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# The Movement Ecology of an Agricultural Pest, Navel Orangeworm, *Amyelois transitella*

By

Stephen Kyle Bayes

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in

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Committee in charge:

Professor Stephen C. Welter, Chair
Professor Nicholas J. Mills
Professor Marc Hellerstein

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Abstract

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Tracking the movement of small organisms is of tremendous importance to understanding the ecology of populations, communities, and ecosystems. However, it remains one of the most difficult challenges facing the field of movement ecology. This dissertation focuses on the movement of an agricultural pest, the navel orangeworm (*Amyelois transitella*, NOW). I first examined intercrop movement of NOW between two agricultural commodities, almonds and walnuts. By using protein markers and an enzyme-linked immunosorbant assay technique to detect markers on recaptured moths, I demonstrated significant male NOW movement (up to 300 meters) and no significant directional preferences of movement based on crop, season, or wind velocity. However, protein marker contamination of control moths within traps was significant, limiting our ability to detect movement patterns. In addition, the scale of this study may have been too small to capture larger scale directional patterns of movement.

In order to overcome some of the observed challenges of protein marking techniques for small organisms, I developed and tested an intrinsic marking technique for tracking NOW using dietary fatty acid profiles as a biomarker. This was accomplished by analyzing fatty acids from NOW moths raised on two different host plants with significantly different fatty acid profiles. Using this data a linear discriminant analysis model was developed and validated to distinguish NOW based on their larval host plant. Results showed that NOW fatty acid profiles are strikingly similar to those of their host plant. Therefore fatty acids can act as a valuable intrinsic marking technique for tracking small organisms, avoiding many of the drawbacks of external markers, and providing a useful tool for the study of movement ecology.

Fatty acid tracking is effective for small organisms, but does not determine movement paths, direction, or distance of movement in a localized setting. In order to draw meaningful conclusions from localized movement data using intrinsic marking techniques, I developed a Gaussian-based dispersal model. This model was applied to field-caught NOW moths from three sites in the central valley of California. Average movement distance was estimated to be about 50 m per generation at two sites and about 600 m per generation at the third site. The study demonstrates that probability-based dispersal models combined with intrinsic marking techniques provides a useful tool for both tracking and understanding the localized movement capabilities of small organisms.

## Dedication

This dissertation is dedicated to my parents, Jane and Kyle Bayes,
for their love and support in this and all of my other endeavors.

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### Chapter 3

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First, I would like to thank Steve Welter, my committee chair, advisor and mentor. Steve encouraged new ideas, giving me the freedom to explore, while still grounding me when I got a little crazy. His enthusiasm and passion for the research was contagious and made the lab a wonderful place to work. In addition, he was always willing to listen and had thoughtful, useful, and pertinent advice for just about every situation. I cannot thank him enough for his dedication, patience and hard work in fostering my development into a fully-fledged scientist.

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## Introduction

Movement and dispersal play a key role in the evolutionary success of any species, allowing individuals to capitalize on spatial and temporal variation in the landscape (Hanski et al. 1999; Clobert et al. 2001; Bowler and Benton 2005; Clobert et al. 2012). Movement gives individuals the ability to access resources (Hanski et al. 2002), escape predation (Gude et al. 2006), and increase their mating opportunities (Hovestadt et al. 2014). As a result, a wide array of movement strategies have evolved, all of which interact with the spatial and temporal structure of the landscape to shape our ecological communities and ecosystems. In a world that is increasingly being altered due to climate change, expanding agriculture, and habitat loss, a better understanding of the interplay between species movement and landscape structure will be of the utmost importance to managing ecosystems and protecting biodiversity.

Agricultural habitats provide a prime testing ground for looking at how landscape level factors influence species movement. Modern agriculture is mostly composed of monocultures, forming a patchwork of habitat types that spatially and temporally separate resources across a variety of scales. These resources will be used to varying degrees by different species, and thus provide an ideal environment for studying movement ecology. Additionally, from an applied perspective, many ecosystem services are provided by insects, and take place in our agricultural habitats. The effective management of pollinators, phytophagous insects, and their natural enemies requires a deep understanding of how these species move across our fragmented agro-ecosystems. Historically, studying insect movement has been a difficult and arduous process due to their small size and short lifespans (Cronin and Reeve 2005). However, new tracking techniques are providing opportunities to examine questions of insect movement that have previously proven difficult to study.

I set out to examine the movement of an agricultural pest, *Amyelois transitella* (Lepidoptera: Pyralidae), also known as the Navel Orangeworm (NOW) because it was first discovered feeding on rotting oranges in Arizona (Wade 1961). NOW is a primary pest of almonds and pistachios in California and attacks a number of other agricultural crops including walnuts, figs, and pomegranates (Meals and Caltagirone 1995). Thus it is a species that can utilize a variety of different resource and habitat types, making it ideal for examining movement dynamics. For effective management of NOW it is important to know whether individuals readily move between agricultural crops and whether their movement is directional being driven by wind velocity or by seasonal changes in resource availability.

In Chapter 1, I examine how crop type, wind velocity, and time of the season affected the movement of NOW between two different cropping systems, walnuts and almonds. I opted to use protein markers paired with enzyme-linked immunosorbant assays (ELISAs) to track the movement of NOW. The ELISA method is a cost effective mass-marking technique for tracking insects across the landscape (Hagler 1997; Jones et al. 2006). The results showed that the false positive signal for NOW marking in the field was quite high, thus greatly reducing the amount of movement I was able to detect. I saw no evidence of directional movement of NOW due to wind, crop type, or season. NOW was easily able to access the most distant traps, suggesting that the scale of my experimental observations was too small.

Due to the shortcomings of the protein marking technique, in Chapter 2, I develop an intrinsic marking technique based on the fatty acid profiles of NOW. The premise being that, through the process of dietary routing (Blem 1976; Pond 1981), NOW adults should have a similar fatty acid composition to the host plant on which they have fed as larvae. This technique

proved to be very successful, showing that NOW truly "are what they eat," and demonstrates that fatty acids can be used as a reliable intrinsic marker to track insect movement. This work also showed that NOW readily moves between walnut and almond orchards, effectively linking these two cropping systems with regard to the management of this pest.

The results from this new marking technique were quite exciting, demonstrating for the first time that fatty acids can be used as intrinsic markers for tracking movement of small organisms such as insects. However, as intrinsic markers, fatty acids also presented novel challenges in terms of the type of movement data obtained. Normally, movement studies deal with movement paths that have a distinct start and end point. In contrast, movement data obtained from intrinsic markers has a distinct end point, but the starting point is unknown and could be from a number of different source locations in the landscape.

For the third and final chapter, I collected instrinsically marked NOW from walnut and almond orchards, and developed a probabilistic dispersal model to examine NOW movement capabilities from this new type of dataset. The model assumes movement via a random walk, estimated by a random diffusion dispersal kernel (Turchin 1998). I applied the model to movement data obtained from trapping NOW in the field at three distinct locations in the Central Valley and analyzing the trapped moths for their fatty acid signatures. The results showed clear variation in the movement patterns of NOW, and the capability of NOW populations to move an average of 594 m per generation.

My success in tracking NOW movement will benefit the field of movement ecology, particularly advancing our ability to track small arthropods in localized landscapes. Although I failed to find directional patterns of NOW movement between crops, my development of a new intrinsic marker paired with a novel modeling approach should prove extremely valuable to furthering our knowledge of NOW movement and that of other small organisms.

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**Chapter 1**
**Intercrop movement of navel orangeworm monitored using enzyme-linked immunosorbant assays**


## Abstract

Insect movement plays a vital role in our agroecosystems and landscape structure can play an important role in how and when insects move between crops. We examined intercrop movement of an agricultural pest, the navel orangeworm (*Amyelois transitella*, NOW), between two agricultural commodities; almonds and walnuts. We used protein markers to mark moths, an enzyme linked immunosorbant assay (ELISA) technique to detect the markers on recaptured moths, and female NOW virgin-baited traps to monitor their movement between these orchard crops. We examined the effects of season, crop type, and wind velocity on NOW movement using both egg and milk protein markers. Marking rates were substantially higher for egg protein than for milk protein. Protein marker contamination of other moths within traps was significant and should be controlled for in future ELISA marking experiments. Our results clearly showed significant male NOW movement, with moths easily moving to the outer edges of the trapping area (300 meters). There was no significant effect of crop, season, or wind velocity on directional preferences of NOW movement. This suggests that the pattern of movement of NOW was by simple diffusion, although the scale of our study may have been too small to capture larger scale directional patterns of movement. The implications of the observed patterns of NOW movement for the management of NOW as an agricultural pest are discussed.

## Introduction

Movement of insects is increasingly being recognized as playing a significant role in agricultural systems. Through movement insects can provide important ecosystem services such as pollination (Klein et al. 2007; Schulp et al. 2014), decomposition (Gessner et al. 2010), and biological control (Vandermeer et al. 2010), but also can be responsible for damage to crops (Costanza et al. 1997; Mazzi and Dorn 2012). Movement plays a vital role in the life history strategy of all insects, allowing individuals to locate resources, find mates and disperse to new habitats (Bowler and Benton 2005). It has been shown to be shaped by a variety of factors including resource competition (Herzig 1995), mate competition (Ikawa et al. 1993), inbreeding avoidance (Wheelwright and Mauck 1998), population age (Ovaskainen et al. 2008), time of year (Chapman et al. 2006) and predator avoidance (Losey and Denno 1998).

A common theme emerging from the literature on trophic interactions and community ecology is that landscape level factors tend to dominate those acting at a more localized scale (e.g. Steffan-Dewenter et al. 2002; Thies et al. 2008; Bennett and Gratton 2012; Chaplin-Kramer et al. 2013). Landscape structure determines the availability of local resources, and by filtering the regional species pool, it influences the diversity and composition of local communities (Tscharntke et al. 2012; Macfadyen and Muller 2013; Rösch et al. 2013). Most agricultural landscapes are patchy in nature, with a mosaic of different crops, natural areas, and other man-made features. Any given insect species will only be able to utilize some of these habitat components, and will thus need to be effective in moving between patches of usable habitat by dispersing through unusable habitat, known as matrix.

Recent studies have focused on insect movement between natural and agricultural habitats (Blitzer et al. 2012; Tscharntke et al. 2012). It is clear from these studies that movement is indeed occurring between these different systems, and is highly significant in structuring local insect communities. In addition to the variation between natural and agricultural areas, there is significant spatial and temporal variation within agricultural habitats due to differences in cropping systems and management practices. How and when insects are moving between these different cropping systems may play an equally important role in structuring local insect communities.

We set out to quantify intercrop movement of an invasive moth, navel orangeworm (*Amyelois transitella*) (Walker) (NOW). In the Central Valley of California, NOW feeds on the fruits of a wide variety of crops, the two most widespread being almonds (*Prunus dulcis*) and walnuts (*Juglans regia*) (Wade 1961). The resources available to NOW vary between these two cropping systems. As NOW larvae are unable to penetrate the hulls and shells of the nuts, the kernels are only available to NOW when they split open close to harvest (almonds), or when they have been damaged in some way (walnuts). Consequently, the significance of NOW as a pest and the intensity of management practices used against it vary between these two orchard crops. NOW is less of an economic concern for walnut growers because in most walnut cultivars NOW larvae are unable to penetrate the hard shell unless it has been damaged, and thus NOW has only an indirect impact on the harvestable crop. In contrast, hull and shell split often occurs before harvest in almond orchards making them particularly susceptible to NOW damage. As a result, walnut growers are less likely to actively manage NOW populations and there is potential for NOW to utilize the spatiotemporal variation in both resource availability and timing of pest management practices across the landscape.

We expect that NOW actively moves between almond and walnut orchards to utilize the variation in resource availability and habitat suitability. We hypothesize that NOW populations are able to build in walnut orchards early in the season on damaged walnuts left over from the previous year. As orchard sanitation is more common in almond orchards there are fewer left over resources for NOW to utilize early in the season. We expect NOW to move from walnut into almond orchards when the almond hull and shell split occurs, and finally move back from almond to walnut orchards to overwinter on the left over nuts. To track NOW movement between almond and walnut orchards we elected to use enzyme-linked immunosorbant assays (ELISA) of protein-marked adult moths (Hagler 1997; Jones et al. 2006). The application of ELISAs to the study of insect movement was developed to be able to detect very low concentrations of specific vertebrate proteins using commercially available protein-specific antibodies. Vertebrate proteins can be applied to large areas of vegetation using commercial spray equipment and any adult insects that are either present in or subsequently visit the area may pick up the vertebrate protein markers on their body surface. Adult insects can then be captured from the surrounding landscape and assayed to determine whether they carry the markers that were applied to different areas, providing valuable information on the dispersal patterns of marked individuals. We used protein markers and ELISA to address two separate questions, one, to evaluate whether this approach could be used successfully to monitor movement of NOW, and two, to determine seasonal patterns of directional movement of NOW between almond and walnut orchards.

## Materials and Methods

*Field Sites*

Field sites in the Central Valley of California were chosen using the following criteria: availability of adjacent 0.4 ha blocks of walnuts and almonds, availability of growers willing to apply protein markers in the two blocks, and sufficient NOW pressure to support the study. Orchards were located in Patterson, Oakdale, Escalon and Winters. A protein marking solution of either 10% egg white (Michael's Foods, Minnetonka, MN, USA), or 20% milk (Lucerne Foods, Pleasanton, CA, USA) in water was applied to the trees within the 0.4 ha blocks of the two adjacent crops using commercial spray equipment. Additional 0.4 ha blocks on the non-adjacent sides of the sprayed blocks were left without protein application to allow us to monitor movement within each crop as well as between crops (Fig. 1).

One crop (walnuts or almonds) was sprayed with the egg protein marker, and the other crop was sprayed with the milk protein marker. We randomized which protein was applied to a given crop between orchard sites, however the crop assignment was kept the same for all spatial and temporal replicates within an orchard. Protein marker application started in June and continued at intervals through to early October (Table 1). Either one to two spatial replicates were set up at each of the five orchard sites (Table 1) separated by at least 215m. We evaluated the independence of the spatial replicates within an orchard site by placing traps half way between the two replicates. If a significant number of marked male moths were caught in these traps it would suggest that the spatial replicates were not independent of one another. As movement of NOW males between the adjacent crops could be influenced by the timing of crop maturity, hull split, or harvest date, we divided the growing season into three different periods (early, mid, and late season, Table 1).

Six white delta traps (Suterra, Bend, OR) were placed in each block, for a total of 24 traps per replicate, enabling us to evaluate movement within a crop in comparison to the inter-crop movement. Each trap was baited with three laboratory-reared virgin NOW females contained within a 7 cm square fiberglass sleeve. Trap liners and virgin females were replaced every seven days. Captured male moths were covered in clean plastic film and brought back to the laboratory for analysis.

To estimate the potential for false positives in the field, a single identifiable laboratory-reared male NOW (FP-NOW) was attached to each new trap liner before they were placed out in the field. Our concern was whether wild moths that became marked by their activity in a protein-sprayed block might contaminate unmarked moths on the trap liners before being immobilized by the adhesive. Marker proteins blowing in the wind or carried into the traps by other insects were also a potential concern. The FP-NOW individuals recovered from trap liners in the field were put through the same screening as all field caught NOW, thus the FP-NOW individuals served as a control for both contamination within a trap and the entire field collection process.

*Enzyme Linked Immunosorbant Assays*

We used two protein specific ELISAs following the methods of Jones et al. (2006). Clean laboratory-reared male moths were put through the same process as field-collected male moths to serve as negative controls. Each male moth was removed from the trap liners using wooden toothpicks (Diamond, Danville, IN) and placed individually into 1 ml plastic tubes (USA Scientific, Ocala, FL, USA) with 1 ml tris-buffered saline (TBS) (pH 8.0) (Sigma-Aldrich, St. Louis, MO, USA) with 0.3 mg of ethylenediamine tetra acetate (EDTA) (Sigma-Aldrich, St. Louis, MO, USA). The type of plastic tube used for the sample insects was carefully selected and standardized as the protein markers tend to adhere to certain plastics and can thus reduce the signal of the assays. Moths were submerged and rotated at (80 revolution/min) on a laboratory rotator for 3 min before being removed from the solution.

Aliquots of 80 µl of each solution from a trapped male moth was transferred into individual wells of a 96-well microplate (Nunc Polysorp; Nalge Nunc, Naperville, IL) for the egg albumin ELISA and a 96-well microplate (Maxisorp; VWR International, Visalia, CA, USA) for the milk assay. Each plate of sample solutions included seven negative control wells that contained solutions from unmarked laboratory-reared NOW and used to control for the variability commonly seen between ELISA microplates (Sivakoff et al. 2011). The samples were incubated for 2 h at 37 °C , followed by five washes of phosphate-buffered saline (PBS)(Sigma-Aldrich, St. Louis, MO, USA) with 0.9% Triton-X100 (PBST) (MP Biomedicals, Santa Ana, CA, USA). Each well was then given 300 µl of blocker solution consisting of PBS with 20% bovine serum (Sigma-Aldrich, St. Louis, MO, USA) and 1300 ppm Silwet (PBSS-BS20) (Helena Chemical Company, Collierville, TN, USA). Plates were subsequently incubated for an additional 1 h at 37 °C before 80 µl of the primary antibody was added to each well. For the milk protein marker, the primary antibody was anti-casein protein (Biodesign International, Saco, ME, USA) diluted in PBSS-BS20. For the egg protein marker, the primary antibody was anti-chicken protein (Sigma-Aldrich, St. Louis, MO, USA) diluted in PBS with 30% bovine serum and 1300 ppm Silwet (PBSS-BS30). All antibody dilutions were determined with a checkerboard titration assay (Crowther 2001). Plates were further incubated at 37 °C for 30 min and washed five times with PBST. Then 80 µl of secondary antibody was added to each well.

For milk protein plates, the casein secondary antibody was a donkey anti-sheep IgG peroxidase conjugate (Sigma-Aldrich, St. Louis, MO, USA). For egg protein plates, the egg albumin secondary antibody was donkey anti-rabbit IgG (H+L) with a peroxidase conjugate (Pierce Biotechnology, Rockford, IL, USA). Secondary antibodies were diluted in PBSS-BS20 and PBSS-BS30 for milk and egg proteins respectively.

Plates were again incubated at 37 °C for 2 h before being washed. Milk protein plates were washed three times with PBS with 2.3g/l of sodium dodecyl sulfate (SDS) (Sigma-Aldrich, St. Louis, MO, USA), followed by three times with PBST. Egg protein plates were washed five times with PBST. Next, 80 µl of TMB (ImmunoPure Ultra TMB substrate kit 34028; Pierce Biotechnology, Rockford, IL, USA) was added to each well and the plate was put on an orbital shaker in the dark for 5 min at 100 revolutions/min. To stop the reaction, 80 µl of 2N H2SO4 was added to each well. Optical densities were read immediately with a dual wavelength plate reader (Emax; Molecular Devices, Sunnyvale, CA) at 450 nm using 490 nm as a reference.

*Data Analysis*

Long-distance dispersal studies require a very low threshold for false positives, as dispersal events become exponentially more difficult to detect through area-dilution effects with greater distance from the source (Turchin 1998). We determined which of the male moths were marked by following the methods of Sivakoff et al. (2011). To normalize for the variation among plates, a standard normal variate transformation was used to change optical densities into z-scores. We then used a marking threshold that was based on the mean z-score of the negative controls plus three times the mean standard deviation for all negative control z-scores. This threshold allows for a higher marking percentage than a threshold representing the highest negative control score for all plates (recommended by Sivakoff et al. 2011), while keeping the false positive rate relatively low. As we were not measuring long-distance dispersal, our ability to detect a greater number of marked moths greatly outweighed the small increase in false positives.

All analyses were performed using the R project software (version 2.15.1). To answer the question of whether we were able to detect movement of NOW using protein markers, we used generalized linear models (GLM) with binomial errors to compare marking rates for field-trapped NOW and the FP-NOW controls. This analysis was run separately for the milk and egg marking proteins with moth type (NOW vs. FP-NOW), period of the season (early, mid, late), crop origin (almond, walnut), and area captured (sprayed with marker or not sprayed with marker) as fixed effects. We compared means for different levels of significant factors using pairwise t-tests with Holm adjustment (Crawley 2013). Marking rates were used in this analysis because of the small number of FP-NOW relative to the field-trapped NOW. A non-significant difference in marking rates between NOW and FP-NOW would indicate that we were unable to successfully detect NOW movement due to contamination of field-trapped moths within traps.

We used GLM to examine directional movement of NOW between the two crops. The measurement variable was the number of marked moths in blocks to either side of a protein marker sprayed block. Numbers of marked moths (egg and milk combined) were used for this analysis, rather than marking rate, because unmarked moths do not provide any information on moth movement. To correct for variation in numbers of moths trapped in each orchard and crop type, as well as for differences between marking rates of the two markers, we transformed the measurement variable, the absolute number of marked moths, into a z-score. The fixed factors of

the model were period of the season and patterns of inter and intra crop movement (almond to almond, almond to walnut, walnut to almond and walnut to walnut). We also examined directional movement events in relation to wind velocity during the course of the study. We expected that male moths would move up wind in response to the sex pheromones emitted from the virgin female lures in each trap. Wind velocity data was obtained from the California Irrigation Management Information System (http://www.cimis.water.ca.gov). The mean wind velocity was calculated from hourly data for each trapping period for each replicate. Average velocities were broken down into their x and y components, with the y component denoting the velocity of wind blowing from the spray blocks. Numbers of marked NOW were transformed into z-scores for the blocks to either side of the spray blocks and paired with the y-component of the wind velocities for the specific time period. We ran a Pearson's product-moment correlation analysis between the z-scores and wind velocities.

Finally, to examine the potential for asymmetrical NOW movement between orchards due to differences in moth population levels between the different crops we analyzed our overall trap catch within each crop type at the three sites, regardless of marking. We used GLM with Poisson errors, with numbers of moths as the response variable and period of the season and crop type as fixed effects.

## Results

*Marking Rates and Contamination*

A total of 18,812 male moths were collected over the course of the study. The placement of the traps used for testing the independence of spatial replicates ranged from about 150-320 meters from the closest protein marker spray area. Marked NOW catches in these traps were not uncommon (19% marked with egg protein and 1% marked with milk protein, n = 758). Therefore, it is likely that the two spatial replicates within an orchard site were not completely independent of each other. Although we did not place traps further than 320 m from the protein spray blocks, it is probable that NOW is capable of flying much larger distances given the high level of marked moths captured at this distance for both protein markers (Fig. 2). In view of these results, the data from spatial replicates within blocks were pooled for further analysis. To maximize the marking potential we choose to assay only those NOW trapped during the first week after each application of the protein markers at the orchard sites. We assayed 5,712 NOW from the field, plus an additional 483 FP-NOW controls. Egg protein marking rates were higher than milk protein marking rates both within sprayed blocks and across all blocks (Fig. 3). The FP-NOW controls showed higher marking rates than the negative ELISA plate controls which were 1.6% (n = 571) for the egg protein and 0.7% (n = 571) for the milk protein. This indicates that protein marker contamination and thus false positives occurred in the field before ELISA analysis of the field-trapped NOW, and that consequently not all of the male moths from the traps that were classified as marked would necessarily have originated from the protein treated blocks.

The binomial GLM to determine if movement could be successfully monitored using protein markers and ELISA showed significant differences in marking rates of field-trapped NOW compared to FP-NOW for both egg and milk protein markers, with whether the moth was captured in a sprayed area or not, and period of the season also having significant effects (Table 2). Higher marking rates of field-trapped NOW shows that we were successfully able to detect

marked individuals from the field, and that false positives were not overriding the movement signal. The late season trap catch showed a significantly higher marking rate than the early or mid-season trap catch, suggesting greater NOW movement at this period of the season. As expected, there was a significantly higher marking rate within the spray area, indicating that marked moths were recaptured more frequently within the spray area compared to outside of the spray area.

*Directional Movement*

We saw no significant effect of the movement pattern of NOW between crops (Table 3), and no significant effect of the interaction between movement pattern and season. This indicates that there was no evidence for a seasonal pattern of NOW movement between almond and walnut and that NOW movement was non-directional occurring equally frequently within and between crops. However, there was a significant effect of season on NOW movement, with more movement occurring late in the season than either early or mid-season ($p < 0.001$, pairwise t-test). Wind velocity was not correlated with the directional movement of NOW between orchard blocks ($r = -0.06$, $df = 116$, $p = 0.51$).

*Overall Trap Catch*

We found that overall trap catch, regardless of marking, was higher in walnut orchards compared to almond orchards (Fig. 4), although the difference was not significant. Overall trap catch did vary with season, however, with more moths trapped in late season than either early or mid-season ($p < 0.01$, pairwise t-test) (Table 3).

## Discussion

We have demonstrated that external protein markers can be successfully used to monitor movement of male NOW in the field and that the marking rate for egg protein was notably greater than for milk protein. Recapture of protein-marked NOW indicated that there was significant movement occurring between adjacent almond and walnut orchards at the scale of this study, but we found no evidence for any directional movement that could be attributed to crop, period of the season, or wind velocity. Thus the pattern of movement of the male NOW observed in our study appeared non-directional, approximating simple diffusion. However, even in the absence of any directional bias due to preferential movement of NOW towards a particular crop, it is still possible for there to be asymmetry in terms of absolute numbers moving between these two crops. If NOW were more abundant in walnuts than almonds and their movement occurred through simple diffusion, then we would expect a larger flow of NOW from walnuts into almond orchards. In other words, orchards with higher populations of NOW would be expected to have differential spillover into nearby habitats with lower or no NOW populations. For the orchards included in our study we did not see any significant difference in overall NOW trap catch between walnuts and almonds; however, the close proximity of the adjacent blocks and the high degree of movement are likely to have contributed to a more uniform trap catch between crops.

Although this study did not set out to quantify the maximum range of movement of male NOW, it is suggested by our results that these moths can move far beyond the distances

considered in this study. We observed large numbers of marked NOW in our traps that were furthest from the protein-sprayed blocks, suggesting that moths moved in significant numbers far beyond the 320 m maximum distance considered. The expectation in dispersal studies is that the rate of recapture becomes exponentially smaller with distance from the point of origin due to an area-dilution effect (Turchin 1998). However, we still observed significant numbers of NOW captured at 320 m from the point of origin, again suggesting that the movement distance must have been considerable. A similar capacity for dispersal was observed in a study of NOW movement based on the mark-recapture of NOW females marked with red dye and monitored using almond meal baited egg-laying traps (Andrews et al. 1980).

We encountered a significant number of false positives from field contamination of moths in the delta traps baited with virgin-females. By placing dead laboratory-reared NOW in the traps in the field (FP-NOW), we found that a significant number of these moths became marked with the egg protein while they were exposed in the traps (Fig. 3). In contrast, we saw very low marking rates from laboratory reared negative controls on the ELISA plates. The level of contamination for the milk protein appeared to be much lower, but as the overall marking rate was also much lower for this marker, it remains unclear whether this was actually the case. Although most studies that have used the ELISA marking technique have not included false positive controls in the field (e.g. Hagler 1997; Jones et al. 2006; Sivakoff et al. 2012) we would argue that they are necessary to estimate the potential for contamination. In their absence, our results would suggest that studies that have used ELISAs for measuring insect movement may have experienced a much higher degree of false positives than they have reported and potentially implied higher levels of movement. It is possible that the potential for contamination between individuals in delta traps could be greater for Lepidoptera than for other insect taxa as protein-marked scales are easily detached from their bodies and wings. Nonetheless, we recommend that future studies of insect movement using the ELISA marking technique should include a control for within-trap contamination.

In addition, we found that the type of plastic used in vials for rinsing the protein markers from the recaptured moths had a significant effect on the strength of the ELISA signal. We suspect that some protein markers can bind with certain plastics, thus reducing or eliminating the ELISA signal. Our lab work also suggested that the greater the length of time that the recaptured moths were stored in a freezer before being rinsed to recover the protein markers also reduced the ELISA signal (data not presented). These factors should be taken into careful consideration when designing a dispersal or movement study using protein markers.

If we assume that movement of female NOW matches that of males, the results from our study have clear implications for the management of NOW. Currently, orchards are managed individually for control of NOW, often with little or no collaboration between neighboring growers. We have demonstrated that the movement of NOW causes them to readily cross orchard boundaries and consequently that pest control efforts would benefit from a more coordinated landscape approach. In addition, native habitats should be considered as potential sources of NOW as they are known to have a broad diet range (Wade 1961). We have observed NOW developing on acorns (*Quercus agrifolia*) in the laboratory, and it is likely that NOW, as a generalist, is adept at utilizing many other wild plants. The scale of our study was clearly too small to allow a full understanding of the movement patterns and capabilities of NOW. However, the more important finding is that crops used by NOW are inter-linked by adult moth dispersal and that effective management of this pest will require decisions that are based on landscape-level considerations and multiple crops. If the goal of management is to reduce NOW

populations on a regional scale such as with areawide pheromone mating disruption systems, then management practices will need to consider inter-crop movement and spillover as significant concerns.

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| Timing | Marker application date | Orchard # | Location |
|---|---|---|---|
| Early season | 5/11/2010 | 1 | Patterson |
| | 6/30/2010 | 1 | Patterson |
| | 6/8/2010 | 2 | Winters |
| | 6/9/2010 | 3 | Oakdale 1 |
| | 6/24/2010 | 4 | Oakdale 2 |
| | 6/29/2010 | 5 | Escalon |
| Mid season | 7/16/2010 | 1 | Patterson |
| | 7/6/2010 | 2 | Winters |
| Late season | 8/30/2010 | 3 | Oakdale 1 |
| | 8/30/2010 | 5 | Escalon |
| | 9/6/2010 | 1 | Patterson |
| | 10/4/2010 | 4 | Oakdale 2 |

**Table 1:** The timing of protein marker applications in each of the orchard sites in relation to period of season.

| Marker protein | df | F | p |
|---|---|---|---|
| **Egg** | | | |
| Moth type | 1 | 6.366 | 0.013 * |
| Season | 2 | 9.374 | <0.001 *** |
| Area captured | 1 | 46.882 | <0.001 *** |
| Crop type | 1 | 0.287 | 0.59 |
| | | | |
| **Milk** | | | |
| Moth type | 1 | 9.722 | 0.003 ** |
| Season | 2 | 70.615 | <0.001 *** |
| Area captured | 1 | 11.542 | 0.001 ** |
| Crop type | 1 | 0.481 | 0.49 |

**Table 2:** Results from the binomial GLM to test whether NOW movement could be successfully monitored using either egg or milk-marking proteins using false positive controls within traps placed in the field.

| | df | F | p |
|---|---|---|---|
| **Directional movement** | | | |
| Movement pattern | 1 | 2.082 | 0.12 |
| Season | 2 | 15.046 | <0.001 *** |
| Movement pattern x Season | 1 | 0.489 | 0.81 |
| | | | |
| **Overall trap catch** | | | |
| Crop type | 1 | 1.155 | 0.29 |
| Season | 2 | 7.908 | 0.002 ** |
| Crop type x Season | 2 | 0.138 | 0.87 |

**Table 3:** Results from the GLM to test for directional movement of NOW between almond and walnut blocks, and for differences between the overall trap catch in almond versus walnut orchards.

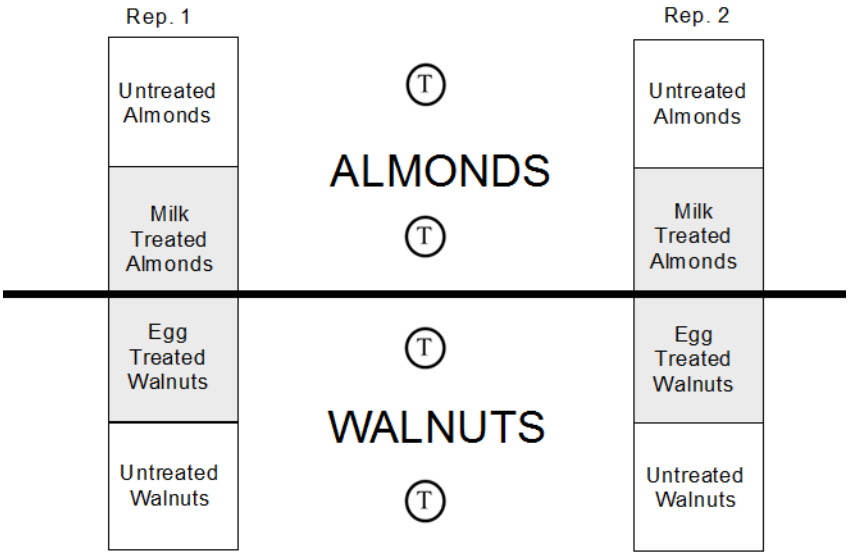


**Figure 1:** A schematic representation of the experimental layout for an orchard site with two spatial replicates. Protein treatments were randomized between crops at different orchard sites, but remained the same for each plot within orchard sites for the different application dates. The circled "T" shows where traps were placed to test for the independence of replicates.

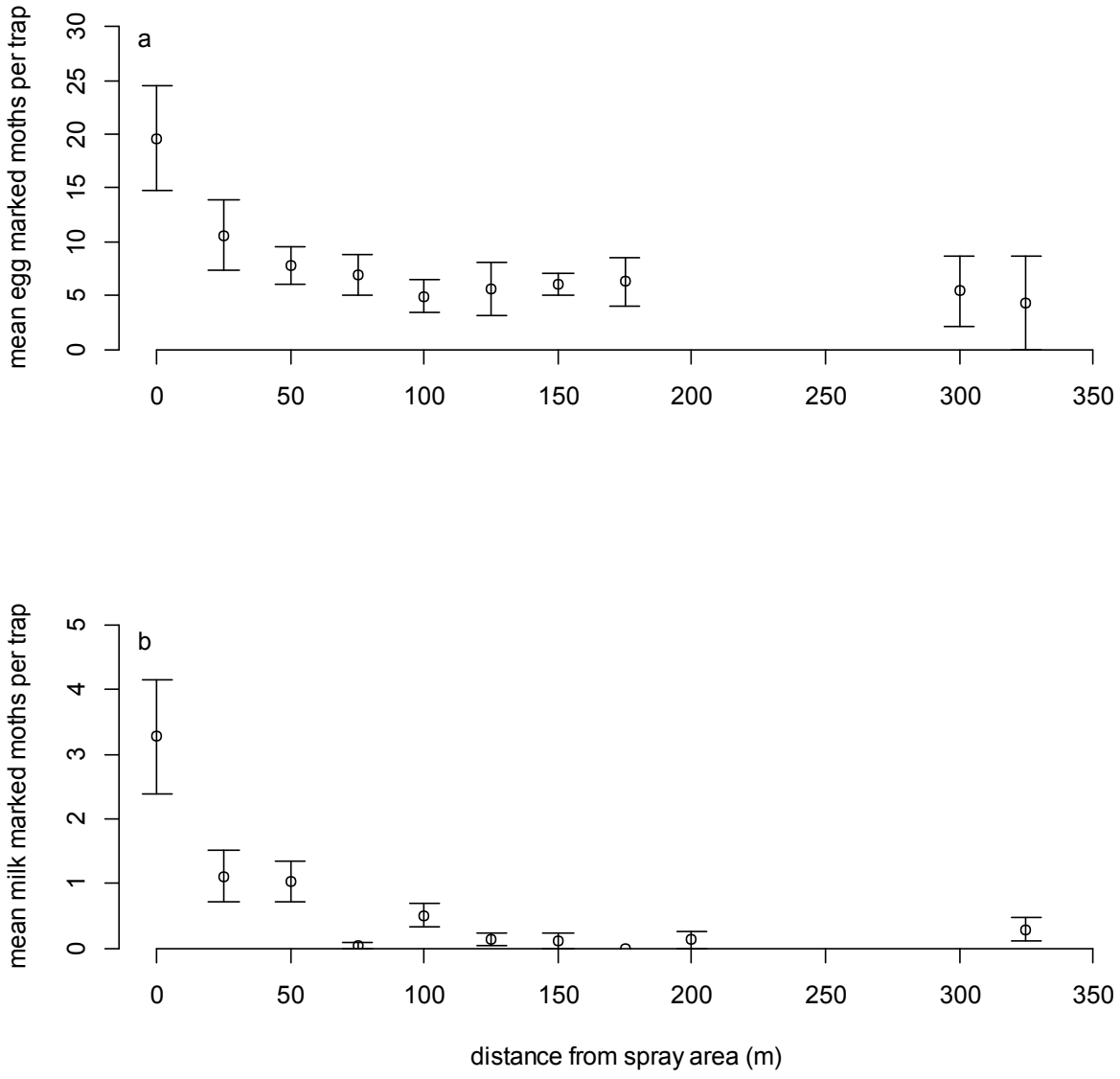


**Figure 2:** Mean (±SE) number of marked moths per trap measured using ELISA for a) egg protein and b) milk protein for traps placed at different distances from plots where protein markers were applied.

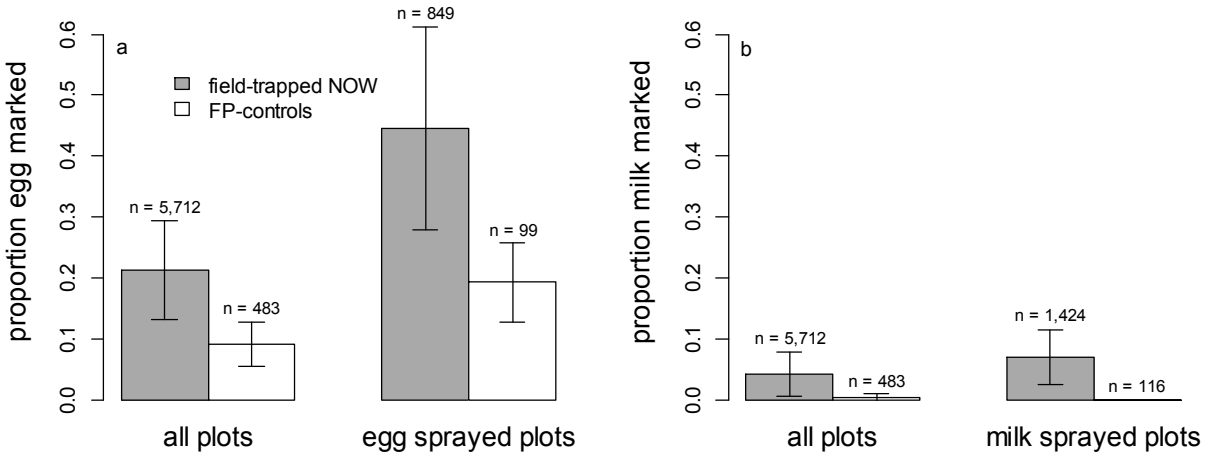


**Figure 3:** The mean (±SE) proportion of moths marked with a) egg protein and b) milk protein for wild caught NOW in orchard sites (gray), and FP-NOW placed in the orchard sites to control for field contamination (white).

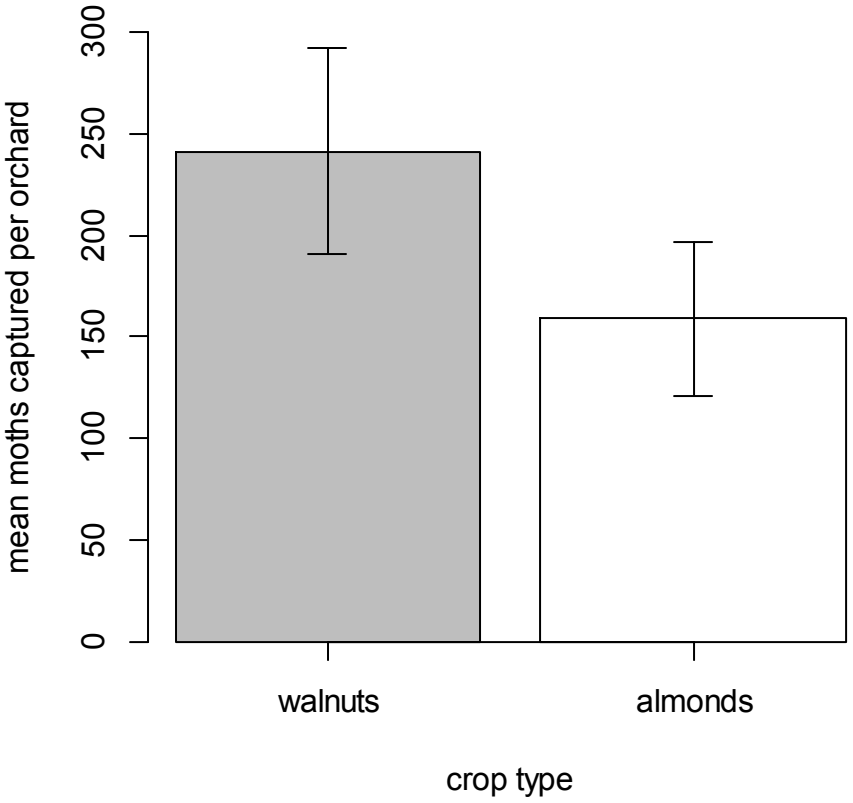


**Figure 4:** The mean (±SE) numbers of total moths caught in walnut (gray) and almond (white) orchards.

## Chapter 2

## You are what you eat: fatty acid profiles as a method to track the habitat movement of an insect


### Abstract

Tracking the movement of small organisms is of tremendous importance to understanding the ecology of populations, communities, and ecosystems. However, it remains one of the most difficult challenges facing the field of movement ecology. We developed an intrinsic marking technique for tracking small organisms using dietary fatty acid profiles as a biomarker as well as for clarifying source-sink dynamics between populations on a landscape level. Navel orangeworm moths (NOW), *Amyelois transitella* (Walker) (Lepidoptera: Pyralidae), raised on two different host plants with significantly different fatty acid profiles, were used to develop a model that distinguishes NOW based on their larval host plant. Wild NOW from both known and unknown host plants were used to validate the model. NOW fatty acid profiles showed striking similarities to the fatty acid profile of their host plant demonstrating that fatty acids can act as an intrinsic marking technique for quantifying the movement of small organisms. We anticipate that given sufficient spatial variation in dietary fatty acids this technique will be useful in studying the movement of arthropods and other invertebrates particularly when addressing questions of source-sink dynamics.

## Introduction

Organismal movement, defined as the movement of a whole organism through space, has wide ranging effects on populations, communities and ecosystems (Turchin 1998; Clobert et al. 2001; Kokko and Lopez 2006; Murakami et al. 2008; Clobert et al. 2012; Bonelli et al. 2013). Understanding the capabilities and motivations for animal movement, and the consequences of such movement are of great importance for ecology, in general, and in particular for pressing issues such as habitat loss and fragmentation (Debinski and Holt 2000; Fahrig 2007; Stevens and Baguette 2008; Soomers et al. 2013), climate change (Leroux et al. 2013), invasions (Gaither et al. 2013) and conservation planning (Hodgson et al. 2009; Rayfield et al. 2011). A movement ecology paradigm has been identified with the hope of creating a more comprehensive view of the role of organismal movement in ecology and evolution (Nathan et al. 2008). Within this paradigm, the tremendous diversity of organisms necessitates the development of a wide range of techniques for studying movement that accommodate differences in body size, spatial and temporal scales, mode of dispersal (e.g. passive versus active), and dispersal medium (e.g. air, water, etc.). Global positioning system technology has revolutionized the field of movement ecology for any animal that is large enough to carry a tracking device (Hebblewhite and Haydon 2010). However, tracking the movement of smaller organisms remains a much more difficult challenge (Lavandero et al. 2004; Cronin and Reeve 2005; Wikelski et al. 2007).

Current techniques for tracking the movement of small organisms fall broadly into two categories: extrinsic and intrinsic marking techniques (Rubenstein and Hobson 2004). Extrinsic marking techniques encompass the majority of marking tools currently used and involve any approach that places an identifiable mark on an organism. In contrast, intrinsic marking techniques, also known as self-marking techniques, use information that is already on or inside the organism to infer something about its movement. We set out to develop an intrinsic marking tool for tracking the movement of small organisms. The proposed method is based on the premise that different food sources contain a wide variety of different fatty acids (Maguire et al. 2004), and that through a process known as dietary routing (Blem 1976; Pond 1986), it is more efficient for consumers to retain the fatty acids from their food unchanged within their own body tissues (Stanley-Samuelson et al. 1988; Selvan et al. 1993; Dalsgaard et al. 2003; Chamberlain et al. 2005; Budge et al. 2007; Reuss and Chamberlain 2010; Haubert et al. 2011).

Lipids play a vital role in all organisms where they are used as a structural component of cell membranes and as a source of energy. The direct incorporation of dietary fatty acids into body tissues through dietary routing has enabled fatty acid profiles to be used in many studies of food web ecology (e.g. Iverson et al. 2004; Theimann et al. 2008), particularly in soil (Zelles et al. 1999, Ruess et al. 2002; Ruess et al. 2004; Chamberlain et al. 2005) and marine ecosystems (Ederington et al., 1995; Meziane et al. 1997; Müller-Navarra et al., 2000; Nelson 2001, Dalsgaard 2003; Daly et al. 2010). For fatty acids to be effective as intrinsic markers for food web studies Reuss and Chamberlain (2010) note that they must be subject to dietary routing with minor metabolic modification, unique to a specific food source, and have limited biosynthesis in consumer metabolism. These same constraints also apply to the application of fatty acids as intrinsic markers for the study of organismal movement.

The use of fatty acids as a marking technique has the advantage of eliminating the cost, time and labor of marking a large number of individuals, and does not run the risk of interfering with the movement of an organism. Thus it is highly desirable for field research particularly for organisms that are difficult to mark due to their size, short life span, or elusive nature. However,

like other intrinsic marking techniques, fatty acid profiles are limited by the opportunity of finding a profile that is indicative of movement. For example, tracking movement using stable isotopes requires natural isotopic variation in the environment which can be hard to find on small scales (Hobson 1999; West et al. 2006). We expect that the applicability of fatty acid markers will depend on the presence of sufficient spatial variation in dietary fatty acids, but that given such variation, it will provide an additional intrinsic marking technique that may prove very useful in answering previously difficult to answer questions regarding the habitat movement of arthropods.

To investigate this, we chose to use an invasive moth, *Amyelois transitella* (Walker), known as the navel orangeworm (NOW). NOW was first seen in California in 1942 and was widespread in California by 1943 (Wade 1961). The moth has a very diverse diet and is able to feed on a large variety of fruits and nuts (Wade 1961). NOW is relatively immobile in its larval stage and therefore each individual eats only one source of food over the course of its juvenile development. Since NOW larvae consume all of their fats in the larval stage, the adult NOW should bear the fatty acid profile of the larval host plant unless there is substantial endogenous synthesis of fatty acids in the larval, pupal, or adult stages. Using gas chromatography, we tested this hypothesis with laboratory and field collected NOW raised on known host plants. We then verified the technique on wild caught male NOW of unknown host plant origin.

## Material and Methods

*Host plant effect on fatty acid profiles of NOW in the laboratory*

NOW larvae were collected from infested walnuts in Fresno, CA in 2001. These NOW were reared on a meridic diet (Tebbets 1978) consisting of wheat bran, glycerol, honey, yeast, vitamins and water for 120 generations. Rearing procedures for the NOW colony involved placing 300 NOW eggs in a 3.8 liter jar with 750 g of diet. Jars were covered with a paper towel lid secured by a rubber band to allow ventilation, and maintained at 25°C with a photoperiod of 16L:8D h. Larvae hatching from the eggs consumed the diet and pupated in the diet before eclosing to mate and lay eggs inside the jar. The majority of the eggs were laid on the paper towel lid of the jar which was then removed to add eggs to the next jar. Each generation lasted for approximately one month, and the colony was maintained as discrete generation cohorts.

A total of 10 jars were set up for the laboratory-based host plant effect experiment with 500 g of either conventionally grown walnuts or almonds (Berkeley Bowl Marketplace, Berkeley, CA). Each jar received 200 NOW eggs from the colony and was covered with a paper towel. The NOW larvae were allowed to pupate in the jars and were then checked every 3 days to collect 20 newly emerged adults from each food source. Each adult NOW was placed in a 1.7 ml polypropylene tube (Danville Scientific, Metuchen, NJ, USA) with 450 µl $CHCl_3$ and ground with a plastic pestle (Fisher Scientific Co., Pittsburgh, PA). After thorough grinding, the material was made into a 2:1 $CHCl_3$:MeOH (v/v) solution by adding 900 µl of 1:1 $CHCl_3$:MeOH (v/v). In addition, 50 µl of 2% butylated hydroxytoluene (Sigma Chemical Co., St. Louis, MO, USA) in $CHCl_3$ was added to prevent the autoxidation of unsaturated fatty acids. The samples were stored at -20°C for further analysis. A set of 10 samples of 5g, taken from individual nuts of both host plants, were run through the same process to examine the differences in fatty acid profiles between the NOW males and their host plants.

*Host plant effect on fatty acid profiles of NOW in the field*

To confirm that NOW feeding on almonds and walnuts in the field produced similar fatty acid profiles to NOW raised in the laboratory, we collected nuts containing NOW larvae from the Central Valley of California from May through August of 2011. Infested nuts were collected from five different locations that included Patterson, Escalon, Hughson, Modesto and Riverbank. Nuts were placed in 3.8L glass jars covered with paper towels and checked daily for emerging adult moths. A set of 25 adult NOW from almond and 18 from walnut were recovered from the jars. As with the laboratory reared NOW, these adults were ground individually and placed in a 2:1 $CHCl_3$:MeOH (v/v) solution with 50µL of 2% butylated hydroxytoluene and stored at -20°C for further analysis.

*Testing the fatty acid profiles of NOW from the field with unknown host plant origin*

For proof of concept of this method as an effective technique for tracking insects, we collected adult male NOW of unknown host plant origin from Oakdale, California, in white plastic delta traps with sticky liners (Suterra, Bend, OR, USA). Three virgin females that would release sex pheromone were enclosed in a 1 inch square fiberglass mesh cage as the lure in each trap. A set of 16 traps were placed in the field on 30-Aug-2011 and left for 24 h. Male moths were individually removed from the traps and ground in a 2:1 $CHCl_3$:MeOH (v/v) solution with 50 µl of 2% butylated hydroxytoluene before storage at -20°C for further analysis. The glue from the sticky liner used to trap the male NOW was run on the gas chromatograph, as described below, and was shown to contain no detectable amounts of fatty acids.

*Lipid extractions and analysis of fatty acid methyl esters*

Total lipids were extracted from NOW and nuts using the method of Bligh and Dyer (1959). Previously frozen samples were extracted with 0.75 ml of 2:1 $CHCl_3$:MeOH (v/v) for 1h at room temperature. Following brief centrifugation to settle insoluble material the supernatant was transferred to a clean microvial and phases separated by the addition of 0.75 ml of 1M NaCl. The samples were centrifuged at 10,000x G for 10 min and approximately 400µl of the lower organic layer were placed in a new tube, taking special care to exclude the aqueous layer from the transfer. The transferred lipid extracts were dried under a stream of nitrogen gas and resolubilized with 200µl $CHCl_3$. Fatty acid methyl esters (FAMEs) were generated from the free fatty acids and complex lipids by adding 200µl of 3N HCl in methanol (Sigma Chemical Co., St. Louis, MO, USA) and incubating at 55°C for 1h. The resulting FAMEs were recovered by adding 200 µl water, 300 µl hexane, and 100µl $CHCl_3$, mixing thoroughly and centrifuging for 5 min at 7,000x G. Approximately 200 µl of the bottom organic layer were transferred to a glass gas chromatograph vial (Fisher Scientific Co., Pittsburgh, PA) and dried down under nitrogen. The FAMEs were re-suspended in 1ml heptane, capped tightly, and stored at -20°C until analysis.

FAMEs were analyzed on an Agilent 6890N gas chromatograph/mass spectrometer (Agilent 5073) equipped with a flame ionization detector (FID), DB-225 capillary column (50% cyanopropylphenyl-dimethypolysilozane, 0.25 mm x 30 m x 250 µm film thickness, Agilent) and an automatic injector. The temperature program ran at 40°C/ min from 110 to 220°C after an initial 2 min hold. After initial confirmation of the identities of peaks produced from FAME

standards (Restek, Bellafonte, PA, USA) and selected NOW samples, quantitative analyses of FAMEs from samples was based on identification of peaks in comparison to retention times of the standards. Baseline separation of *trans*-9 18:1 (methyl elaidate) and *cis*-9 18:1 (methyl oleate) was not achieved. For simplicity we refer to the compounds in our samples coeluting with the cis-9 18:1 standard as "oleate" although other isomers may be present.

*Statistical analysis*

Two-way ANOVAs were used to compare the composition of the FAMEs between the host plants and NOW adults that had been raised as larvae on the host plants. All FAMEs were converted to a proportion of total fatty acids and square root transformed to meet the assumption of homogeneity of variance. Tukey's test was used to examine pairwise differences in the proportional composition of the FAMEs for both nuts and NOW adults from the two host plants.

The square root transformed FAME profiles were subsequently analyzed by linear discriminate analysis (LDA) (Crawley 2007) using the R software (version 2.15.1) function lda in the MASS package. To avoid collinearity between fatty acids in the LDA, stearate was removed from the analysis because it had a high degree of correlation with myristate. The 40 adults from the host plant effect on fatty acid profiles of NOW in the laboratory experiment were used as a training dataset to estimate a basic LDA model that could identify moths based on their larval host plant. The 43 field collected moths from known host plant served as a validation dataset to test the accuracy of the basic LDA model. To further estimate error in our model, all 83 NOW from known host plants were resampled using a jackknifing approach in which one half of the individuals were selected at random to create a model, while the remaining half were used to validate the model. This process was run 10,000 times and an average error rate was calculated (Tukey 1958).

A full LDA model was then created using all 83 moths of known host plant origin from the laboratory and field samples. Equal prior probabilities were assigned to each host plant. The full model was applied to the field collected NOW of unknown host plant origin. A posterior was produced for each NOW giving the probability of assignment to a particular host plant. In order to reduce the number of false positives, we removed any moths that had a posterior of less than 0.999 from the final analysis on field collect moths of unknown origin.

## Results

*Host plant effect on fatty acid profiles of NOW in the laboratory*

A total of 40 NOW adults (20 raised on almonds and 20 raised on walnuts), and 20 nuts (10 almonds and 10 walnuts) were analyzed from the host plant effect experiment in the laboratory. Example GC/FID FAME profiles for NOW adults raised on the two host plants are presented in Figure S1 (Online Resource 1). FAMEs that occurred at very low levels or that were not found consistently across samples were excluded from further analysis. There were seven different FAMEs consistently found in both the moths and the nuts from the two host plants: myristate, palmitate, palmitoleate, stearate, oleate, linoleate, and α-linolenate (Fig. 1). Overall FAME profiles of nuts from the host plants were significantly different from each other (ANOVA, $F_{1,6} = 54.82$, $P < 0.001$, Fig. 1a). In addition, Tukey's test identified significant pairwise differences between almonds and walnuts for three FAMEs: oleate, linoleate, and alpha-linolenate (Fig. 1a). The FAME profiles of the NOW adults showed the exact same pattern with

significant differences between NOW adults raised on almonds versus walnuts (ANOVA, $F_{1,6}$ =24.43, $P < 0.001$), and significant pairwise differences between the same three FAMEs (Fig. 1b).

The FAME profiles of the 40 NOW adults reared in the laboratory on nuts from known host plant were used as a training dataset to create a basic LDA model, excluding stearate due to high collinearity with myristate (Fig. 2a). The LDA model showed a clear separation of the adult moths reared as larvae on almonds and walnuts.

*Host plant effect on fatty acid profiles of NOW in the field*

A total of 43 NOW adults, 25 from almonds and 18 from walnuts, were successfully reared from the field collected nuts. The same seven FAMEs observed in NOW raised in the laboratory on walnuts or almonds were consistently found in all 43 field samples. There were significant differences between almond and walnut fed moths (ANOVA, $F_{1,6} = 24.43$, $P < 0.001$). Tukey's test showed significant pairwise differences in 3 FAMEs: oleate, linoleate and α--linolenate ($P < 0.001$).

The data from the 43 NOW adults of known host plant origin from the field were used as a validation dataset (after exclusion of stearate) for the basic LDA model developed from the laboratory reared moths. The model correctly identified the host plant of all 43 samples from the validation dataset with zero percent error (Fig. 2b). To estimate an actual error rate, we ran 10,000 jackknife resampling runs with randomly selected training and validation datasets from the combined set of 83 NOW adults from known host plants (laboratory reared plus field collected). We obtained an estimated error rate of 0.0036%. A full model was then constructed using all 83 moths from the known host plant experiments (again excluding stearate) to use for predicting the larval host plant of field-collected adult NOW (Fig. 3).

*Testing the fatty acid profiles of NOW from the field with unknown host plant origin*

We collected a total of 668 adult male NOW over a 24 h period from the 16 virgin female-baited traps. The full LDA model, based on the 83 adult NOW from known larval host plants, indicated that 432 field-collected male NOW were of almond origin, while 236 were of walnut origin (Fig. 4a). To address the concern that age, metabolism or diet quality may have altered the fatty acid profiles of field caught moths and causing misclassification, we used a highly conservative approach and removed any NOW that had a posterior of less than 0.999. A total of 23 NOW were therefore removed from the analysis to reduce misclassifications (Fig. 4b). The majority, 79%, of the male NOW were classified as having developed as larvae on the same host plant as was present in the orchard in which they were captured (Fig. 5). However, given that 21% of the male moths collected in each orchard did not originate from that host plant it is clear that movement of male moths between orchards was occurring.

## Discussion

The results demonstrate that dietary routing is indeed playing a major role in determining the fatty acid composition of NOW and that variation in host plant fatty acid profiles can be preserved at a higher trophic level and create similar significant variation in an insect herbivore. The LDA model showed a zero percent error rate in predicting the diet between adult NOW that

had eaten almonds compared to those that had eaten walnuts during their larval development. This model when applied to unknown field data gave a clear bimodal distribution suggesting that dietary routing is the overriding factor determining the fatty acid profiles of NOW males. Captured moths tended to have a fatty acid profile that matched the food source in the orchard in which they were captured. However, we also demonstrated that considerable movement occurred between different cropping systems, confirming that the movement of NOW adults does indeed play a significant role in this orchard ecosystem.

We saw more variation in fatty acid profiles from field collected NOW from unknown host plants than from laboratory raised NOW. It is likely that the error rate from the virgin female-baited traps in the field was slightly higher than the estimated 0.0036% obtained from the laboratory rearing. The fatty acid profile of a field-collected moth is likely to be influenced by a variety of factors including age; temperature; larval competition and parasitism by affecting acquisition of fatty acids from the host plant; and host plant genotype and phenotype. The extremely low error rate verifies that these factors are not playing a significant role in determining host plant origin. Since NOW do not feed on fatty acids as adults, if moths preferentially metabolize certain fatty acids as adults, the overall fatty acid profile could change with age. For other insects, however, starvation appears to have minimal effect on the fatty acid profile (Canavoso et al. 1998; Haubert et al. 2004), with similar results found for krill (Stübing et al. 2003). Thus age should not have had a significant influence on the fatty acid profiles of the NOW adults collected from unknown host plants.

Although NOW is a generalist and is known to feed on a wide range of plants (Wade 1961), there were no other known host plants within a 16 km radius of the field site. It is possible, however, that a few of the NOW males that were collected had developed on an alternative diet and so it is not surprising that our field data showed a wider range of fatty acid profiles than we found among the individuals from known host plants. To account for these potential unknown influences on fatty acid profiles we took a very conservative approach and excluded from our analysis any NOW that had a posterior probability of assignment of less than 0.999. The strong bimodal distribution combined with very few moths being excluded with our conservative approach make it clear that the majority of field-captured NOW had developed as larvae on either walnuts or almonds, and that the host plant was the primary factor affecting the fatty acid profiles of the adults tested.

There are some drawbacks related to the use of fatty acid profiles as a tool for the analysis of organismal movement. As seen in Fig. 5, while the larval host plant can be identified for the NOW adults and thus habitat movement can readily be tracked; it is not possible to directly track the movement of individuals as the exact location of origin of the trapped adults remains unknown. However, probabilistic models when applied to this type of data should allow for very accurate predictions of average movement patterns for the species. The successful application of this methodology to other small invertebrates will depend on distinct fatty acid profiles in the food sources of interest combined with significant landscape-specific variation in those food sources. If the variation in fatty acids is not tied to the landscape, fatty acid profiles will reveal no information on habitat movement. Stable isotopes show similar limitations when used as an intrinsic marker, but nonetheless have proved to be an invaluable technique for tracking organismal movement (Rubenstein and Hobson 2004; West et al. 2006). Thus fatty acids and potentially other intrinsic markers, such as waste products (Mitlin and Vickers 1964) and n-alkanes (Mayes et al. 1986), can serve as valuable trophic biomarkers and contribute to the study of organismal movement.

Fatty acid profiles have been shown to be extremely useful biomarkers for the study of trophic interactions particularly in soil and marine systems (e.g. Chamberlain et al. 2005; Ruess & Chamberlain 2010; Haubert et al. 2011; Kelly et al. 2012). However, we would argue that this technique is largely underutilized in insect ecology. In general, insects get the majority of their fats in the larval stage, thus giving them a high potential for unique fatty acid profiles. In addition, many species of parasitoid have lost the ability to synthesize their own lipids (Visser et al. 2010), thus increasing the likelihood that these profiles can be detected through multiple trophic levels. The data presented here strongly suggest that larval NOW exhibit dietary routing with low rates of endogenous synthesis of fatty acids and, indeed, "are what they eat" with respect to fatty acid profiles. Fatty acid profiles as intrinsic markers have considerable potential for use in the field and may greatly aid in our ability to monitor the movement of arthropods with distinct fatty acid profiles.

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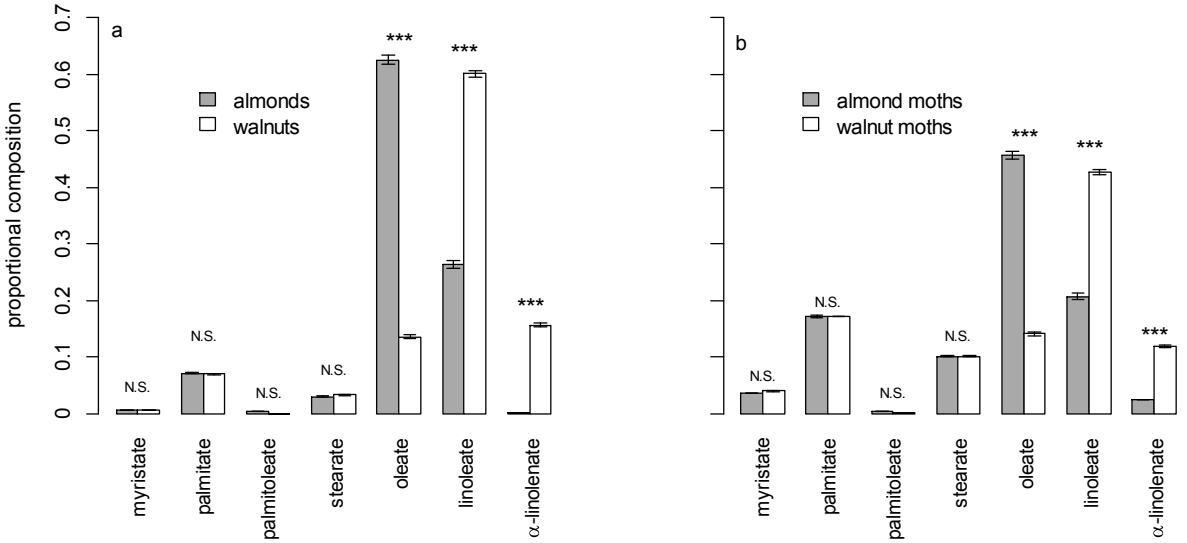


**Figure 1:** Fatty acid profiles from the laboratory showing (a) mean (±SE) proportional composition of FAMEs from the nuts of two host plants, almonds and walnuts ($n$ = 10), and (b) mean (±SE) FAME proportional composition of FAMEs from *Amyelois transitella* (NOW) adults reared on nuts from known host plants (n = 20). *** $P < 0.001$, N.S. not significant for host plant effect (Tukey test).

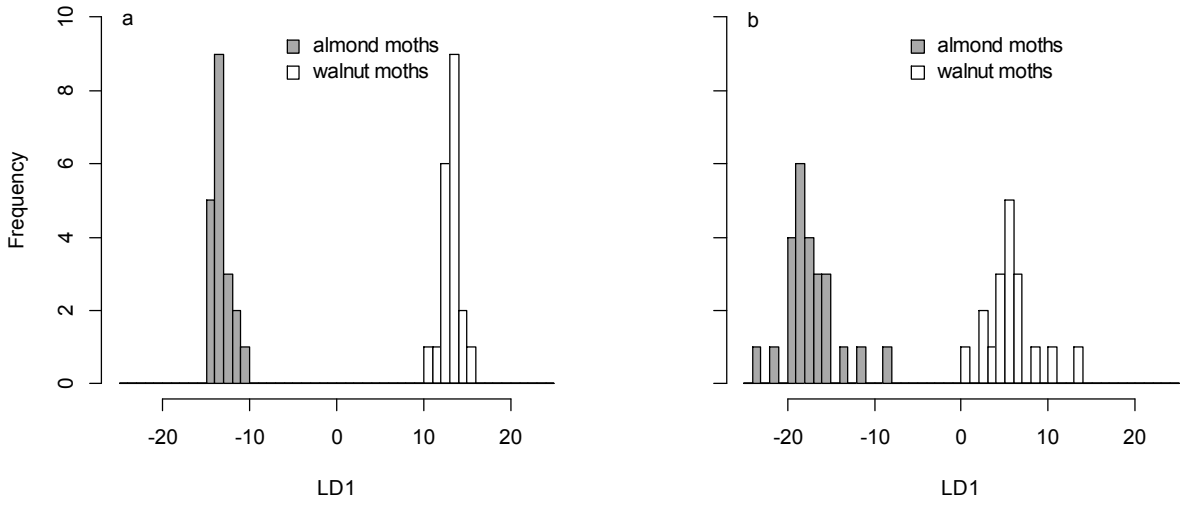


**Figure 2:** FAME profiles for NOW along the first linear discriminant axis (LD1) of the LDA model (a) estimated from the training data set of adults reared as larvae on nuts of the two host plants in the laboratory, and (b) based on the validation data set of adults reared as larvae on collected nuts from known host plants in the field.

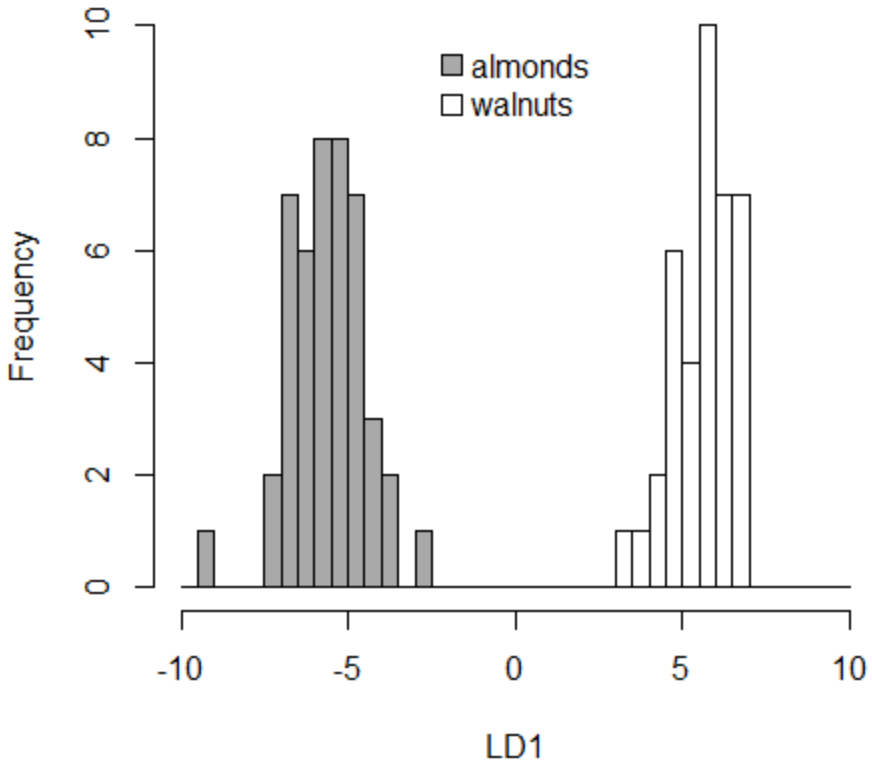


**Figure 3:** FAME profiles for NOW adults along the first linear discriminant axis (LD1) of the full LDA model for the combined laboratory and field samples from known host plants. All almond individuals had an LD1 of less than zero, while all walnut individuals had an LD1 of more than zero.

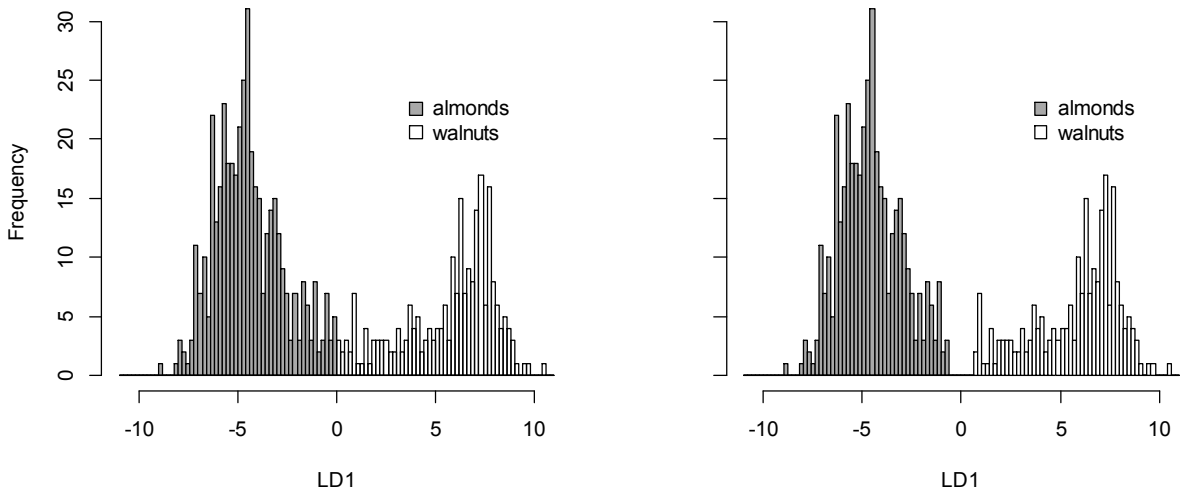


**Figure 4:** FAME profiles of adult male NOW along the first linear discriminant axis (LD1) of the full LDA model for individuals of unknown host plant origin collected in virgin female-baited traps, showing (a) all trapped males ($n$ = 668) and (b) all trapped males excluding 23 individuals with a posterior of less than 0.999 ($n$ = 645).

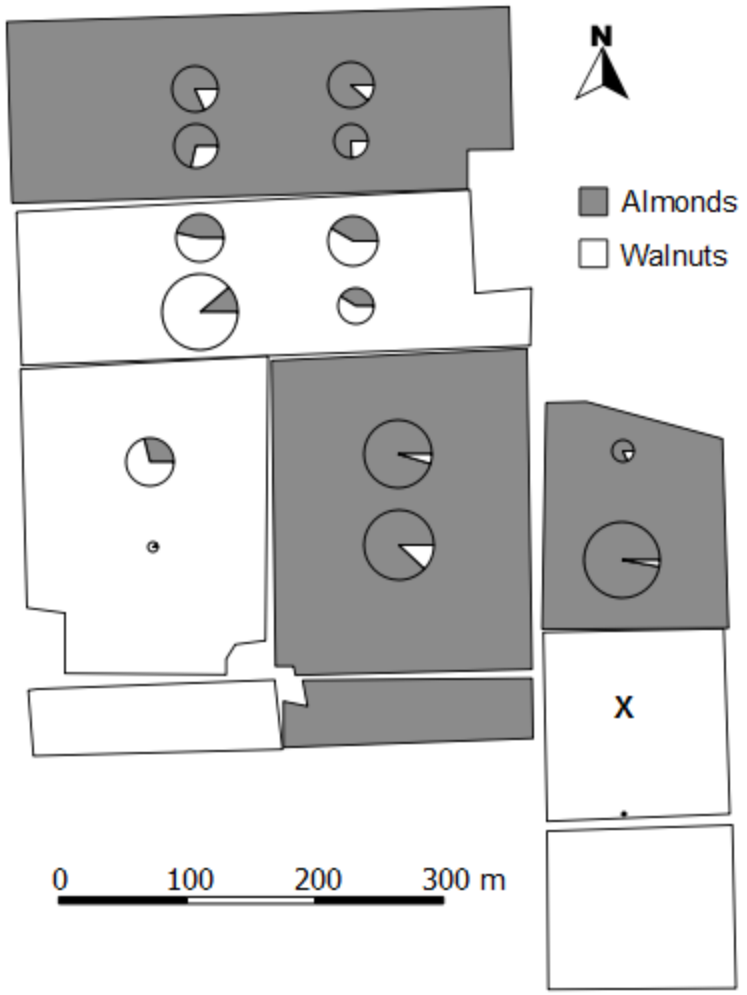


**Figure 5:** A map showing the movement of NOW across the landscape. The blocks represent almond and walnut orchards. Each pie chart represents a virgin female-baited trap, where the size of the pie chart corresponds to the number of NOW caught in the trap and the shading correspond to the FAME profile assignment of the moths in each trap. The "X" marks a trap with no NOW caught.

## Chapter 3

## Estimating the dispersal of an intrinsically marked insect from known larval food sources: application to movement of navel orangeworm


### Abstract

Intrinsic marking techniques can be extremely effective tools for studying animal movement. They avoid many of the drawbacks of applied external markers, but as exact movement paths cannot be determined, they typically require high levels of spatial variation in marker signal across the landscape and have been utilized most frequently for detecting large scale migration events. In order to measure more localized movement from unknown area sources, we developed a Gaussian-based dispersal model as a tool to analyze movement patterns using data from intrinsic marking techniques. This model was applied to field-caught navel orangeworm moths (*Amyelois transitella*, NOW) intrinsically marked with unique fatty acid signatures that, through dietary routing, are characteristic of their larval food plants. A number of NOW traps baited with virgin females were placed at 3 sites in the central valley of California. The application of the model worked well for all three sites, with average movement distance estimated to be approximately 50 m per generation for two of the sites, and approximately 600 m per generation for the third site. NOW showed different patterns of movement based on crop origin for the third site, suggesting that the model assumption of movement by random diffusion may have been violated. Implications of this anomaly are discussed. Probability-based dispersal models provide a valuable approach to analyzing movement data from intrinsic markers and can be readily adapted to a variety of dispersal kernels.

## Introduction

Spatial processes play a key role in ecology. They shape population dynamics and species distributions, as well as being a major factor driving the evolution of populations (Colbert et al. 2001, 2012; Bullock et al. 2002; Bowler and Benton 2005; Kokko and Lopez 2013). With increasing habitat fragmentation (Lindenmayer et al. 2012) and changes in climatic conditions (Rosenzweig et al. 2008), the study of spatial processes and movement ecology have never been more critical, and will be vital to protecting and promoting biodiversity, mitigating species invasions, and effectively managing ecosystems. While movement is important to all organisms, and has been the subject of numerous publications, the motivations and capabilities of movement for most species are still not well understood (Holyoak et al. 2008). Insects, in particular, remain a difficult group to study due to their small size, short lifespan and large capacity for movement (Cronin and Reeve 2005).

New methodologies are leading the way in the study of insect movement. Intrinsic or self-marking techniques, such as stable isotopes (e.g. Gratton et al. 2008; Schallhart 2009), pollen (Silberbauer et al. 2004), and fatty acids (Chapter 2), are proving to be effective tools for measuring insect movement on a large scale, without many of the costs and complications involved with marking individuals. However, intrinsic marking techniques produce datasets where the precise starting location of an individual is often unclear. Instead of analyzing an exact movement path, these techniques rely on sufficient spatial variation of a marker across the landscape to detect general movement patterns (e.g., Sellick et al. 2009). As a result, intrinsic marking techniques are not suitable for all situations, and have been used more frequently to study large scale movement events (Rubenstein and Hobson 2004; West et al. 2006; Gratton et al. 2008) than to monitor more localized patterns of movement.

Our first objective in this study was to develop an approach to the estimation of movement from datasets derived using intrinsic marking techniques, and secondly, to apply the approach to movement of navel orangeworm (*Amyelois transitella*) (NOW) across a more localized agricultural landscape using fatty acid signatures as intrinsic markers (Chapter 2). In California NOW is a pest of almonds and walnuts, which are larval food sources that carry unique fatty acid signatures (Maguire et al. 2004). Through dietary routing (Blem 1976; Pond 1981), NOW adults retain the fatty acid signature of their larval food plant (Chapter 2). Thus fatty acid signatures can be used to elucidate patterns of intercrop movement by NOW, but a lack of information on starting location and exact movement paths makes the estimation of dispersal rates much more difficult.

In an attempt to estimate dispersal rates using data representing patterns of movement determined from use of intrinsic markers, we developed a probabilistic dispersal model for estimating the average linear displacement of individuals from known habitat sources. We then applied the model to our intrinsic marker dataset to predict the most probable dispersal rates of NOW. Our model assumes a constant rate of movement over the course of adult life. This assumption ignores many of the factors known to influence individual movement such as resource competition, inbreeding avoidance, or kin competition (Clobert et al. 2001). Nonetheless, the analysis provides useful insights into the population-level movement of NOW, and can easily be adapted to other intrinsic marking techniques and to assumptions of other probability distributions for movement.

**Dispersal Model for Intrinsic Marker Datasets**

While models with leptokurtic distributions are thought to provide a better representation of dispersal in natural populations (Kot et al. 1996; Chapman et al. 2007) they can be sensitive to the particular functions used (Kot et al. 1996) and often include additional parameters that can be difficult to estimate from field data. For simplicity, we opted here to use a model based on a Gaussian probability distribution, closely approximating movement via a random walk (Turchin 1998), because it requires fewer parameters and provides a good baseline for examining movement. Assuming movement by random diffusion (Turchin 1998), then *N* marked individuals released at a central point at time 0 would have a spatio-temporal density distribution at time *t* that is described by:

$$\mu(x, y, t) = \frac{N}{4\pi Dt} \; e^{-\frac{x^2+y^2}{4Dt}}$$

where *x* and *y* are orthogonal distances from a central release point, *D* = diffusion rate, and *t* = time since release (Fig. 1). When using intrinsic rather than extrinsic marking techniques to study movement there are likely to be multiple release points in the landscape acting as sources of the marker, rather than one central release point, and the time since release is represented by the age of the captured individuals. We are interested in estimating the diffusion rate (*D*), but are not able to measure the age of the captured individuals (*t*), so we combined these two variables into a movement coefficient (*L*) representing the average distance moved by an individual between its source location and capture location:

$$L = \sqrt{Dt}$$

Thus the movement coefficient is an estimate of the average linear displacement between a source location and the point of death resulting from capture. The average linear displacement is not a measure of individual movement capabilities, but rather a measure of the diffusion distance of a population at mean age of capture. Actual pathways of individual movement during a species' lifetime are not constrained to be linear and so would be considerably larger than the movement coefficient.

We are interested in the most probable value of the movement coefficient for a particular species given an observed distribution of marked individuals captured at specific locations in the landscape. The landscape is defined by a set radius around each capture location. To estimate the movement coefficient, first we need to calculate the probability of finding a marked individual at a particular capture location ($\gamma$), for a specific landscape ($\theta$) and movement coefficient (*L*):

$$P(\text{capture} \mid L, \theta, \gamma) = \iint \frac{1}{4\pi L^2} \; e^{-\frac{x^2+y^2}{4L^2}} \, dxdy$$

This equation specifies the area under the probability distribution curve for finding a marked individual at a particular capture location as influenced by the movement coefficient of the population and the distances between all potential source locations in the landscape and that

particular capture location. The probability of finding multiple marked individuals (*n*) at the same capture location is simply the product of the probability of each individual capture:

$$P(\text{all captures } |L, \theta, \gamma) = \prod_{1}^{n} P(\text{capture } |L, \theta, \gamma)$$

The product of these probabilities for all individuals captured across the complete set of capture locations (*m*) deployed in the landscape then generates a probability for a specific movement coefficient for the entire population of marked individuals:

$$P(\text{all captures } |L, \theta) = \prod_{1}^{m} P(\text{all captures } |L, \theta, \gamma)$$

By repeating these calculations for all possible values of the movement coefficient we can then examine which value has the highest probability for the entire population of marked individuals. It is important to note that in practice, the total numbers of individuals captured will vary considerably between species, landscapes and number of capture locations, and the combined area of source locations within a landscape can also be very different. While both will influence the absolute probabilities of the movement coefficients, neither will change the relative probabilities or the peak likelihood of the movement coefficient.

## Materials and Methods

*Trap catch of NOW in the field*

To obtain landscape level data on movement of NOW in the field, collection of adult male moths was accomplished following the methods of Chapter 2. Male NOW of unknown food plant origin were trapped in white plastic delta traps with sticky liners (Suterra, Bend, OR, USA). As a lure, three laboratory-reared virgin females were placed inside a 2.5cm square fiberglass mesh cage inside each trap. A total of 40 traps were deployed on 30-Aug-2011 in walnut and almond orchards at three sites in the Central Valley of California, Escalon, Oakdale, and Patterson. Each site had both walnut and almond orchards and traps were placed in both orchard types. The Escalon site had 13 traps, the Oakdale site had 16 traps and the Patterson site had 11 traps (Fig. 2). Late August is just before almond harvest and coincides with the period of increased NOW movement in both crops (Chapter 2). Traps were placed in the orchards between 10:00am and 2:00pm and hung at a height of 2m in the lower canopy of trees separated by at least 40m. The virgin female-baited traps were left in the orchards for 24 h, and then trap liners were removed, placed in a cooler, and returned to the laboratory. Male moths were removed from the liners and individually ground in a 2:1 $CHCl_3$:MeOH (v/v) solution with 50 µl of 2% butylated hydroxytoluene before storage at -20°C for further analysis.

*Fatty Acid Analysis*

Fatty acid analysis was conducted following the methods of Chapter 2. Total lipids were extracted from individual NOW males using the method of Bligh and Dyer (1959). Samples were extracted in the storage solution of 2:1 MeOH (v/v) followed by brief centrifugation to settle insoluble material. Phases were separated and the organic layer was transferred to a new microvial, dried under a stream of nitrogen gas, and resolubilized with $CHCl_3$. We then added 3N HCl in methanol (Sigma Chemical Co., St. Louis, MO, USA) and incubated to generate fatty acid methyl esters (FAMEs) from the free fatty acids and complex lipids. The FAMEs were recovered and dried under nitrogen. The FAMEs were re-suspended in heptane and stored at -20°C for further analysis.

FAMEs were analyzed on an Agilent 6890N gas chromatograph/mass spectrometer (Agilent 5073) equipped with a flame ionization detector (FID), DB-225 capillary column and an automatic injector. After initial confirmation of peak identities of FAME standards (Restek, Bellafonte, PA, USA) and of selected NOW samples, quantitation was carried out by FID. FAMEs were identified during quantitative analyses by comparing retention times to those of the standards.

FAME profiles were converted to a proportion of total fatty acids and square root transformed to meet the assumption of homogeneity of variance. Transformed FAME profiles were analyzed by linear discriminate analysis (LDA) (Crawley 2013) using the R project software (version 2.15.1) function lda in the MASS package. Stearate was removed from the analysis due to a high degree of correlation with myristate. The lda model developed in Chapter 2 to classify moths as having fed as larvae on almond or walnut was used to identify the larval food source of each captured moth. Individuals originating from almond orchards are referred to as almond moths and those originating from walnut are referred to as walnut moths. A posterior was produced for each NOW individual giving the probability of assignment to a particular host plant. We removed any moths that had a posterior of less than 0.999 from the final analysis to reduce chances of false positives.

*Data analysis*

Using the R project software (version 2.15.1) we used a generalized linear model to analyze the number of male NOW caught with larval food source (almond or walnut), crop type (almond or walnut) and site as factors. Numbers of male NOW caught were converted to z-scores to standardize for differences in abundance between sites.

*Application of the dispersal model to NOW movement*

The probabilistic dispersal model was used to combine the captured moths at each field site and the distances of each trap from all potential larval food sources of those moths classified as originating from either almonds or walnuts. The two markers allowed us to estimate the most probable movement coefficient for the NOW population based on a combination of the two distinct larval food sources in the landscape. Barring a perfectly symmetrical landscape, the probability of a walnut moth capture will differ from the probability of an almond moth capture for any given trap. Taking the product of these probabilities across almond and walnut moth captures for the complete set of traps gives us the best overall probability of any given value for the movement coefficient ($L$):

$$P(\text{all captures } |L, \theta) = \prod P(\text{walnut moth}) * \prod P(\text{almond moth})$$

For each of the three sites separately, we estimated the probabilities for the entire population of marked moths for all values of *L* between 1 and 10,000 m. The highest probability for the observed field captures at each site then corresponds to the most probable movement coefficient for NOW.

To estimate the distances between traps and larval food sources, accurate maps of the distribution of almond and walnut orchards within a 10 km radius of each of the three field sites were obtained from agricultural land use data for 2011 from Stanislaus County Department of Weights and Measures (http://www.stanag.org/weights-measures.shtm) and the San Joaquin County Agricultural Commissioner's Office (http://www.sjgov.org/agcomm). We used the software package Quantum GIS (1.8.0) to manipulate and summarize the data layers. The land use data used to describe the landscape (θ) was ground truthed for an 8 km radius around each of the field sites confirming that there were no discrepancies between the GIS layers and actual land use coverage. The positions of each trap were added to the land use maps, and the polygons and trap locations were exported to R software (version 2.15.1) for analysis. Integrations were carried out using the R software function polyCub in the polyCub package (Meyer and Held, 2014). A logarithmic format was used for all probabilities (and thus sums rather than products for combining the entire population of marked moths), due to the limitation of the R software in handling very small numbers.

The potential range of values for the movement coefficient is heavily influenced by the landscape and the scale of trap placement. For example, if there are numerous nearby sources of one particular food plant, the chances of identifying long-range dispersal of moths developing as larvae on another food plant becomes very unlikely because the model weights short distance movement much more heavily. In other words, since it is more probable that a trapped insect would have come from nearby food sources than from distant food sources, the model would underestimate the actual movement of any individuals that arrive at traps from distant locations. To better understand our estimates of the movement coefficient for NOW, we need to place them into the context of the possible range of values (maxima and minima) that the model can detect for a particular landscape.

The maximum measureable movement coefficient was calculated for each of the three field sites in the Central Valley from the most probable value for the movement coefficient, assuming that one male moth from an almond source was caught in every trap located in a walnut orchard, and that one male moth from a walnut source was caught in every trap located in an almond orchard. This maximum represents the extreme scenario where all moths leave the locations of their larval food plants and use longer distance dispersal to seek locations that support an alternative food plant. The measureable minimum was calculated by taking the most probable value for the movement coefficient, assuming that one male moth from an almond source was caught in every trap located in an almond orchard, and that one male moth from a walnut source was caught in every trap located in a walnut orchard. This minimum represents the extreme where all moths are restricted in their movement such that they do not move out of locations supporting their larval food plant. Thus any estimated value of the movement coefficient that falls close to one of these two extremes would indicate that the true movement coefficient value was either higher or lower than could be measured by the diffusion model for a particular landscape. In addition, a "complete mixing" scenario was calculated by assuming that

one male moth from each food source was caught in each trap irrespective of whether the trap was located in an almond or walnut orchard. Complete mixing represents an intermediate level of movement where moths move freely across the landscape irrespective of the food plants present.

## Results

*Trap catch of NOW and fatty acid analysis*

A total of 1195 male NOW were collected from the three sites in the Central Valley, of which 599 were classified as almond moths, 546 were classified as walnut moths, and 50 individuals could not be classified by the linear discriminant analysis model for fatty acid signatures due to a posterior of less than 0.999. These 50 moths were excluded from further analysis. We observed bidirectional movement of NOW between walnut and almond blocks at Oakdale and Patterson, but only unidirectional movement of NOW from almonds to walnuts at Escalon (Fig. 2). Nonetheless, NOW movement clearly occurred at the scale represented by the distance between traps at all three sites. Although we caught almost equal numbers of almond and walnut moths overall, the relative abundance of the two different sources of moths varied between crops and between sites (Fig. 3). There were significant interactions between larval food source and crop type, showing that moths were more likely to be caught in their crop of origin (Table 1). There was also a significant interaction between larval food source and site, showing that Patterson had more walnut moths than almond moths, and Escalon and Oakdale had more almond moths than walnut moths. Finally, there was a significant interaction between crop type and site, showing that Patterson and Escalon had more moths captured in walnut orchards while Oakdale had more moths captured in almond orchards.

*Application of the diffusion model to NOW movement*

There was a clear peak for the most probable value of the movement coefficient ($L$) for NOW in the landscapes at all three sites (Fig. 4). The estimated value was very similar for two of the sites, 44 m at Oakdale and 69 m at Patterson, but was much greater at the third site, 594 m at Escalon. These estimates fell within the boundaries of our measureable maxima and minima for all three sites (Fig. 5a). Those for the Oakdale and Patterson sites fell between our measureable minima and the complete mixing scenarios for these landscapes. However, the estimate for the Escalon site fell beyond the complete mixing scenario and approached the maximum measureable value for the landscape.

To further investigate the movement coefficients we developed separate estimates for almond and walnut moths (Fig. 5b). The estimates were different for moths from the two different larval food sources at Escalon ($L$ = 14 m for walnut moths, $L$ = 852 m for almond moths) and both were at the measureable limits for the landscape at that site. In contrast, the separate estimates for almond and walnut moths were very similar for Oakdale ($L$ = 48 m for walnut moths, $L$ = 41 m for almond moths), and Patterson ($L$ = 72 m for walnut moths, $L$= 38 m for almond moths).

## Discussion

In this study we have been able to demonstrate that NOW consistently moved between walnut and almond orchards in California. In addition, a Gaussian dispersal model was used to estimate the average linear displacement of NOW from known larval food plant sources, as detected by intrinsic fatty acid signatures, in the form of a movement coefficient (*L*). The dispersal model provided clear peaks for the most probable outcomes of the movement coefficient for NOW at all three orchard sites. In two of these landscapes the movement coefficient was estimated to be around 50 m per generation, while a third landscape suggested a much larger movement coefficient of 594 m per generation. This larger estimate was driven entirely by the movement of almond moths with little to no movement of walnut moths. The practical significance of such extensive movement of male NOW can be seen from the relative likelihood of movement predicted by the dispersal model between neighboring orchard blocks in a landscape (Fig. 6).

The movement coefficients estimated at these three orchard sites represent average linear displacements during the lifetime of the male NOW. However, as the "lifetime" of the male NOW in our study is the time from adult eclosion to being caught in a virgin female-baited trap, these estimated movement coefficients do not fully reflect the actual dispersal capabilities of NOW. Actual dispersal capabilities will clearly depend on the realized lifespan of an insect (*t*) such as NOW and its diffusion rate (*D*). The relationship between these two parameters is nonlinear for any given movement coefficient. An estimated movement coefficient of 594 m per generation for the Escalon site would correspond to a diffusion rate that is orders of magnitude greater than the corresponding rates from the other two sites regardless of potential differences in the lifespans of the moths (Fig. 7).

Thus our analysis suggests that NOW is capable of flying very long distances. The spatial scale of our trap captures constrained the range of values of the movement coefficient that we were able to detect. However, at the Escalon site our analysis estimated a value that approached the maximum detectable limit for the landscape. This estimate suggests that at least in some cases NOW males, half-way through their adult life, were on average about 600 m away from where they fed as a larva. Assuming that the likelihood of movement does not change with adult age, this would mean that some NOW males could end up 1.2 km away from their larval food plant at the end of their lifetime. In addition, we must consider that Gaussian based models of movement are likely to underestimate the amount of longer distance dispersal (Kot et al. 1996), suggesting that NOW movement capabilities may extend well beyond 1.2 km per generation when considering invasion potential. Given that NOW has 3-4 generations per year in California (Luedeling et al. 2011), this rate of movement is very similar to the average rate of radial expansion of invasive forest insect and pathogen populations in the USA, which was estimated to be 5.2 km/year (Liebhold et al. 2013).

The difference in estimated movement coefficients for almond and walnut moths at the Escalon site was surprising and could have been due to a number of factors. Hypothetically, almond moths could have a larger capacity for movement across the landscape than walnut moths. However, since we did not see the same pattern at the other two sites this seems unlikely. Alternatively, the almond moths at the Escalon site might have been much older before being captured in the traps than the walnut moths, which coupled with a similar diffusion rate, could have contributed to the difference in estimated movement coefficients (Fig. 7).

A more likely explanation, however, is that the almond moths at the Escalon site violated one or more of the assumptions of the Gaussian dispersal model. If the population of almond moths had moved directionally rather than randomly into the traps at this site, it would have greatly inflated our estimated value of the movement coefficient. It is possible that the walnut blocks at this site supported nuts that were particularly attractive to the almond NOW at the time of trapping as the walnut moths within these same blocks did not appear to move out of them. Similarly, if the dispersal kernel for NOW more closely approximated a leptokurtic distribution than a Gaussian distribution, the simple diffusion model would not provide a good fit to the data. Leptokurtic distributions can arise from subsets of a population having different Gaussian diffusion rates (Skalski and Gilliam 2003). It is possible that many of the almond moths at the Escalon site were members of a group of "movers" with a very high movement coefficient, while the walnut moths belonged to a more stationary group and therefore had a much smaller movement coefficient. However, it remains unknown whether NOW has two different movement states, a stationary state with small amounts of random diffusion and a dispersal state with much larger movements.

The Escalon site had larger detection limits than the other two sites (Fig. 5a), a result of the landscape properties and the placement of traps. Traps at the Escalon site were placed further from each other than at the other two sites, and at the center of blocks of walnuts and almonds. Trap placement in the landscape, and landscape properties, such as patch size and shape, can greatly affect the detection range of this model. In particular, the distances of the traps from potential sources greatly influences the maximum detection limits. For future studies, we would suggest placing traps at a variety of spatial scales, in order to increase the accuracy and detection limits of the model. In addition, we would highly recommend inclusion of an estimate of the age of the individual moths trapped. Having an estimate for age would allow for a finer scale analysis of the movement data.

In this study we have been able to show that probability-based models paired with intrinsic marking techniques have great potential for furthering our knowledge of dispersal processes for small animals, such as insects, that can be difficult to track as individuals. By applying such an approach to NOW movement we were able to obtain clear estimates of movement coefficients from intrinsically marked individuals in a relatively small scale and localized landscape. In our study, we choose to use a Gaussian dispersal kernel for the model; however, alternative dispersal kernels could readily be applied to the same modeling approach. To be successful, the approach does require a sufficient amount of spatial variation in the intrinsic marker across the landscape, as without that variation, the limits of movement detection would be very small. Therefore, the scale at which the movement of small animals can be accurately estimated in a particular landscape will vary with the availability of suitably informative intrinsic makers. However, if an appropriate intrinsic marker can be found, probability-based models provide a powerful analytical tool for estimating dispersal parameters.

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| | df | Deviance | p |
|---|---|---|---|
| Larval food source | 1 | 0.256 | 0.48 |
| Crop type | 1 | 0.820 | 0.21 |
| Site | 2 | 0.061 | 0.94 |
| Larval food * Crop type | 1 | 18.159 | < 0.001 *** |
| Larval food * Site | 2 | 12.213 | < 0.001 *** |
| Crop type * Site | 2 | 5.1387 | 0.007 ** |

**Table 1:** Results from the GLM to test the influence of larval food source, crop type, and site on the number of male NOW caught in virgin-baited traps.

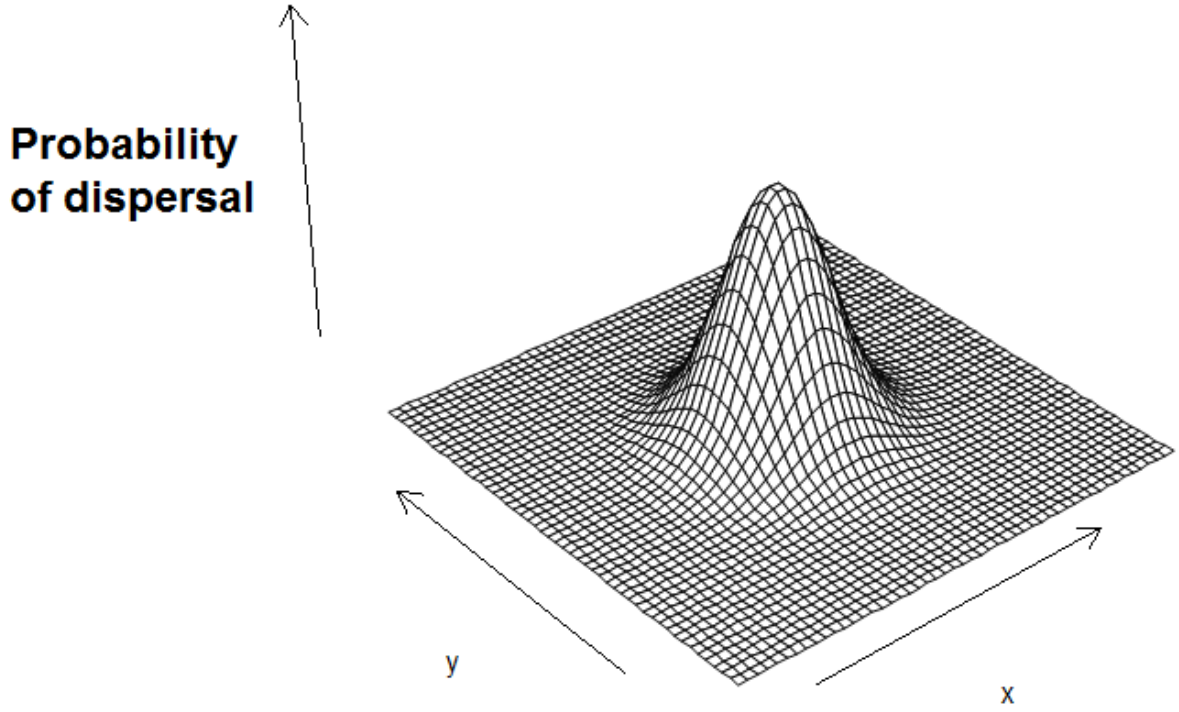



**Figure 1:** Probability of dispersal across a landscape from a central point under random diffusion. This curve gives the probability of an individual being observed at any point in the landscape, given the diffusion rate, and time since release.

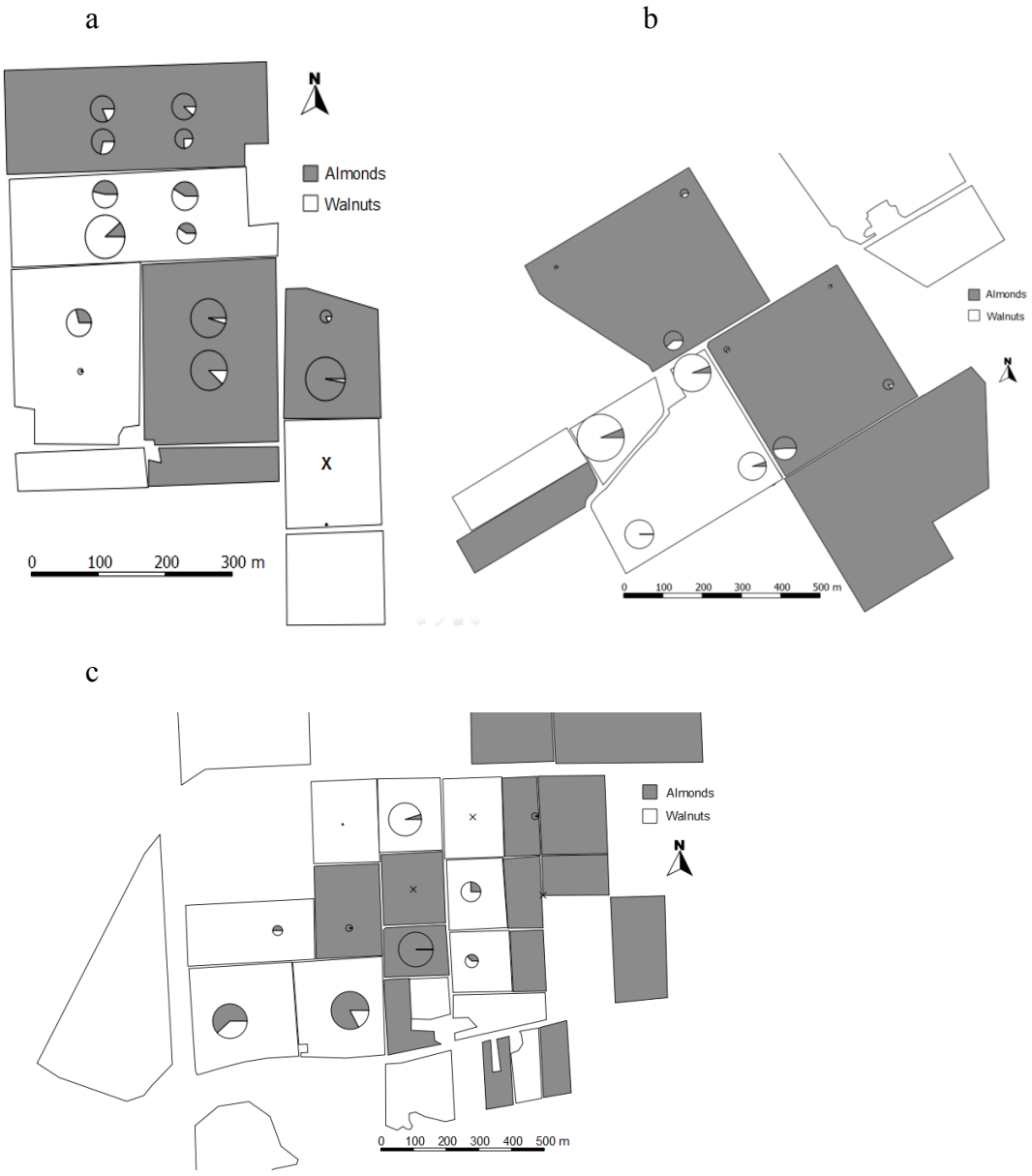



**Figure 2:** Maps of the orchard sites located in (a) Oakdale, (b) Patterson, and (c) Escalon, showing the movement of NOW across these landscapes. The blocks represent almond and walnut orchards. Each pie chart represents a virgin female-baited trap, where the size of the pie chart corresponds to the number of NOW caught in the trap and the shading corresponds to the larval food source assignment of the moths in each trap. The "X" marks a trap with no NOW caught.

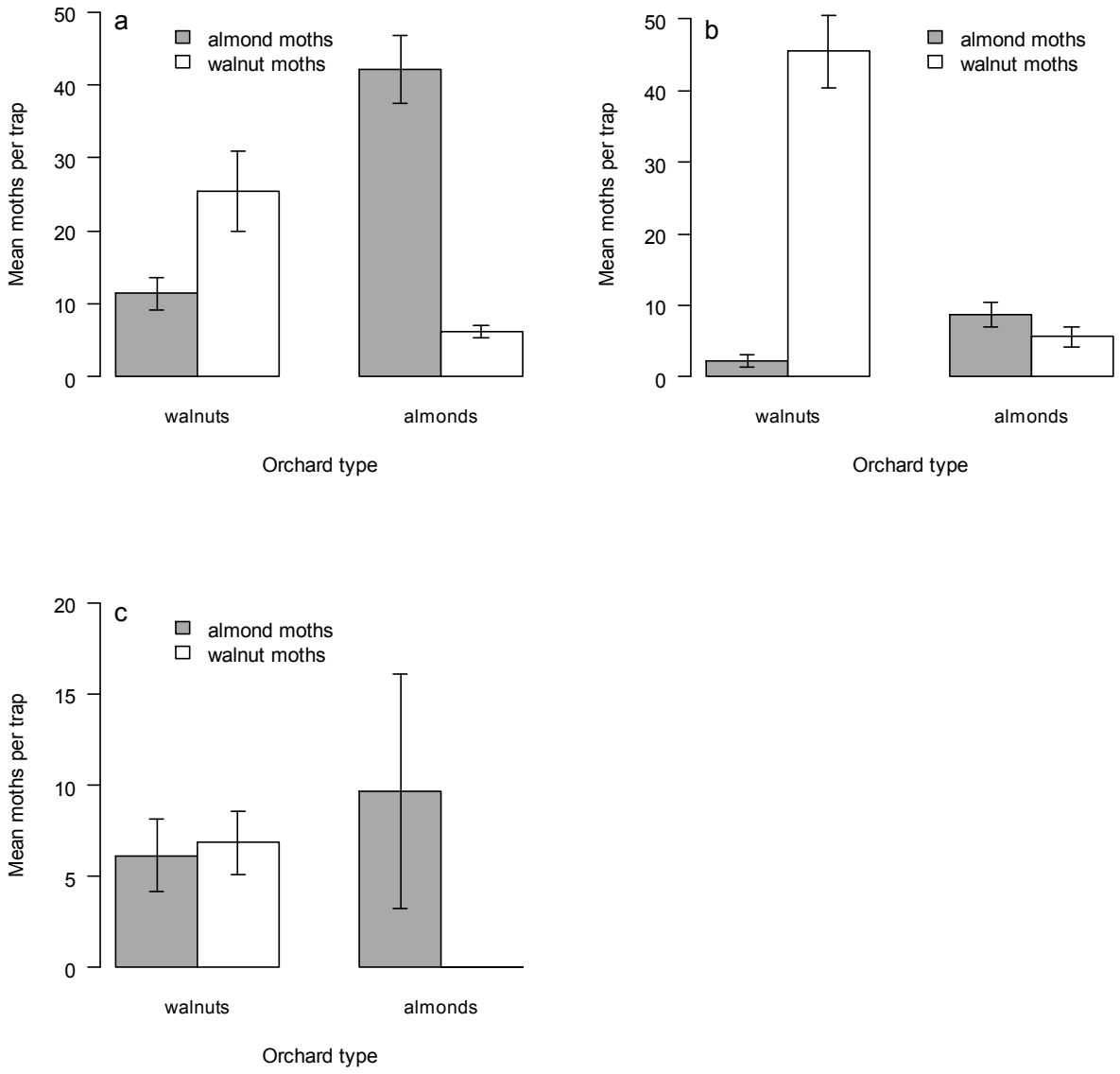



**Figure 3:** Mean (±SE) almond and walnut moths caught per trap in (a) Oakdale (almond blocks n = 8, walnut blocks n = 8), (b) Patterson (almond blocks n = 7, walnut blocks n = 4), and (c) Escalon (almond blocks n = 5, walnut blocks n = 8).

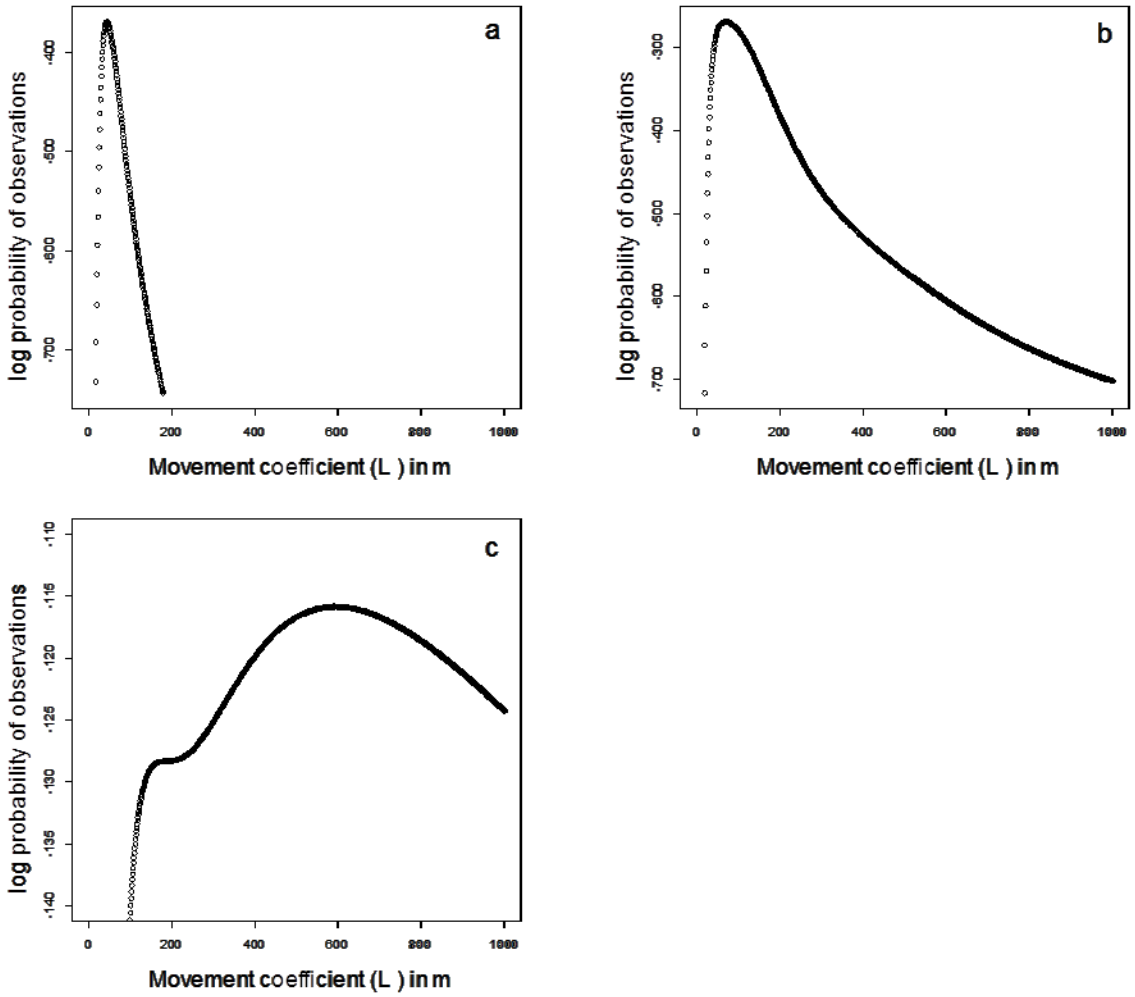
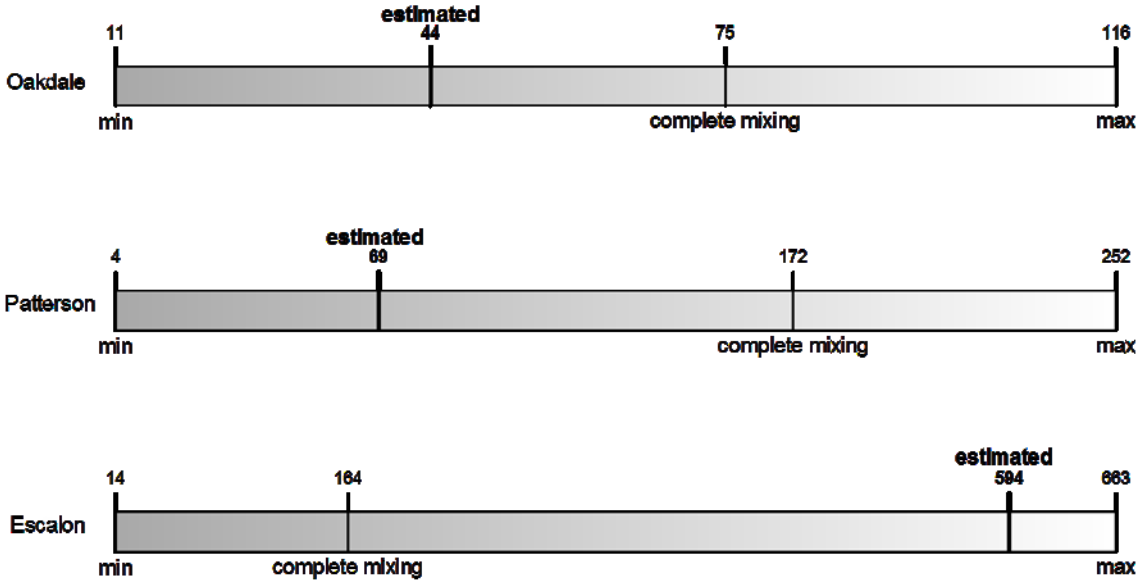



**Figure 4:** Probability distributions for the estimated movement coefficients (*L*) for NOW in (a) Oakdale (668 male moths trapped), (b) Patterson (292 male moths trapped), and (c) Escalon (120 male moths trapped).

a.




b.

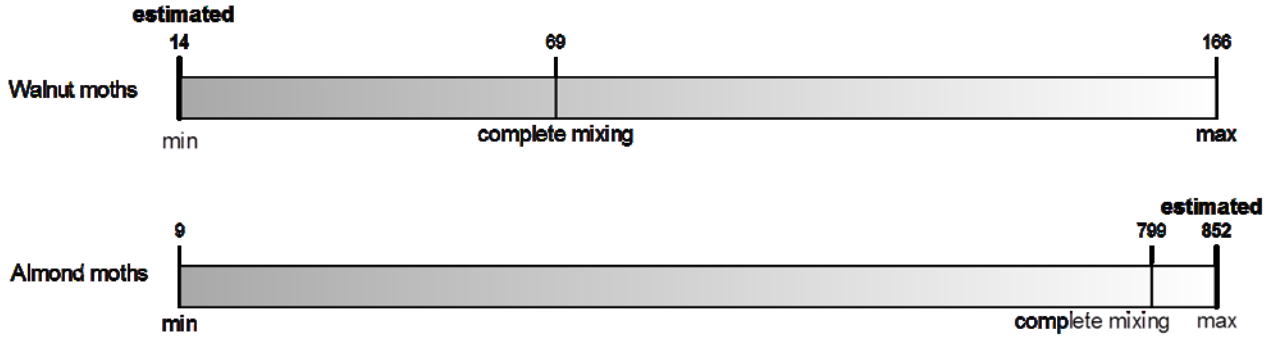



**Figure 5:** Estimated most probable values of the movement coefficient (*L*) for NOW (a) at all three orchard sites, and( b) for almond versus walnut moths trapped at the Escalon site, placed in the context of the maximum and minimum measureable values of *L* for each landscape and the complete mixing scenario.

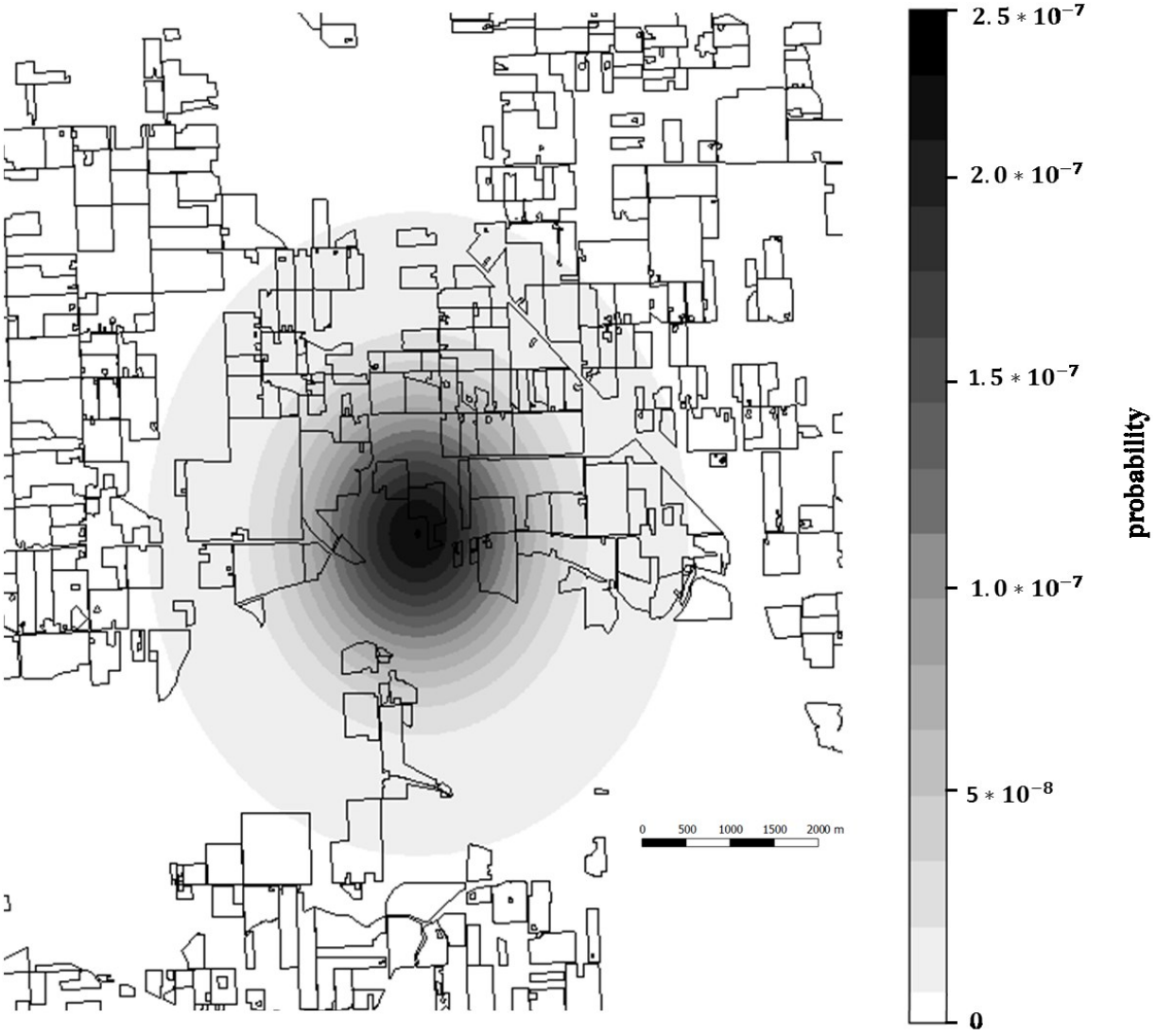



**Figure 6:** Map showing a random dispersal probability distribution with $L = 594$ for an individual NOW starting at the center, plotted onto a hypothetical landscape with different orchard blocks.

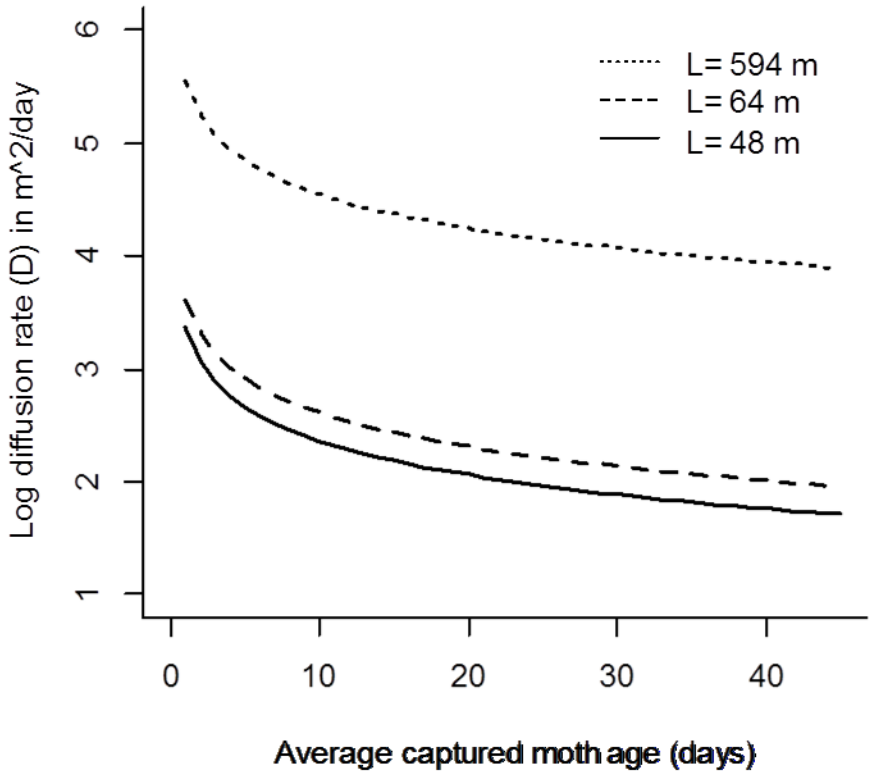



**Figure 7:** Relationship between diffusion rate (*D*) and adult moth age (*t*) for the estimated values of the movement coefficient (*L*) for NOW at each of the three sites.

## Conclusions

The movement of insects remains a difficult topic to study. Questions of how crop type and season effect NOW movement were not fully answered in my work due to problems that arose with the use of protein marking techniques, and instead my research turned towards developing a novel intrinsic marker for tracking NOW movement. The marker development was successful and provides a useful tool to examine movement of small organisms that have spatial variation in their dietary fatty acids. We used the fatty acid biomarkers, paired with probabilistic dispersal models to show that NOW can move an average of about 600 m in a single generation.

There are three potential avenues of future research. First, I would suggest more closely examining the scale of NOW movement. Although we addressed this topic in detail in Chapter 3, my experimental design limited the range of movement that could be detected by the dispersal model. An initial analysis of specific landscapes could allow for more effective trap placement and thus maximize the range of detectible movement. Such an approach would better capture NOW movement capabilities across a wider range of scales.

Second, I would examine intercrop movement throughout the growing season. Although we failed to detect any directional movement events between crops in Chapter 1, it is possible that it is still occurring in NOW populations. Fatty acid biomarkers would allow us to examine the interplay between season and crop type, thus informing effective population level management of this agricultural pest. In addition, this research could be combined with examination of the scale of NOW movement to determine if there is temporal variation in the extent of NOW movement.

Finally, it would be interesting to examine the spatial dynamics of NOW parasitoids. Preliminary work suggests that NOW parasitoids can also be identified by their fatty acid profiles. Capturing NOW parasitoids across the landscape would allow us to answer the same questions of movement patterns across trophic levels. Classical biological control of NOW in California has been largely ineffective, and it is possible that this failure in part results from differential movement capabilities of NOW and its parasitoids. Research into the movement dynamics of entomophagous insects and their natural enemies will be necessary for a deeper understanding of how movement ecology helps structure populations, communities, and ecosystems.