae. Larvae were transferred to cups with fresh diet every two to three days and the density of larvae per cup was also reduced as the larvae developed so each cup held approximately 50 second instar larvae, 30 third instar larvae, 15 fourth instar larvae, or 5 fifth or sixth instar larvae.

In these conditions, it took larvae approximately four weeks to develop the fifth instar. To reduce mold in diet containers, the diet was autoclaved before feeding.

Evaluating the movement of gypsy moth larvae

We released fifth and sixth instar gypsy moth larvae at our field site July 18 – July 26th in 2016 and June 20th – June 27th in 2017. Larvae were released simultaneously each day of the study at four locations along the perimeter of the buffer zone (Fig. 2.1). Between 12 and 24 larvae were released each day, with roughly equal amounts at each release point and treatment each day. We released 103 and 171 larvae in 2016 and 2017, respectively. Larvae were released at 0600h in 2016 and 0700h in 2017. Positions of larvae were recorded every hour in 2016 and every 30 minutes in 2017 for 10 hours, at which time all released larvae were recovered and retired. Likewise, the positions of any larvae that died during the day were recorded. During each location recording, we recorded air temperature with an anemometer (Extech Instruments 45160) and ground temperature next to the larvae with an IR thermometer (EnnoLogic eT650D).

We used harmonic radar to locate the larvae, which works by following a high frequency signal returned from a small diode worn as a tracking tag by the insect to a handheld radar unit (RECCO R9 Detector, Lindgö, Sweden) wielded by the investigator (Boiteau and Colpitts 2004, Machial et al. 2012). Diodes (RECCO R56, Lindgö, Sweden) were glued to the 6th or 7th abdominal segment of a fifth or sixth instar larvae of gypsy moth the night prior to release using cyanoacrylate glue (Loctite super glue gel), which showed no toxic effects to insects in a previous study in our lab (Machial et al. 2012). The diodes measure 6.5 cm in length and are made of a flexible plastic which prevent them from causing the larvae to get stuck on other objects while moving. Numbered bright orange queen bee tags (Queen Marker Kits, The Bee Works) approximately 2 mm in diameter were also glued dorsally to the 2nd or 3rd thoracic segment to uniquely identify larvae. In 2016, we had to reuse diodes each day due to a manufacturer shortage. By the last two days of the study in 2016, we switched to visual location of larval gypsy moth as we were concerned that accumulating layers of cyanoacrylate glue on the diodes were reducing the effectiveness of the harmonic radar. Harmonic radar diodes were still attached to all larvae to remain consistent with previous protocols. In 2017, larvae were tracked with harmonic radar throughout the duration of the experiment and new diodes were utilized each day.

To record the location of larvae, their distance from the center of the plot was measured with an electronic range finder (Nikon Forestry Pro 8381). A compass was used to record the azimuth of the larvae (i.e. the angle subtended between the north-south line running through the center of our plot and the position of the larvae, measured clockwise from north). This information was used to calculate their location on an *x-y* coordinate plane centered on the log deck. We were able to use these coordinates to recreate the movement paths and calculate total path distance and absolute distance moved. This information was also used to calculate the bearing and turn angles for each larvae. Bearing is the absolute direction in degrees a larvae moved between two sampling times and is between 0° and 360°, with 0°/360° corresponding to north. The turn angle of a movement is the change in bearing between two successive moves. Turn angles are between -180° and 180°: negative values indicate a left turn relative to the bearing of the previous move, positive values indicate a right turn, and 0° indicates no turn, or straight movement.

Experimental treatments

In 2016, we examined how the presence or absence of host vegetation at the edge of the buffer zone affected the movement of larvae. To do this, we randomly selected two of the four release points each day and placed three red oak (*Quercus rubra*) and three quaking aspen (*Populus tremuloides*) saplings at each of those release points (six trees per chosen release site). These species are preferred and suitable hosts for gypsy moth (Mauffette and Lechowicz 1984, Liebhold et al. 1997). We kept the saplings in 10-gallon nursery pots to facilitate moving them between release points each day. Once the saplings were in position, we covered the pots with double shredded wood mulch. Half the larvae we released each day were placed directly onto the branches of saplings and the other half were placed on the ground at the other two release points. Larvae released on the ground were placed several feet apart around the release point to reduce the likelihood that they might follow one another. In 2017, we repeated the presence/absence of host vegetation treatment. An additional treatment was added in 2017 in which half of the larvae released were starved for 24 hours prior to release. This treatment was added to examine how limited availability of food, which may occur during outbreaks of gypsy moth, might affect the movement of gypsy moth larvae.

Simulating Movement

We used our empirical field results to parameterize a simulation model of the movement of gypsy moth larvae across buffer zones of six different sizes (25 foot intervals from 75 to 200 feet) and with log decks or wood product staging areas 25 feet, 50 feet, or 75 feet in diameter (i.e. 18 buffer different zone/log deck combinations). The model was used to evaluate the efficacy of current regulatory practice in reducing the risk of gypsy moth larvae to encounter central staging areas within a buffer zone. In our model, the log deck and buffer zone were simulated as a circle within a circle (Fig. 2.2), where the buffer zone distance was the distance between the circumference of the outer perimeter and the circumference of the inner staging area. The three radii of simulated inner staging areas were 25 feet, 50 feet, and 75 feet.

To simulate movement paths, we first examined our field data to determine if larvae disperse via a correlated random walk or uncorrelated random walk pattern. Both dispersal modes are commonly used to describe movement by ground-crawling insects (Bell 1991). In an uncorrelated random walk, there is no directional persistence in the movement of the organism; the direction of movement in a time step is not associated with the direction in which an organism was previously moving. In a correlated random walk, there is some degree of autocorrelation or “memory” in the direction moved by an organism between time stops. We hypothesized that gypsy moth larval movement would follow a correlated random walk based on previous research showing strong directional movement (Doane and Leonard 1975).

To evaluate this hypothesis, we calculated expected and predicted net squared displacement based on our field data (Kareiva and Shigesada 1983, Turchin 1998). If observed net squared displacement increases linearly with time and falls within the 95% confidence intervals of the expected net squared displacement, a correlated random walk can be an appropriate way to model the movement of the gypsy moth (Kareiva and Shigesada 1983, Turchin 1998). Because very few paths in 2016 consisted of more than four or five observations, only movement paths from 2017 were used to calculate net squared displacement. When calculating net squared displacement only larvae from the group released in the absence of foliage were used as movement parameters may change in heterogeneous habitat patches (Turchin 1998, Goodwin and Fahrig 2002).

Autocorrelation in the absolute direction, or bearing, of movement was calculated by averaging the cosines of the difference in bearing between two moves separated by a lag l up to $l = 15$ (Turchin 1998). Statistical significance in autocorrelation, indicating a correlated random walk pattern, was determined by 95% confidence intervals that did not overlap zero. The movement of the larvae in the field matched a correlated random walk pattern (see Results). Thus, we simulated movement paths for larvae based on a correlated random walk protocol, in which we generated 20 successive steps to reflect our field experiments recording larval positions every 30 minutes for 10 hours.

To simulate the movement path of a larva, we randomly selected a point on the perimeter of the buffer zone from where the path would start. We used a Bernoulli distribution fit to our empirical data to determine the probability that a larva would move at each step of the simulated movement path. All distribution fitting was done using maximum likelihood estimation via the fitdistrplus package in R (Delignette-Muller et al. 2015). To determine if a larva moved or stayed still at any time step in the movement path, we drew from a Bernoulli distribution where the estimated probability of moving was 0.65 (based on field data). If the larva moved, we then drew a turn angle and a distance moved for that step, unless it was the first step. For the first step, instead of drawing a turn angle, we drew a random movement direction from a uniform circular distribution. The turn angle for other moves was drawn from a Gaussian distribution ~ $N(-2.36, 91.44)$, with parameters derived from the empirical turn angle data. If the turn angle drawn was greater than 180° or less than -180°, a new angle was drawn. The movement distance was drawn from a Gamma distribution constructed from empirical movement distances and had a shape parameter of 1.40 and a scale parameter of 3.49. The *x*,*y* coordinates of the larva were recorded after each step and used as the starting location for the next step. After each step, the location of the larva was checked to see if it was inside the circumference of the inner circle (i.e. on the log deck) or if it would have passed through the inner circle to reach its current location (i.e. passed through the hypothetical wood staging area). If the larva passed through or ended within the staging area during a time step, it was counted as reaching the log deck and the simulation for that path was ended. We simulated 10,000 movement paths for each combination of log deck and buffer zone size. All simulations were conducted in R (R Core Team 2016).

Statistical analysis

Due to the differences in ground temperatures and overall movement of the larvae (see Results), we analyzed each year separately in all analyses. To test whether treatments affected the probability of larvae moving, we used logistic regression with movement (i.e. moved or stationary) as a binomial response. To compare the average total path distance between treatments, we used a one-way (2016) or multiple (2017) ANOVA with square root transformed distances to normalize and ensure equal variances of residuals. Larvae that did not move were excluded from analyses (39 and 56 larvae in 2016 and 2017, respectively). Analysis of circular parameters, such as bearing (i.e. compass direction), follows Batschelet (1981) and Pesey et al. (2013) using the R package “circular” (Agostinelli and Lund 2013). To test if larvae released at the same release point tended to move in the same direction, we used Kuiper’s Test of Uniformity

(Batschelet 1981) to determine if the distribution of average movement directions differed from a circular uniform distribution. In this test, θ is the average bearing of all larvae released at a particular release point and r represents the degree of clustering around θ; $r = 1$ would represent all larvae moving in the same direction and $r = 0$ would represent no clustering. This analysis used only larvae that moved during the observation period. All statistical analyses were carried out in R (R Core Team 2016). All means are reported ± one standard deviation.

Results

None of the larvae out of 103 released in 2016 moved farther than 100 feet; the longest total path distance for any larva in 2016 was 63.0 feet (Fig. 2.3A). This latter distance was traversed in four hours. In 2017, 23 out of 171 larvae released moved 100 feet or more; four larvae moved over 200 feet and three larvae moved over 300 feet (Fig. 2.3B). These larvae were released directly on the ground. No larvae out of 103 released in 2016 reached the log deck. In 2017, three larvae out of the 171 released reached the log deck. All three of these larvae were released on the ground and represent approximately 4% of the larvae released on the ground, or approximately 2% of all larvae released.

The ambient air temperature was higher during the study period in 2016 vs 2017. In 2016, the average high air temperature was 32.2 ± 3.9 °C while the average high air temperature in 2017 was 24.7 ± 4.4 °C. Average hourly ground temperatures in 2016 ranged from 18.2 – 48.6 °C, peaking at 1300h. The average hourly ground temperatures in 2017 tended to be lower, ranging from 15.3 – 34.7 °C and would peak between 1300h and 1400h. Due to the high ground temperatures in 2016, 75 of the 103 larvae released in 2016 died before the end of the day, with mortality occurring as early as 1100h. In 2017, larval mortality was lower; only 5 of the 171 larvae released died before the end of the daily experiment was terminated 10 hours after release.

Ninety-eight percent of all larvae placed on the ground at the start of a trial moved to new positions by the end of the daily experiments in both 2016 and 2017. Larvae released directly onto the ground in 2016 moved significantly farther than larvae released in trees (Fig. 2.4A, Table 1). When larvae released in the absence of foliage encountered a sheltered area within the buffer zone, such as a large piece of bark or a shaded area underneath rocks, they would often stop moving and would remain there for the duration of the observations. Feeding status and the interaction of feeding status and year had no significant effect on the proportion of insects moving. The larvae released on the ground in 2017 also moved significantly farther than those placed in trees; there was no significant effect of starvation on the total path distance of the larvae (Fig. 2.4B, Table 1).

Indeed, larvae placed in trees were more likely to remain in place than larvae initially placed on the ground. In 2016, 73% of larvae placed in a tree were still present in that tree 10 hours later, as were 67% of larvae in 2017. Larvae placed into trees sometimes left the tree if, for example, they were blown out of the foliage by a wind gust, but would frequently return. For example in 2017, of 24 larvae that left the trees during the experiments, 18 returned to them later in the day while the remaining six did not return. In 2016, thirteen larvae released into trees left their tree, but did not return as they appeared to quickly succumb to the high ground temperatures.

In 2016, larvae at three of the four release points (SE, SW, and NW) tended to displace in the same directions, according to Kuiper's Test of Uniformity (Table 2). In other words, the directions traveled by the larvae at three of the four release points were not uniformly distributed between 0° and 360° and instead clustered around one direction (Table 2). Similarly, in 2017, the larvae at all release points tended to displace in the same direction within release point groups (Table 2). Observed net squared displacement fell within the 95% confidence bands for predicted net squared displacement for 6 of 20 time steps but still increased linearly with time, indicating that the larvae are moving in a correlated random walk but with only moderate amounts of correlation between time steps (Fig. 2.5). This pattern is further supported by the significant autocorrelation in the direction moved by the larvae, which shows moderate amounts of significant correlation between moves separated by one, two, or three time steps (i.e. error bars do not overlap 0) (Fig. 2.6). In other words, a larva is likely to continue moving in a similar direction for up to three time steps after any given position recording.

Simulating movement

The percentage of larvae reaching the log deck increased with increasing log deck radius while this percentage dropped as buffer zone size increased (Fig. 2.7). In all simulated log deck sizes, the percentage of larvae reaching the center of the simulated buffer zone area decreased exponentially as buffer zone distance increased and reached a minimum of 0.5% for a log deck 25 feet in radius and a 200 foot buffer zone; all simulations had some percentage of larvae reach the simulated center. The maximum percentage of larvae reaching the log deck was approximately 12.6% with a buffer zone distance of 75 feet and a log deck radius of 75 feet. The simulation that most closely approximated our field site (a buffer zone radius of 100 feet and a log deck radius of 25 feet) also closely matched the percentage of larvae we saw reach the log deck, with approximately 4% of larvae in both the simulation and field study reaching the log deck.

Discussion

Our results that gypsy moth larvae do not often reach the log deck, despite being able to move over 300 feet in 10 hours, and that the number of larvae reaching the log deck decreases as the buffer zone size increases, demonstrate that the buffer zone practice effectively reduces the likelihood that larvae will encounter the log deck. We saw that larvae were unlikely to leave host vegetation or shelter once it was acquired, suggesting that forays into the buffer zone will be infrequent. When forays away from the trees did occur though, the larvae returned 70% of the time. Furthermore, because our simulations showed that increases in buffer zone size from the current practice of 100 feet result in diminishing returns in effectiveness, the current practice is likely sufficient.

Our observation that larvae could move over 300 feet is consistent with Doane and Leonard (1975), who observed gypsy moth larvae in a grassland field as far as 125 m (410 ft) away from the woodland edge. Total path distance, however, is not enough to consider when evaluating whether the buffer zone is effective. How larvae move within the buffer zone (e.g. turn angles, path straightness, movement direction) will also affect whether they reach the staged wood materials. Moderate variance in turn angles (N ~ (-2.36, 91.44)), autocorrelation in bearing up to the third time lag (Fig. 2.6), and net squared displacement increasing linearly with time reflected a correlated random walk pattern characterized by directional persistence through time. Correlated random walks are a common search strategy when an organism does not know where resources are, as it strikes a balance between thoroughly exploring its environment versus re-treading old ground too often (Bell 1991, Turchin 1998). To evaluate the efficacy of regulatory practices in limiting the spread of invasive insects, it is important to have relevant biological information (Andersen et al. 2004). By incorporating these patterns into our simulation, we have shown that, all other factors being equal, the log deck at the center of the buffer zone will be encountered infrequently despite the overall movement ability of larvae.

The results of our simulation are likely conservative. We expect that fewer larvae would enter the wood staging area than our simulation showed, as we did not incorporate the potential effects of host vegetation around the perimeter of the buffer zone on the reduced propensity for larvae to enter the buffer zone. Larvae on the ground were approximately four times more likely to move than larvae released in trees, suggesting that if food and pupation sites are nearby, larvae will not enter buffer zones. Once larvae are in a tree, they seldom leave except to look for shelter, food, or pupation sites (Campbell et al. 1975, Weseloh 1997) consistent with settling behaviors we observed. Such behaviors are consistent with previous studies that have shown gypsy moth tend to navigate towards tall objects within two or three meters of them (Doane and Leonard 1975, Roden et al. 1992). Thus, surrounding forest composition may be an important determinant of dispersal into and residency times in buffer zones.

Feeding status did not affect the probability that an insect would leave its tree, or how far an insect moved once it left the tree. It is possible that our starvation treatment was not severe enough and a longer period of starvation would have produced a stronger response as noted in laboratory studies of movement (Chapter 1). Capinera and Barbosa (1977), for example, showed that starved larvae were more likely to disperse. Studying the effects of starvation in the buffer zone environment could be an avenue for future work, as the availability of food in the surrounding area might affect the propensity of larvae to enter the buffer zone which could, in turn, require adjusting the buffer zone regulatory practice to the conditions in the local environment.

Another factor that might alter the effectiveness of the buffer zone is the density of gypsy moth in the surrounding area. Larvae from high density populations are active throughout the day and night and are more likely to leave their trees (Campbell and Sloan 1977). Additionally, in high density populations, larvae may occupy all or most available bark flaps, where they will rest or pupate. When all bark flaps are occupied, larvae seek other sheltered areas, leading to increased numbers of pupae in the leaf litter and surrounding areas (Campbell et al. 1975). As such, it is possible that a lack of sheltered resting sites in high density populations will cause more larvae to enter the buffer zone. Moreover, more larvae may encounter the log deck simply due to random diffusion of the higher number of larvae present. Similarly, the number of larvae encountering that object will increase as the size of the object at the center of the buffer zone increases (Fig. 2.7). Increasing the buffer zone as the size of the stored objects increases or setting limits on the size of the stored objects may retain higher levels of effectiveness.

At seven out of the eight release points (four per year), larvae tended to move in the same direction as the distribution of directions moved by larvae differed significantly from a uniform distribution. Larvae were released in groups of four to five each day and spaced several feet apart. It is unlikely that similar movement directions are due to larvae following a “leader” (Liebhold et al. 1986). The average direction of movement varied slightly among most release points, except for the NE release point in 2016 and the NW release point in 2017, which were much different from the others (Table 2). Both Doane and Leonard (1975) and Weseloh (1985) observed that larvae moved mostly in a NE or

SW direction, while our larvae moved mostly NW or E. This movement coincided with features on the landscape at our site. For example, the larvae at the NE release point in 2017 moved mostly towards a hill with host vegetation located beyond the buffer zone area. Gypsy moth larvae will move towards objects that subtend the largest angle in their field of vision (Roden et al. 1992) and use visual cues in host selection (Roden et al. 1992, Smitley et al. 1993). It is unlikely that visual perception played a role here as it is thought that crude visual perception of larval Lepidoptera extends no more than three meters (Roden et al. 1992). Larvae released in our study were released much farther than three meters from these other objects. Liebhold et al. (1986) suggest that silk trails may be important in host location for gypsy moth similar to other Lepidoptera species (Fitzgerald and Peterson 1988) . We did not visually observe any silk trails and cannot identify the mechanism leading to this observation. Because only larvae released at the SE release point in 2017 tended to move towards the staging area, it seems unlikely that larvae were specifically seeking the log deck in general. Other larvae that moved towards the log deck ended up moving past it or changing directions to move away from the log deck.

The substrate of the buffer zone may also alter how likely it is for larvae to move within the buffer zone and how far they travel within the buffer zone. We saw high levels of mortality in 2016 due to the high ground temperatures. Much of the surface of our buffer zone consisted of dark colored materials, which absorb more solar radiation than light colored surfaces, and reached temperatures as high as 48.6 °C. Covering the buffer zone with dark colored substrates such as wood mulch may increase thermal mortality of larvae that are in the buffer zone during the day or discourage movement in the buffer zone during the heat of the day. While this practice would only be effective during the hottest parts of the day or year, it could be a low-cost and low-effort change that could improve the effectiveness of the buffer zone.

While this study provides insights into effectiveness of buffer zones and host vegetation into reducing movement of late instar larvae over short time intervals, it will be up to managers and policy makers to decide acceptable levels of risk. Increasing the size of the buffer zone from 100 feet to 125 feet would provide the largest increase in effectiveness before there are strong diminishing returns in effectiveness (Fig. 2.7). Additionally, managers may wish to consider varying buffer zone size based on the size of the staging area or current population levels of the gypsy moth. In the future, this study can provide an example of how to evaluate the movement of ground crawling insects and use that data to support management decisions.

Table 2.1. Effect of different treatments on total path distance of gypsy moth larvae released in central Wisconsin, USA in the summers of 2016 and 2017.

Year	Treatment	*F*-value	DF	*P*-value
2016	Host vegetation (present/absent)	14.67	1, 62	< 0.0001
2017	Host vegetation (present/absent)	32.86	1, 111	< 0.0001
2017	Feeding treatment (fed/starved)	1.07	1, 111	0.3029
2017	Host vegetation x feeding treatment	0.03	1,111	0.8569

Table 2.2. Results of Kuiper's Test of Uniformity calculated to determine if the average direction of displacement for gypsy moth larvae clustered in a particular direction and was not uniformly distributed between 0° and 360°. Larvae were released and followed in a field plot in central Wisconsin, USA in the summers of 2016 and 2017.

	2016 Release Quadrant				2017 Release Quadrant			
Parameter	NE	NW	SE	SW	NE	NW	SE	SW
θ	26	264	284	265	302	98	326	313
r	0.01	0.74	0.73	0.44	0.92	0.31	0.66	0.55
V	0.84	2.24**	2.10**	1.75*	3.36**	1.82*	2.55**	2.38**
n	18	9	10	13	15	23	20	26

θ is the average direction of displacement in degrees.

r is the length of the resultant vector for the average direction θ. This represents a measure of concentration about θ. A value of one would indicate all average directions of displacement are in the same direction, and a value of zero indicates the average directions are uniformly distributed between 0° and 360°

V is the test statistic for Kuiper's Test of Uniformity with statistical significant denoted with asterisks (* $0.025 < p < 0.05$, ** $p < 0.01$)

n is the number of individuals in each sample.

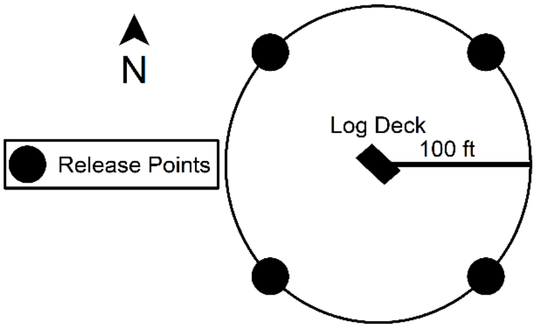

Figure 2.1. A schematic showing the layout of the field site where we released gypsy moth larvae in central Wisconsin, USA in the summers of 2016 and 2017. A log deck was staged in the middle of a granite quarry. We measured 100 ft out from the center of this log deck to establish the perimeter of a vegetation-free buffer zone and released larvae at the intercardinal directions (NE, SE, SW, NW) along the perimeter of this buffer zone (see text). (Note: figure is not to scale.)

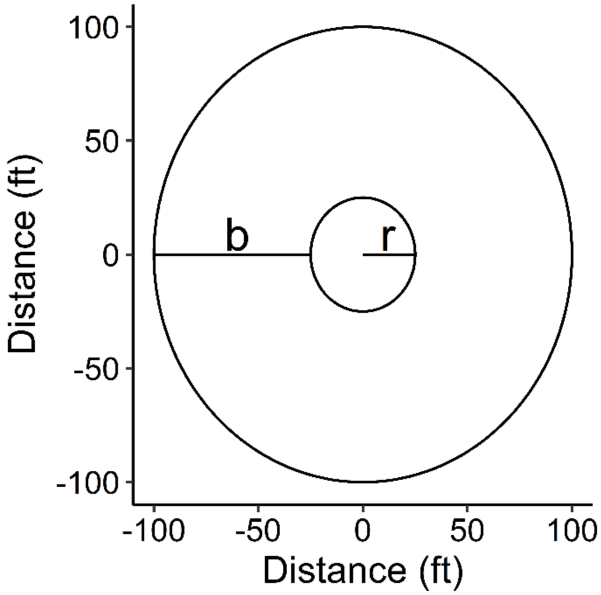

Figure 2.2. An example schematic image of the log deck and buffer zone used in our simulations. We used three different radii, *r*, of wood product stage areas (25, 50, and 75 feet) and six different buffer zone distances, *b*, (75, 100, 125, 150, 175, and 200 feet) in our simulation. In this figure *b* = 75 ft and *r* = 25 ft. We simulated movement of 10,000 gypsy moth larvae for all combinations of *r* and *b*.

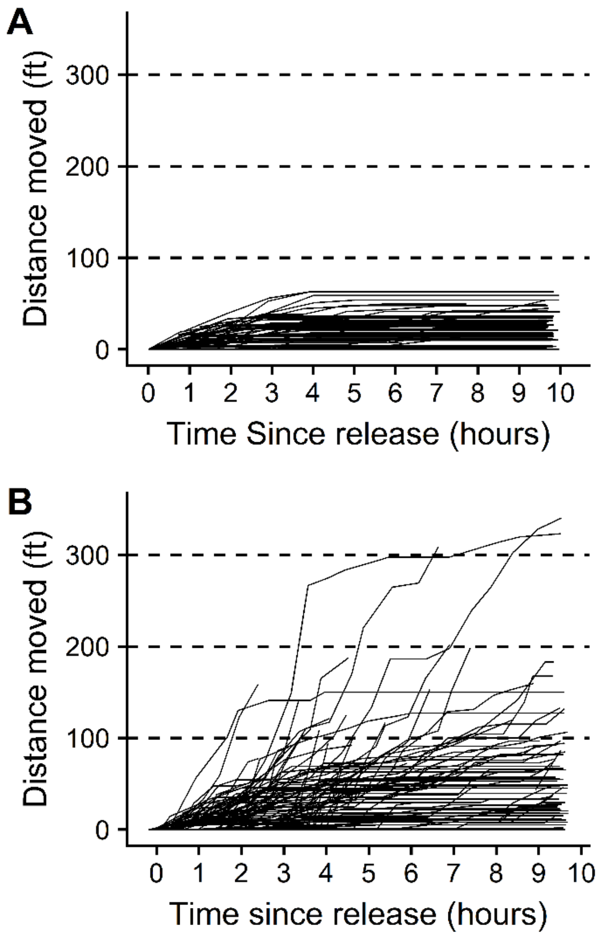

Figure 2.3. Cumulative distance moved over time by gypsy moth larvae released on the edge of a vegetation-free area, or buffer zone, in central Wisconsin in A) 2016 (*n* = 101) and B) 2017 (*n* = 171). One hundred feet is the size of the standard buffer zone radius currently used.

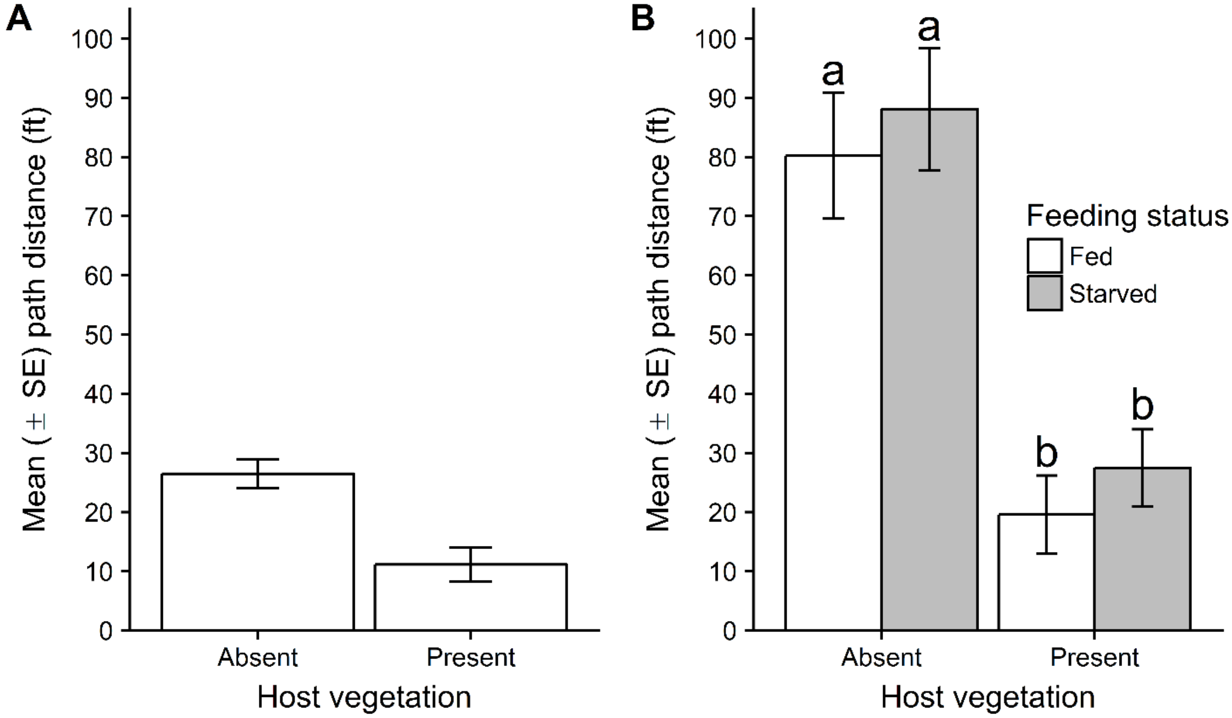

Figure 2.4. A) Average distance traveled by gypsy moth larvae released in trees (host vegetation present, *n* = 10) and on the ground (host vegetation absent, *n* = 50) in 2016. B) Average distance traveled by gypsy moth larvae that were released in host vegetation and fed prior to release (*n* = 12), released into trees and starved for 24 h prior to release (*n* = 14), released on the ground and fed (*n* = 49), and released on the ground and starved for 24 h prior to release (*n* = 40) in 2017. In both years, larvae that did not move at all were excluded from analyses.

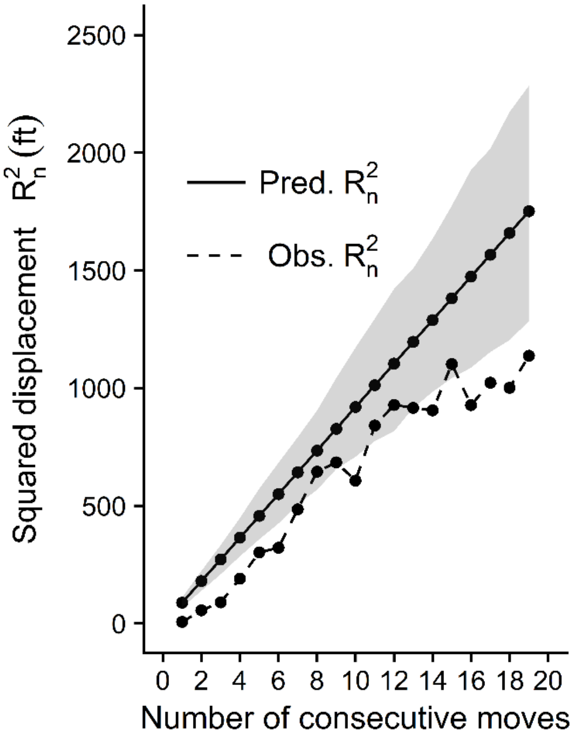

Figure 2.5. Predicted and observed net squared displacement (R_n^2) calculated from movement parameters from gypsy moth larvae released in central Wisconsin in June, 2017 (per Kareiva and Shigesada 1983; Turchin 1998). If gypsy moth larvae movement is a correlated random walk, observed R_n^2 will increase linearly with time and will fall within the 95% confidence interval of the predicted R_n^2 (in grey in the figure above).

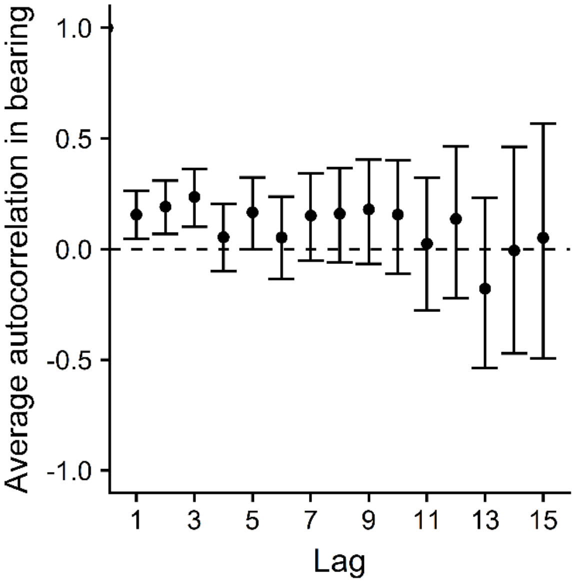

Figure 2.6. Average autocorrelation in compass direction traveled (bearing) for gypsy moth larvae released in a vegetation-free area in Wisconsin over seven days in June 2017. Calculated according to Turchin (1998). Positive autocorrelation at lag *l* indicates that a larvae moving in a direction at a time *t* will likely continue moving in a similar direction for *t* + *l* time steps.

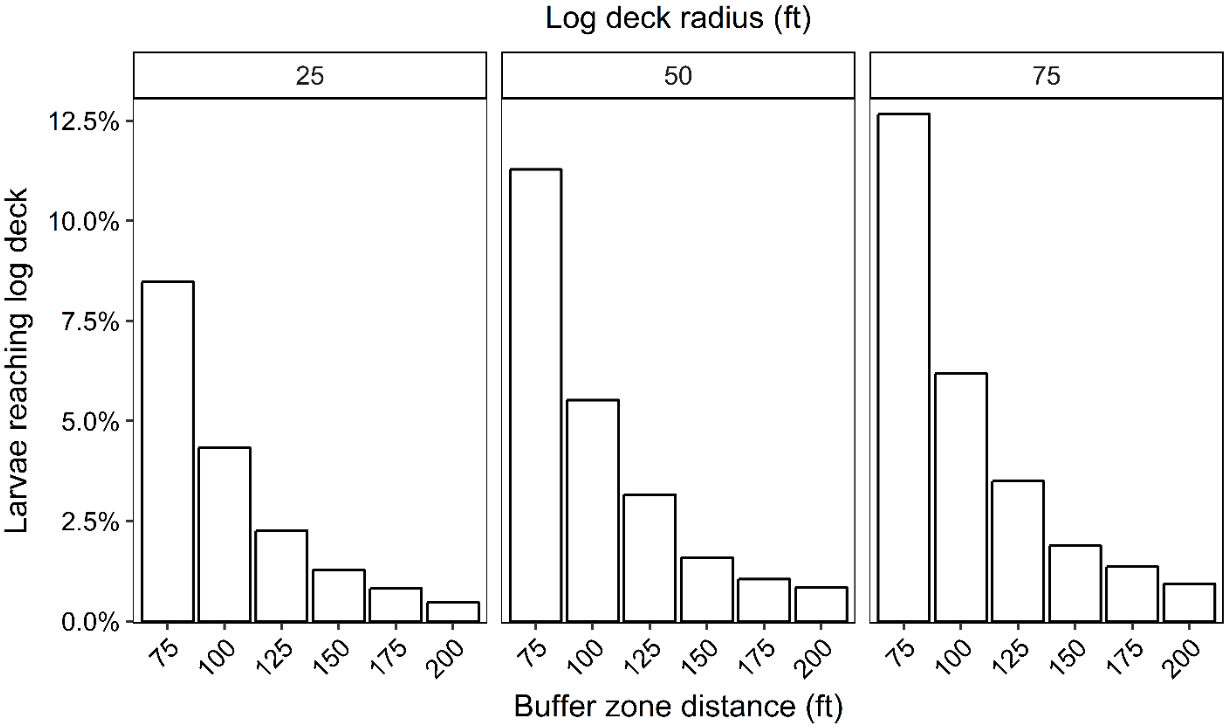

Figure 2.7. The results of Monte Carlo simulations evaluating how frequently gypsy moth larvae encounter a log deck at the center of a vegetation-free buffer zone if they begin moving at the edge of the buffer zone. The simulations were parameterized based on field data collected by following the movement of gypsy moth larvae released in a vegetation-free area in central Wisconsin in June 2017. We simulated the movement of 10,000 larvae under 18 different log deck radii and buffer zone distance combinations.

Thesis Conclusions

Chapter 1

1) **Larvae that feed on more suitable or preferred hosts may be better equipped to search for food during periods of food deprivation.** Larvae that fed on foliage (as opposed to the artificial diet) had the same initial capacity for movement. Only larvae that fed on bur oak, a preferred food source, moved farther as they went longer without food. This increase in distance moved by the bur oak-fed larvae was mainly due to an increase in mean velocity as starvation time increased.
2) **Feeding on less preferred or less suitable hosts increases the probability larvae will move.** Larvae that fed on larch and artificial diet were more likely to move than those that fed on bur oak. This response, however, is also affected by starvation time; as starvation time increased from zero hours to 48 hours, all larvae became likely to move.
3) **Managers may need to consider adjusting the size of the buffer zone depending on the type of host plants on the perimeter and expected populations of gypsy moth.** Buffer zones with preferred host plants along the perimeter may have a lower likelihood of intrusion by larvae than those with less preferred hosts nearby. During outbreaks of gypsy moth, it may be more likely that larvae will move into the buffer zone as they deplete food sources in the immediate vicinity.

Chapter 2

1) **Larvae are able to reach products staged at the center of a 100-foot buffer zone but appear unlikely to do so.** When no host vegetation was present, 4% of the larvae released reached the wood staged at the center of the buffer zone. No larvae

that were released in the presence of host vegetation reached the center of the buffer zone.

2) **Increasing the size of the buffer zone will decrease the likelihood larvae will reach objects staged at the center, but with diminishing returns.** According to simulation models of different buffer zone sizes, increases in the efficacy of the buffer zone diminish quickly after a 125-foot radius. This result is, however, dependent on the size of the objects staged at the center of the buffer zone. Larger staging areas may require larger buffer zones to remain efficacious.

3) **The buffer zone practice is likely achieving the desired impact.** Our simulation and field studies show small numbers of gypsy moth larvae will reach the center of the buffer zone. Under conditions where hosts are available on the outside of the buffer zone and populations of gypsy moth are low, it is unlikely that larvae will enter the buffer zone.

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