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Movement and Nutritional Ecology of the Navel Orangeworm, *Amyelois transitella* (Lepidoptera: Pyralidae)

A Dissertation submitted in partial satisfaction
of the requirements for the degree of

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in

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by

Joshua Eugene Reger

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Dissertation Committee:
Dr. Houston Wilson, Chairperson
Dr. Kerry Mauck
Dr. Matt Daugherty

The Dissertation of Joshua Eugene Reger is approved:

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Committee Chairperson

University of California, Riverside

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Dedication

This dissertation is dedicated to my fiancée, Kayla, and to any future entomology apostle that finds guidance in these words like stars in the night sky.

ABSTRACT OF THE DISSERTATION

Movement and Nutritional Ecology of the Navel Orangeworm, *Amyelois transitella* (Lepidoptera: Pyralidae)

by

Joshua Eugene Reger

Doctor of Philosophy, Graduate Program in Entomology
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Dr. Houston Wilson, Chairperson

The navel orangeworm, *Amyelois transitella* Walker (Lepidoptera: Pyralidae), is the most significant insect pest of almonds, pistachios, and walnuts. Tolerance for *A. transitella* feeding damage is < 2% due to yield loss, increased processing costs, and its association with aflatoxins. To maintain this level of control, several integrated pest management (IPM) strategies are used. However, these strategies are not coordinated among growers or applied uniformly. Consequently, gaps remain in management that can be exploited by migrating *A. transitella*. These gaps can be better managed by understanding *A. transitella's* dispersal ability and movement between orchards. The goals of this dissertation were to (i) examine *A. transitella* dispersal in the field, (ii) discover potential intrinsic biomarkers obtained from larval diet, and (iii) evaluate the influence of larval diet on flight capacity and nutritional profiles of adults.

Mark-release-recapture methods evolved over four years in a series of studies. Trap comparison studies demonstrated that *A. transitella* was more effectively captured

from a greater distance with pheromone lures rather than blacklights. Additionally, mass-reared A. *transitella* were recovered in fewer numbers than locally-reared moths. Several new methods to trace the movement of *A. transitella* through intrinsic biomarkers sequestered in the larval diet were compared. Non-polar compounds showed greater classification accuracy than polar metabolites and stable isotopes of amino acids, predicting the larval host of *A. transitella* in controlled settings. Accuracy with lipids declined when applied to field-collected insects of unknown origin. A carotenoid, lutein, was shown to be highly concentrated in both pistachio kernels and pistachio-reared *A. transitella,* showing promise for future targeted studies. Common larval diets showed diverse nutritional profiles. However, these differences were not as clear in adult *A. transitella* macronutrient composition and generally did not influence flight performance. *Amyelois transitella's* ability to compensate for differences in diet quality to reduce negative impacts on flight capacity is not surprising for a polyphagous insect. This dissertation project highlighted the role of larval diet in dispersal and provided insight into the prospects and limitations of tracing moth movement at the landscape scale to improve IPM programs.

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Introduction

Integrated pest management (IPM) is generally defined as a strategy that uses cultural, biological, and chemical tools to keep insect pest populations and damage below economic thresholds in agricultural systems (Stern et al. 1959, Bottrell 1979, FAO 1996). It is an ecological and environmental approach developed in direct response to the excessive use of synthetic insecticides, which became widely available after World War II and the Green Revolution (Bosch 1978, Jain 2010). However, intense production and profitability remain the main drivers in agricultural systems (Horlings and Marsden 2011), and many current IPM program designs reflect this focus on chemical strategies (Deguine et al. 2021). Modern agriculture is at a crossroads, facing increased food demand and a global population concerned about the environmental cost of meeting it.

In 2023, California adopted a roadmap for accelerating sustainable pest management. A key takeaway from this plan is the elimination of priority pesticides (i.e., active ingredients highly hazardous to humans and the environment) by 2050 (Sustainable Pest Management Work Group 2023). Yield losses due to insect damage remain high (Thomas 1999, Manosathiyadevan et al. 2017), and their management is a major cost for growers and the environment with priority insecticides. Therefore, sustainably reducing yield loss from insect damage is vital to meeting the world's nutritional demands. Improving IPM programs and promoting more sustainable agricultural practices are necessary to make agriculture more efficient. Coordinating pest management efforts from multiple growers can improve program results compared to field-to-field practices.

Area-wide IPM (AW-IPM) is a coordinated, large-scale approach to suppressing entire pest populations across a defined geographic area, rather than on a field-by-field basis (Hendrichs et al. 2021). This variant of IPM is particularly useful with highly mobile and polyphagous pests, built as a suite of tools to address pest populations and damage on multiple fronts. (Vreysen et al. 2007). It has been successfully applied to eradicate pest populations (Van der Vloedt and Klassen 1991, Tabashnik et al. 2021), enhance biological control (Wyckhuys et al. 2021, Yu et al. 2021), and manage the spread of insect-vectored plant diseases (García-Ávila et al. 2021, Figuera et al. 2022). In recent years, the California tree nut industry has been interested in implementing AW-IPM tactics targeted at one of its most important insect pests (Wilson et al. 2021).

The navel orangeworm, *Amyelois transitella* Walker (Lepidoptera: Pyralidae), is a polyphagous pest that was first reported in Arizona infesting split citrus fruit (Mote 1922, Glick 1923, Lockwood 1931). Populations of *A. transitella* migrated into California, and by the 1940s, it was present as far north as the Sacramento Valley. The rapid spread of *A. transitella* was likely aided by the vast plantings of walnuts, in which it became known to be a pest of rather than citrus (Michelbacher and Ross 1955, 1957, Michelbacher and Davis 1961). Modern tree nut acreage has expanded to cover more than 2.2 million cultivated acres of tree nuts in California (CDFA 2024). *Amyelois transitella* is ubiquitous across almond, pistachio, and walnut systems, causing economic damage as well as in alternative hosts such as figs. Damage from *A. transitella* is direct through larval feeding on nut kernels or fruit. Feeding reduces biomass, and crops are contaminated with webbing and frass, rendering them unmarketable (Wade 1961).

Infestation is also associated with aflatoxin-producing fungi, *Aspergillus flavus*. Aflatoxins are highly regulated in key export markets. Thus, growers aim to keep *A. transitella* damage below 2%.

Seasonal Phenology

The first generation of *A. transitella* develops exclusively on remnant "mummy" nuts (i.e., unharvested nuts). Remnant nuts are the sole oviposition sites for the first generation because *A. transitella* larvae cannot penetrate the hulls and husks of developing tree nuts. The emergence of second-generation adults in late June or early July, depending on temperature, coincides with hull split in almonds (Mid-June to early July) and the degradation of hull integrity. At this time, new crop almonds become vulnerable to *A. transitella* infestation. The protective hull or husk splits, allowing eggs laid on the nut to easily access the kernel. Following almonds, pistachios and walnuts become vulnerable in August and September, respectively. Notable exceptions are pea-split pistachios, which allow for earlier *A. transitella* infestation (Kuenen and Siegel 2011), and codling moth (*Cydia pomonella*; Lepidoptera: Tortricidae) damage in walnuts (Michelbacher and Ross 1957). The third and fourth flights are most damaging in terms of yield loss for new crop nuts, as these are the largest *A. transitella* generations. As nighttime temperatures drop in the fall, *A. transitella* activity decreases, coinciding with the end of nut harvest. After harvest, mummy nuts serve as overwintering sites for *A. transitella,* which will become the first flight in the following spring.

Management of *A. transitella* relies on integrated strategies including winter sanitation, monitoring, mating disruption, insecticides, and timely harvest (Wilson et al. 2020). Together, these tools can provide adequate control of current pest populations.

Orchard Sanitation

The foundation of *A. transitella* management is winter sanitation of mummy nuts, but there are several challenges associated with it. The rising cost of labor and equipment is a factor limiting the adoption of orchard sanitation in all crops. Sanitation also tends to be less effective in pistachios due to the smaller nuts that are hard to reach in the berms of a row. Additionally, rainy winter conditions prevent or delay workers and equipment from entering orchards. As such, regional orchard sanitation has not been optimized, making some orchards net exporters of *A. transitella* moths, which can undermine or disincentivize local efforts to sanitize individual blocks when *A. transitella* immigrates in from nearby orchards.

Monitoring

Monitoring *A. transitella* is conducted with two major types of lures. The first, ovibait lures, are used to attract gravid females and encourage egg deposition (Rice 1976, Rice et al. 1976). Some consist of 50 g almond meal and 10% crude almond oil (Sanderson and Barnes 1990, Kuenen et al. 2008). Alternatively, Peterson lures utilize ground pistachio mummies with a sticky bottom wing trap and is an effective alternative

design (Nay et al., 2012). This design is effective at monitoring gravid females but is not designed to indicate egg deposition.

A commercial synthetic pheromone lure to attract *A. transitella* males has been available since 2013 (Higbee et al. 2014). Since the debut of the lure, the trap design and density recommendations have been refined to wing or delta traps placed in orchards at a rate of 1 per 20 ha (Burks & Higbee, 2015). Lures are used to monitor the emergence of new generations of *A. transitella* and time insecticide sprays, particularly around the nut peak vulnerability. The lure is compatible with mating disruption when paired with phenyl propionate (PPO) (Burks et al., 2016). Mating disruption is an effective but relatively expensive tool that can significantly reduce crop damage, particularly when implemented over large contiguous orchard blocks. However, its success may be reduced by immigration of mated females from surrounding orchards and inconsistent grower adoption.

Chemical Control

Insecticide sprays for *A. transitella* control primarily consist of two applications of pyrethroids (e.g., bifenthrin, lambda-cyhalothrin), diamides (chlorantraniliprole), and/or insect growth regulators (methoxyfenozide) timed to coincide with declining hull integrity of new crop nuts (i.e., July, August, and September for almonds, pistachios, and walnuts, respectively). While pyrethroids are the most cost-effective active ingredient, *A. transitella* resistance to the pyrethroid bifenthrin has recently been documented (Demkovich et al. 2015). The need for area-wide management is increasing as *A.*

transitella populations are expected to rise with warming temperatures. Because *A. transitella* can disperse more than 10 km in a single night, orchard-level management is strongly influenced by surrounding landscapes and neighboring orchard practices. Crop phenology and harvest timing create shifting patterns of host availability that likely drive moth movement between orchards. As a result, poorly managed orchards may act as population sources that compromise local control efforts.

Timely Harvest

Harvesting quickly aims to reduce *A. transitella* infestation by removing nuts from the field. It has been demonstrated in almonds (Connell et al., 1989) and walnuts (Olson et al., 1975). Like winter sanitation, this process can be hindered by equipment and labor availability. While pistachios and walnuts are simultaneously harvested and removed from the orchard, almonds are typically shaken from trees and then allowed to dry for 4–10 days on the orchard floor before removal.

Need for AW-IPM

When the above strategies are effectively used in combination, growers can typically achieve the desired level of *A. transitella* control (<2% infestation) for current populations. Average temperatures are expected to increase in the coming decades, and with them *A. transitella* populations (Pathak et al., 2021), thus optimized strategies are going to be needed. Unfortunately, practices have not been equally adopted across all regions. Growers are at a crossroads with *A. transitella* management. There is a pressing

need to increase the use of non-chemical strategies, but the greater adoption and optimization of these tools currently require additional information about *A. transitella* movement between orchards.

A. transitella is a highly mobile pest with an average dispersal capacity of over 10 km in a single night, and for females, this range is extended when mated (Rovnyak et al., 2018; Sappington & Burks, 2014). The widespread cultivation of its host material, namely tree nuts, is extensive across California. However, the dynamic nature of tree nut phenology introduces a constantly changing environment for *A. transitella,* where the availability of suitable hosts fluctuates. The movement of *A. transitella* between orchards is likely a crucial mechanism for the persistence of its populations. Although dispersal between orchards has been observed, there is a lack of characterization regarding the precise timing, frequency, and extent of such movements, as well as the factors driving them.

In any given orchard, the likelihood of moth immigration is probably influenced by the quantity and quality of management in neighboring potential *A. transitella* sources. Colonization events can potentially undermine local management efforts, as demonstrated by a study conducted in almond orchards, which revealed that proximity to pistachio orchards was a key factor in *A. transitella* infestation (Higbee & Siegel, 2009). Pistachio orchards, being likely sources of *A. transitella* due to inadequate crop sanitation, present a challenge due to the difficulty in gathering and destroying mummy pistachios.

Poor or uncoordinated management in certain orchards may turn them into sources of *A. transitella*. Additionally, the timing of crop development and harvest activities can catalyze moth movement. While there are nearly 2 million acres of tree nuts in California, the vulnerability of these hosts to *A. transitella* is influenced by hull integrity, staggered across almonds, pistachios, and walnuts due to differences in phenology. The decline in hull integrity generally increases the availability of new crop nuts over the season, but this can be countered by harvest events, where significant quantities of suitable host material are rapidly removed from the landscape. For example, both pistachio hull degradation and almond harvest occur in August, leading to a simultaneous increase and decrease in host availability. At the landscape scale, this net gain or loss of host material is likely a key driver of *A. transitella* dispersal between orchards. Harvest events in certain landscapes may compel *A. transitella* to disperse in search of new hosts, resulting in highly temporally specific colonization events in nearby areas.

Similar approaches have proven effective for other major lepidopteran pests like *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae), *Pectinophora gossypiella* (Lepidoptera: Gelichiidae), and *Cydia pomonella* (Hofmeyr et al., 2019; Tabashnik et al., 2021; Thistlewood & Judd, 2019), which were built on a firm understanding of pests' dispersal and landscape ecology.

Current management practices, in combination, targeted at *A. transitella* can typically achieve the desired level of control. However, not all are optimized across growing regions and can be undermined at the local level by immigrating *A. transitella*.

A better characterization of this inter-orchard movement may allow growers to coordinate better strategies against this pest. To improve our understanding of *A. transitella* movement at the landscape scale, my dissertation project will (1) characterize *A. transitella* dispersal within the orchard, (2) explore the use of novel markers to trace adult *A. transitella* back to their larval host, and (3) quantify the influence of *A. transitella* larval host on adult moth nutritional status and flight.

What is the field dispersal range of *A. transitella* in pistachio orchards?

Chapter 1 addresses an important question about the dispersal ability of *A. transitella* in the field. It is reported that *A. transitella* is capable of inter-orchard movement and is a strong flyer in laboratory settings, yet few studies have been able to document its full dispersal potential in the field. This information is critical to the implementation of some area-wide strategies, like sterile insect releases. In collaboration with the USDA Animal and Plant Health Inspection Service (APHIS), Agricultural Research Service (ARS), and tree nut growers, a multi-year series of mark-release-recapture experiments was able to be conducted across California.

From 2022-2025, experimental designs and methods evolved year-to-year to improve the recapture of *A. transitella*. Initially, in a 2022 pilot study, a single blacklight trap was tested in a fallow field at the University of California, Riverside (UCR) because no tree nut plots were present. However, that setting was not representative of *A. transitella* agroecosystems, so the trial was repeated the next year in pistachio orchards at a larger scale, comparing the blacklight to an industry-standard pheromone lure. The

pheromone lure proved to be the better tool, so a new grid design with multiple pheromone lures was used in 2024 and 2025.

The source and handling of test insects also changed from year to year. In 2022, *A. transitella* larvae were driven from the USDA APHIS mass-rearing facility in Phoenix, AZ, to UCR. When the releases were moved to commercial pistachio orchards, the larvae were flown to a small airport in Shafter, CA, and driven to the University of California Kearney Agriculture Research and Extension Center (UC KARE) via FedEx in 2023 and 2024. Unfortunately, in 2025, the USDA APHIS facility was unable to provide any insects, so *A. transitella* was reared from lab colonies at the USDA ARS Science Joaquin Valley Agricultural Sciences Center in Parlier, CA. The USDA ARS strain was different than the one from USDA APHIS in Phoenix, AZ, but known equally fit when reared locally (Reger et al. 2021), so recapture rates were not suspected to be negatively affected. Studies in 2022, 2023, and 2025 worked with a single strain and have been grouped as “Trapping Radius and Dispersal Studies”.

In 2024, an opportunity to directly compare the dispersal of locally-reared and mass-reared *A. transitella* was pursued. Locally-reared *A. transitella* were released over three nights per release-recapture period. Mass-reared adult *A. transitella* were released in addition to locally-reared adults. Sterilized moths were produced in the same mass-rearing facility and shipped under similar conditions as larvae. Flight fitness between the two treatments was measured both in the field and in the lab with flight cylinders.

What other intrinsic markers from tree nuts are present in *A. transitella*?

A key bit of scholarship that influenced my dissertation was the work with fatty acid methyl esters and *A. transitella* published by Bayes et al. (2014). The fatty acid profiles of almonds, walnuts, and *A. transitella* that developed on them could be identified using gas chromatography. However, pistachio and almond profiles were too similar, which severely limited the application of this technique in future studies. Chapter 2 is an exploration of untargeted mass spectrometry techniques and targeted analysis of a carotenoid, lutein, to attempt to find compounds that can distinguish all three tree nuts as larval sources for *A. transitella*. Results from this study provide a critical tool for tracing *A. transitella* movement at the landscape level.

What is the influence of *A. transitella* larval diet on adult dispersal?

Remnant nuts have been reported to be of poorer quality as a host (Siegel et al. 2010, Kuenen and Siegel 2011), and dispersal has been determined on *A. transitella* reared on an artificial diet (Sappington and Burks 2014, Rovnyak et al. 2018), which leaves the influence of new crop tree nuts on dispersal unknown. The study in Chapter 3 further explored the effects of different host crops. I decided to conduct a study that would evaluate the macronutrients provided by specific diets and the flight ability of *A. transitella* reared on those same diets. Information generated from this work provides insight into the importance of larval diet quality and *A. transitella* ecology.

References

Bayes SK, Hellerstein MK, Fitch M, et al. 2014. You are what you eat: fatty acid profiles as a method to track the habitat movement of an insect. Oecologia. 175(4):1073–1080.

Bosch RVD. 1978. The Pesticide Conspiracy. University of California Press. https://doi.org/10.1525/9780520909748

Bottrell DG. 1979. Integrated pest management. Council on Environmental Quality.

CDFA. 2024. California Agricultural Statistics Review, 2023-2024. California Department of Food and Agriculture, Sacramento, CA.

Connell JH, Labavitch JM, Sibbett GS, et al. 1989 July 1. Early Harvest of Almonds to Circumvent Late Infestation by Navel Orangeworm. https://doi.org/10.21273/JASHS.114.4.595

Deguine J-P, Aubertot J-N, Flor RJ, et al. 2021. Integrated pest management: good intentions, hard realities. A review. Agron. Sustain. Dev. 41(3):38. https://doi.org/10.1007/s13593-021-00689-w

Demkovich M, Siegel JP, Higbee BS, et al. 2015. Mechanism of resistance acquisition and potential associated fitness costs in Amyelois transitella (Lepidoptera: Pyralidae) exposed to pyrethroid insecticides. Environ. Entomol. 44(3):855–863.

Figuera SG, Babcock B, Lubell M, et al. 2022. Collective action in the area-wide management of an invasive plant disease. Ecol. Soc. 27(2). https://doi.org/10.5751/ES-13217-270212

(FAO) Food and Agriculture Organization of the United Nations. 1996. Food, agriculture and food security: developments since the World Food Conference and prospects. Technical background document no. 1 for the World Food Summit. FAO, Rome, Italy.

García-Ávila CJ, Trujillo-Arriaga FJ, Quezada-Salinas A, et al. 2021. Holistic area-wide approach for successfully managing citrus greening (Huanglongbing) in Mexico. In: Area-Wide Integrated Pest Management. CRC Press. p. 33–49.

Glick PA. 1923. The Survey of Myelois venipars, Dyar, in Arizona. Arizona Commission of Agriculture and Horticulture. 14th Annual Report: 78–97. Arizona Commission of Agriculture and Horticulture.

Hendrichs J, Pereira R, Vreysen MJ. 2021. Area-wide integrated pest management: development and field application. CRC Press.

Higbee, B., & Siegel, J. (2009). New navel orangeworm sanitation standards could reduce almond damage. California Agriculture, 63(1), 24–28.

Higbee BS, Burks CS, Larsen TE. 2014. Demonstration and Characterization of a Persistent Pheromone Lure for the Navel Orangeworm, Amyelois transitella (Lepidoptera: Pyralidae). Insects. 5(3):596–608. https://doi.org/10.3390/insects5030596

Horlings LG, Marsden TK. 2011. Towards the real green revolution? Exploring the conceptual dimensions of a new ecological modernisation of agriculture that could 'feed the world.' Glob. Environ. Change. 21(2):441–452. https://doi.org/10.1016/j.gloenvcha.2011.01.004

Jain HK. 2010. Green revolution: history, impact and future. The Green Revolution: Histor.

Kuenen LPS (Bas), Bentley W, Rowe H, et al. 2008. Bait formulations and longevity of navel orangeworm egg traps tested. Calif. Agric. 62(1).

Kuenen LPS, Siegel JP. 2011. Variable Developmental Rate and Survival of Navel Orangeworm (Lepidoptera: Pyralidae) on Pistachio. J. Econ. Entomol. 104(2):532–539. https://doi.org/10.1603/EC10177

Lockwood S. 1931. An economic Survey of the Navel Orange Worm, Myelois venipars, Dyar, in Arizona. Calif. Dept. Agr. Bull. 10: 655–660.

Manosathiyadevan M, Bhuvaneshwari V, Latha R. 2017. Impact of Insects and Pests in loss of Crop Production: A Review. In: Dhanarajan A, editor. Sustainable Agriculture towards Food Security. Singapore: Springer. p. 57–67. https://doi.org/10.1007/978-981-10-6647-4_4

Michelbacher A, Ross N. 1955. Navel orangeworm: field control of walnut pest in northern California aided by restrictive measures. Calif. Agric. 9(3):4–4.

Michelbacher A, Ross N. 1957. Navel orangeworm: summer infestations of codling moth on walnuts favorable to navel orangeworm. Calif. Agric. 11(7):12–12.

Michelbacher AE, Davis CS. 1961. The navel orangeworm in northern California. J. Econ. Entomol. 54(3):559–562.

Mote D.C. 1922. New Orange Pest in Arizona. Calif. Dept. Agr. Bull. 11: 628–633.

Olson, W. H., Hendricks, L. C., Sibbett, G. S., Davis, C. S., & Ramos, D. E. 1975. Navel orangeworm control through early harvest. Calif. Agr. 29: 3.

Pathak TB, Maskey ML, Rijal JP. 2021. Impact of climate change on navel orangeworm, a major pest of tree nuts in California. Sci. Total Environ. 755:142657. https://doi.org/10.1016/j.scitotenv.2020.142657

Reger JE, Wenger JA, Brar G, et al. 2021. Evaluating flight performance of mass-reared and irradiated navel orangeworm (Lepidoptera: Pyralidae) for sterile insect technique. J. Econ. Entomol. 114(4):1542–1548.

Rice RE. 1976. A comparison of monitoring techniques for the navel orangeworm. J. Econ. Entomol. 69(1):25–28.

Rice RE, Sadler LL, Hoffmann ML, et al. 1976. Egg Traps for the Navel Orangeworm, Paramyelois transitella (Walker) 1. Environ. Entomol. 5(4):697–700. https://doi.org/10.1093/ee/5.4.697

Rovnyak AM, Burks CS, Gassmann AJ, et al. 2018. Interrelation of mating, flight, and fecundity in navel orangeworm females. Entomol. Exp. Appl. 166(4):304–315.

Sanderson JP, Barnes MM. 1990. Ability of egg traps to detect the onset of second-generation navel orangeworm (Lepidoptera: Pyralidae) moth activity in almond orchards. J. Econ. Entomol. 83(2):570–573.

Sappington TW, Burks CS. 2014. Patterns of flight behavior and capacity of unmated navel orangeworm (Lepidoptera: Pyralidae) adults related to age, gender, and wing size. Environ. Entomol. 43(3):696–705.

Siegel JP, Bas Kuenen LPS, Ledbetter C. 2010. Variable development rate and survival of navel orangeworm (Lepidoptera: Pyralidae) on wheat bran diet and almonds. J. Econ. Entomol. 103(4):1250–1257.

Stern V, Smith R, Van den Bosch R, et al. 1959. The integration of chemical and biological control of the spotted alfalfa aphid: the integrated control concept. Hilgardia. 29(2):81–101.

Sustainable Pest Management Work Group. (2023). *Accelerating sustainable pest management: A roadmap for California*. California Department of Pesticide Regulation. https://www.cdpr.ca.gov/docs/sustainable_pest_management_roadmap/

Tabashnik BE, Liesner LR, Ellsworth PC, et al. 2021. Transgenic cotton and sterile insect releases synergize eradication of pink bollworm a century after it invaded the United States. Proc. Natl. Acad. Sci. 118(1).

Thomas MB. 1999. Ecological approaches and the development of "truly integrated" pest management. Proc. Natl. Acad. Sci. 96(11):5944–5951. https://doi.org/10.1073/pnas.96.11.5944

Van der Vloedt AM, Klassen W. 1991. The development and application of the sterile insect technique (SIT) for New World screwworm eradication. World Anim. Rev. :42–49.

Vreysen MJB, Robinson AS, Hendrichs J, et al. 2007. Area-wide integrated pest management (AW-IPM): principles, practice and prospects. Area-Wide Control Insect Pests. :3–33.

Wade W. 1961. Biology of the navel orangeworm, Paramyelois transitella (Walker), on almonds and walnuts in northern California. Hilgardia. 31(6):129–171.

Wilson H, Burks CS, Reger JE, et al. 2020. Biology and management of navel orangeworm (Lepidoptera: Pyralidae) in California. J. Integr. Pest Manag. 11(1):25.

Wilson H, Hansen S, Culbert N. 2021. Is Navel Orangeworm a Candidate for Area-Wide Integrated Pest Management in California? | West Coast Nut.

Wyckhuys KAG, Orankanok W, Ketelaar JW, et al. 2021. Biological control: Cornerstone of area-wide-integrated pest management for the cassava mealybug in tropical Asia. In: Area-wide integrated pest management. CRC Press. p. 17–32.

Yu YB, Yang HL, Lin Z, et al. 2021. Development and Area-wide application of biological control using the parasitoid Aphidius gifuensis against Myzus persicae in China. In: Area-Wide Integrated Pest Management. CRC Press. p. 3–16.

Chapter 1: Dispersal potential of *Amyelois transitella* in pistachio orchards measured with mark-release-recapture techniques

Abstract

California's $7.5 billion tree nut industry faces significant economic pressure from the navel orangeworm, *Amyelois transitella* Walker (Lepidoptera: Pyralidae), a key pest of almonds, pistachios, and walnuts. Larval feeding on nut kernels reduces crop yield and quality, increases processing times, lowers payments to growers, and, notably, increases the risk of aflatoxin contamination – thus prompting growers to aim for infestation levels <2%. Although *A. transitella* is a strong flyer capable of traveling several kilometers per night in laboratory assays, its baseline field dispersal range remains unclear. A better understanding of this parameter would help refine current monitoring and management strategies. Furthermore, the pistachio industry recently made a significant investment in the development of sterile insect technique (SIT) for *A. transitella* that leverages the availability of a mass-rearing and irradiation facility in Phoenix, AZ. A clear understanding of *A. transitella* dispersal will be critical to the success of this new program. As such, here, mark-release-recapture (MRR) techniques were used to (i) compare the trapping radius of two different trap types and (ii) estimate field dispersal of both locally-reared and mass-reared *A. transitella* in commercial pistachio orchards. The trap comparison studies demonstrated that blacklight traps had a small, localized attraction radius (<10 m). In contrast, wing traps baited with a pheromone and phenyl propionate (PPO) lure attracted moths from greater distances (10-100 m). In the field dispersal study, recovery of *A. transitella* tended to average around 0.47%, and individuals dispersed up to 205 m. Finally, comparison of mass-reared and locally-reared *A. transitella* found lower recovery of the

mass-reared moths (0.13%) relative to the locally-reared moths (0.37%), and that mass-reared moths tended to be recovered much closer to the release point (8 m, as versus 184 m for locally-reared). Associated flight cylinder assays found similar patterns, with fewer of the mass-reared moths (63%) exiting the cylinders over a 24-hr period compared to the locally-reared moths (97%). Taken together, these studies provide new information on the relative trapping radius of blacklight traps and wing traps with a pheromone and PPO lure, field dispersal of *A. transitella*, and the relative performance of a novel mass-reared *A. transitella* moth.

Introduction

California is the leading agricultural state in the United States, with approximately 23.8 million acres dedicated to agriculture, which generates a farmgate value of $59.4 billion (CDFA 2024). Among the top commodities, tree nuts (i.e., almonds, pistachios, and walnuts) account for 2.2 million cultivated acres and a combined annual value of $7.5 billion (CDFA 2024). Almond, *Prunus dulcis* (Rosales: Rosaceae) is the most widespread, with 1.3 million acres. Pistachio, *Pistacia vera* (Sapindales: Anacardiaceae), and walnut, *Juglans regia* (Fagales: Juglandaceae) follow with 462,000 acres and 385,000 acres, respectively.

The primary insect pest of almonds and pistachios, as well as a serious pest of walnuts, is the navel orangeworm, *Amyelois transitella* Walker (Lepidoptera: Pyralidae). Adult moths deposit eggs onto the new crop nuts, and the larvae that emerge feed directly on the kernels. This leads to reduced crop yield and quality, as well as increased processing time and lower bonus payments to growers (Wilson et al. 2020). Infestation of *A. transitella* is also associated with the presence of *Aspergillus flavus* Link (Eurotiales: Aspergillaceae) (Palumbo et al. 2014), which produces aflatoxin, a known human carcinogen that is heavily regulated in key markets. As such, growers and processors have a low tolerance for *A. transitella* populations and typically strive for <2% crop infestation.

Amyelois transitella overwinters as larvae or pupae within remnant "mummy" nuts left behind in orchards after harvest (Wade 1961). In the spring, increasing temperatures and photoperiods trigger adult emergence from overwintering sites, typically beginning in April. Since new crop nuts are not yet vulnerable to *A. transitella* infestation due to high

hull integrity, these first flight moths must oviposit on the same remnant mummy nuts from which they emerged. The second flight generally occurs in late June, coinciding with the availability of new crop almonds. Infestation of these nuts depends largely on hull integrity, since the larvae are unable to penetrate the hulls on their own. In almonds, hull split typically begins in early July, aligning closely with the second flight in early summer. Starting with almonds, hull degradation progresses sequentially across crops, with pistachios and walnuts becoming vulnerable in approximately August and September, respectively. This creates a staggered availability of susceptible new crop nuts. The onset of new crop development represents a substantial increase in both the abundance and quality of host material. Larvae develop more rapidly on these higher-quality new nuts compared to remnant mummy nuts (Siegel et al. 2010, Kuenen and Siegel 2011). Consequently, population densities increase markedly during the third and fourth flights in August and September, respectively, coinciding with nut maturation and harvest in almonds, pistachios, and walnuts (Wilson et al. 2020).

Amyelois transitella is a strong flyer compared to other lepidopteran orchard pests. Flight mill assays have shown *A. transitella* capable of flying 7-15 km in a single night (Sappington and Burks 2014), and mated females tend to fly further than unmated ones (Rovnyak et al. 2018). Inter-orchard movement of *A. transitella* was first demonstrated by Meals and Caltagirone (1971). In the field, reported dispersal distances of *A. transitella* are well below those reported on the flight mill. Andrews et al. (1980) reported upwind movement of *A. transitella* up to 375 m. Pistachios bordered by almonds had increased damage up to 500 m into an orchard due to *A. transitella* immigration (Andrews and Barnes

1982). Indirect evidence of longer flights has been reported in almonds, which were shown to be at a significantly higher risk of *A. transitella* damage if they were within 5 km of pistachio orchards (Higbee and Siegel 2009). Modern techniques have been developed to track the movement of natural populations of *A. transitella* by characterizing fatty acid profiles of adults, which can reflect their larval hosts (Bayes et al. 2014). However, knowledge of larval origin alone does not necessarily help determine the field dispersal range of *A. transitella*, since tree nut orchards are widespread. As such, while the baseline field dispersal range of *A. transitella* remains unknown, it could be studied using mark-release-recapture (MRR) methods.

Mark-release-recapture is a common approach for assessing insect dispersal. The MRR method consists of releasing insects with either an internal marker (e.g., dye incorporated into the larval diet) or an external marker (e.g., fluorescent powder) and then recapturing them at some distance away from the release point. Different designs can be used to target shorter (single trap) or longer (multiple traps) dispersal ranges (Turchin and Odendaal 1996). Mark-release-recapture methods have been used to measure dispersal capabilities of sterilized insects (Suckling et al. 2005, Judd et al. 2011), invasive pests (Kirkpatrick et al. 2018, 2019), study gene transfer (Margaritopoulos et al. 2012), insect conservation (Beck and Linsenmair 2006, Merckx and Slade 2014), and to establish trap radius (Adams et al. 2017). Insects are recaptured with various lures and trap combinations, depending on the species and study system.

Insects can be attracted to visual and odor cues. Light traps attract nocturnal insects through positive phototaxis (i.e., movement towards light). In Lepidoptera, shorter light

wavelengths, UV, blue, and green elicit stronger positive phototaxis (Sambaraju and Phillips 2008, Park and Lee 2017). These light traps are reported to have a small attractive radius, which does not alter natural movement patterns from a great distance (Truxa and Fiedler 2012). However, they require a power source and are generally attractive to a wide range of insects. This can increase the bycatch of non-target species and make sample processing more time- and labor-intensive. Pheromones, particularly synthetic pheromone lures, are an attractive alternative for their ease of use and species specificity. Pheromones are carried on the wind and detected by insect olfactory organs. Sex pheromones are commonly used in these types of lures (Cardé 2007), but aggregation pheromones are available for some organisms, such as *Euschistus* spp. (Heteroptera: Pentatomidae) (Aldrich et al. 1991) and *Leptoglossus zonatus* Dallas (Heteroptera: Coreidae) (Millar et al. 2022). The development of a synthetic pheromone lure is a complex process, typically carried out only for economically important pests.

Mark-release-recapture studies require large numbers of the target organism and an effective lure or trapping system. A synthetic sex pheromone lure for *A. transitella* has been commercially available since 2013 (Higbee et al. 2014). In pistachios, these lures are recommended for monitoring *A. transitella* by placing them in either a wing or delta trap with a sticky liner (Burks and Higbee 2015) at a density of one trap per two ha (five ac) (Haviland et al. 2023). Pheromone lures for *A. transitella* are known to interfere with one another at distances up to 400 m (Burks and Higbee 2013). Furthermore, pheromone lures lose their attractiveness in or near orchards under mating disruption (Burks et al. 2016), which entails flooding an orchard with partial blends of synthetic *A. transitella* sex

pheromones to reduce the ability of male moths to locate females and mate. Currently, it is estimated that approximately 500,000 acres of tree nuts in California use mating disruption (Burks and Thomson 2020). Recently, phenyl propionate (PPO) paired with pheromone lures (i.e. "combo" lures) have been shown to maintain attractiveness to *A. transitella* near or in orchards under mating disruption (Burks 2017), and these have become an important tool for monitoring this pest as the adoption of mating disruption for *A. transitella* continues to expand.

Given the current lack of knowledge of *A. transitella* dispersal in the field, a series of experiments was conducted to (i) compare the trapping radius of blacklight traps to wing traps baited with a combined pheromone and phenyl propionate (PPO) lure (i.e. "combo" lure), (ii) estimate *A. transitella* dispersal in commercial pistachio orchards and (iii) compare the dispersal of locally-reared *A. transitella* to a novel mass-reared *A. transitella* moth. To evaluate the trapping radius, a single centralized trap was used with multiple insect release points radiating outward at increasing distances. In contrast, field dispersal of *A. transitella* was measured with a single insect release point surrounded by a grid of wing traps with the combo lure. These projects aimed to characterize the trapping radius of wing traps baited with the new combo lure, measure *A. transitella* field dispersal, and evaluate the performance of a new mass-reared *A. transitella* for use in a sterile insect technique (SIT) program. Findings from these studies will contribute new information to improve the monitoring and management of this pest.

Materials and Methods

Study #1 - Trapping Radius and Field Dispersal Studies

Field Study 2022: Blacklight Trapping Radius

For this study, mixed-sex (50:50 M:F) *A. transitella* larvae internally marked with Calco red dye N-1700 (Oil Red 2144, Royce Global, East Rutherford, NJ) were acquired from a mass-rearing and irradiation facility in Phoenix, AZ, operated by the USDA Animal Plant Health Inspection Service (APHIS). Late-stage larvae, between their 4th and final 5th instar, were driven (4 h) in a temperature-controlled cooler held at 4 °C from Phoenix, AZ, to UC Riverside (UCR). At UCR, the larvae were reared on standard *A. transitella* diet (Finney and Brinkman 1967) to adulthood within large 60 x 30 x 15 cm plastic trays (RU units) inside 60 x 60 x 60 cm cages (Bug Dorm 2M120, MegaView Science Co., Ltd., Taiwan) in a climate-controlled room set to 26 °C, 60% RH, and a 14:10 (L:D) photoperiod. Adults began to emerge after approximately ten days, and the largest single emergence occurred after twelve days. Adult moths that emerged before the twelfth day were discarded. Only moths on the twelfth day were used in the releases to remove age as a factor. One thousand *A. transitella* were collected with a vacuum-powered aspirator into twenty 16 oz. plastic deli cups (i.e., 50 moths per deli cup). The weight of the moths was recorded to calculate the amount of UV powder needed. All adult *A. transitella* were externally marked with a UV powder (DayGlo Color, Cleveland, OH) at a rate of 1 mg powder for every 200 mg of insects. Before marking, the moths were immobilized via exposure to -20 °C for 4 min, then marked with UV powder by rolling them in a 16 oz. deli

cup. Five UV powder colors were used to represent each of the five specific release distances.

In a fallow field, around a centralized blacklight trap, release points were set at increasing distances up to 45 m in all four cardinal directions (3 m, 6 m, 15 m, 30 m, and 45 m) (Figure 1.1A). Release distances were based on distances from Trux and Konrad (2012b), in which Pyraloidea species were only recaptured with a blacklight at distances <50 m. The blacklight trap (2851M, Bioquip, Rancho Dominguez, CA) used in this study consisted of a five-gallon bucket, a metal funnel, three plastic light-baffling vanes, a 22W circular blacklight bulb (FC8T9/BL), and electronic cords. The bulb was powered by a 12V 26AH DC battery (SigmasTek, Bridgeview, IL) and hung on a shepherd's hook (Shep92HookBlk, Home Depot, Atlanta, GA) at a height of 1.5 m. The blacklight trap was baited with a Vapor Tape II strip (Aberdeen Road Company, Emigsville, PA) at the bottom of the bucket to increase insect retention. The blacklight bulb was on from dusk until dawn (approx. 1930 h – 0600 h), and the battery was charged each day during the three-night recapture period following each release event. Two release events took place, one on 8/1/22 and the other on 8/8/22, with 1,000 *A. transitella* adults released on each date across 20 total release points (i.e., 50 moths per release point per release event).

On the day of the release, 50 *A. transitella* adults were put into a 13 x 8 x 25 cm paper grocery bag that was placed on the ground at each of the 20 release points. Moth recapture in the blacklight trap was then monitored for each of three nights after the release. Each morning at approximately 0600 h, all insects captured in the blacklight trap were

removed and examined with a UV flashlight. All identified marked *A. transitella* were sexed, and release distance was determined by the color of the external dye.

Field Study 2023: Comparing Trapping Radius of Blacklight and Wing Trap with Combo Lure

Here, the trapping radius of a blacklight trap was compared to a wing trap baited with a paired pheromone (Biolure, Suterra, Bend, OR) and PPO lure (PHEROCON®, Trécé Inc., Adair, OK) ("combo lure" hereafter). *Amyelois transitella* larvae were again sourced from the USDA APHIS mass-rearing facility in Phoenix, AZ. In contrast to the previous study, larvae were packaged in an insulated cooler chilled to 4 °C and shipped via FedEx to the UC Kearney Agricultural Research and Extension Center in Parlier, CA. Larvae developed into adults on the standard diet in a climate-controlled greenhouse (26.0 °C ± 3.4 °C, 64.2% ± 8.8%). The greenhouse was exposed to the natural photoperiod, which was 14:10 (L:D) in peak summer and fell to 12:12 in the fall. Adult *A. transitella* were collected and marked with the same methods as in 2022. Only moths that emerged on the twelfth day post-delivery were used. As before, moths were collected with a vacuum-powered aspirator and marked with one of five unique colors in deli cups.

Release distances were increased in this second study, and a second trap and lure type was added to compare trapping radii. The study took place in two similar commercial pistachio orchards that were four miles apart near San Joaquin, CA. Each orchard consisted of 40 ac of 'Golden Hills' variety pistachios under standard irrigation, crop nutrition, pest, and disease control practices. In the center of each 40 ac site, one of two traps was placed.

One site was equipped with a blacklight trap identical to the one used in 2022, and the other with a wing trap containing a combo lure. Release distances ranged up to 150 m (10 m, 20 m, 50 m, 100 m, 150 m) (Figure 1.1B) based on recapture data from 2022.

Six release events took place in each orchard (6 releases x 2 sites = 12 releases total) between July-September 2023 (7/11/23, 7/24/23, 8/14/23, 8/30/23, 9/15/23, and 9/25/23). Release events were staggered every two weeks within a given orchard to prevent overlap with any previously released moths. While the goal for each release event was to have 50 *A. transitella* adults at each of the 20 release points (i.e., 1,000 moths total per release event per site), actual numbers tended to be lower, and so the number of moths per release bag was adjusted accordingly for each event based on the total number of available moths. Following collection and marking of adults for each release point, the marked *A. transitella* were transported to the field in a cooler with ice packs (~1 hr drive time). At each release point, moths were placed into a small 13 x 8 x 25 cm paper grocery bag and suspended at ~1.5 m height in the tree canopy. For each release event, blacklight and wing traps with combo lures were monitored over three consecutive nights. Each day, the traps were checked at ~0900 h. During each trap check, the blacklight was emptied, and the battery was swapped with a fully charged replacement, while for the wing traps, a new sticky liner was installed. The lures of the wing trap were replaced every six weeks.

Field Study 2025: Field Dispersal of A. transitella in Pistachio Orchard

Here, in contrast to the trap radius studies, field dispersal of *A. transitella* was measured with a single point release in the center of a grid of traps to measure outward dispersal.

Adult *A. transitella* moths used in this study were reared in Parlier, CA, by the USDA Agricultural Research Service (ARS). While the ARS moths used here in 2025 were a different strain of *A. transitella* than the ones provided by the USDA APHIS in 2022-2023 (ARS was "Mendota" strain, APHIS was "Phoenix" strain), previous studies have shown that these two strains have equivalent flight performance when reared locally (Reger et al. 2019, 2020). As larvae approached the 4-5th instar, they were moved from the USDA ARS to a greenhouse at UC KARE and held in twelve 1-gal glass jars. The jars were housed in 60 x 60 x 120 cm cages (Bug Dorm 6M620, MegaView Science Co., Ltd., Taiwan) and allowed to move out of the jars into the cages as adults emerged. To collect adult moths, insect cages were moved into a walk-in cooler set at 4 °C for 15 min. Immobilized insects were then poured into a deli cup, weighed, and marked with UV powder as before.

Moth releases took place in a 105 ac commercial pistachio orchard ('Golden Hills' variety) under standard management practices. The trapping grid consisted of 20 wing traps baited with combo lures hung in trees at a height of ~1.5 m. Traps were placed at 8 m, 126 m, 205 m, 297 m, and 338 m away from the release point, with each trap covering approximately 5.25 ac. per trap (Figure 1.2B). Trap liners were switched weekly, and lures were replaced every six weeks. Eight release events, two weeks apart, occurred from April

to October (4/30/25, 5/12/25, 5/27/25, 6/9/25, 6/23/25, 7/7/25, 7/21/25, and 9/15/25). No releases took place in August due to harvest activity in the block.

All marked *A. transitella* adults were transported to field sites in a refrigerated cooler set to 4 °C (Atlas SE, Euhomy, Sacramento, CA). Drive time to sites was 1 h from UC KARE. Moths were placed into a single large 18 x 30 x 40 cm paper grocery bag with approximately twenty-five 4" diameter cardboard tubes positioned vertically within the bag, to provide more vertical resting space for the moths. The paper bag was hung from a tree at ~1.5 m height in the center of the orchard. Moths were released within 2 h before sunset.

Study #2 - Comparing Field Dispersal of Mass-Reared and Locally-Reared *A. transitella*

Field Study 2024

This study was conducted to compare the relative performance of locally-reared *A. transitella* with novel mass-reared and irradiated *A. transitella* that were commercially shipped to UC KARE. The locally-reared moths were derived from APHIS facility larvae shipped to Parlier, CA, as in the 2022-2023 studies. Two studies were conducted in 2024. The density of traps in the trapping grid surrounding the release point differed between these two studies. See Table 1.1 for details comparing the two study designs.

In both studies, mass-reared moths were produced by the USDA APHIS mass-rearing facility in Phoenix, AZ. Larvae were reared on a standard NOW diet. Adults were collected in a custom cold collection system and held at between 2-10 °C for up to 24 h before irradiation via exposure to 300 Gy of gamma radiation from a $Cobalt^{60}$ core in

vented paper deli cups. The mass-reared and irradiated moths were then shipped overnight (30 h) via a commercial carrier. Mass-reared adults arrived chilled and were weighed and marked with UV powder upon arrival. Mass-reared moths were released on a single night because they only shipped to UC KARE once day a week. They were released with locally-reared moths on the second night of the release period.

For both studies, locally-reared moths were derived from late-stage larvae shipped to UC KARE (Parlier, CA) from the USDA APHIS mass-rearing facility in Phoenix, AZ. As before, these larvae completed development in a climate-controlled greenhouse at UC KARE. Adults were collected and released on the tenth, eleventh, and twelfth day after delivery. The collection of locally-reared moths was altered by placing the cage of freshly emerged adults in a walk-in cooler set at 4 °C for 15 min. Immobilized insects were poured into a deli cup, weighed, and marked with UV powder.

Two colors of powder were used to denote the locally- and mass-reared moths. Release populations from each treatment were roughly equal in abundance, as determined by weight. Subsamples of moths were collected to evaluate the sex ratio (local- and mass-reared), as well as the mating status of females (locally-reared only). All marked *A. transitella* adults were transported to field sites in a refrigerated cooler set to 4 °C (Atlas SE, Euhomy, Sacramento, CA). Drive time to sites was 1 h from UC KARE. Moths were placed into two 18 x 30 x 40 cm paper grocery bags (one bag each for locally- and mass-reared moths) filled with twenty 4” diameter cardboard tubes, which were hung from a tree at a height of ~1.5 m in the center of the orchard. Cardboard tubes were added to increase

the surface area for moths to perch on before flight. Moths were released within 2 h of sunset.

At the field site, a single release point surrounded by a grid of wing traps baited with combo lures was used to track *A. transitella* dispersal (Figure 1.2A). Five release events took place from August to October (8/12/24, 8/26/24, 9/9/24, 9/23/24, and 10/7/24) in a 155 ac 'Golden Hills' variety pistachio orchard under standard management practices. For the first study (two release events), 16 traps were placed at 8 m, 126 m, 205 m, 297 m, and 338 m away from the release point, with each trap covering approximately 9.6 ac. per trap (low-density grid, Figure 1.2A). For the second study (three release events), 28 traps were positioned at 8 m, 128 m, 184 m, 260 m, 280 m, 302 m, and 392 m from the release point, with each trap covering approximately 5.5 ac per trap (high-density grid, Figure 1.2A). Following each release event, the traps were checked every 7 days over a 2-week period. The combo lures were placed every six weeks. During each trap check, the sticky liners were replaced, and all collected moths were counted, sexed, and determined to be either wild, locally- or mass-reared.

In addition to these field assays, the flight ability of the locally- and mass-reared moths was evaluated in flight cylinder assays. Flight cylinders consisted of PVC tubes 20 x 20 cm internally coated with Insect-a-Slip (Bioquip, Rancho Dominguez, CA) placed within a 30 x 30 x 30 cm cage (Bug Dorm 4H3030, MegaView Science Co., Ltd., Taiwan). In each cylinder, 10 male *A. transitella* of a single type (i.e., locally- or mass-reared) were placed. After 24 h, the number of moths that had escaped the cylinder was recorded. An assay was conducted with each cohort from each release event, and each treatment was

replicated three times per assay. All assays were conducted under a 14:10 L:D photoperiod with an ambient temperature of 28 °C and 50%RH.

Statistical Analysis

Study #1 - Trapping Radius and Field Dispersal Studies

Data from the studies in 2022-2023 to evaluate trapping radius were summarized, but no statistical tests were performed due to low recapture rates. In the field dispersal study in 2025, logistic regression was used to evaluate the proportion of marked moths recaptured at each sample distance with a binomial distribution and logit link function (*glmer* function in the 'lme4' package) in the R statistical program, version 4.5.1 (https://www.r-project.org/). Trap distance was a categorical fixed effect with release period included as a random effect. The effect of trap distance on moth recapture was evaluated with likelihood ratio tests using the *drop1* function, which generated the χ^2 and *P*-values reported here.

Study #2 - Comparing Field Dispersal of Mass-Reared and Locally-Reared A. transitella

For the field comparison of mass-reared and locally-reared moths in 2024, only data from the last three releases with the higher trap density grid were compared because not all recapture distances were present in the two low trap density release periods. Logistic regression with a binomial error distribution and logit link function was again used to evaluate the proportion of recaptured marked moths (*glmer* function in the 'lme4' package). While the initial model included an interaction effect between "trap distance"

and “moth type” (i.e., mass-reared or locally-reared), no interaction was detected and so these were modeled instead as individual fixed effects, with release period as a random effect. The effect of trap distance and moth type on moth recapture was evaluated with likelihood ratio tests using the *drop1* function, which generated the χ^2 and *P*-values reported here.

Flight cylinder data in 2024 on emergence of local and mass-reared *A. transitella* was compared across all release cohorts with a two-sample t-test (*t.test* function in base R).

Results

Study #1 - Trapping Radius and Field Dispersal Studies

Trapping Radius – Blacklight Trap Alone (2022)

In 2022, a total of 2,000 (1,000 per release period) *A. transitella* were released. All recaptures of *A. transitella* took place on the first night following release. For the first release event, 0.9% of the total *A. transitella* released were recaptured, and 25% never left the release bags (Table 1.2). Moths were recaptured at only two of the distances, 0.8% at 6 m, and 0.1% at 15 m, based on the total number of moths released. Following the second release event, the total recapture rate increased to 4.4%, with some recaptures occurring at all five distances, and only 2.9% left in the release bags. The highest recapture rates occurred at 3 m and 6 m distances, 1.6% and 1.8%, respectively. The remaining 0.8% were divided among the 15 m (0.5%), 30 m (0.1%), and 45 m (0.2%) trapping points.

Trapping Radius – Blacklight vs. Wing Trap with Combo Lure (2023)

In 2023, a total of 8,840 *A. transitella* were released (737 ± 105 moths per release event per orchard). Only the first, third, and fifth (7/11/23, 8/14/23, and 9/15/23) release

events resulted in recapture of any released *A. transitella* (Tables 1.3-1.4). The combo lure attracted *A. transitella* from 10 m, 50 m, and 100 m compared to only 10 m for the blacklight (Table 1.4).

Field Dispersal (2025)

In 2025, a total of 20,513 *A. transitella* were released with an average of 2,564 ± 620 moths per release event. In the final model there was a significant effect of trap distance on moth recapture ($\chi^2 = 179.6$, df = 4, $P < 0.001$). 70.2% of the females released were mated, and there was a 1:1.2 (M:F) sex ratio. Overall recapture rate was 0.41%, which ranged from 0.09% to 2.02% (Table 1.5). No *A. transitella* were recaptured at a trap distance beyond 205 m.

Study #2 - Comparing Field Dispersal of Mass-Reared and Locally-Reared *A. transitella*

In 2024, a total of 61,478 (29,330 locally-reared and 32,418 mass-reared) *A. transitella* were released across five release events in a single orchard (10,998 ± 2,472 locally-reared and 13,081 ± 6,579 mass-reared per release event). 52.7% of locally-reared females were found to be mated (mass-reared moths were not checked for mating status), with a M:F sex ratio of 1:1.2 (locally-reared) and 1:0.8 (mass-reared). Across all five releases, 0.38% of locally-reared and 0.11% of mass-reared adults were recaptured.

For the first two release events (using a low-density trap grid), a total of 20,322 *A. transitella* were released (7,332 locally-reared and 12,991 mass-reared). Overall recapture

rate with the low-density trap grid was 0.43% and 0.18% for locally-reared and mass-reared moths, respectively (Table 1.6). For the last three releases (using a high-density trap grid), a total of 41,156 *A. transitella* were released (21,998 locally-reared and 19,456 mass-reared). The average recapture rate with the high-density trap grid was 0.38% and 0.11% for locally-reared and mass-reared moths, respectively (Table 1.7).

For the high-density trapping, significantly more locally-reared *A. transitella* were recaptured than the mass-reared moths ($\chi^2 = 25.633$, df = 1, $P < 0.001$). For both types of moths, there was a significant effect of trap distance ($\chi^2 = 129.449$, df = 6, $P < 0.001$), with more recaptured closer to the release point than at greater distances (Table 1.7)

Results from the flight cylinder assays found apparent performance differences between the locally-reared and mass-reared moths. On average, 97% of locally-reared adults flew out of the flight cylinders after 24 h, which was significantly higher than mass-reared adults at 63% ($t = 5.389$, df = 14, $P < 0.001$).

Discussion

Mark-release-recapture studies can be a useful way to better understand insect dispersal and improve monitoring strategies. Here, a series of field studies demonstrated how different moth types, release methods, and trapping strategies can influence the recovery of marked *A. transitella* adults in commercial pistachio orchards.

Trapping Radius Studies (2022-2023)

Low attractive radius of the blacklight trap

Findings from the trap radius studies demonstrated that blacklight traps have a very small range of attraction compared to wing traps baited with a pheromone and phenyl propionate "combo" lure. All *A. transitella* recaptured in the blacklight traps were from distances <50 m, with the majority from <10 m. This follows previous MRR studies with blacklight traps, where recapture rates have been shown to diminish below 1% at 50 m for moth species from several lepidopteran families, such as Noctuidae and Sphingidae (Beck and Linsenmair 2006), Geometridae and Pyraloidea (Truxa and Fiedler 2012b), and other macro-moth families (Merckx and Slade 2014). Wing traps baited with combo lures were able to attract *A. transitella* for distances 10-fold (i.e., 100 m) greater than the blacklight in the same period. While these results highlight the limited attractive range of blacklight traps, environmental context may further influence trap performance.

Differential response to the blacklight trap in the fallow field vs. the orchard environment

In contrast to Merckx and Slade (2014), who also released insects in a fallow field, our study found more moths recaptured in the blacklight trap in the fallow field than in an orchard. One possible explanation is that more *A. transitella* were released at <50 m in the fallow field than in the pistachio orchard (Table 1.1). Alternatively, *A. transitella* flight behavior could have influenced recapture. The height at which *Manduca* spp. were caught in blacklight traps was a good indicator of the height at which they were flying (Stewart and Lam Jr 1968). In other words, moths were attracted to the light they could see most directly while in flight. In 2022, moth release bags were on the ground instead of hanging in the trees. As moths left the bags, they often flew upward, which may have brought them

into direct view of the blacklight trap in the fallow field. In contrast, *A. transitella* in an orchard would have flown above the light trap because they were released at the same height as the blacklight. Beyond structural differences between habitats, factors such as temperature may also influence moth activity and recapture rates.

Merckx and Slade (2014) proposed that temperature played a role in their study, with cooler nights reducing recapture. However, in our study, nighttime temperatures were similar at both sites (23.32 ± 0.25 °C) and regularly exceeded the 13 °C threshold for *A. transitella* flight (Landolt and Curtis 1982). During cooler nighttime temperatures, female *A. transitella* will call, and males will fly earlier (Burks et al. 2011). However, the warm nights in all locations indicate *A. transitella* should have behaved similarly. Given the similarity in temperature conditions between sites, other ecological factors likely contributed to the observed differences in recapture.

Competition was likely more intense in the orchard than in the fallow field due to the prior presence of wild *A. transitella* and host plant cues present in orchard sites. Calling females of wild *A. transitella* compete with traps while ripening nuts produce plant volatiles that are attractive to male and gravid female *A. transitella*, respectively (Beck et al. 2012, Burks and Higbee 2013). Pheromones and kairomones have been reported to elicit a stronger response than light from studies with other pyralids. For instance, significantly higher numbers of *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae) were caught with pheromone lures and food bait compared to UV light traps (Sambaraju and Phillips 2008). This is likely the cause for the greater attraction radius of the combo lure compared to the blacklight in this study.

Variability in field recapture rates

There was high variability in recapture rates across the various release events. Aside from variability caused by insect behavior following release, human handling of the insects in a conventionally managed agroecosystem could have hampered moth recapture, as follows.

In 2022, after the first release, the low recapture rate and high mortality may have been due to physical damage caused by the vacuum-powered aspirator. The force of the aspirator was reduced from -0.98 kPa to -0.40 kPa for the second release. Second release mortality reduced from 25% to 2.9%, and the percentage recapture of *A. transitella* increased by 3.3% (from 0.9% to 4.4%). Beyond physical handling, chemical management practices in the field may have also further impacted moth survival and recapture.

Forty-eight hours before the fourth release event in 2023, the insecticides methoxyfenozide (Intrepid 2F®, Corteva) and bifenthrin (Sniper®, Loveland Products) were applied to the pistachio trees. Bifenthrin is a contact insecticide with a strong residual effect, which may have contributed to the lack of recapture for that release event.

Field Dispersal of *A. transitella* (2025)

The mean total percent recapture across all release events was 0.41%, and the furthest distance of recapture was 205 m (Table 1.5). This was an improvement and clearer demonstration of *A. transitella* dispersal potential in the field compared to previous years. However, it was lower compared to similar studies with lepidopteran pests. In one study, codling moth *Cydia pomonella* L. (Lepidoptera: Tortricidae) percent recapture ranged from

10-30% (male) and 3.5-20% (female) with pheromone and pear ester lures, respectively (Margaritopoulos et al. 2012). In the Margaritopoulos et al. study, sexes were released separately, which may have accounted for the high percent recapture. In another study (Judd et al. 2011), mixed-sex release of mass-reared and irradiated *C. pomonella* were recaptured at a rate of 3.9-5.0% in 10-13 ac apple orchards. In flight mill assays, *A. transitella* flew a greater distance in a single night than *C. pomonella* (Schumacher et al. 1997, Sappington and Burks 2014), so the lower recapture rate found here was unexpected.

Trap density is an important factor in MRR studies. Wing traps with combo lures were used at a rate of 5.25 ac/trap. Other lepidopteran studies with higher percent recapture had higher trap densities (Sanders 1983, Judd et al. 2011). In contrast, lower densities such as 1 trap / 20 ac (*C. pomonella*) (Adams et al. 2017) and 1 trap / 71 ac (*T. anartoides*) (Suckling et al. 2005) have resulted in similar or higher recapture rates relative to those in the present study. At the current trap density in this study, lures were likely interfering with each other (Burks and Higbee 2015) and potentially lowering the recapture rate per trap. A higher trap density may have increased recapture, but it was not feasible due to the time and effort to replace liners in the field.

Comparison of Locally-Reared and Mass-Reared Moths (2024)

Recapture Rates

We observed a total percent recapture rate of 0.38% and 0.11% for locally-reared and mass-reared *A. transitella*, respectively. While some individuals of both moth types were recaptured at the furthest trap distance (392 m), locally-reared moths were found more

frequently (0.03%) than mass-reared (0.01%). The flight cylinder assays are in line with field data and validate that mass-reared moths appeared less fit, since fewer of them exited the cylinders. However, the mechanisms for these negative effects remain unclear.

Performance of Mass-Reared Moths- temperature

Locally-reared moths appeared to outperform mass-reared moths in both field and laboratory assays. Previous studies have shown that both the flight potential and mate-seeking behavior of similarly mass-reared *A. transitella* adults were reduced (Reger et al. 2020, 2021). The exact mechanisms for this reduced fitness are unknown, and the effects of mass-rearing, exposure to cold temperatures, and irradiation have not been parsed. Both local and mass-reared *A. transitella* were reared at constant optimal temperatures, but upon emergence in the USDA APHIS facility, the mass-reared adult *A. transitella* was aggregated through a vacuum collection system and then rapidly chilled and held at 2–10 ◦C for over 24 h. Furthermore, mass-reared moths were held for roughly 30 h at temperatures <5 °C. Cold temperatures have been observed to negatively affect male *Thaumatotibia leucotreta* Meyrick (Lepidoptera: Torticidae), where the flight performance of males was reduced under field conditions after exposure to cold storage temperatures between 4 °C and 6 °C (Boersma and Carpenter 2016). Adult *Manduca sexta* L. (Lepidoptera: Sphingidae) were more cold-tolerant than larvae; recovery time from chill comas increased with prolonged exposure to 0 °C (Andersen et al. 2017). Mass-reared *A. transitella* in this study were held under chilled conditions for over 30 h until released in the field, so a delayed response from mass-reared moths may be present. In contrast,

locally-reared adults were only held for 3 h before release. However, no *A. transitella* from either treatment was recaptured after the first week. *Teia anartoides* Walker (Lepidoptera: Lymantriidae) recovery declined from 30% to 10% over a week (Suckling et al. 2005), so perhaps checking traps more frequently would have been a better approach.

Conclusions

These studies provide new insights into the local sampling radius of both blacklight traps and wing traps baited with a combo lure, as well as the dispersal capabilities of *A. transitella* in the field and the relative performance of a novel mass-reared *A. transitella* moth.

Blacklight traps can be useful tools for hyperlocal (<10 m radius) sampling, but they may not be well-suited for studying the dispersal of *A. transitella*, especially in an orchard setting where perception of the blacklight trap is significantly reduced relative to a fallow field. Wing traps with a combo lure, in contrast, appear to attract *A. transitella* from as far as 100 m away, and as seen here, were used in subsequent field dispersal studies. Additional studies would be useful to explore the relative trapping radius of different trap types (e.g., wing vs. delta traps) and lures (e.g., ovibait, pheromone alone, and in combination with PPO), as well as to compare outcomes in different orchard types (e.g., almond, pistachio, walnut).

The observed dispersal distances of *A. transitella* in the field were consistently below the potential nightly flight ranges reported from flight mill studies, which is not surprising given the artificial environment of a flight mill assay. However, the overall low

recapture rate of *A. transitella* in this study, which may have, in some cases, been due to moth handling methods, prevents us from drawing definitive conclusions about the true field dispersal range of *A. transitella* across this series of studies. Additional work with an increased number of release events and fewer confounding variables is still needed to fully characterize *A. transitella* movement under field conditions.

Finally, a novel mass-reared *A. transitella* moth used for an SIT program did appear to exhibit reduced performance compared to a locally-reared moth. Efficacy of SIT is contingent on the ability of mass-reared sterile moths to compete with wild moths. While here the mass-reared moths were not compared directly to wild *A. transitella* moths, but rather a locally-reared laboratory strain, it does raise questions about the competitive abilities of these mass-reared *A. transitella*. Future studies in this area will need to differentiate the effects of the mass-rearing, irradiation, and transportation processes on the performance of these novel sterile moths. Notably, the dispersal of the locally-reared moths in the 2024 and 2025 studies remained consistent.

Tables and Figures

Table 1.1. Summary of experimental design and *A. transitella* source 2022 to 2025

Study	Year	Site	Trap	Trap Design	Insect Source and Handling				Dates	No. of Moths
					Larvae	Adults	Irr	Ship		
#1	2022	Fallow	B[3]	Center	Phx	Local	N	N	8/1-8/8/22	1,000 ± 0
	2023	Pistachio	B+C[4]	Center	Phx	Local	N	N	7/11-9/30/23	1,473 ± 210
#1	2025	Pistachio	C	Grid[2]	Local	Local	N	N	4/30-10/13/25	2,564 ± 620
#2	2024	Pistachio	C	Grid[1]	Phx	Local	N	N	8/12-8/26/24	3,666 ± 802
					Phx	Phoenix	Y	Y	8/12-8/26/24	6,496 ± 3,696
	2024		C	Grid[2]	Phx	Local	N	N	9/11-10/7/24	7,332 ± 1,670
					Phx	Phoenix	Y	Y	9/11-10/7/24	6,585 ± 2,883

[1]low-density 9.6 acres/trap; [2]high-density 5.5 acres/traps; [3]blacklight trap; [4]wing trap with combo lure.

Table 1.2. Summary percent recovery of the 2022 single-trap multiple-release study: blacklight only

Date	No. of NOW Released	Percent Recaptured					
		3 m	6 m	15 m	30 m	45 m	Total
8/1/22	1,000	-	0.8	0.1	-	-	0.9
8/8/22	1,000	1.6	1.8	0.7	0.1	0.2	4.4
Total	2,000	1.6	2.6	0.8	0.1	0.2	5.8

Dashes indicated zero percent recapture.

Table 1.3. Percent *A. transitella* recaptured in blacklight traps at increasing distances away from the release point after a single night.

Date	No. of moths	Percent Recaptured					
		10 m	25 m	50 m	100 m	150 m	Total
7/11/23	1,000	-	-	-	-	-	-
7/24/23	250	-	-	-	-	-	-
8/14/23	725	0.21	-	-	-	-	0.21
8/30/23	975	-	-	-	-	-	-
9/15/23	750	0.13	-	-	-	-	0.13
9/30/23	720	-	-	-	-	-	-
Total	4,420	0.33	-	-	-	-	0.89

Dashes indicated zero percent recapture.

Table 1.4. Percent *A. transitella* recaptured in wing traps with combo lure at increasing distances away from the release point after a single night.

Date	No. of moths	Percent recaptured					
		10 m	25 m	50 m	100 m	150 m	Total
7/11/23	1,000	0.20	-	-	-	-	0.20
7/24/23	250	-	-	-	-	-	-
8/14/23	725	-	-	0.14	0.21	-	0.35
8/30/23	975	-	-	-	-	-	-
9/15/23	750	-	-	-	-	-	-
9/30/23	720	-	-	-	-	-	-
Total	4,420	0.20	-	0.14	0.21	-	0.89

Dashes indicated zero percent recapture.

Table 1.5. Summary of the 2025 *A. transitella* field dispersal study

		Percent recaptured at:					
Date	**No. of moths**	**8 m**	**126 m**	**205 m**	**297 m**	**338 m**	**Total**
4/30/25	643	2.02	-	-	-	-	2.02
5/12/25	257	0.39	-	-	-	-	0.39
5/27/25	2,313	0.09	-	-	-	-	0.09
6/9/25	1,085	0.28	-	0.09	-	-	0.37
6/23/25	2,648	0.19	0.04	-	-	-	0.23
7/7/25	3,106	0.26	-	-	-	-	0.26
7/21/25	5,651	0.20	0.05	-	-	-	0.25
9/15/25	4,810	0.59	0.10	0.08	-	-	0.77
Total	20,513	0.35	0.04	0.02	0.00	0.00	0.41

Dashes indicated zero percent recapture.

Table 1.6. Percent locally-reared and mass-reared *A. transitella* recaptured in wing traps with combo lure in 2024 (low-density grid) at increasing distances away from the release point after a single night.

Date	No. of moths	Moth Type	Percent recaptured at:							Total
			8 m	128 m	184 m	260 m	280 m	302 m	392 m	
8/12/24	4,468	Local	-	0.05	-	-	0.11	0.07	0.13	0.36
8/26/24	2,864	Local	-	-	-	-	0.07	-	-	0.07
Total	7,332		-	0.05	-	-	0.18	0.07	0.13	0.43
8/12/24	10,191	Mass	-	0.02	-	-	0.03	-	0.02	0.07
8/26/24	2,800	Mass	-	-	-	-	0.11	-	-	0.11
Total	12,991		-	0.02	-	-	0.14	-	0.02	0.18

*Data were not sufficient for analysis. Dashes indicated zero percent recapture.

Table 1.7. Percent locally-reared and mass-reared *A. transitella* recaptured in wing traps with combo lure in 2024 (high-density grid) at increasing distances away from the release point after a single night.

Date	No. of moths	Moth Type	Percent recaptured at: 8 m	128 m	184 m	260 m	280 m	302 m	392 m	Total
9/11/24	5,668	Local	0.07	-	-	-	-	-	-	0.07
9/23/24	10,675	Local	0.34	0.11	0.13	-	-	0.01	0.01	0.60
10/7/24	5,655	Local	0.14	0.14	0.04	0.02	0.07	0.02	0.04	0.46
Total	29,330		0.16	0.08	0.06	0.00	0.04	0.02	0.03	0.38
9/11/24	3,636	Mass	0.03	-	-	-	-	-	-	0.03
9/23/24	3,565	Mass	0.17	-	0.03	-	-	-	0.03	0.23
10/7/24	12,255	Mass	0.05	0.01	0.02	-	0.02	0.02	0.01	0.13
Total	32,418		0.04	0.01	0.01	-	0.02	0.02	0.01	0.11

Dashes indicated zero percent recapture.

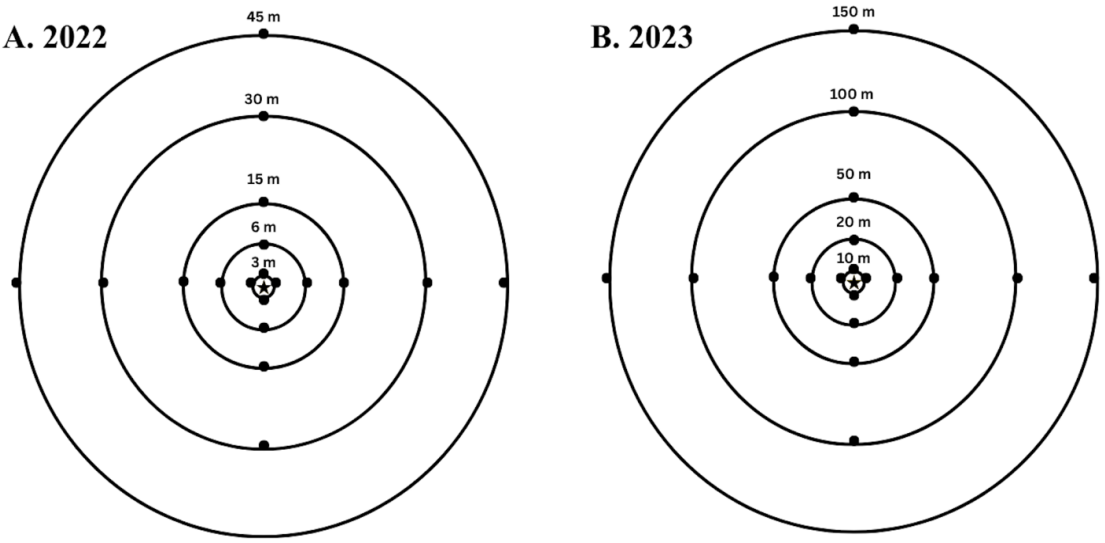

Figure 1.1. Trap layout for single-trap studies in (A) 2022 in a fallow field and (B) 2023 in a pistachio orchard.

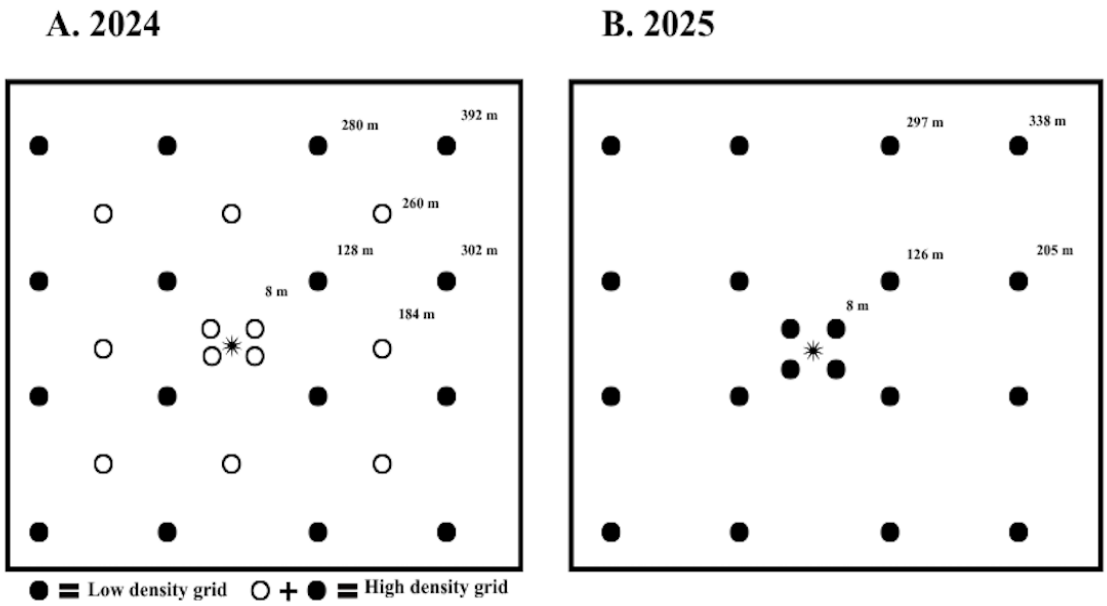

Figure 1.2. Trap layout for multiple-trap-single-release studies in (A) 2024, high trap density, and (B) 2025. The star in the center of each design represents the release point.

References

Adams CG, Schenker JH, McGhee PS, et al. 2017. Maximizing information yield from pheromone-baited monitoring traps: estimating plume reach, trapping radius, and absolute density of *Cydia pomonella* (Lepidoptera: Tortricidae) in Michigan apple. *J. Econ. Entomol.* 110:305–318.

Aldrich JR, Hoffmann MP, Kochansky JP, et al. 1991. Identification and attractiveness of a major pheromone component for Nearctic *Euschistus* spp. stink bugs (Heteroptera: Pentatomidae). *Environ. Entomol.* 20:477–483.

Andersen MK, Jensen SO, Overgaard J. 2017. Physiological correlates of chill susceptibility in Lepidoptera. *J. Insect Physiol.* 98:317–326.

Andrews KL, Barnes MM. 1982. Invasion of pistachio orchards by navel orangeworm moths from almond orchards. *Environ. Entomol.* 11:278–279.

Andrews KL, Barnes MM, Josserand SA. 1980. Dispersal and oviposition by navel orangeworm moths. *Environ. Entomol.* 9:525–529.

Bayes, S. K., M. K. Hellerstein, M. Fitch, et al. 2014. You are what you eat: fatty acid profiles as a method to track the habitat movement of an insect. Oecologia. 175: 1073–1080.

Beck J, Linsenmair KE. 2006. Feasibility of light-trapping in community research on moths. *J. Res. Lepidoptera.* 39:18–37.

Beck JJ, Higbee BS, Light DM, et al. 2012. Hull split and damaged almond volatiles attract male and female navel orangeworm moths. *J. Agric. Food Chem.* 60:8090–8096.

Boersma N, Carpenter JE. 2016. Influence of holding temperature and irradiation on field performance of mass-reared *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae). *Fla. Entomol.* 99:215–221.

Burks CS. 2017. Combination phenyl propionate/pheromone traps for monitoring navel orangeworm in almonds in the vicinity of mating disruption. *J. Econ. Entomol.* 110:438–446.

Burks CS, Brandl DG, Higbee BS. 2011. Effect of photoperiod and temperature on mating in navel orangeworm. *J. Insect Sci.* 11:48.

Burks CS, Higbee BS. 2013. Effect of abundance on sampling range and trap interference. *Environ. Entomol.* 42:143–149.

Burks CS, Higbee BS. 2015. Impact of trap design and density on pheromone monitoring. *J. Econ. Entomol.* 108:600–610.

Burks CS, Kuenen LPS, Daane KM. 2016. Phenyl propionate and sex pheromone for monitoring navel orangeworm. *J. Econ. Entomol.* 109:958–961.

Burks CS, Thomson DR. 2020. Factors affecting mating disruption using aerosol dispensers. *J. Econ. Entomol.* 113:1290–1298.

Cardé RT. 2007. Using pheromones to disrupt mating of moth pests. In: *Perspectives in ecological theory and integrated pest management*, pp. 122–169.

CDFA) California Department of Food and Agriculture. 2024. California Agricultural Statistics Review 2023-2024. California Department of Food and Agriculture, Sacramento, CA.

Demkovich M, Dana CE, Siegel JP, et al. 2015a. Effect of piperonyl butoxide on insecticide toxicity. *J. Econ. Entomol.* 108:2753–2760.

Demkovich M, Siegel JP, Higbee BS, Berenbaum MR. 2015b. Mechanism of resistance acquisition in *Amyelois transitella*. *Environ. Entomol.* 44:855–863.

Finney GL, Brinkman D. 1967. Rearing the navel orangeworm in the laboratory. *J. Econ. Entomol.* 60:1109–1111.

Higbee BS, Burks CS, Larsen TE. 2014. Persistent pheromone lure for navel orangeworm. *Insects.* 5:596–608.

Higbee BS, Siegel JP. 2009. New sanitation standards could reduce almond damage. *Calif. Agric.* 63:24-28.

Judd GJR, Arthur S, Deglow K, et al. 2011. Mark–release–recapture tests of codling moth. *Can. Entomol.* 143:300–316.

Kirkpatrick DM, Acebes-Doria AL, Rice KB, et al. 2019. Estimating trap plume reach for *Halyomorpha halys*. *Environ. Entomol.* 48:1104–1112.

Kirkpatrick DM, Gut LJ, Miller JR. 2018. Trap plume reach for *Drosophila suzukii*. *J. Econ. Entomol.* 111:1285–1289.

Kuenen LPS, Siegel JP. 2011. Developmental rate of navel orangeworm on pistachio. *J. Econ. Entomol.* 104:532–539.

Landolt PJ, Curtis CE. 1982. Temperature effects on circadian rhythm. *Environ. Entomol.* 11:107–110.

Light DM, Ovchinnikova I, Jackson ES, et al. 2015. Effects of X-ray irradiation on navel orangeworm. *J. Econ. Entomol.* 108:2200–2212.

Margaritopoulos JT, Voudouris CC, Olivares J, et al. 2012. Dispersal ability in codling moth. *Agric. For. Entomol.* 14:399–407.

Meals DW, Caltagirone LE. 1971. Invasion rate of navel orangeworm. *J. Econ. Entomol.* 64:90–92.

Merckx T, Slade EM. 2014. Macro-moth attraction to light. *Insect Conserv. Divers.* 7:453–461.

Millar JG, Zou Y, Hall DR, et al. 2022. Identification of leptotriene in leaffooted bugs. *J. Nat. Prod.* 85:2062–2070.

Palumbo JD, Mahoney NE, Light DM, et al. 2014. Spread of *Aspergillus flavus* by navel orangeworm. *Plant Dis.* 98:1194–1199.

Park JH, Lee HS. 2017. Phototactic response of insects to LEDs. *Appl. Biol. Chem.* 60:137–144.

Reger JE, Wenger J, Brar G, et al. 2020. Response of irradiated navel orangeworm to pheromone extract. *Insects.* 11:703.

Reger JE, Wenger JA, Brar G, et al. 2021. Flight performance of irradiated navel orangeworm. *J. Econ. Entomol.* 114:1542–1548.

Rovnyak AM, Burks CS, Gassmann AJ, et al. 2018. Mating, flight, and fecundity relationships. *Entomol. Exp. Appl.* 166:304–315.

Sambaraju KR, Phillips TW. 2008. Responses of *Plodia interpunctella* to light. *J. Insect Behav.* 21:422–439.

Sanders CJ. 1983. Local dispersal of spruce budworm. *Can. Entomol.* 115:1065–1070.

Sappington TW, Burks CS. 2014. Flight behavior of navel orangeworm. *Environ. Entomol.* 43:696–705.

Schumacher P, Weyeneth A, Weber DC, et al. 1997. Flight mill studies in *Cydia pomonella*. *Physiol. Entomol.* 22:149–160.

Siegel JP, Kuenen LPS, Ledbetter C. 2010. Development rate on wheat bran and almonds. *J. Econ. Entomol.* 103:1250–1257.

Stewart PA, Lam JJ Jr. 1968. Insect catch at different trap heights. *J. Econ. Entomol.* 61:1227–1230.

Suckling DM, Charles J, Allan D, et al. 2005. Performance of irradiated *Teia anartoides*. *J. Econ. Entomol.* 98:1531–1538.

Truxa C, Fiedler K. 2012. Attraction to light in moths. *Eur. J. Entomol.* 109:77–84.

Turchin P, Odendaal FJ. 1996. Sampling area of pheromone traps. *Environ. Entomol.* 25:582–588.

Wade W. 1961. Biology of the navel orangeworm. *Hilgardia.* 31:129–171.

Wilson H, Burks CS, Reger JE, et al. 2020. Biology and management of navel orangeworm. *J. Integr. Pest Manag.* 11:25.

Chapter 2: Evaluation of novel intrinsic markers to identify the larval host plant of adult *Amyelois transitella* (Lepidoptera: Pyralidae)

Abstract

Understanding insect movement is critical for the development of effective integrated pest management (IPM) programs, especially for highly mobile polyphagous pests such as the navel orangeworm, *Amyelois transitella* (Lepidoptera: Pyralidae). While mark-release-recapture (MRR) studies can be used to characterize movement, they can be time and labor-intensive to rear and mark many insects or the plants they interact with, plus artificial markers can interfere with insect behavior. One alternative is the use of intrinsic markers, which rely on detecting a unique signature in the organism associated with its larval host. While previous studies have used fatty-acid profiles to identify *A. transitella* adults that developed on walnuts, they could not differentiate almond and pistachio signatures. As such, to fully leverage this tool for research, additional methods are needed. Here, multiple intrinsic markers were evaluated for their ability to clearly determine the larval host plant of *A. transitella* reared on almond, pistachio, and walnut. Methods included compound-specific isotope analysis (CSIA) of amino acids, untargeted metabolomics and lipidomics, and targeted quantification of lutein. CSIA revealed significant differences in $\delta^{13}C$ and $\delta^{15}N$ amino acid profiles among host plant material and the *A. transitella* reared on those plants. However, isotopic variation was influenced by environmental and management factors, limiting its reliability for field applications. Metabolomics and lipidomics identified hundreds to thousands of features. While polar metabolites provided moderate to high classification accuracy for plant and insect samples, nonpolar lipids were the strongest classifier. Unfortunately, accuracy with lipids declined

when the model was applied to field-collected insects of unknown origin, likely due to variation in age, diet, and insecticide exposure. Finally, lutein was shown to be highly concentrated in both pistachio kernels and pistachio-reared *A. transitella*. High-performance liquid chromatography (HPLC) offered greater sensitivity and specificity than spectrophotometric methods, although the latter offers a more rapid and cost-effective approach. Overall, no single method reliably determined larval host origin across all conditions. However, combining targeted biomarkers such as lutein with broader molecular approaches, such as fatty-acid profiles, offers a promising framework for tracing pest origin and movement. These findings provide new tools for studying *A. transitella* dispersal to improve regional IPM strategies for this pest in California tree nut systems.

Introduction

Effective integrated pest management (IPM) relies on a clear understanding of insect biology, phenology, and economic injury thresholds paired with accurate monitoring that then informs the timely implementation of cultural, mechanical, biological, and/or chemical management interventions (Stern et al. 1959, Radcliffe et al. 2009). Unfortunately, for any individual grower, even the most effective on-farm management strategies can be undermined by colonization of pests from nearby locations, especially when dealing with a highly mobile polyphagous species. In response, area-wide IPM programs have been developed to monitor and manage arthropod pests at the regional or landscape scale (Vreysen et al. 2007). Key to this approach is a clear understanding of the timing, extent, and catalyzing forces of insect dispersal.

To do this, many researchers have conducted mark-release-recapture (MRR) studies that rely on the use of artificial internal and/or external markers (Hagler and Jackson 2001), such as fluorescent powders (Logan and Proverbs 1975, Arredondo et al. 2017), fat-soluble dyes (Naranjo 1990), protein immunomarkers (Hagler 2019), harmonic radar (Walter et al. 2021, Siderhurst et al. 2025), physical marking (Morton 1982), or UV-reactive fluorophores (Hagler et al. 2024, Liu et al. 2025). Yet, these methods have many challenges and limitations. For instance, they can have detrimental effects on insect behavior and fitness (Moffitt and Albano 1972), typically require large numbers of insects for marking (Adams et al. 2017), and the markers may interfere with insect behavior or degrade in the environment over time (Hagler et al. 2024, Liu et al. 2025). Alternatively, markers can also be applied to the environment and picked up by insects as they interact

with the marked plants (Hagler and Machtley 2020). However, this too can be very labor-intensive, as well as difficult to scale over large areas with a wide range of potential hosts. In contrast, studying existing insect populations using intrinsic biomarkers from their natural diet may offer a way to overcome such limitations.

The navel orangeworm, *Amyelois transitella* Walker (Lepidoptera: Pyralidae), is the most significant insect pest in California almond, pistachio, and walnut production (Wilson et al. 2020). Larvae hatch from eggs laid on the developing new crop nuts and feed directly on the kernel. This damage reduces crop yield and quality, increases crop vulnerability to aflatoxin contamination, and can lead to reduced bonus payments to growers. For all these reasons, growers have an extremely low tolerance for *A. transitella* damage (i.e., <2%).

To maintain such low levels of damage, growers employ a suite of IPM tools that includes orchard sanitation (i.e., the removal of unharvested nuts), mating disruption, insecticides, and timely harvest. All of this is used with a monitoring program, with various traps and lures available to track the activity of adult males, mated females, and egg deposition. When used together, these practices can effectively reduce crop damage from *A. transitella*, but even the most aggressive management program can be undermined by colonization of *A. transitella* from nearby farms.

Amyelois transitella is a strong flyer (Sappington and Burks 2014) that is known to disperse between orchards (Andrews and Barnes 1982, Bayes et al. 2014). For instance, proximity to pistachio orchards, where winter sanitation is difficult due to the small size and durability of remnant pistachios, is a key predictor of *A. transitella* damage in almond

orchards (Siegel and Higbee 2009). However, the timing, extent, and catalyzing forces of *A. transitella* dispersal between orchards are still poorly understood.

Amyelois transitella is a capital breeder that does not feed (other than water) as an adult. Therefore, all the resources it will need to mate and disperse are obtained as a larva from a singular host plant, which may result in a distinct signature in adult moths. To date, fatty acid methyl esters (FAME) have been used to distinguish *A. transitella* adults that developed on walnut from those that developed on almonds and pistachios (Bayes et al. 2014, Burks et al. 2020). However, the FAME profiles of *A. transitella* reared on almonds and pistachios were too similar and could not be separated, thus limiting the utility of this tool. Here, we set out to explore additional intrinsic markers for *A. transitella* that would allow adult moths to be traced back to their larval host plant and differentiate among almond, pistachio, and walnut hosts. This included compound-specific isotope analysis, metabolomics, lipidomics, and lutein assays.

Compound-specific isotope analysis (CSIA) is a form of stable isotope analysis that examines the ratios of light isotopes (e.g., carbon and nitrogen) in specific tissues like amino acids (Quinby et al. 2020). Essential amino acids like histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), threonine (Thr), and valine (Val) must be obtained from an insect's diet. Nonessential amino acids such as alanine (Ala), asparagine (Asx), glutamine (Glx), glycine (Gly), proline (Pro), serine (Ser), and tyrosine (Tyr) can be synthesized *de novo* by insects. CSIA offers several advantages over standard bulk isotope analysis, for example, revealing the source of carbon from larval and adult diet sources to construct essential and non-essential amino acids (O'Brien et al.

2002). For instance, the abundance and natal origin of *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) across heterogeneous cotton-growing landscapes were determined by analyzing the ratio of ^{13}C to ^{12}C ($\delta^{13}C$) (Tsafack et al. 2016). In plants, the photosynthetic pathway (i.e., C3, C4, and CAM) can alter $\delta^{13}C$. Drought and water-use efficiency can alter the ratio of these isotopes, so even plants with the same photosynthetic pathway can produce different values (Farquhar et al. 1989). Nitrogen-15 variation is broader than ^{13}C in primary producers like plants because it is more easily influenced by environmental factors, such as fertilizer applications and soil composition (Bateman and Kelly 2007, Chung et al. 2019). However, it can still be used to examine trophic positions and food web complexity, particularly when paired with carbon isotope analysis (Adams et al. 2016, Pollierer et al. 2019). In entomology, the application of CSIA is growing, along with other complementary molecular techniques to examine the influence of diet on insect physiology.

Metabolomics is the comprehensive study of metabolites, which serve as the functional readout of cells and offer a snapshot of an organism's physiological state. Compared to CSIA, which targets select isotopes (e.g., $\delta^{13}C$, $\delta^{15}N$, $\delta^{2}H$, and $\delta^{18}O$), metabolomics can detect thousands of metabolic compounds. Like isotopes, these compounds can be influenced by diet and detected in insect tissue (Snart et al. 2015, Dong et al. 2017). Untargeted methods scan all metabolite species, while targeted approaches focus on one or a few. For instance, untargeted metabolomics revealed unique metabolic fingerprints of five closely related host plants in *Pieris rapae* L. (Lepidoptera: Pieridae) (Riach et al. 2019). Untargeted metabolomics also characterized differences in the

metabolome of predatory *Arma chinensis* Fallou (Hemiptera: Pentatomidae) and phytophagous *Halyomorpha halys* Stål (Hemiptera: Pentatomidae) based on feeding strategies (Su et al. 2026).

Lipidomics, a subfield of metabolomics, investigates the total lipid profile of organisms and generates both polar and non-polar lipids. What differs between polar and non-polar lipids is their molecular structure and biological use. Polar phospholipids are structural molecules that form cell membrane bilayers with glycerol attached to a polar phosphate group. In contrast, nonpolar lipids like triglycerides are often used as sources of energy. Due to these differences, polar primary and secondary metabolites are best analyzed with gas chromatography-mass spectrometry (GC-MS), while non-polar lipid metabolites are typically detected using liquid chromatography-mass spectrometry (LC-MS). Both metabolomics and lipidomics are powerful and often complementary tools that can be applied to a single insect or plant sample to yield a broad array of potential biomarkers (Kim et al. 2020).

While untargeted approaches may lead to the discovery of more biomarkers that can distinguish all tree nuts in a single analysis, a possible biomarker for pistachios may already be known. Lutein is a carotenoid that is known for its benefits for eyesight (Scott et al. 2025). Significantly higher concentrations of lutein are found in pistachios relative to almonds or walnuts (Stuetz et al. 2017). Lutein can be sequestered in both insect tissues (Eichenseer et al. 2002, Nguyen et al. 2019) and cocoons (Manupa et al. 2023) through feeding on host material. Quantification of lutein in plant and insect material is typically carried out with thin-layer chromatography (TLC) (Nguyen et al. 2019) or high-

performance liquid chromatography (HPLC) (Eichenseer et al. 2002). With HPLC, the UV absorbance of samples at 440-450nm is measured to quantify lutein content. However, a spectrometer can also be used to measure the UV absorbency of lutein and other carotenoids (Lee et al. 2009, Nguyen et al. 2019). Spectrometers are quicker and less technical, which may provide a faster and cheaper option to quantify lutein in plant and insect material.

In what follows, multiple methods were evaluated for their potential utility to trace *A. transitella* adults back to their larval host plant. First, CSIA was used to compare $\delta^{13}C$ and $\delta^{15}N$ in amino acid profiles in plant and insect tissues. Second, metabolomics and lipidomics were used similarly to detect polar secondary metabolites and non-polar lipids, respectively, in plant and insect tissue. Finally, lutein, a known biomarker in pistachios, was quantified in plant and insect tissues using a novel extraction method. The advantages and challenges of each method are then discussed, followed by suggestions for future work to refine and optimize the most promising approaches. Findings from this project provide new insight into the influence of host crops on *A. transitella* physiology and chart a path for method development to trace this economically important pest.

Materials and Methods

To explore this topic, we first evaluated the ability of three approaches to differentiate larval host based on intrinsic markers (i.e., signatures of larval host material) found in adult *A. transitella* moths reared on known hosts (i.e., Study #1). For this study, cohorts of *A. transitella* were reared from almonds, pistachios, and walnuts in 2023 and

again in 2025, to generate adult *A. transitella* from known hosts. The intrinsic marking systems explored included compound-specific isotope analysis, metabolomics and lipidomics, as well as lutein assays. We then took a subset of the most promising methods to the field by attempting to use lipidomics and lutein assays to identify the larval host of wild *A. transitella* collected across a series of almond, pistachio, and walnut orchards (i.e. Study #2).

Study # 1 - Evaluation of Intrinsic Markers

Field Sites

Field sites used in this study were research plots located at the UC Kearney Agricultural Research and Extension Center (UC KARE) in Parlier, CA (Table 2.1). The nut varieties used in this study were "Nonpareil" (almond), "Kerman" (pistachio), and "Chandler" (walnut). The almond plot was maintained similarly to a commercial orchard with regular applications of synthetic pesticide, herbicide and fertilizer. The walnut and pistachio blocks did not receive any pesticides, herbicides or fertilizers, but were pruned annually and generally maintained in a healthy condition.

Insect Source, Nut Inoculation, and Collection of Insect and Plant Material

Amyelois transitella eggs were sourced from a colony at the USDA Agricultural Research Service (ARS) in Parlier, CA. The colony consisted of a single strain ("Mendota") that has been maintained and used for research since 2012. Egg pads (i.e., paper towels with 3-5 days old fertile eggs) were cut into strips containing ~50 eggs each.

Each strip was then glued (Elmer's glue stick, Newell Brands, Westerville, OH) to a Tyvek® wristband (Uline, Pleasant Prairie, WI). Next, the egg strips were attached to developing new crop almonds, pistachios, and walnuts to encourage neonate larvae to infest the targeted host crop. Egg strips were attached to an individual nut by wrapping the wristband around the circumference of the nut hull or husk. Egg strips were deployed in almonds (7/10/2023 & 7/15/2025), pistachios (8/7/2023 & 9/8/2025), and walnuts (9/4/2023 & 9/8/2025) at times that correlated to peak nut kernel vulnerability (e.g., almond hull-split). In total, 60 nuts (20 per crop) across 10 trees (2 per tree) were inoculated for this study. Inoculated nuts were covered with a fine mesh bag (1,000 microns) to protect them from further damage or infestation.

Four weeks later, once the larvae had reached the pupal stage, the inoculated nuts were collected and moved to a climate-controlled greenhouse (26.0 °C ± 3.4 °C, 64.2% ± 8.8%). Each set of nuts was placed, by crop, into a BugDorm-4F4590 (MegaView Science Co., Ltd, Taiwan). Cages were monitored daily for adult *A. transitella* emergence. All emerged adults were sexed and then prepared for analysis accordingly.

In addition to insect material, nut kernels themselves were also collected from each tree two weeks after inoculation on 7/24/2023 (almonds), 8/21/2023 (pistachio), and 9/22/2025 (walnuts). Then, samples were dried for 72 h at 55 °C to measure the chemical signatures from almond, pistachio, and walnut kernels.

CSIA – Sample Preparation and Analysis

For the compound-specific isotope analysis, all adult *A. transitella* that emerged from inoculated nuts in the greenhouse were sexed and then dried for 48 h at 55°C. In total, 30 *A. transitella* (10 per crop) were weighed, placed in 2 ml microfuge tubes, and pulverized with a steel bead for 30 s at 30 Hz with a TissueLyser LT (Qiagen, Hilden, Germany) before being stored at -20°C. Additionally, after drying, the 15 nut kernels (5 per crop) were ground using a commercial spice grinder (Waring WSG30). Both insect and plant material were then delivered on ice to the UC Davis Stable Isotope Facility (SIF) for amino acid CSIA.

Approximately three mg of each sample was used for GC-C-IRMS analysis. Gas chromatography was performed on a Thermo Trace GC 1310 gas chromatograph coupled to a Thermo Scientific Delta V Advantage isotope-ratio mass spectrometer via a GC IsoLink II combustion interface. To be compatible with GC-C-IRMS, amino acids were first subjected to acid hydrolysis (6 M HCl, 70 min, 150 °C under nitrogen) before derivatization into *N*-acetyl methyl esters (NACME) (Corr et al. 2007). Derivatives were injected at 260 ºC (spitless, 1 min) and separated on an Agilent DB-35 column (60 m x 0.32 mm ID x 1.5 μm film thickness) at a constant flow rate of 2 ml/min under the following temperature program: 70 °C (hold 2 min); 140 °C (15 °C/min, hold 4 min); 240 °C (12 °C/min, hold 5 min); and 255 °C (8 °C/min, hold 35 min).

Isotope measurements were scale-normalized to primary reference materials. Carbon isotope ratios are expressed relative to the Vienna PeeDee Belemnite (VPDB)

standard, and nitrogen isotope ratios against the nitrogen content of air. Final values were displayed as parts per thousand (per mil; ‰) calculated as follows:

$\delta^{13}C = [(^{12}C/^{13}C)\ sample - (^{12}C/^{13}C)\ standard] / (^{12}C/^{13}C)\ standard \times 1000$

$\delta^{15}N = [(^{14}N/^{15}N)\ sample - (^{14}N/^{15}N)\ standard] / (^{14}N/^{15}N)\ standard \times 1000$

Untargeted Metabolomics and Lipidomics – Sample Preparation and Analysis

For the metabolomics and lipidomics analyses, all adult *A. transitella* that emerged from inoculated nuts in the greenhouse were sexed and then were flash frozen in liquid nitrogen and stored at -80 °C before further analysis. In total, 30 *A. transitella* (10 per crop) and 15 nut kernels (5 per crop) were submitted for analysis. Whole-body insect samples were processed at the UC Riverside Metabolomics Core (https://metabolomics.iigb.ucr.edu/). All samples were dried for 72 hours at -84°C using a lyophilizer (Labconco Freezone™ 6L, Kansas City, MO) and then homogenized with an OMNI Bead Ruptor 24 bead mill with cryo unit (Revvity, Kennesaw, GA). During homogenization, samples were run in 1-minute intervals and kept below 5 °C. Once, the homogenized samples were weighed out to 10 ±0.5 mg. Once weighed, samples were freeze-dried in preparation for extraction.

A biphasic extraction method was used, which results in a polar and nonpolar fraction. Samples were freeze-dried and extracted with 100 µL/mg of ice-cold 3: 2 methyl tert-butyl ether: 80% methanol in a glass vial. Each sample was sonicated in a cold-water bath (Model 15337410, Fisher Scientific) for 20 min, followed by a 90 min vortex at 4 ^{o}C.

The 200 µL/mg of LC-MS grade water was added to the extracting vial to induce the phase separation, followed by a 5 min vortex at 4 ^{o}C. After centrifugation for 15 min at 4 ^{o}C at 4000 x g, 200 µL of the top layer (i.e., nonpolar layer) was transferred to a 2 mL glass vial, and 400 µL of the bottom polar layer was transferred to a new 2 mL glass vial. Both fractions were dried under a gentle stream of nitrogen at room temperature. A 20 µl aliquot from each sample was pooled to prepare a quality control sample for lipidomics and polar metabolites assays. The polar fraction was stored at -80 ^{o}C for further analysis by GC-MS. The nonpolar fraction was resuspended in 200 µL of 9:1 methanol: toluene for LC-MS-based lipidomics analysis.

LC-MS lipid analysis was performed on a G2-XS quadrupole time-of-flight mass spectrometer (Waters™, Milford, MA) coupled to an I-class UPLC system (Waters™). Separations were carried out on a CSH C18 column (2.1 x 100 mm, 1.7 µM) (Waters™). The mobile phases were (A) 60:40 acetonitrile: water with 10 mM ammonium formate and 0.1% formic acid and (B) 90:10 isopropanol: acetonitrile with 10 mM ammonium formate and 0.1% formic acid. The flow rate was 400 µL/min, and the column was held at 65 °C. The injection volume was 1 µL. The gradient was as follows: 0 min, 10% B; 1 min, 10% B; 3 min, 20% B; 5 min, 40% B; 16 min, 80% B; 18 min, 99% B; 20 min, 99% B; 20.5 min, 10% B. The MS scan range was (50 to 1600 m/z) with a 100 ms scan time. MS/MS was acquired in a data-dependent fashion. Source and desolvation temperatures were 150 °C and 600 °C, respectively. Desolvation gas was set to 1100 L/h, and cone gas to 150 L/hr. All gases were nitrogen except the collision gas, which was argon. The capillary voltage was 1 kV in positive ion mode. A quality control sample, generated by pooling

equal aliquots of each sample, was analyzed periodically to monitor system stability and performance.

The analysis of small metabolites by GC–MS was performed using Thermo 1300 coupled with Thermo Fisher ISQ7000 mass spectrometers. The dried-down polar fraction was resuspended in 50 µl of methoxyamine hydrochloride solution in GC-grade pyridine (20 mg mL^{-1}) and vortexed for 2 h at 4 °C. Shaking continued for another 30 min after adding 50 µL of MSTFA +1% TMCS solution (ThermoTS48915) (Bhatia et al., 2015). Samples were injected (2 µL) directly into the GC-MS. Chromatographic separations of metabolites were carried out on a 30m x 0.25mm x 0.25 µm Thermo TG5SilMS column. Chromeleon software was used to process the chromatographic and mass spectrometric data. The GC oven temperature was maintained at 50^{o}C for 1 min, then gradually raised at the rate of 7^{o}C min^{-1} to 300^{o}C and maintained for 5 min. The sample was injected in the split mode at a splitting ratio of 1:20. Helium was used as a carrier gas and set at a constant flow rate of 1 mL min^{-1}.

The mass selective detector was run in the electron impact (EI) mode, with an electron energy of 70 eV. The ion source, the transmission line, and the inlet temperature were set to 300^{o}C, 280^{o}C, and 290^{o}C, respectively. Full scan mode (SCAN) was employed, and the mass scan range was 50~700 m/z. A standard alkane mix was used for gas chromatography coupled to mass spectrometry (GC-MS) for quality control and retention index calculations. All samples were analyzed in random order. The QC sample was prepared by mixing aliquots of the samples to create a pooled sample. To monitor the GC-MS system and data acquisition stability, an injection of a QC sample was tested every 10

injections of the random biological samples. The abundances were then normalized to the internal standard, Myristic Acid $D_{27,}$ and the normalized values were used for statistics.

The untargeted metabolomics and lipidomics data processing (peak picking, alignment, deconvolution, integration, and spectral matching) was performed in Progenesis Qi software (Waters™). Ions included for deconvolution were [M+H], [M+NH_4], [M+Na], and [M+H-H_2O] in positive ion mode, and [M-H], [M-H-H2O], and [M-H+HCOOH]. The untargeted data were normalized to the total ion count. Features with a CV greater than 30% across QC injections and an average abundance of less than 500 in quality control injections were removed (Dunn et al. 2011, Barupal et al. 2018). To group features, several mass spectral metabolite databases were searched against, including Metlin (Smith et al. 2005), Lipidmaps (Sud et al. 2007), HMDB (Wishart et al. 2007), Mass Bank of North America (Horai et al. 2010), and an in-house database.

Lutein Assays – Sample Preparation and Analysis

For the lutein assays, all adult *A. transitella* that emerged from inoculated nuts in the greenhouse were sexed, dried for 24 h at 55 °C, and then stored at -20 °C until further analysis. In total 90 *A. transitella* (30 per crop, 15 male and 15 female) were run on the spectrometer along with 15 nut kernels (5 per crop). On the HPLC, 12 *A. transitella* (4 per crop, 3 male and 1 female) with 9 nut kernels (3 per crop) were analyzed.

Lutein was extracted from plant and insect tissues according to Lee et al. (2009) with the following modifications. Insects and plant samples were dried for 24 h and 72 h at 55 °C, respectively. Whole insect and single tree nut kernels were extracted. Nut kernels

were ground, and 100.69 ± 0.20 mg of nut powder was collected. Both plant and insect samples were extracted with 6 mL of acetone in individual 15 mL polypropylene Falcon tubes. Insect samples were crushed with a glass stir rod before the addition of acetone. Samples were mixed on a nutating mixer (Fisherbrand™, Fisher Scientific, Hampton, NH) at 20 rpm for 3 h under aluminum foil to prevent photodegradation of lutein. Insect and plant material were separated from the solvent after the extraction duration. Most samples were filtered with #1 Whatman paper using a Buchner funnel and a vacuum flask.

A subset of samples was directly compared on the spectrometer and HPLC (Agilent 1200 series, Wilmington, DE). These samples were filtered by passing the solvent through a 0.45-μm Millipore MF syringe filter instead of using a Buchner funnel and #1 Whatman paper. This change was made so that samples could be filtered more directly into glass vials. Samples were run on the spectrometer first, then evaporated to complete dryness under nitrogen gas. Samples were resuspended in 100 µL of acetone and placed in a vial insert and capped in 2 mL amber autosampler vials for HPLC analysis.

To quantify lutein, optical density (OD) values were compared against a standard curve generated with five lutein concentrations (1, 0.5, 0.1, 0.05, 0.01 ppm). In addition to the standard runs, quality control injections of dilutions 0.01 and 0.05 ppm were run halfway through the samples. The lutein analytical standard (Millipore Sigma, Darmstadt, Germany) was dissolved in HPLC-grade acetone in a stock solution of 2 ppm. On the spectrometer (SpectraMax® ABS Plus, Molecular Devices, San Jose, CA), 3 mL of solvent was read at 446 nm in a cuvette (BRANDTECH® Scientific, Inc., Essex, CT) using the software SoftMax Pro 7.1.2 (Molecular Devices, San Jose, CA). The background

absorbance of acetone blanks was subtracted from all samples and standards. On the HPLC system, 40 μl of sample was injected in a YMC ODS AM 303 column (5 μm; 250 x 4.6 mm, Waters, Milford, MA). Column temperature was maintained at 30 °C with the mobile phases consisting of solvent A (75% HPLC-grade methanol) and solvent B (100% HPLC-grade ethyl acetate). The following solvent gradient was used for separations: 0 min 70% A, 15 min 10% A, 20 min 70% A. All increases of solvent B were linearly programmed. The flow rate was 1.0 mL/min. Samples were read with a photo diode array detector operating at 446 nm.

Study #2 - Determining the Larval Host of Wild *A. transitella*

Field Sites and Insect Collection

In 2025, wild *A. transitella* were collected from a series of almond, pistachio, and walnut orchards to explore whether lipidomics and lutein assays could be used to determine the larval host of field-collected *A. transitella*. All sites were under standard conventional management practices located in Fresno and Tulare counties.

At each site, adult *A. transitella* were captured using wing traps baited with a pheromone lure (Biolure, Suterra, Bend, OR) combined with a phenyl propionate (PPO) bait (Trece, Adair, OK), which allows the trap to remain attractive even in the presence of mating disruption. Traps were placed on the edge of three commercial walnut orchards (Table 2.1). Sampling took place from 4/1/2025 to 10/20/2025, during which time all lures were replaced every 6 weeks and liners were replaced weekly.

All moths were removed from sticky liners within 24 h after traps were brought back to UC KARE. The glue was dissolved in Histo-Clear (National Diagnostics, Atlanta, GA), allowing intact whole insects to be collected and stored. For the lipidomics assays, moths were held at -80 °C, while for the lutein assays, moths were dried for 24 h at 55 °C and then stored at -20 °C.

For the lipidomics assays, a total of 30 *A. transitella* (10 per site) were collected from each walnut site on two dates (Site 1, 8/21/2025 & 9/4/2025; Site 2, 8/21/2025 & 8/28/2025; Site 3, 8/21/2025 & 9/4/2025). For the lutein assays, a total of 496 moths were collected from 2 almond, 3 pistachio, and 3 walnut orchards over 8 nonconsecutive periods (8/7/2025, 8/13/2025, 8/14/2025, 8/20/2025, 8/31/2025, 9/17/2025, 9/24/2025, and 10/9/2025).

Statistical Analysis

Two statistical programs were used to analyze data. Mass spectra (i.e., metabolomics) and CSIA data were imported into MetaboAnalyst version 6.0 (https://www.metaboanalyst.ca/) (McGruer et al. 2023). With MetaboAnalyst, autoscaling was used to normalize relative abundance and generate principal component analysis (PCA) score plots. All other statistics were conducted in the R Statistical Program (version 4.5.1) (http://www.r-project.org/). In all tests, male and female *A. transitella* were analyzed together because sex was not a significant factor.

CSIA, Metabolomics and Lipidomics

Mass spectra and CSIA data were classified using a random forest model (function and package 'randomForest'). Random forest models were used because they are more robust and less prone to overfitting when the number of variables exceeds the sample size, compared to other discriminant models (Ranganathan and Borges 2010, Gromski et al. 2015). All models were trained on 80% of the samples and tested on the remaining 20% for validation purposes. The minimum number of features for the random forest model ('importance' function in the 'varSelRF' package) that could best classify nut kernel and *A. transitella* samples is reported. In addition to important features, the out-of-bag (OOB) error and the mean decrease in accuracy (MDA) for important features are listed.

δ^{13}C AA and δ^{15}N AA values were compared with a GLM with a Gaussian distribution. Gaussian distribution was used because residuals were normal and datasets contained negative values. Nut kernel and *A. transitella* samples were evaluated separately with the host crop as a fixed effect. Individual fixed effects were evaluated with likelihood ratio tests using the 'drop1' function, which generated the F and P-values reported here.

The mean relative abundances of the top five lipid and metabolite classes for nut kernels and *A. transitella* were compared using a generalized linear model ('glm' function in base R) with a gamma distribution and log link. The host crop was evaluated as a fixed effect with likelihood ratio tests using the 'drop1' function, which generated the χ^2 and P-values reported here. Additionally, the mean relative abundance of the metabolite spinosyn b was compared between 2025 samples with linear mixed-effects models ('lmer' function in the 'lme4' package). Gamma distributions cannot handle zeros as data points, so the

mixed effect model was selected instead. The site was used as a fixed effect with the sample replicate as a random effect.

Lutein Assays

Lutein concentrations were compared between nut kernels and *A. transitella* separately with a GLM (gamma distribution and log link). Fixed effects were the host crop and the dry weight of the sample. Fixed effects were evaluated with the 'drop1' function, and the χ^2 and P-values are reported here. Additionally, lutein concentrations run on both the HPLC and spectrometer were compared with a paired t-test ('t.test' function in base R). Finally, a threshold value for lutein to indicate if *A. transitella* likely originated from pistachios based on spectrometer data was calculated with a receiver operating characteristic (ROC) curve (Florkowski 2008) ('roc' function in the 'pROC' package).

Results

Study # 1 - Evaluation of Intrinsic Markers

CSIA - $\delta^{13}C$ of Amino Acids

Concentrations of $\delta^{13}C_{Hyp}$ (hydroxyproline) were lower than the limit of quantification for all samples, and in nut kernels, $\delta^{13}C_{His}$ was also below the quantification limit. Among all nut kernel AAs, $\delta^{13}C_{Glx}$, $\delta^{13}C_{Pro}$, and $\delta^{13}C_{Ser}$ were similar between crops. In general, almonds were the least depleted (i.e., most positive $\delta^{13}C$) of ^{13}C, and pistachios were the most depleted. However, $\delta^{13}C_{Gly}$, $\delta^{13}C_{Leu}$, $\delta^{13}C_{Lys}$, and $\delta^{13}C_{Tyr,}$ almonds and

walnuts were similar while being significantly more enriched than pistachios. Only $\delta^{13}C_{Met}$ significantly differed in all three crops. Findings are summarized in Table 2.2.

δ^{13}C AA values in *A. transitella* were generally more depleted but followed the trends of tree nut kernels. Notable exceptions were found in non-essential amino acids $\delta^{13}C_{Glx}$, $\delta^{13}C_{Pro}$, and $\delta^{13}C_{Ser,}$ in which pistachio-reared *A. transitella* were more heavily depleted compared to the other groups (Table 2.3). Like tree nut kernels, only $\delta^{13}C_{Met}$ in *A. transitella* was significantly different between all three groups.

In a PCA on tree nut δ^{13}C AA, the first and second components explained 79% and 12.7% of the variance. All crops were significantly separated from each other (Figure 2.1A). In insect samples, 75.4% and 14.8% δ^{13}C AA variation was explained by the first two components (Figure 2.1B). Pistachio and walnut insect samples overlapped, and a single almond outlier overlapped with the pistachio group.

CSIA - δ^{15}N of Amino Acids

Concentrations of $\delta^{15}N_{Hyp}$ were lower than the limit of quantification for all samples, and concentrations of $\delta^{15}N_{His}$ and $\delta^{15}N_{Met}$ were too low in nut kernels to be detected. Among all tree nut amino acids, $\delta^{15}N_{Ala}$, $\delta^{15}N_{Ile}$, and $\delta^{15}N_{Phe}$ were not significantly different. Almonds were the most depleted in all other δ^{15}N AA except for $\delta^{15}N_{Pro,}$ for which walnuts and almonds were similar. (Table 2.4).

δ^{15}N AA values in *A. transitella* were generally more enriched but followed the trends of tree nut kernels. However, $\delta^{15}N_{Thr}$ and $\delta^{15}N_{Tyr}$ were depleted in *A. transitella* relative to the host crop. $\delta^{15}N_{Ile}$ was enriched twice as much in *A. transitella* reared on

walnuts and pistachios compared to almonds. $\delta^{15}N_{Ala}$ and $\delta^{15}N_{Ser}$ were significantly different between all three *A. transitella* groups (Table 2.5).

In the PCA with tree nut kernels $\delta^{15}N$ AA, the first component explained 62.6% of the variation, and the second component explained 18.9% (Figure 2.2A). For *A. transitella*, the first and second components explained 73.1% and 8.1% of the variation in $\delta^{15}N$ for insect samples (Figure 2.2B). In both plots, all groups overlapped.

CSIA – Classification with Random Forest Models

Plant and insect samples were classified using a random forest model, with mixed results. Plant samples $\delta^{13}C$ AA were accurately classified with an out-of-bag error (OOB) of 0. $\delta^{13}C_{Gly}$ had the strongest influence on the model with an MDA of 9.27 (Table 2.6). When samples were classified using $\delta^{15}N$ AA values, the OOB increased to 0.333, indicating that samples were misclassified on average 33% of the time. $\delta^{15}N_{Pro}$ had the highest MDA of 9.17 (Table 2.7). Insect samples had an overall OOB of 0.1 and 0.223 for $\delta^{13}C$ AA and $\delta^{15}N$ AA, respectively. $\delta^{13}C_{Met}$ and $\delta^{15}N_{Ser}$ had the highest MDA within each model used on *A. transitella* samples (Table 2.6-2.7). All models were run with either all $\delta^{13}C$ AA or $\delta^{15}N$ AA as the predictors.

Metabolomics

Untargeted metabolomic analysis detected 605 features. They were divided into 98 tentatively identified (referred to hereafter as “known”) and 507 unknown features in nut kernel and insect samples. Of the known features, 58 metabolite classes were assigned. In

plant samples, the top five most abundant classes were beta-hydroxy acids and tricarboxylic acids in the first two positions, followed by sugar alcohols, hexoses, and non-metal phosphates. Together, these classes accounted for about 50% of the relative abundance in almonds and walnuts, and 42% in pistachios. In insect samples, the top five classes based on relative abundance were hexoses, non-metal pyrophosphates, non-metal phosphates, sugar alcohols, and alpha amino acids. Features from these classes accounted for 60% of the relative abundance of all known features.

There were significant differences in the relative abundance of the top five feature classes determined by a generalized linear model. In plant samples, walnuts contain the highest relative abundance of beta-hydroxy acids ($\chi^2 = 1.01$, df $= 2$, $P < 0.001$), hexoses ($\chi^2 = 7.41$, df $= 2$, $P < 0.001$), and sugar alcohols ($\chi^2 = 2.18$, df $= 2$, $P < 0.001$) while pistachios had the most non-metal phosphates ($\chi^2 = 34.59$, df $= 2$, $P < 0.001$) (Figure 2.3). For insect samples, significant differences were found in the relative abundance of alpha amino acids ($\chi^2 = 1.01$, df $= 2$, $P = 0.01$) and non-metal pyrophosphates ($\chi^2 = 1.06$, df $= 2$, $P = 0.01$) (Figure 2.4).

Initially, a PCA was performed with all features. However, only PCA on known features is reported here because there was no significant difference in the results. In the plant samples, the first two components explained 31.9% and 24.3% of the variation, with all groups being significantly separated (Figure 2.5A). In contrast, the insect samples all overlapped, and the first two components explained 35.9% and 12.5% of the variation (Figure 2.5B).

Plant and insect samples were classified using a random forest model. Plant samples were accurately classified with an OOB error of 0. Two metabolites were selected from the 98 tentatively identified compounds as the most important, with MDA scores of 22.32 and 21.98 (Table 2.8). Insect samples had an OOB error of 0.033 with a single *A. transitella* reared on walnuts misclassified as originating from almonds. Six metabolites were selected as the most important predictors for the model, with MDA values ranging from 11.15 to 5.02 (Table 2.8).

Lipidomics

Untargeted lipidomic analysis detected 6,728 features. They were divided into 721 known and 6007 unknown features. Of the known features, 57 lipid classes were assigned. In both plant and insect samples, the top five lipid classes based on relative abundance consisted of phosphatidylcholines (PC), diglycerides (DG), triglycerides (TG), phosphatidylethanolamines (PE), and sphingomyelins (SM). Together, these classes accounted for >80% of the relative abundance detected in known compounds.

There were significant differences in the relative abundance of the top five feature classes determined by a generalized linear model. In plant samples, walnuts had significantly lower relative abundance of lipid classes PC ($\chi^2 = 3.14$, df = 2, $P < 0.001$), PE ($\chi^2 = 3.46$, df = 2, $P < 0.001$), and TG ($\chi^2 = 0.24$, df = 2, $P < 0.001$). In contrast, walnuts had the highest relative abundance of DG ($\chi^2 = 0.37$, df = 2, $P < 0.001$), and no differences were found among SM (Figure 2.6). For insect samples, *A. transitella* reared on pistachios had significantly lower PC ($\chi^2 = 0.77$, df = 2, $P < 0.001$) and PE ($\chi^2 = 1.74$, df = 2, P <

0.001) values compared to other groups, but significantly higher SM ($\chi^2 = 1.30$, df = 2, P < 0.001) and TG values ($\chi^2 = 1.44$, df = 2, P < 0.001). The difference in relative abundance of DG was significant between all groups ($\chi^2 = 0.36$, df = 2, P < 0.001), with pistachios having the highest levels and almonds the lowest (Figure 2.7).

A PCA was performed with all features. However, only PCA on known features is reported because there was no significant difference in the results. In both plant and insect samples, groups were significantly separated by host crop. In plant samples, the first two components explained 43.1% and 23.7% of the variation (Figure 2.8A). In insect samples, the first two components accounted for 38.0% and 25.6% of the variation (Figure 2.8B).

Plant and insect samples were classified using a random forest model. Plant samples were accurately classified by host crop with an OOB error of 0. Two metabolites were selected from the 721 known features as the most important, with MDA scores of 25.64 and 22.62 (Table 2.9). Insect samples were accurately classified with an OOB error of 0. Fourteen of the 712 known features are the most important predictors for the model, with MDA values ranging from 9.57 to 5.94 (Table 2.9).

Lutein Assays

Lutein levels in pistachio and pistachio-reared *A. transitella* were significantly higher relative to almonds and walnuts. Pistachio kernels (45.18 ± 0.68 μg/g) had significantly higher levels of lutein compared to almonds (15.24 ± 0.73 μg/g) and walnuts (16.30 ± 1.34 μg/g) ($\chi^2 = 4.26$, df = 2, P < 0.001) (Figure 2.9). Pistachio-reared *A. transitella* ($\chi^2 = 75.69$, df = 2, P < 0.001) had twice (38.44 ± 3.15 μg/g) as much lutein as almonds (20.12 ± 1.36 μg/g) and walnuts (15.58 ± 0.72 μg/g) (Figure 2.10). Lutein concentrations

were calculated based on a standard curve value (R^2 = 0.99) after the background absorption of the acetone was subtracted. Walnut-reared dried *A. transitella* averaged 10.11 ± 0.38 mg, which was larger than moths from almonds (4.99 ± 0.43 mg) or pistachios (5.01 ± 0.38 mg) (χ^2 = 48.01, df = 2, P < 0.001).

On the HPLC, lutein was detected in higher concentrations in pistachio samples compared to other groups. For *A. transitella*, lutein was only detected in pistachio-reared moths (38.89 ± 9.15 μg/g). Pistachio nut kernels averaged 25.47 ± 9.77 µg/g of lutein, which was 25-fold higher than that of almonds (0.99 ± 0.00 μg/g) or walnuts (1.06 ± 0.12 μg/g). There was no difference in the mean lutein content in nut kernel samples on either the HPLC or the spectrometer. However, the HPLC reported higher concentrations of lutein in pistachio-reared *A. transitella* than the spectrometer (t = 3.54, df = 3, P = 0.03) (Figure 2.11). Concentrations were similar in nut kernel samples between the spectrometer and HPLC. Standard dilutions were run before and after samples to generate a standard curve (mean R^2 = 0.99). The average retention time of lutein in standards was 11.33 min. Two more runs were made with new samples and standards, but lutein was not detected below 0.1 μg/mL in the standards, so the samples were not included in this report.

Study #2 - Determining the Larval Host of Wild *A. transitella*

Lipidomics

Untargeted lipidomic analysis detected 4,820 features. They were divided into 528 known and 4292 unknown features. The most abundant lipid class within identified features was TG, which ranged from 73-12% relative abundance across samples. Samples

collected from the walnut site 1 (Wal1) had significantly higher relative abundance of DG ($\chi^2 = 56.16$, df = 2, $P < 0.001$) and TG ($\chi^2 = 43.32$, df = 2, $P < 0.001$) (Figure 2.12). The metabolite spinosyn B was detected in 2025 samples. Relative abundance was significantly different ($\chi^2 = 34.34$, df = 2, $P < 0.001$) between sites, with the greatest abundance in samples from Wal2, followed by Wal3 and Wal1 (Figure 2.13).

The predictive power of the random forest model, trained on *A. transitella* samples collected in 2023, was applied to field samples collected in 2025. Only features present in both years were used. Between the two sets of known features, 57 were present in both years. With these shared features, the OOB error for 2023 samples remained at 0. However, the model predicted the larval host crop of all *A. transitella* from 2025 as either almond or walnut. The probability of membership (i.e., accuracy) for these predictions ranged from 0.50 to 0.37 (Figure 2.14).

Lutein Assays

Due to a lack of replication with the HPLC, a receiver operating characteristic (ROC) curve-based *A. transitella* lutein concentrations determined by a spectrometer calculated a value of 32 μg/g of lutein would indicate pistachio as a larval source (Figure 2.15). From *A. transitella* analyzed from various field sites, 15.92 % (79/496) likely developed on pistachios (Figure 2.16). However, this method indicated that some *A. transitella* recaptured in walnut sites, Din, Swall, and Jared, originated from pistachios, whereas the LC random forest model only predicted almond or walnut as possible larval sources.

Discussion

The signatures of $\delta^{13}C$ AA, $\delta^{15}N$ AA, metabolome, and lipidome in California tree nuts and in *A. transitella* have been understudied. This has left gaps in the application of these signatures to trace insect movement. Here, a series of experiments demonstrated the benefits and limitations of various methods for detecting potential intrinsic biomarkers in *A. transitella* from their larval host crop. Non-polar compounds like lutein in the lipidome may offer the most robust and reproducible avenues for future research when combined with existing approaches.

Compound-Specific Isotope Analysis (CSIA)

Values for $\delta^{13}C$ AA and $\delta^{15}N$ AA have recently been reported to authenticate the country of origin for walnuts (Athaillah et al. 2023). Here, the ranges for $\delta^{13}C$ AA and $\delta^{15}N$ AA overlap with the values reported by Athaillah et al. (2023). Additionally, our study reports for the first time the $\delta^{13}C$ AA and $\delta^{15}N$ AA values for almonds and pistachios. As C3 plants, the photosynthetic pathway is the same for all three crops, which should limit variation in $\delta^{13}C$ values (Larsen et al. 2009). While differences among tree nut kernels could be attributed to genetic variation, other factors may be in play.

Water use efficiency (WUE) is the ratio of plant biomass (i.e., carbon fixation) and water used by a plant. The WUE and $\delta^{13}C$ tend to be positively correlated, and lower WUE (i.e., less carbon fixation per unit of water) results in a lower $\delta^{13}C$. In hybrid genotypes from the almond genus, *Prunus,* and pistachio rootstocks, drought stress led to reduced $\delta^{13}C$ values (Esmaeilpour et al. 2016, Mininni et al. 2022). However, the relationship

between WUE and $\delta^{13}C$ is complex and influenced by many factors. The kernel developmental stage can also impact WUE. In California almonds, WUE was lowest during fruit development in July (Peddinti and Kisekka 2025). Water use efficiency of 'Kerman' pistachios is reported to be higher than 'Golden Hills' and 'Lost Hills' varieties (Núñez et al. 2026). Tree size and structure are also important factors. In walnut leaves, the spatial distribution of WUE varied significantly at different heights and light gradients (Le Roux et al. 2001).

A common application of stable isotope analysis is to distinguish organic vs conventional or pesticide-free products (Chung et al. 2019, Bontempo et al. 2020). While all the research plots at UC KARE were conventionally (albeit minimally) managed, the almond plot was most closely worked as a conventional commercial orchard with regular applications of synthetic insecticides, fungicides, fertilizers, and herbicides. In contrast, the pistachio and walnut plots were pesticide-free and minimally managed. In general, almonds had the highest $\delta^{13}C$ AA and the lowest $\delta^{15}N$ AA, a trend supported in the literature for conventional systems. For instance, in shiitake mushroom cultivation, mushrooms from conventional systems had higher $\delta^{13}C$ AA and lower $\delta^{15}N$ AA values than those from organic and pesticide-free systems (Kim et al. 2022).

$\delta^{13}C$ AA was a better classifier than $\delta^{15}N$ AA in both plant and insect samples. Increased variation in $\delta^{13}C$ AA profiles may be attributed to both WUE and field management, whereas $\delta^{15}N$ AA is likely only affected by the latter. These differences in plants can then be passed on to *A. transitella,* which allows for tracing the larval host crop. Insects, unlike plants, need essential amino acids from their diet. Essential amino acids are

often directly assimilated into tissue since the insects cannot synthesize them *de novo*. In this study, eight essential amino acids: histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), threonine (Thr), and valine (Val) were quantified. For δ^{13}C AA, differences in nut kernels were passed directly to *A. transitella* (Tables 2.2-2.3). For δ^{15}N AA, $\delta^{15}N_{Ile}$ and $\delta^{15}N_{Thr}$ trends differed between plants and insects (Tables 2.4-2.5). $\delta^{15}N_{Thr}$ was depleted to similar levels in insects, losing significant differences seen in plant tissue. This is in line with earlier studies on other invertebrates (Pollierer et al. 2019) related to the metabolism of threonine. For *A. transitella* reared on almonds, $\delta^{15}N_{Ile}$ was not as enriched as that from pistachios or walnuts. While the reason for this is unclear, possibilities could be attributed to the metabolic fate of nitrogen. Exchange of nitrogen between 'trophic' (i.e., Asx, Ile, Leu, Pro, Val) AAs is done through a single chemical step involving glutamic acid (O'Connell 2017). Glutamic acid ($\delta^{15}N_{Glx}$) was lowest in almonds and almond-reared *A. transitella*. This may have limited the enrichment of $\delta^{15}N_{Ile}$ but would require additional research.

Compound-specific isotope analysis of amino acid profiles is a powerful tool to authenticate crop origins, management practices, and trophic positions. However, for tracing the larval source of *A. transitella,* it may be too sensitive to environmental factors and have low reproducibility. Differences in insects were often entirely dependent on the difference being present in the host crop. While δ^{13}C AA appeared to be the better candidate, a robust evaluation of baseline δ^{13}C AA is needed across all crops, varieties, and management practices (i.e., organic & conventional) to develop a database of expected values. The random forest model predicted $\delta^{13}C_{Met}$ to be the most important feature in

classifying *A. transitella* and was the only variable significantly different in plant and insect profiles, which appears to make it a potential biomarker. It is important to consider that $\delta^{13}C_{Met}$ and $\delta^{15}N_{Met}$ were below the quantification limit in Athaillah et al. (2023) and in this study ($\delta^{15}N_{Met}$ only). This indicates that it may have limited success in detection and, therefore, use as a biomarker.

Metabolomics

Profiles of polar secondary metabolites were more diverse than those of amino acids in tree nut kernel and *A. transitella* samples. No standards were run to confirm the identity of any compounds, so conclusions that can be drawn about tentatively identified ("known") compounds are limited. However, the object of the untargeted approach was to get a broad view of potential biomarkers of tree nuts. Random forest models classified plant samples with no error, and insect samples with a 3% error rate based on GC/MS-identified features. The important features selected to classify samples were often common metabolites in plants.

Tree nut samples were accurately classified using as few as two features. The feature with the highest MDA was tentatively identified as conduritol B epoxide and was found at significantly higher levels in walnuts. Conduritol B epoxide is not a widely recognized endogenous metabolite in plants, but can be found (Salazar and Furlan 2007). The molecular formula of conduritol B epoxide is $C_6H_{10}O_5$, which is also the formula for the repeating unit of major polysaccharides. Walnuts had significantly higher levels of hexoses, six-carbon monosaccharides, which may be the source of the $C_6H_{10}O_5$ molecule

detected, but more research is needed. The second important feature was 3-aminopropionitrile, found in the highest levels in pistachio nuts. This feature is a product of cyano-amino acid metabolism (Zhou et al. 2020). There is limited research on this compound in tree nuts, but the amino acid metabolism in pistachios increases in August and September to boost protein content in kernels (Adaskaveg et al. 2025). Further research about the influence of phenology and metabolite profiles is needed.

More polar metabolite features were required to classify insect samples than to classify plants. Plants produce diverse primary and secondary metabolites, including phenols, flavonoids, terpenoids, sterols, fatty acids, and sugars, that function in defense, signaling, and structural integrity (Bocso and Butnariu 2022, Ahlawat et al. 2024). This may account for the presence of xylitol on the VIP list. However, insect guts may be more selective for beneficial polar compounds to assimilate with detoxification (Kshatriya and Gershenzon 2024). Isothreonic acid is a precursor to L-ascorbic acid, which is vital to insect growth (Kramer and Seib 1982). Other compounds, such as D-fructose, uric acid, and citric acid, are needed in energy metabolism or are products of the Krebs cycle (Shirvani et al. 2025). The most influential compound according to the random forest model and the only one with an increased fold change in *A. transitella* in all crops was D-arabitol. D-arabitol is a common polyol found in fungi like *Aspergillus niger* Tiegh (Eurotiales: Aspergillacae) as a carbon source (Lewis and Smith 1967, Diano et al. 2006). *Aspergillus spp.* of fungi are prevalent in tree nut orchards and infest damaged nut kernels (Bayman et al. 2002, Ortega-Beltran et al. 2018). While *A. transitella* is a known carrier of *A.*

flavus (Palumbo et al. 2014), it is possible that moth samples carried *A. niger,* which resulted in the high D-arabitol signal. (Sanz et al. 2008*)*

A major limitation of this use of GC/MS in this study was its single application. The features detected were able to accurately classify plant samples and insect samples with a low error rate. Continued exploration may have yielded a more reliable list of features, reducing the error rate. However, due to time and cost constraints, LC/MS appeared to be the better candidate since it had an 0% error rate. Additionally, GC/MS can detect fewer compounds than LC/MS since they need to be volatilized for GC compatibility. Meaning the list of known features may not increase much with continued use. The use of standards to definitively identify important features is necessary for future research with this method.

Lipidomics

Nonpolar metabolites were the largest and most diverse analyzed in this study. No standards were run to confirm the identity of any compounds, so conclusions that can be drawn about tentatively identified ("known") compounds are limited. In 2023, random forest models classified plant and insect samples with no error based on LC/MS-identified features. This method was repeated on *A. transitella* collected from walnut sites in 2025, from unknown sources. Overall lipid classes and quantity were reduced in 2025 samples, possibly due to insecticide activity. The probability of correct classification of unknown samples to larval host crops is low.

Nut kernel lipid profiles were correctly classified with as few as two features, like the polar metabolite random forest model. The two features were TG (16:0/16:1(9Z)/18:2(9Z,12Z)) and FAHFA (18:0/12-O-18:0). Pistachios had the highest relative abundance of TG (16:0/16:1(9Z)/18:2(9Z,12Z)). In contrast, FAHFA (18:0/12-O-18:0) was significantly concentrated in almonds. Walnuts had the lowest relative abundance of both compounds. Lipid profiles are complex matrices that change with kernel development. In Australia, Nonpareil almonds rapidly accumulate lipids in the kernels 115 d after flowering, with a slight increase again 167 d close to harvest (Zhu et al. 2017). Kerman variety pistachios accumulate mono and polyunsaturated fatty acids between July and August before levels stabilize towards the end of August (Adaskaveg et al. 2025). Walnuts grown in China also accumulate large amounts of unsaturated fatty acids 88 d after pollination, which would be in July in California, but oil content continues to increase until harvest (Jin et al. 2023). The shifting accumulation of lipids is an important consideration in future studies.

More non-polar features were needed to classify insect samples than plant samples. Unlike nut samples, a mix of fatty acids, phospholipids, and storage lipids was an important feature. Non-polar compounds like lipids are heavily influenced by diet. Diets high in lipids lead to increased lipid storage across insect genera, including *Apis mellifera* L. (Hymenoptera: Apidae) (Wang et al. 2021), the diamondback moth *Plutella xylostella* L. (Lepidoptera: Plutellidae) (Warbrick-Smith et al. 2006), the black field cricket *Teleogryllus commodus* Walker (Orthoptera: Gryllidae) (Rapkin et al. 2018), the brown planthopper *Nilaparvata lugens* Stål (Hemiptera: Delphacidae) (Zhou et al. 2018), and *Drosophila*

melanogaster Meigen (Diptera: Drosophilidae) (Grönke et al. 2005, Wat et al. 2020). Varying dietary content affects not just glycerolipids like TG but also phospholipids, sphingolipids, and sterols (Carvalho et al. 2012, Knittelfelder et al. 2020).

Fewer features were detected in the wild collected *A. transitella* compared to those reared on a known host. With the wild moths, there was a 28% reduction in total features and 26% reduction in known features. Only one of the nine important features from the known host samples, DG (18:2(9Z,12Z)/18:3(9Z,12Z,15Z)/0:0) was present in the wild caught moths. This reduction may be attributed to several factors. With the moths from known hosts, both insect and plant samples were run together to enable direct comparisons, which may have increased the number of detected features. However, no known features were completely absent in *A. transitella* and present in tree nuts, so this is unlikely to be the cause. Other physiological factors may be the cause

The wild *A. transitella* collected were of unknown age, and some may have been over a week old. In the trap, the insect had no access to food or water. While *A. transitella* does not feed as an adult, reduced access to water has been shown to decrease both fertility and longevity in females (Burks 2014). Here, this may have also led to changes in their carbohydrate and lipid profiles. For instance, extended periods of dehydration led to depleted carbohydrate and lipid reserves in the Antarctic midge, *Belgica antarica* Jacobs (Diptera: Chironomidae) (Teets et al. 2012), and starvation led to reduced fat body size in adult *Manduca sexta* (Lepidoptera: Sphingidae) (Ziegler 1991). Liquid chromatography MS revealed reductions in neutral and phospholipids in *Goniozus legneri* Gordh (Hymenoptera: Bethylidae), indicating these factors are important considerations in

LC/MS approaches. Starvation is also known to alter lipid utilization, with saturated fatty acids favored over unsaturated fatty acids in *Spodoptera litura* (Lepidoptera: Noctuidae). In this way, starvation may have altered the lipidome of the wild caught *A. transitella* as well.

Diglycerides and triglycerides were some of the most abundant and important features detected in 2023 and 2025 across all samples. The DG and TG are neutral lipids and the preferred source of fuel for flight in lepidopteran species (Arrese and Soulages 2010). Decline of unsaturated C16-C18 fatty acids correlated with flight duration in 12 h of flight mill assays with *S. litura* (Murata and Tojo 2002). Patterns of lipid mobilization during flight can differ between individual moths, depending on whether or not they are dispersing locally or over long distances (Sappington et al. 1995). The distance and duration of flight from captured *A. transitella* were unknown and may be a source of year-to-year variation in lipid profiles.

Spinosyn B was detected in *A. transitella* captured in 2025. Spinosyn B is a metabolite of Spinosyn A, the main active ingredient in Spinosad. Spinosad is marketed as an organic-approved product under the tradename Entrust®. Spinosad is reported to have sublethal effects that reduce DG, TG, and CL lipid classes in insects (Piri et al. 2014, Martelli et al. 2022). The relative abundance of spinosyn B varied between sites, with Swall having significantly lower concentrations than Jared. Consequently, *A. transitella* capture at Swall on average had significantly higher relative abundances of DG and TG features. No records of Spinosad applications were reported in walnut test sites, indicating moths immigrated from treated areas. Enhanced detoxification is reported in some *A. transitella*

populations due to cytochrome P450 (Demkovich et al. 2015), which has implications for the use of this product, particularly in organic production (Wilson and Gress 2022).

Liquid chromatography and mass spectrometry can detect the largest array of metabolites, which is important in untargeted approaches such as this study. However, lipid variation from year-to-year poses a significant limitation to the application of tracing A. transitella to larval host crops. Submitting reference samples from the current year could improve clarity, but would add cost to an already expensive approach. The use of targeted HPLC may offer many of the strengths of LC/MS while reducing the cost and technical expertise required to run samples.

Lutein Assays

Among the three host plants evaluated, pistachios and pistachio-reared *A. transitella* consistently exhibited the highest lutein concentrations, a pattern confirmed by both spectrophotometric and HPLC analyses. This supports the hypothesis that host plant carotenoid content is reflected in the insect and reinforces the potential of lutein as a biomarker for larval host origin. Although HPLC analysis was conducted with a limited sample size, it consistently detected higher lutein concentrations and produced no false negatives, in contrast to the spectrometer, which exhibited a 40% false negative rate. This suggests that HPLC provides greater sensitivity and specificity, despite potential limitations in throughput and reproducibility associated with smaller sample sizes and more complex sample preparation.

In insects, carotenoids such as lutein are typically obtained through the larval diet (Liu et al. 2025). Lutein is found in relatively high concentrations in pistachios, with reported kernel values ranging from 27.57–35.43 μg/g (Liu et al. 2014, Stuetz et al. 2017), consistent with the concentrations observed in this study. Lutein is sequestered at varying rates across lepidopteran species. Lutein concentrations in *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae) reflect those of their host plants (Eichenseer et al. 2002), while *Trichoplusia ni* Hübner (Lepidoptera: Noctuidae) has been shown to concentrate dietary carotenoids up to 20-fold relative to the host crop (Nguyen et al. 2019). In the present study, lutein concentrations in pistachio-reared *A. transitella* were approximately 56% higher than in the host crop, indicating moderate sequestration. While this level of enrichment is lower than that reported for some lepidopteran species, the strong correspondence between host and insect lutein concentrations supports its utility as a pistachio-associated biomarker.

Direct comparison of analytical methods revealed important differences in detection accuracy. While both methods identified pistachios as having the highest lutein concentrations, the spectrometer substantially overestimated lutein levels in almond- and walnut-reared *A. transitella*. This inflation resulted in necessitating a higher classification threshold and contributed to a high false negative rate (40%) of pistachio-reared *A. transitella*. In contrast, HPLC analysis detected negligible lutein in almond- and walnut-reared insects, resulting in clear separation among host groups and improved classification accuracy. The elevated optical density observed in spectrometer-only samples likely reflects interference from other compounds absorbing within the 380–500 nm range, where

carotenoids are typically detected. Although almonds and walnuts are not known to contain high concentrations of alternative carotenoids (Stuetz et al. 2017), co-extracted pigments or other metabolites may contribute to this signal.

Taken together, these results demonstrate that lutein is a promising biomarker for determining larval host origin in *A. transitella*, particularly when quantified using HPLC. Further optimization with the HPLC is required to locate steps in the protocol that are limiting the detection of lutein at low concentrations consistently. In this experiment, the column was not new and had been used in a previous experiment. Replacing it may be a quick and reliable fix for obtaining reproducible results (Patil 2017, Abdu Hussen 2022). However, additional experimentation with other extraction solvents such as hexane (Liu et al. 2014), different mobile phases like acetonitrile (Miura et al. 2020), and ultrasonic extraction methods (Ahmadi et al. 2024) may be required to improve lutein detection on the HPLC. In contrast, lutein quantification on the spectrometer appears to be a reproducible method, but the error rate needs to be reduced. Single-use syringe filters reduce the noise in almond and walnut samples, which improves the error rate of false negatives in pistachio-reared A. transitella. Ultimately, the spectrometer may be the fastest and most economical method for lutein quantification.

Conclusions

Overall, each analytical approach evaluated in this study presents distinct advantages and limitations for identifying larval host origin in *A. transitella*. Compound-specific isotope analysis provides mechanistic insight into trophic transfer of amino acids,

but its sensitivity to external factors such as water use and other management practices limits reproducibility and practical application. Untargeted metabolomics offers broad chemical coverage and high classification accuracy, particularly for plant samples. However, this approach is costly and not as accurate as other methods. While lipidomics demonstrated the highest classification accuracy and captured biologically relevant diet-driven variation, there were strong influences of insect physiological state, environmental exposure, and year-to-year variability that reduced its reliability for field-collected insects. Additionally, it is also a very expensive method, and more targeted approaches would be more economical. Lutein quantification represents a simple, targeted approach with clear biological relevance and strong host association, particularly when measured using HPLC. However, its utility requires further optimization for consistent detection of lutein at low concentrations (i.e., < 0.1 ppm). Collectively, these findings suggest that while no single method is sufficient alone, targeted biomarkers such as lutein, combined with other approaches such as FAME, may provide the most practical and robust framework for tracing larval host origin in *A. transitella*. That is, samples could be extracted, and the sample could be divided and submitted for FAME analysis to differentiate walnut-originated moths from almond and pistachio, and then lutein could be used to separate between almond and pistachio hosts.

The goal of a single extraction and analysis to trace *A. transitella* is a more obtainable goal than before, with clear next steps. First, optimize lutein extraction and quantification to conclusively validate its use as a pistachio-associated biomarker. Second, combining the lutein analysis with the FAME approach (Bayes et al. 2014) to provide a

dichotomous system to determine the *A. transitella* larval source. If lutein is present, it would indicate the larval source is pistachio. If not, the FAMEs could be analyzed to determine whether the moth originated from almonds or walnuts. The challenge will be to run samples on a single machine. Lutein is not volatile and is sensitive to degradation from heat and light, so detection on a GC machine may be difficult. Fatty acids can be quantified on an HPLC (Nishiyama-Naruke et al. 1998, Guarrasi et al. 2010) but are often derivatized for GC approaches. Alternatively, separate aliquots of individual samples could be run on a GC and HPLC in sequence.

Tables and Figures

Table 2.1. Summary of field sites and *A. transitella* source 2023 and 2025.

Site	Crop	Variety	Size (acres)	Age (years)	Experiment	Collection Period	Collection Method
1.	Almond	Nonpareil & pollinators	3	20	CSIA, GC, LC	July 2023	inoculation
					Lutein	July 2025	inoculation
2.	Pistachio	Kerman	2	20	CSIA, GC, LC	Aug. 2023	inoculation
					Lutein	Aug. 2025	inoculation
3.	Walnut	Chandler	3	20	CSIA, GC, LC	Sept. 2023	inoculation
					Lutein	Sept. 2025	inoculation
4. (Alm1)	Almond	Nonpareil & pollinators	159	20	Lutein	Aug. & Sept 2025	wing trap & Biolure
5. (Alm2)	Almond	Nonpareil & pollinators	524	11	Lutein	Aug. 2025	wing trap & Biolure
6. (Pist1)	Pistachio	Golden Hills	157	17	Lutein	Aug. & Sept 2025	wing trap & Biolure
7. (Pist2)	Pistachio	Golden Hills	628	12	Lutein	Aug. 2025	wing trap & Biolure
8. (Pist3)	Pistachio	Golden Hills	763	13	Lutein	Aug. & Sept 2025	wing trap & Biolure
9. (Wal1)	Walnut	Vina	37	10+	LC & Lutein	Oct 2025	wing trap & Biolure
10. (Wal2)	Walnut	Serr	42	10+	LC & Lutein	Oct 2025	wing trap & Biolure
11. (Wal3)	Walnut	Vina	75	10+	LC & Lutein	Aug. & Oct 2025	wing trap & Biolure

Table 2.2. Summary of $\delta^{13}C$ (mean ± SE) in tree nut kernels amino acids (n = 5).

Amino acid	Almond	Pistachio	Walnut	F
Ala	-22.94 ± 0.76 **ab**	-25.85 ± 1.06 **a**	-22.48 ± 0.36 **b**	5.47*
Asx	-21.37 ± 0.64 **b**	-25.70 ± 0.93 **a**	-23.18 ± 0.54 **ab**	9.07**
Glx	-22.92 ± 0.64 **a**	-24.24 ± 1.06 **a**	-23.14 ± 0.26 **a**	0.98
Gly	-20.90 ± 0.96 **b**	-25.71 ± 0.76 **a**	-21.75 ± 0.33 **b**	12.24**
His	-	-	-	-
Ile	-22.03 ± 0.55 **b**	-27.93 ± 0.81 **a**	-25.95 ± 0.46 **a**	23.09***
Leu	-31.22 ± 0.61 **b**	-34.74 ± 0.96 **a**	-31.02 ± 0.38 **b**	9.15**
Lys	-21.89 ± 0.71 **b**	-28.39 ± 0.80 **a**	-24.26 ± 0.90 **b**	24.97***
Met	-24.71 ± 0.60 **c**	-30.75 ± 1.05 **b**	-34.12 ± 0.77 **a**	33.33***
Phe	-32.94 ± 0.55 **b**	-35.43 ± 0.66 **a**	-33.61 ± 0.35 **ab**	5.80*
Pro	-20.80 ± 0.41 **a**	-23.06 ± 0.94 **a**	-23.11 ± 0.40 **a**	4.26*
Ser	-14.98 ± 0.90 **a**	-16.96 ± 0.83 **a**	-16.24 ± 0.55 **a**	1.65
Thr	-9.99 ± 0.54 **b**	-15.46 ± 0.87 **a**	-14.83 ± 0.51 **a**	20.70***
Tyr	-32.35 ± 0.51 **b**	-35.06 ± 0.75 **a**	-30.98 ± 0.43 **b**	12.89**
Val	-31.09 ± 0.60 **b**	-34.94 ± 0.90 **a**	-32.74 ± 0.29 **ab**	8.95**

Letters represent differences between the three groups for a given amino acid. P value determined by Tukey HSD post hoc test. *P <0.05, **P <0.01, ***P <0.001

Table 2.3. Summary of $\delta^{13}C$ (mean ± SE) in *A. transitella* amino acids (n = 10).

Amino acid	Almond	Pistachio	Walnut	F stat
Ala	-26.66 ± 0.49 **b**	-32.06 ± 0.48 **a**	-31.74 ± 0.50 **a**	38.45***
Asx	-22.35 ± 0.42 **b**	-26.12 ± 0.28 **a**	-25.42 ± 0.41 **a**	28.27***
Glx	-23.31 ± 0.45 **b**	-26.86 ± 0.37 **a**	-25.39 ± 0.45 **a**	17.63***
Gly	-18.70 ± 0.56 **b**	-24.27 ± 0.48 **a**	-22.66 ± 0.56 **a**	28.89***
His	-21.26 ± 0.35 **b**	-26.38 ± 0.55 **a**	-25.81 ± 0.63 **a**	29.09***
Ile	-23.00 ± 0.41 **b**	-28.14 ± 0.68 **a**	-28.89 ± 0.55 **a**	32.93***
Leu	-30.99 ± 0.38 **b**	-33.65 ± 0.31 **a**	-30.54 ± 0.38 **b**	22.17***
Lys	-20.15 ± 0.36 **b**	-25.71 ± 0.72 **a**	-25.42 ± 0.48 **a**	33.21***
Met	-27.67 ± 0.41 **c**	-31.01 ± 0.31 **b**	-37.69 ± 0.50 **a**	151.34***
Phe	-30.54 ± 0.25 **b**	-34.65 ± 0.72 **a**	-35.51 ± 0.44 **a**	27.35***
Pro	-22.10 ± 0.48 **b**	-24.72 ± 0.44 **a**	-22.36 ± 0.38 **b**	11.04***
Ser	-15.74 ± 0.53 **b**	-19.62 ± 0.36 **a**	-19.56 ± 0.40 **a**	26.02***
Thr	-11.84 ± 0.39 **b**	-14.84 ± 0.45 **a**	-13.96 ± 0.47 **a**	12.23***
Tyr	-30.67 ± 0.20 **b**	-34.22 ± 0.56 **a**	-34.11 ± 0.45 **a**	22.36***
Val	-28.04 ± 0.25 **b**	-31.77 ± 0.78 **a**	-32.32 ± 0.37 **a**	20.17***

Letters represent differences between the three groups for a given amino acid. P value determined by Tukey HSD post hoc test. **P* <0.05, ***P* <0.01, ****P* <0.001

Table 2.4. Summary of $\delta^{15}N$ (mean ± SE) in tree nut kernels amino acids (n = 5).

Amino acid	Almond	Pistachio	Walnut	F
Ala	-0.36 ± 0.31 **a**	0.41 ± 0.92 **a**	-1.22 ± 0.63 **a**	1.49
Asx	-0.80 ± 0.31 **a**	1.13 ± 0.51 **b**	1.83 ± 0.33 **b**	12.12**
Glx	-0.47 ± 0.19 **a**	2.35 ± 0.68 **b**	1.71 ± 0.22 **b**	11.96**
Gly	-1.28 ± 0.35 **a**	4.12 ± 1.15 **b**	2.62 ± 0.53 **b**	13.46***
His	-	-	-	-
Ile	-1.31 ± 0.33 **a**	-0.60 ± 0.81 **a**	-1.59 ± 0.72 **a**	0.61
Leu	-5.28 ± 0.20 **a**	-2.02 ± 0.82 **b**	-0.57 ± 0.29 **b**	21.89***
Lys	-2.37 ± 0.54 **a**	0.75 ± 0.75 **b**	1.65 ± 0.75 **b**	9.35**
Met	-	-	-	-
Phe	-1.02 ± 0.22 **a**	-0.09 ± 0.57 **a**	-0.27 ± 0.82 **a**	0.71
Pro	4.54 ± 0.71 **ab**	6.32 ± 0.67 **b**	3.64 ± 0.21 **a**	5.53*
Ser	-0.74 ± 0.20 **a**	2.39 ± 0.74 **b**	2.11 ± 0.41 **b**	11.90**
Thr	-3.30 ± 0.83 **a**	2.01 ± 0.91 **b**	1.76 ± 0.92 **b**	11.40**
Tyr	-0.17 ± 0.39 **a**	0.99 ± 0.70 **ab**	3.16 ± 0.60 **b**	8.53**
Val	1.97 ± 0.24 **a**	3.94 ± 0.60 **b**	3.95 ± 0.25 **b**	8.16**

Letters represent differences between the three groups for a given amino acid. P value determined by Tukey HSD post hoc test. *P <0.05, **P <0.01, ***P <0.001

Table 2.5. Summary of $\delta^{15}N$ (mean ± SE) in *A. transitella* amino acids (n = 10).

Amino acid	Almond	Pistachio	Walnut	F
Ala	6.20 ± 0.41 **a**	8.04 ± 0.48 **b**	9.62 ± 0.41 **c**	15.50***
Asx	6.16 ± 0.26 **a**	7.77 ± 0.31 **b**	8.64 ± 0.32 **b**	17.95***
Glx	7.89 ± 0.38 **a**	10.00 ± 0.49 **b**	11.40 ± 0.38 **b**	17.53***
Gly	5.52 ± 0.35 **a**	7.22 ± 0.39 **b**	7.93 ± 0.42 **b**	10.13***
Ile	6.98 ± 0.83 **a**	12.65 ± 0.96 **b**	12.85 ± 0.67 **b**	16.22***
Leu	2.25 ± 0.61 **a**	6.83 ± 0.69 **b**	8.51 ± 0.50 **b**	28.70***
Lys	-0.26 ± 0.28 **a**	1.02 ± 0.29 **b**	1.71 ± 0.27 **b**	12.71***
Met	1.69 ± 0.35 **a**	4.66 ± 0.66 **b**	6.30 ± 0.56 **b**	18.97***
Phe	0.89 ± 0.25 **a**	1.89 ± 0.40 **a**	1.85 ± 0.38 **a**	2.66
Pro	15.85 ± 0.50 **a**	18.54 ± 0.54 **b**	19.65 ± 0.41 **b**	16.03***
Ser	5.49 ± 0.39 **a**	7.32 ± 0.40 **b**	9.49 ± 0.36 **c**	26.68***
Thr	-12.91 ± 0.39 **a**	-14.06 ± 0.73 **a**	-13.42 ± 0.61 **a**	0.94
Tyr	-2.87 ± 0.31 **ab**	-3.49 ± 0.52 **a**	-1.74 ± 0.53 **b**	3.65*
Val	4.75 ± 0.57 **a**	9.08 ± 0.64 **b**	9.06 ± 0.45 **b**	19.85***

Letters represent differences between the three groups for a given amino acid. P value determined by Tukey HSD post hoc test. *P <0.05, **P <0.01, ***P <0.001

Table 2.6. Summary of the mean decrease in accuracy (MDA) of $\delta^{13}C$ random forest models, for which larger values equate to more influence on model accuracy.

Plant $\delta^{13}C$ AA	**MDA**	**Insect $\delta^{13}C$ AA**	**MDA**
Gly	**9.27**	Met	**14.52**
Thr	**8.51**	Leu	**10.65**
Lys	**8.23**	Tyr	**8.56**
Met	**8.21**	Phe	**8.45**
Ile	**7.87**	Lys	**7.36**
Tyr	**7.01**	Pro	**7.26**
Leu	**6.35**	Gly	**7.09**
Pro	**5.07**	Asx	**6.73**
Ala	**4.80**	His	**6.32**
Asx	**3.91**	Ile	**6.26**
Val	**3.58**	Ala	**6.05**
Glx	**1.96**	Ser	**5.98**
Phe	**0.38**	Glx	**5.62**
Ser	**-0.27**	Val	**4.68**
-	-	Thr	**4.63**

Table 2.7. Summary of the mean decrease in accuracy (MDA) of $\delta^{15}N$ random forest models for which larger values equate to more influence on model accuracy.

Plant $\delta^{15}N$ AA	**MDA**	**Insect $\delta^{15}N$ AA**	**MDA**
Pro	**9.17**	Ser	**12.34**
Gly	**7.82**	Met	**8.50**
Glx	**7.09**	Leu	**7.66**
Leu	**6.60**	Ile	**7.30**
Tyr	**6.53**	Glx	**6.87**
Ser	**5.37**	Pro	**5.86**
Val	**5.20**	Val	**5.68**
Lys	**4.34**	Asx	**5.51**
Asx	**2.67**	Ala	**4.63**
Thr	**0.89**	Lys	**4.05**
Ala	**0.85**	Tyr	**3.29**
Phe	**-1.82**	Thr	**0.76**
Ile	**-2.56**	Phe	**-0.96**
-	-	Gly	**-1.91**

Table 2.8. Summary of the mean decrease in accuracy (MDA) of metabolomics (GC/MS) important features in random forest models.

Tissue	Tentative ID	Retention Time (min)	m/z	MDA
Nut kernel (n =5)	Conduritol β epoxide	15.01	318.074	**22.33**
	3-Aminopropionitrile	9.73	245.038	**21.98**
A. transitella (n = 10)	Arabitol	12.019	73.0296	**13.51**
	Uric Acid	15.896	73.003	**14.75**
	Isothreonic Acid [iso4]	11.057	292.135	**12.66**
	D (-) Fructose	13.361	73.021	**13.40**
	Xylitol	12.05	245.972	**13.24**
	Citric Acid	12.918	73.004	**13.09**

Table 2.9. Summary of the mean decrease in accuracy (MDA) of lipidomics (LC/MS) important features in random forest models.

Tissue	Tentative ID	Retention Time (min)	m/z	MDA
Nut kernel (n = 5)	TG (16:0/16:1(9Z)/18:2(9Z,12Z))	15.79	846.75	**23.91**
	FAHFA (18:0/12-O-18:0)	16.02	589.52	**19.98**
A. transitella (n = 10)	DG (18:0/18:3(9Z,12Z,15Z)/0:0) [iso2]	12.11	601.51	**11.42**
	TG (16:1(9Z)/18:4(6Z,9Z,12Z,15Z)/18:3(6Z,9Z,12Z))	15.18	864.70	**11.07**
	(8R,9S,10S,13S,14S,17R)-17-Ethyl-3,17-dihydroxy-10,13-dimethyl-3,4,5,6,7,8,9,11,12,14,15,16-dodecahydro-1H-cyclopenta[a]phenanthren-2-one *	12.35	335.25	**10.55**
	PE (18:2(9Z,12Z)/16:0)	9.18	738.50	**10.97**
	DG (18:2(9Z,12Z)/18:3(9Z,12Z,15Z)/0:0) [iso2] *	12.35	632.52	**10.22**
	(8R,9S,10S,13S,14S,17R)-17-Ethyl-3,17-dihydroxy-10,13-dimethyl-3,4,5,6,7,8,9,11,12,14,15,16-dodecahydro-1H-cyclopenta[a]phenanthren-2-one*	15.56	317.24	**10.41**
	DG (16:0/18:3(9Z,12Z,15Z)/0:0) [iso2] *	15.84	573.48	**10.20**
	DG (18:2(9Z,12Z)/18:3(9Z,12Z,15Z)/0:0)	15.56	597.48	**8.97**
	MG (18:1(11E)/0:0/0:0)	4.59	357.29	**7.16**

*Compound classified as "similar to" the tentative ID

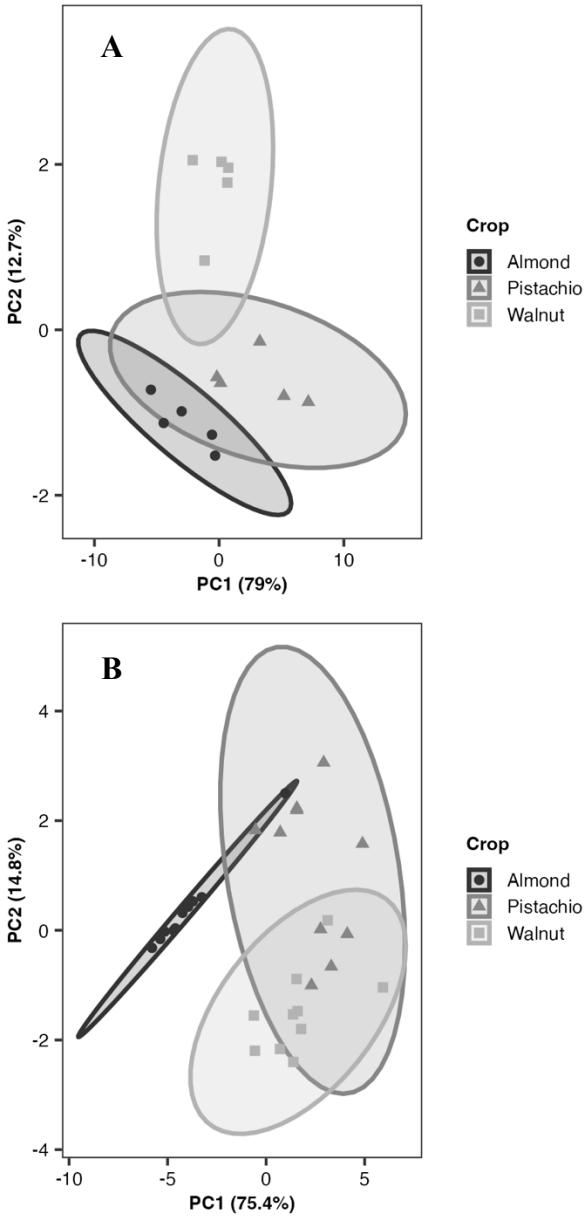

Figure 2.1. Principal component analysis (PCA) considers all $\delta^{13}C$ AA values for (A) nut kernels and (B) *A. transitella*. Highlighted areas represent 95% confidence regions.

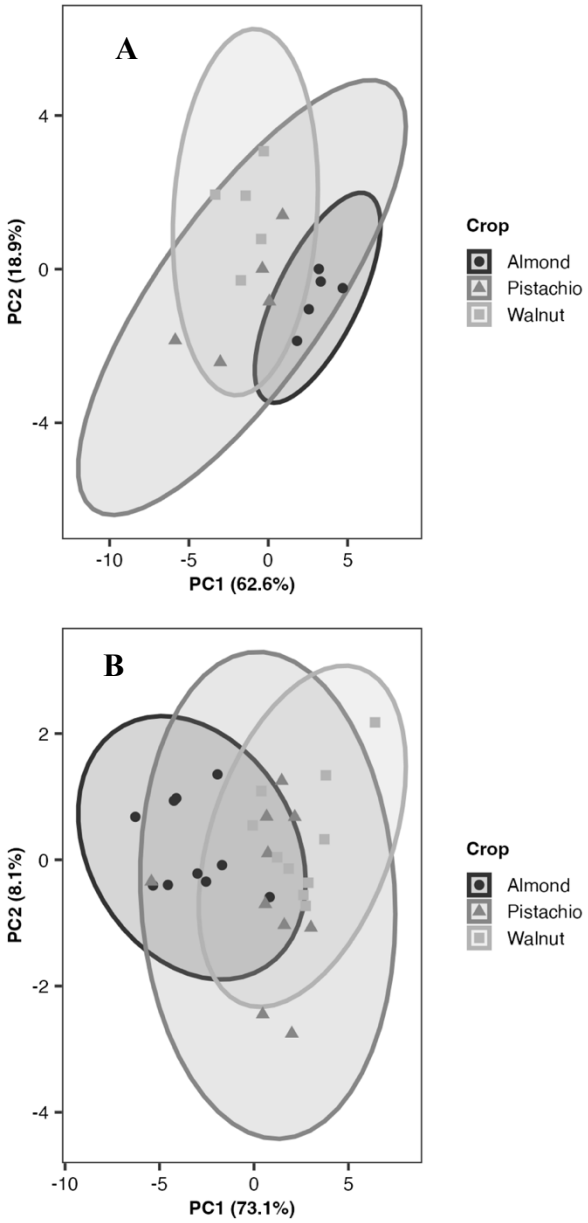

Figure 2.2. Principal component analysis (PCA) considers all values for $\delta^{15}N$ AA (A) nut kernels and (B) *A. transitella*. Highlighted areas represent 95% confidence regions.

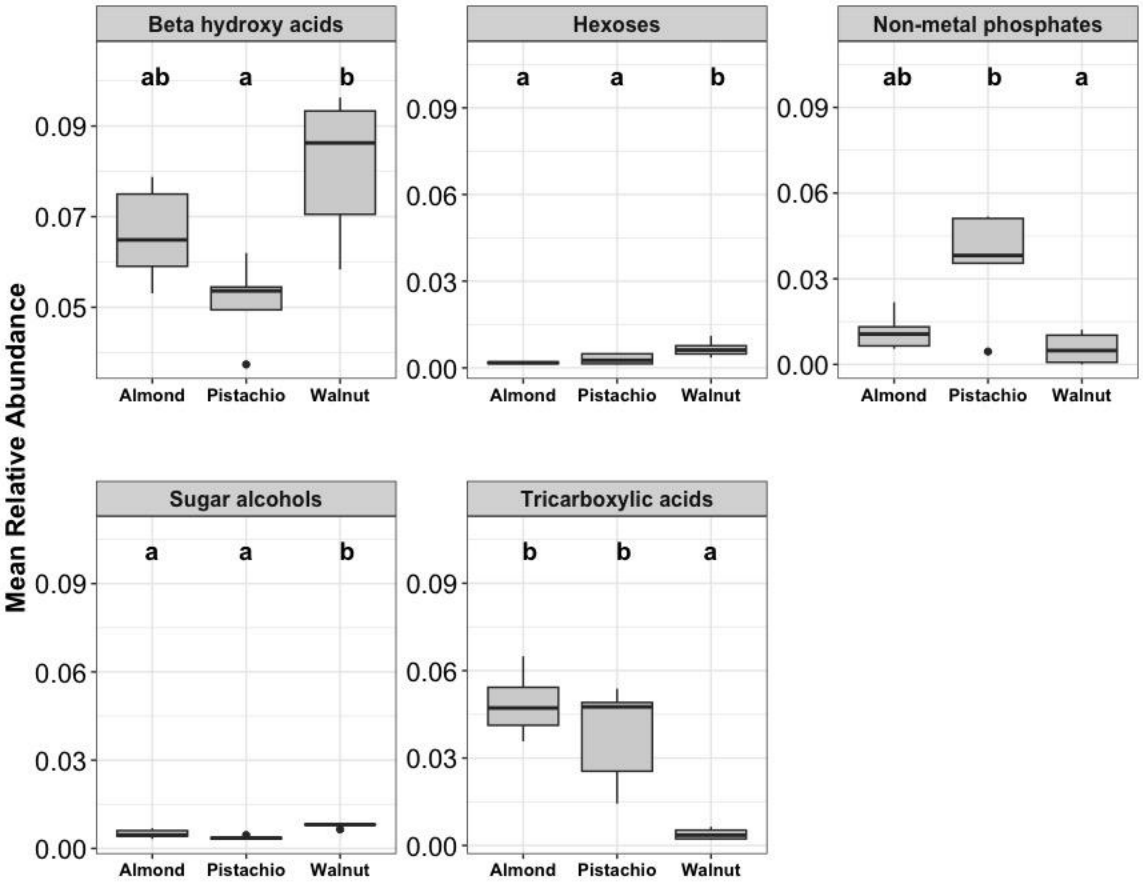

Figure 2.3. Mean relative abundance of the top 5 metabolite classes in 2023 nut kernels. Boxplots that have different letters indicate significant differences, Tukey HSD, $P < 0.05$.

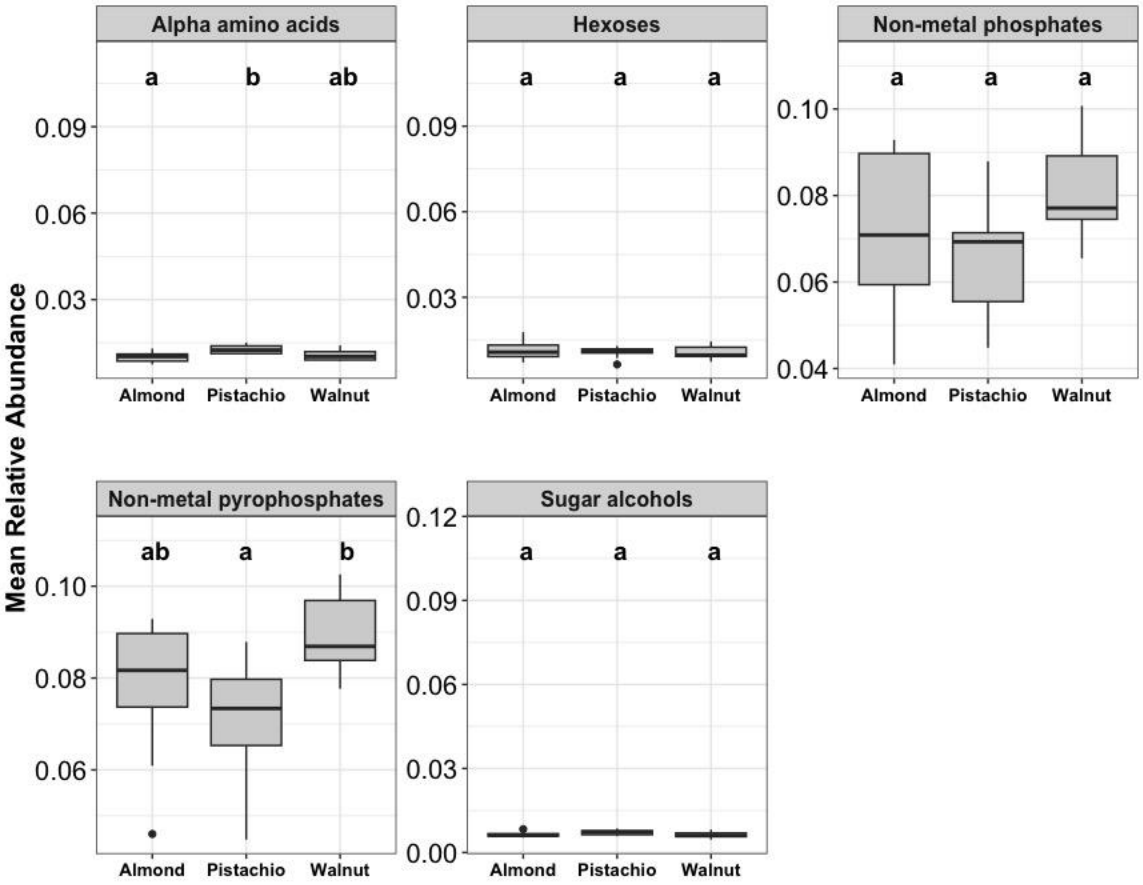

Figure 2.4. Mean relative abundance of the top 5 metabolite classes in 2023 *A. transitella*. Boxplots that have different letters indicate significant differences, Tukey HSD, P <0.05.

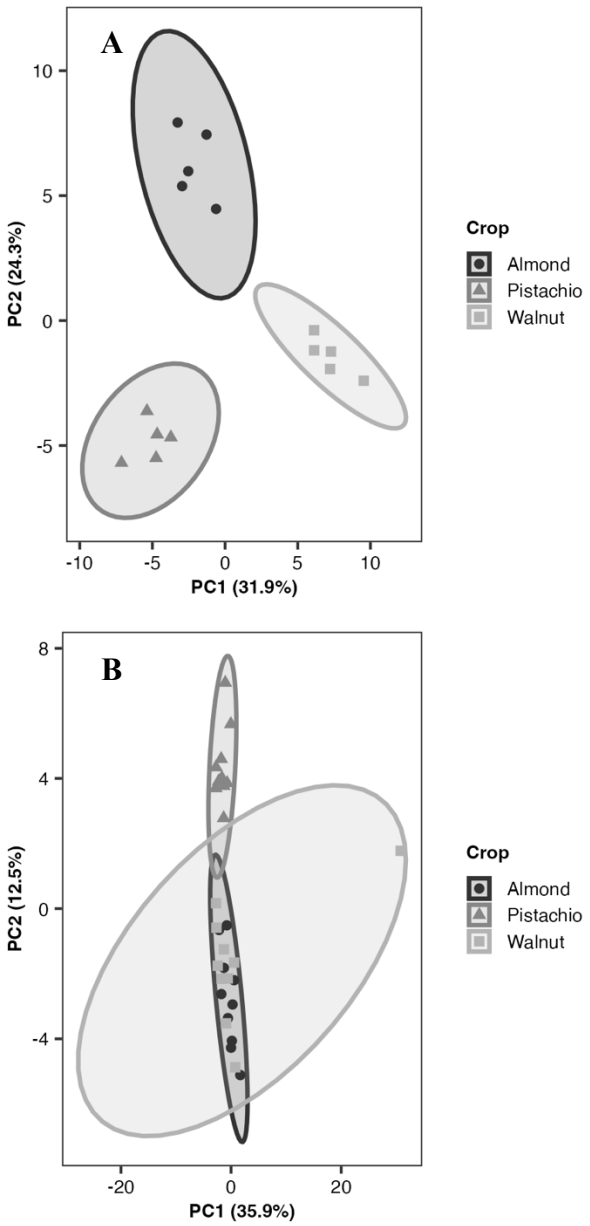

Figure 2.5. Principal component analysis (PCA) considers 98 known metabolites for 2023 (A), nut kernels, and (B) *A. transitella*. Highlighted areas represent 95% confidence regions.

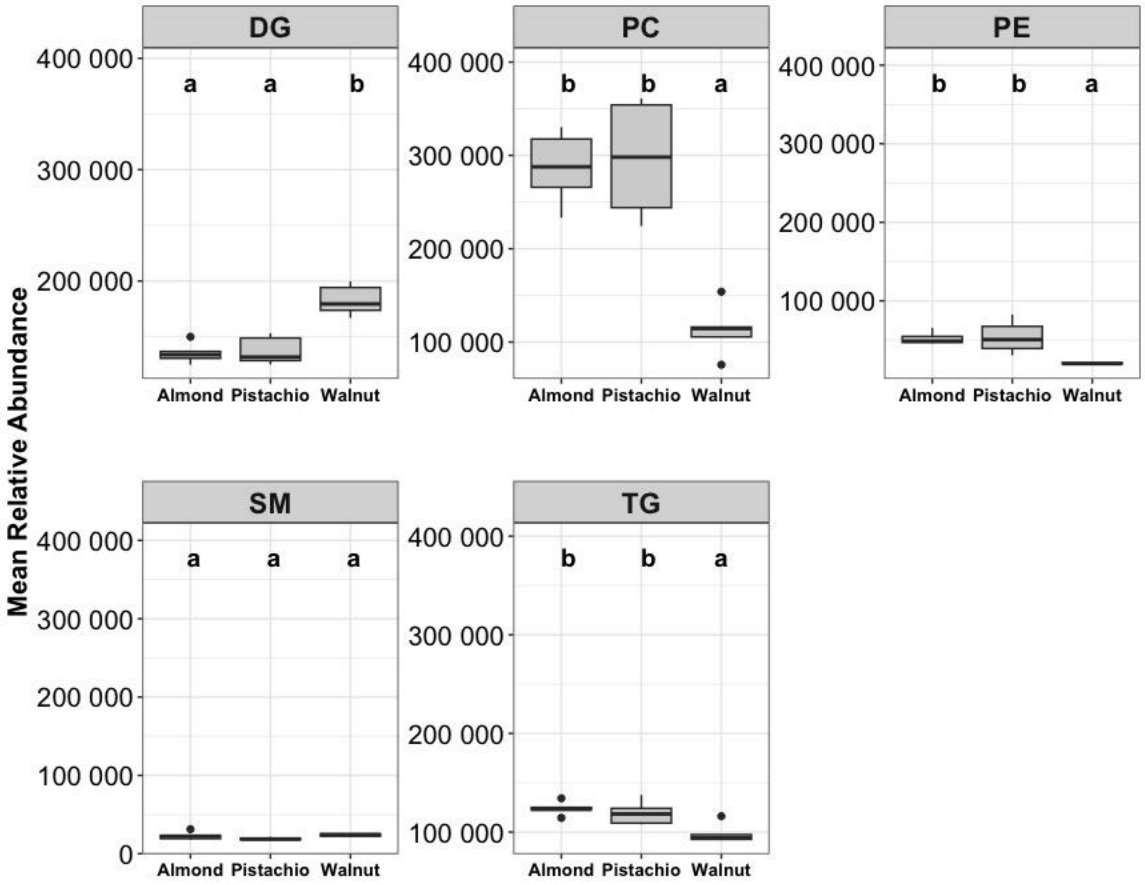

Figure 2.6. Mean relative abundance of the top 5 lipid classes in 2023 nut kernels.
Boxplots that have different letters indicate significant differences, Tukey HSD, P <0.05.

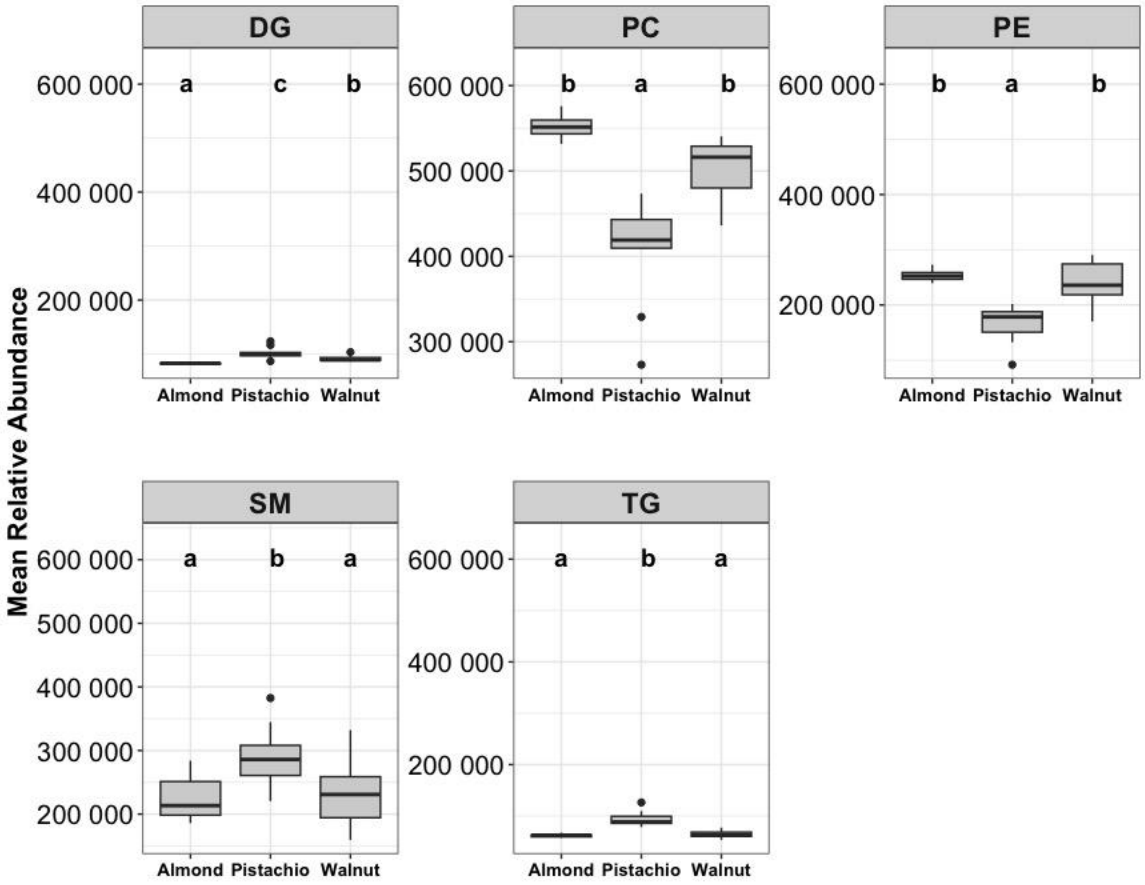

Figure 2.7. Mean relative abundance of the top 5 lipid classes in 2023 *A. transitella*. Boxplots that have different letters indicate significant differences, Tukey HSD, P <0.05.

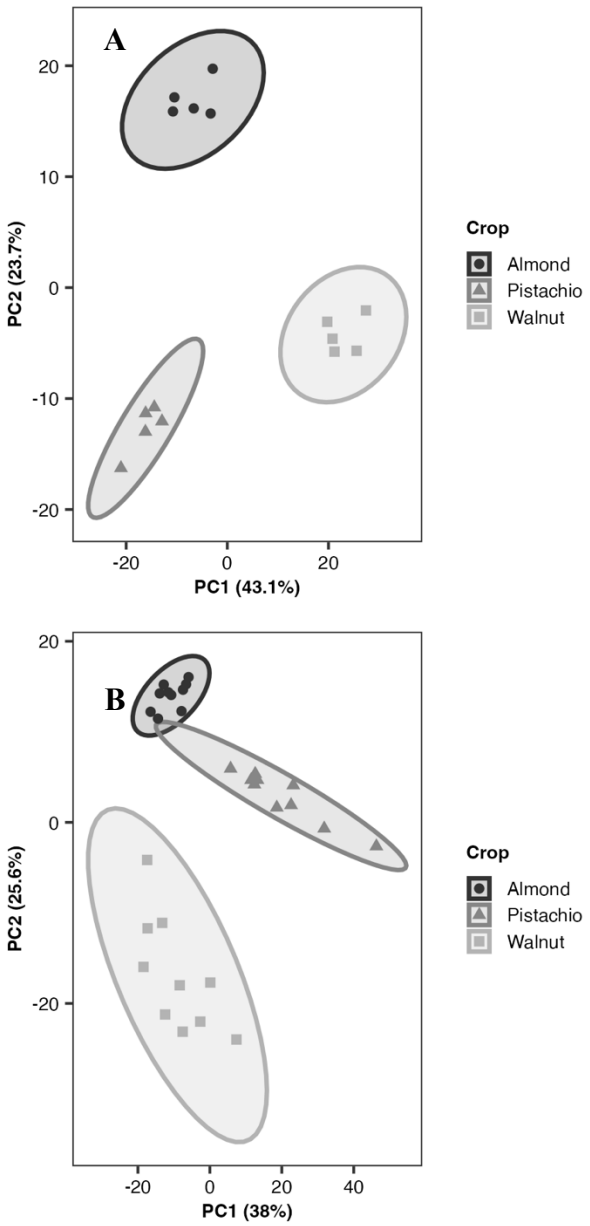

Figure 2.8. Principal component analysis (PCA) considers 712 known lipids for 2023 (A), nut kernels, and (B) *A. transitella*. Highlighted areas represent 95% confidence regions.

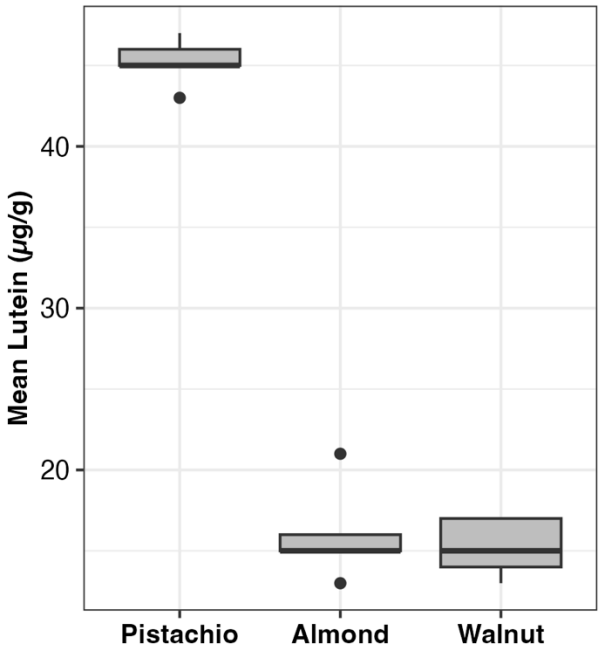

Figure 2.9. Mean concentration of lutein in nut kernels (n = 5) expressed in μg/g measured on the spectrometer.

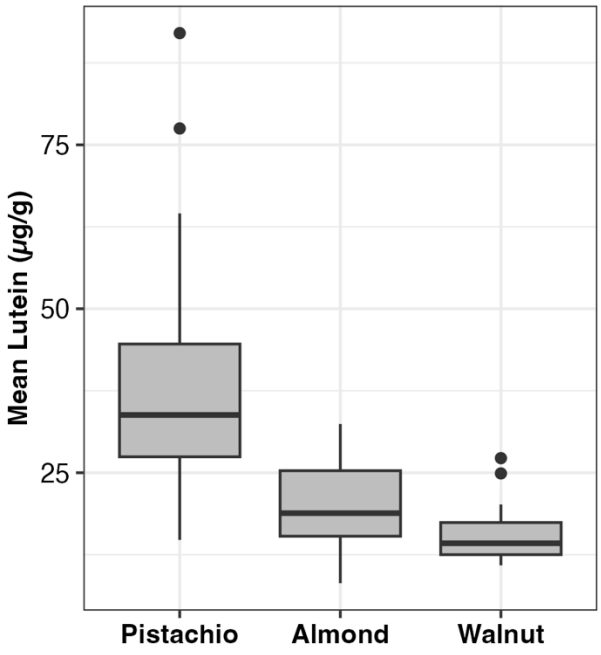

Figure 2.10. Mean concentration of lutein in *A. transitella* (n = 30) expressed in µg/g measured on the spectrometer.

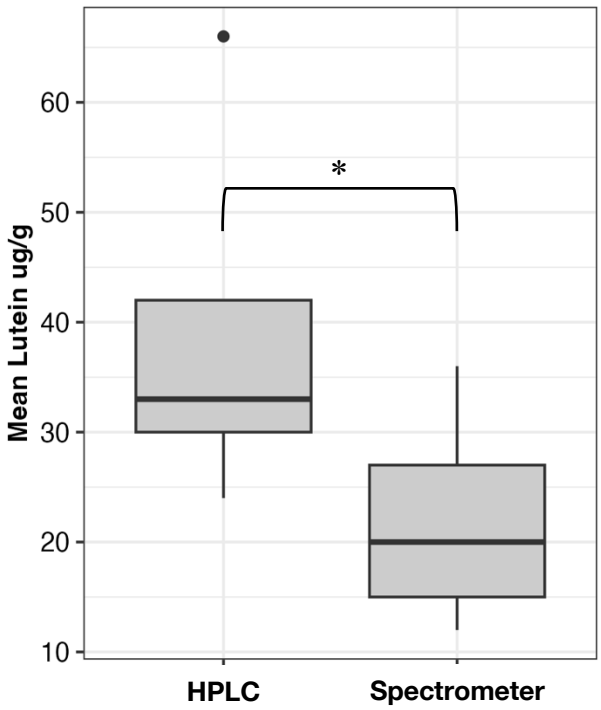

Figure 2.11. Mean concentration of lutein in *A. transitella* measured directly on the HPLC (n = 4) and spectrometer (n = 30). Data analyzed by paired t-test; *P <0.05.

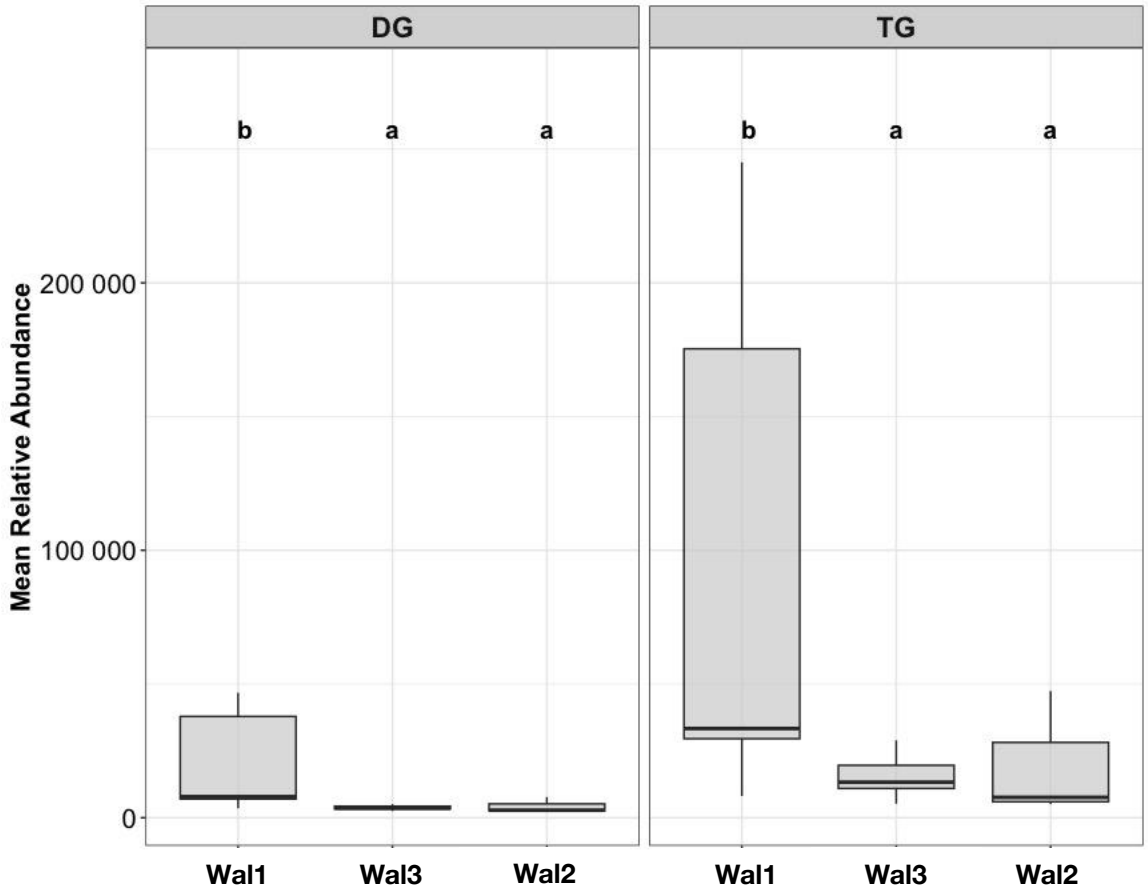

Figure 2.12. Mean relative abundance of diglycerides (DG) and triglycerides (TG) for *A. transitella* capture in the 2025 walnut sites. Boxplots that have different letters indicate significant differences, Tukey HSD, P <0.05 (n = 10).

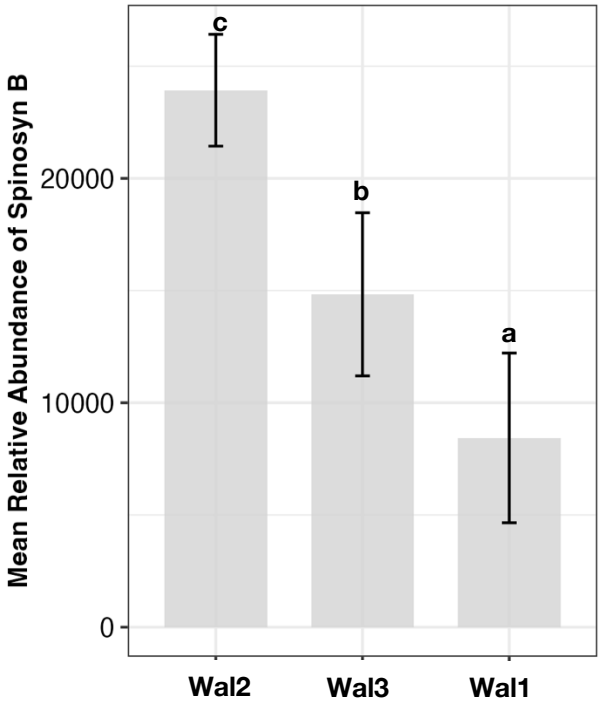

Figure 2.13. Mean relative abundance of Spinosyn B for *A. transitella* capture in the 2025 walnut sites. Boxplots that have different letters indicate significant differences, Tukey HSD, P <0.05 (n= 10).

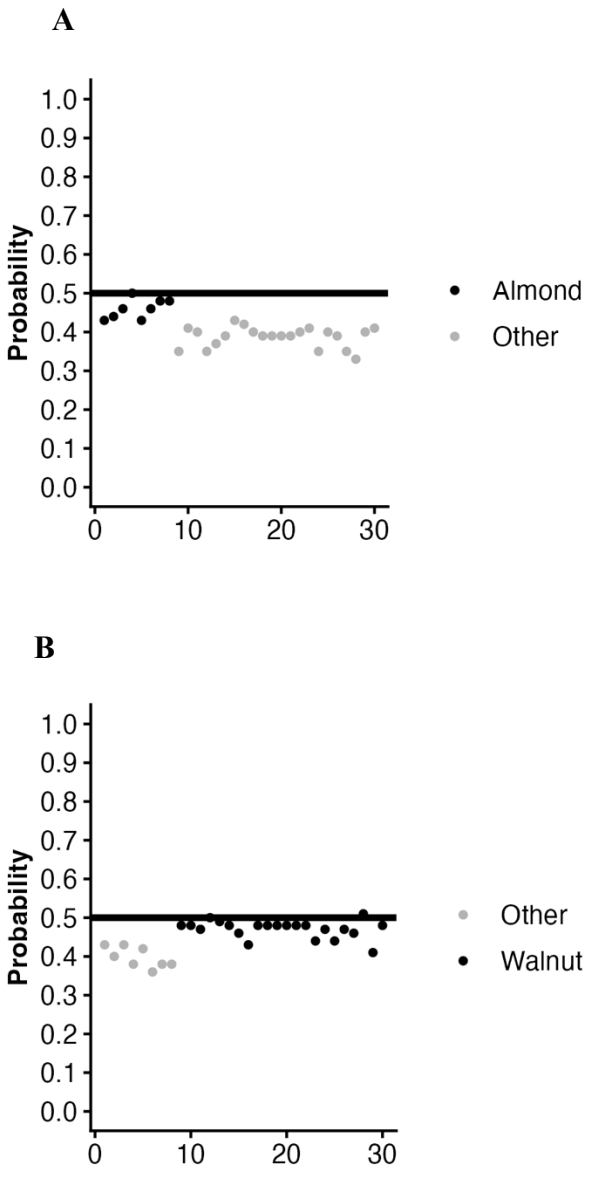

Figure 2.14. The average probability of membership. Samples that have a distinct lipid profile would have an OOB probability close to one. Samples in the group of interest are indicated by black circles, almond (A) and walnut (B); samples constituting the others are indicated by grey circles.

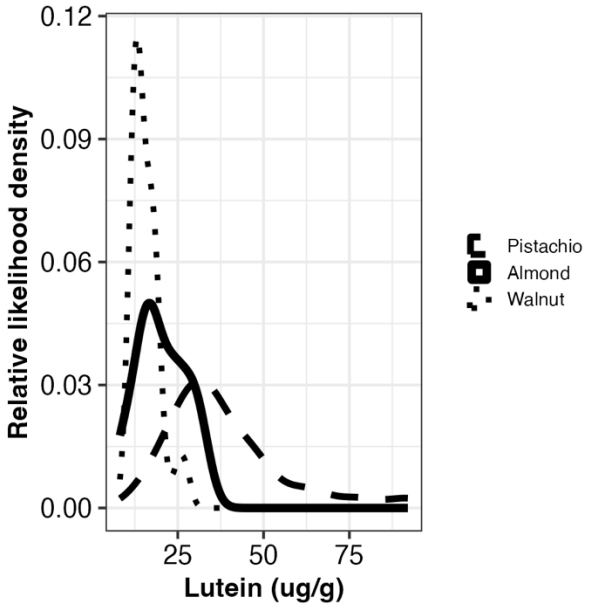

Figure 2.15. Density plot of the distribution of lutein in *A. transitella* (n = 30) measured on the spectrometer.

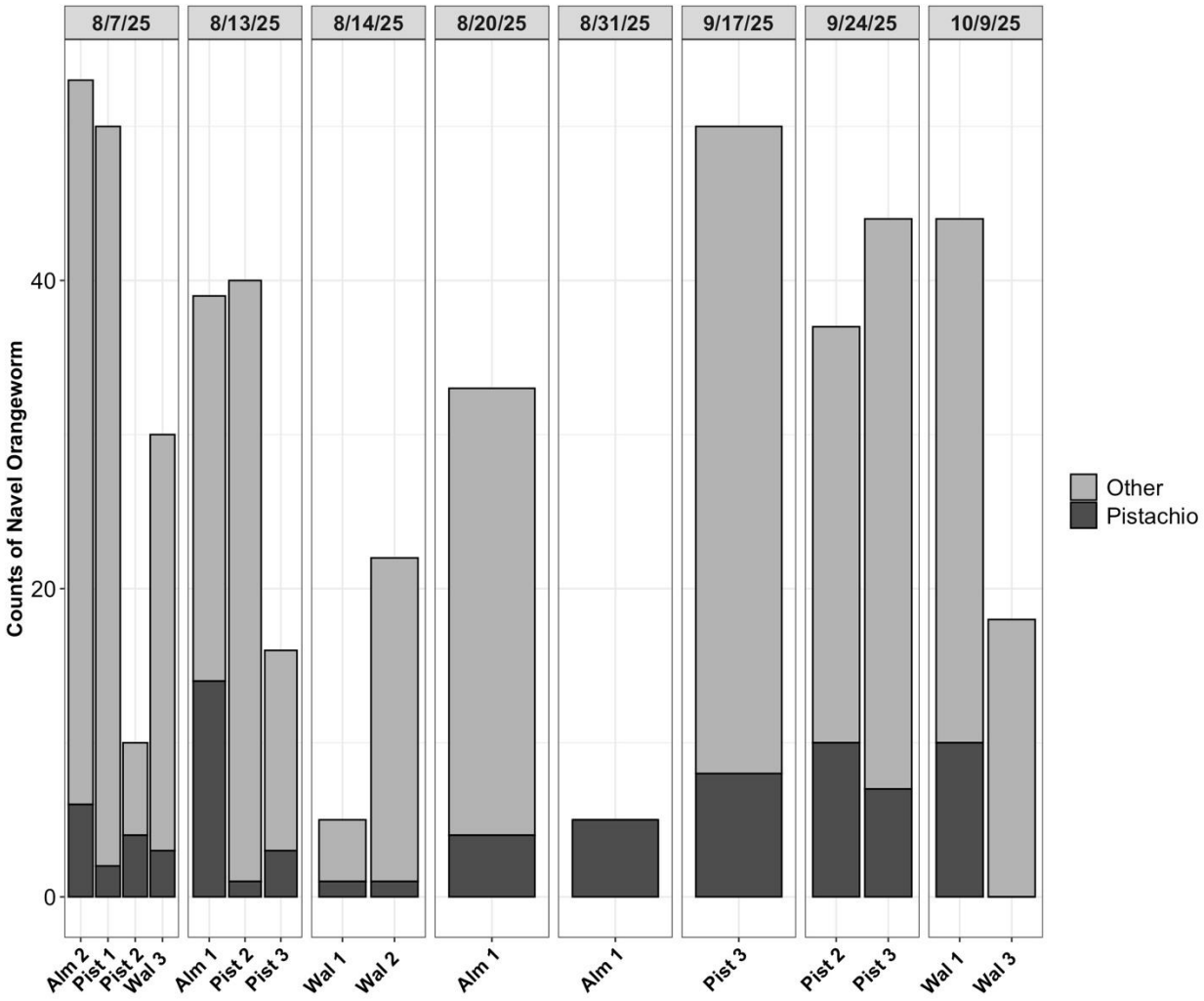

Figure 2.16. The counts of *A. transitella* with a larval source of pistachio or other from field sites in 2025.

References

Abdu Hussen A. 2022. High-Performance Liquid Chromatography (HPLC): A review. Ann. Adv. Chem. 6(1):010–020. https://doi.org/10.29328/journal.aac.1001026

Adams CG, Schenker JH, McGhee PS, et al. 2017. Maximizing Information Yield From Pheromone-Baited Monitoring Traps: Estimating Plume Reach, Trapping Radius, and Absolute Density of Cydia pomonella (Lepidoptera: Tortricidae) in Michigan Apple. J. Econ. Entomol. 110(2):305–318. https://doi.org/10.1093/jee/tow258

Adams M-O, Seifert CL, Lehner L, et al. 2016. Stable isotope signatures reflect dietary diversity in European forest moths. Front. Zool. 13(1):37. https://doi.org/10.1186/s12983-016-0170-0

Adaskaveg JA, Lee C, Wei Y, et al. 2025. In a nutshell: pistachio genome and kernel development. New Phytol. 246(3):1032–1048. https://doi.org/10.1111/nph.70060

Ahlawat YK, Singh M, Manorama K, et al. 2024. Plant phenolics: neglected secondary metabolites in plant stress tolerance. Braz. J. Bot. 47(3):703–721. https://doi.org/10.1007/s40415-023-00949-x

Ahmadi R, Honarvar M, Ghavami M, et al. 2024. Optimization of Lutein Extraction from Pistachio Waste Using Experimental Design and Ultrasonic Method. Waste Biomass Valorization. 15(5):3077–3091. https://doi.org/10.1007/s12649-023-02363-6

Arredondo J, Ruiz L, López G, et al. 2017. Visibility and Persistence of Marker Dyes and Effect on the Quality and Mating Competitiveness of Mass-Reared Flies (Diptera: Tephritidae): Anastrepha obliqua and Bisexual and Genetic Sexing (Tapachula-7) Strains of A. ludens. J. Econ. Entomol. 110(4):1653–1657. https://doi.org/10.1093/jee/tox154

Arrese EL, Soulages JL. 2010. Insect fat body: energy, metabolism, and regulation. Annu. Rev. Entomol. 55:207–225.

Athaillah ZA, Yarnes C, Wang SC. 2023. Bulk and Compound-Specific Stable Isotope Analysis for the Authentication of Walnuts (Juglans regia) Origins. J. Agric. Food Chem. 71(45):16939–16949. https://doi.org/10.1021/acs.jafc.3c03770

Barupal DK, Fan S, Wancewicz B, et al. 2018. Generation and quality control of lipidomics data for the alzheimer's disease neuroimaging initiative cohort. Sci. Data. 5(1):1–13.

Bateman AS, Kelly SD. 2007. Fertilizer nitrogen isotope signatures. Isotopes Environ. Health Stud. 43(3):237–247. https://doi.org/10.1080/10256010701550732

Bayes SK, Hellerstein MK, Fitch M, et al. 2014. You are what you eat: fatty acid profiles as a method to track the habitat movement of an insect. Oecologia. 175(4):1073–1080.

Bayman P, Baker JL, Mahoney NE. 2002. Aspergillus on tree nuts: incidence and associations. Mycopathologia. 155(3):161–169.

Bocso N-S, Butnariu M. 2022. The biological role of primary and secondary plants metabolites. J. Nutr. Food Process. 5(3):1–7.

Bontempo L, Van Leeuwen KA, Paolini M, et al. 2020. Bulk and compound-specific stable isotope ratio analysis for authenticity testing of organically grown tomatoes. Food Chem. 318:126426.

Burks CS. 2014. Effects of Delayed Mating and Access to Water on Oviposition and Longevity in Female *Amyelois transitella*. Int. J. Insect Sci. 6:IJIS.S20688. https://doi.org/10.4137/IJIS.S20688

Burks CS, Rijal JP, Wilson H. 2020. Movement and Monitoring of Navel Orangeworm (Amyelois transitella) in Walnuts: 2020. Annu. Rep. Calif. Walnut Board.

Carvalho M, Sampaio JL, Palm W, et al. 2012. Effects of diet and development on the Drosophila lipidome. Mol. Syst. Biol. 8(1):MSB201229. https://doi.org/10.1038/msb.2012.29

Chung I-M, Kim J-K, An Y-J, et al. 2019. Compound-specific δ13C and δ15N analyses of fatty acids and amino acids for discrimination of organic, pesticide-free, and conventional rice (Oryza sativa L.). Food Chem. 283:305–314.

Corr LT, Berstan R, Evershed RP. 2007. Optimisation of derivatisation procedures for the determination of δ^{13}C values of amino acids by gas chromatography/combustion/isotope ratio mass spectrometry. Rapid Commun. Mass Spectrom. 21(23):3759–3771. https://doi.org/10.1002/rcm.3252

Demkovich M, Dana CE, Siegel JP, et al. 2015. Effect of Piperonyl Butoxide on the Toxicity of Four Classes of Insecticides to Navel Orangeworm (Amyelois transitella) (Lepidoptera: Pyralidae). J. Econ. Entomol. 108(6):2753–2760. https://doi.org/10.1093/jee/tov237

Diano A, Bekker-Jensen S, Dynesen J, et al. 2006. Polyol synthesis in *Aspergillus niger* : Influence of oxygen availability, carbon and nitrogen sources on the metabolism. Biotechnol. Bioeng. 94(5):899–908. https://doi.org/10.1002/bit.20915

Dong H-L, Zhang S-X, Tao H, et al. 2017. Metabolomics differences between silkworms (Bombyx mori) reared on fresh mulberry (Morus) leaves or artificial diets. Sci. Rep. 7(1):1–16.

Dunn WB, Broadhurst D, Begley P, et al. 2011. Procedures for large-scale metabolic profiling of serum and plasma using gas chromatography and liquid chromatography coupled to mass spectrometry. Nat. Protoc. 6(7):1060–1083. https://doi.org/10.1038/nprot.2011.335

Eichenseer H, Murphy JB, Felton GW. 2002. Sequestration of host plant carotenoids in the larval tissues of *Helicoverpa zea*. J. Insect Physiol. 48(3):311–318. https://doi.org/10.1016/S0022-1910(01)00178-0

Esmaeilpour A, Van Labeke MC, Boeckx P, et al. 2016. Impact of osmotic drought stress on carbon isotope discrimination and growth parameters in three pistachio rootstocks (Pistacia spp., Anacardiaceae). In: Options Mediterraneennes 119 XVI GREMPA Meeting on Almonds and Pistachios.

Farquhar GD, Ehleringer JR, Hubick KT. 1989. Carbon Isotope Discrimination and Photosynthesis. Annu. Rev. Plant Physiol. Plant Mol. Biol. 40(1):503–537. https://doi.org/10.1146/annurev.pp.40.060189.002443

Florkowski CM. 2008. Sensitivity, Specificity, Receiver-Operating Characteristic (ROC) Curves and Likelihood Ratios: Communicating the Performance of Diagnostic Tests. Clin. Biochem. Rev. 29(Suppl 1):S83–S87.

Gromski PS, Muhamadali H, Ellis DI, et al. 2015. A tutorial review: Metabolomics and partial least squares-discriminant analysis – a marriage of convenience or a shotgun wedding. Anal. Chim. Acta. 879:10–23. https://doi.org/10.1016/j.aca.2015.02.012

Grönke S, Mildner A, Fellert S, et al. 2005. Brummer lipase is an evolutionary conserved fat storage regulator in *Drosophila*. Cell Metab. 1(5):323–330. https://doi.org/10.1016/j.cmet.2005.04.003

Guarrasi V, Mangione MR, Sanfratello V, et al. 2010. Quantification of Underivatized Fatty Acids From Vegetable Oils by HPLC with UV Detection. J. Chromatogr. Sci. 48(8):663–668. https://doi.org/10.1093/chromsci/48.8.663

Hagler JR. 2019. Super Mark It! A Review of the Protein Immunomarking Technique. Ann. Entomol. Soc. Am. 112(3):200–210. https://doi.org/10.1093/aesa/say039

Hagler JR, Casey MT, Machtley SA, et al. 2024. Marking Helicoverpa zea (Lepidoptera: Noctuidae) with fluorophores for use in mark-release-recapture research. Ann. Entomol. Soc. Am. 117(6):332–339.

Hagler JR, Jackson CG. 2001. Methods for Marking Insects: Current Techniques and Future Prospects. Annu. Rev. Entomol. 46(Volume 46, 2001):511–543. https://doi.org/10.1146/annurev.ento.46.1.511

Hagler JR, Machtley SA. 2020. Refinement of the Protein Immunomarking Technique for Mark-Capture Research. J. Insect Sci. 20(2):8. https://doi.org/10.1093/jisesa/ieaa015

Horai H, Arita M, Kanaya S, et al. 2010. MassBank: a public repository for sharing mass spectral data for life sciences. J. Mass Spectrom. JMS. 45(7):703–714. https://doi.org/10.1002/jms.1777

Jin F, Zhou Y, Zhang P, et al. 2023. Identification of Key Lipogenesis Stages and Proteins Involved in Walnut Kernel Development. J. Agric. Food Chem. 71(10):4306–4318. https://doi.org/10.1021/acs.jafc.2c08680

Kim JH, Lagojda A, Kuehne D, et al. 2020. Determination of Cotton as a Larval Feeding Source for Lepidopteran Moths Using a Derivative from Cotton Metabolites as a Marker by LC-MS/MS Method. J. Chem. Ecol. 46(10):956–966.

Kim S-H, Moon J-K, Jo H-W, et al. 2022. Ecofriendly shiitake authentication using bulk and amino acid-specific stable isotope models. Food Chem. 397:133819. https://doi.org/10.1016/j.foodchem.2022.133819

Knittelfelder O, Prince E, Sales S, et al. 2020. Sterols as dietary markers for *Drosophila melanogaster*. Biochim. Biophys. Acta BBA - Mol. Cell Biol. Lipids. 1865(7):158683. https://doi.org/10.1016/j.bbalip.2020.158683

Kramer KJ, Seib PA. 1982. Ascorbic acid and the growth and development of insects. Adv Chem Ser. 200:275–291.

Kshatriya K, Gershenzon J. 2024. Disarming the defenses: Insect detoxification of plant defense-related specialized metabolites. Curr. Opin. Plant Biol. 81:102577. https://doi.org/10.1016/j.pbi.2024.102577

Larsen T, Taylor DL, Leigh MB, et al. 2009. Stable isotope fingerprinting: a novel method for identifying plant, fungal, or bacterial origins of amino acids. Ecology. 90(12):3526–3535. https://doi.org/10.1890/08-1695.1

Le Roux X, Bariac T, Sinoquet H, et al. 2001. Spatial distribution of leaf water-use efficiency and carbon isotope discrimination within an isolated tree crown. Plant Cell Environ. 24(10):1021–1032. https://doi.org/10.1046/j.0016-8025.2001.00756.x

Lee J-D, Shannon JG, So Y-S, et al. 2009. Environmental effects on lutein content and relationship of lutein and other seed components in soybean. Plant Breed. 128(1):97–100. https://doi.org/10.1111/j.1439-0523.2008.01547.x

Lewis DH, Smith DC. 1967. Sugar Alcohols (Polyols) in Fungi and Green Plants. I. Distribution, Physiology and Metabolism. New Phytol. 66(2):143–184.

Liu T-C (Jean), Burks CS, Wilson H. 2025. Evaluation of a fluorophore for marking navel orangeworm (Lepidoptera: Pyralidae). Mohamed A, editor. J. Insect Sci. 25(1):12. https://doi.org/10.1093/jisesa/ieaf015

Liu Y, Blumberg JB, Chen C-YO. 2014. Quantification and Bioaccessibility of California Pistachio Bioactives. J. Agric. Food Chem. 62(7):1550–1556. https://doi.org/10.1021/jf4046864

Logan DM, Proverbs MD. 1975. A device for marking adult codling moths (Lepidoptera: Olethreutidae) with fluorescent powders. Can. Entomol. 107(8):879–881. https://doi.org/10.4039/Ent107879-8

Manupa W, Wongthanyakram J, Jeencham R, et al. 2023. Storage stability and antioxidant activities of lutein extracted from yellow silk cocoons (Bombyx mori) in Thailand. Heliyon. 9(6). https://doi.org/10.1016/j.heliyon.2023.e16805

Martelli F, Hernandes NH, Zuo Z, et al. 2022. Low doses of the organic insecticide spinosad trigger lysosomal defects, elevated ROS, lipid dysregulation, and neurodegeneration in flies. Elife. 11:e73812.

McGruer V, Bhatia A, Magnuson JT, et al. 2023. Lipid Profile Altered in Phenanthrene Exposed Zebrafish Embryos with Implications for Neurological Development and Early Life Nutritional Status. Environ. Health. 1(1):32–40. https://doi.org/10.1021/envhealth.3c00002

Mininni AN, Tuzio AC, Brugnoli E, et al. 2022. Carbon isotope discrimination and water use efficiency in interspecific *Prunus* hybrids subjected to drought stress. Plant Physiol. Biochem. 175:33–43. https://doi.org/10.1016/j.plaphy.2022.01.030

Miura M, Sakai M, Nogami M, et al. 2020. A rapid LC–MS/MS method for lutein quantification in spinach (*Spinacia oleracea*). Microchem. J. 153:104470. https://doi.org/10.1016/j.microc.2019.104470

Moffitt HR, Albano DJ. 1972. Codling Moths1: Fluorescent Powders as Markers2. Environ. Entomol. 1(6):750–753. https://doi.org/10.1093/ee/1.6.750

Morton AC. 1982. The effects of marking and capture on recapture frequencies of butterflies. Oecologia. 53(1):105–110. https://doi.org/10.1007/BF00377143

Murata M, Tojo S. 2002. Utilization of lipid for flight and reproduction in Spodoptera litura (Lepidoptera: Noctuidae). Eur. J. Entomol. 99(2):221–224.

Naranjo S. 1990. Influence of Two Mass-Marking Techniques on Survival and Flight Behavior of Diabrotica virgifera virgifera (Coleoptera: Chrysomelidae). J. Econ. Entomol. 83. https://doi.org/10.1093/jee/83.4.1360

Nguyen K-O, Al-Rashid S, Clarke Miller M, et al. 2019. Trichoplusia ni (Lepidoptera: Noctuidae) Qualitative and Quantitative Sequestration of Host Plant Carotenoids. Environ. Entomol. 48(3):540–545. https://doi.org/10.1093/ee/nvz029

Nishiyama-Naruke A, Souza JA, Carnelós M, et al. 1998. HPLC Determination of Underivatized Fatty Acids Saponified at 37°C Analysis of Fatty Acids in Oils and Tissues. Anal. Lett. 31(14):2565–2576. https://doi.org/10.1080/00032719808005325

Núñez L, Álvarez S, Mirás-Avalos JM. 2026. Stomatal response and intrinsic water use efficiency of three pistachio cultivars grown in Central Spain: a relevant criterion for plant selection. J. Hortic. Sci. Biotechnol. 101(2):341–353. https://doi.org/10.1080/14620316.2025.2551114

O'Brien DM, Fogel ML, Boggs CL. 2002. Renewable and nonrenewable resources: amino acid turnover and allocation to reproduction in Lepidoptera. Proc. Natl. Acad. Sci. 99(7):4413–4418.

O'Connell TC. 2017. 'Trophic' and 'source' amino acids in trophic estimation: a likely metabolic explanation. Oecologia. 184(2):317–326. https://doi.org/10.1007/s00442-017-3881-9

Ortega-Beltran A, Moral J, Puckett RD, et al. 2018. Fungal communities associated with almond throughout crop development: Implications for aflatoxin biocontrol management in California. PLoS One. 13(6):e0199127.

Palumbo JD, Mahoney NE, Light DM, et al. 2014. Spread of Aspergillus flavus by navel orangeworm (Amyelois transitella) on almond. Plant Dis. 98(9):1194–1199.

Patil, M. P. N. 2017. HPLC Method Development–A Review. J. Pharm. Res. and Edu. 1(2), 243-260.

Peddinti SR, Kisekka I. 2025. Carbon–water coupling in California almond orchards: a multi-scale assessment of ecosystem water use efficiency using eddy covariance and remote sensing. Sci. Total Environ. 990:179914. https://doi.org/10.1016/j.scitotenv.2025.179914

Piri F, Sahragard A, Ghadamyari M. 2014. Sublethal effects of spinosad on some biochemical and biological parameters of Glyphodes pyloalis Walker (Lepidoptera: Pyralidae). Plant Prot. Sci. 50(3):135–144.

Pollierer MM, Larsen T, Potapov A, et al. 2019. Compound-specific isotope analysis of amino acids as a new tool to uncover trophic chains in soil food webs. Ecol. Monogr. 89(4):e01384. https://doi.org/10.1002/ecm.1384

Quinby BM, Creighton JC, Flaherty EA. 2020. Stable isotope ecology in insects: a review. Ecol. Entomol. 45(6):1231–1246. https://doi.org/10.1111/een.12934

Radcliffe EB, Hutchison WD, Cancelado RE. 2009. Integrated pest management: concepts, tactics, strategies and case studies. Cambridge University Press.

Ranganathan Y, Borges RM. 2010. Reducing the babel in plant volatile communication: using the forest to see the trees. Plant Biol. 12(5):735–742. https://doi.org/10.1111/j.1438-8677.2009.00278.x

Rapkin J, Jensen K, House CM, et al. 2018. Genotype-by-sex-by-diet interactions for nutritional preference, dietary consumption, and lipid deposition in a field cricket. Heredity. 121(4):361–373. https://doi.org/10.1038/s41437-018-0130-x

Riach AC, Perera MVL, Florance HV, et al. 2019. Metabolic fingerprints reveal how an insect metabolome is affected by different larval host plant species. Arthropod-Plant Interact. 13(4):571–579.

Salazar MO, Furlan RLE. 2007. A rapid TLC autographic method for the detection of glucosidase inhibitors. Phytochem. Anal. 18(3):209–212. https://doi.org/10.1002/pca.971

Sanz ML, Martínez-Castro I, Moreno-Arribas MV. 2008. Identification of the origin of commercial enological tannins by the analysis of monosaccharides and polyalcohols. Food Chem. 111(3):778–783. https://doi.org/10.1016/j.foodchem.2008.04.050

Sappington TW, Burks CS. 2014. Patterns of flight behavior and capacity of unmated navel orangeworm (Lepidoptera: Pyralidae) adults related to age, gender, and wing size. Environ. Entomol. 43(3):696–705.

Sappington TW, Fescemyer HW, Showers WB. 1995. Lipid and carbohydrate utilization during flight of the migratory moth, Agrotis ipsilon (Lepidoptera: Noctuidae). Arch. Insect Biochem. Physiol. 29(4):397–414.

Scott TM, Ogunbodede O, McKay DL, et al. 2025. Pistachio Consumption Increases Macular Pigment Optical Density in Healthy Adults: A Randomized Controlled Trial. J. Nutr. 155(1):168–174. https://doi.org/10.1016/j.tjnut.2024.10.022

Shirvani H, Fatehi F, Hejri S, et al. 2025. Metabolic Reprogramming and Amino Acid Adjustments in Pistachio (Pistacia vera L.) Under Salinity Stress. Horticulturae. 11(10). https://doi.org/10.3390/horticulturae11101201

Siderhurst MS, Hurst AL, Ladizinsky N, et al. 2025. Using harmonic radar to compare movement behavior between Bactrocera dorsalis and Ceratitis capitata (Diptera: Tephritidae). J. Insect Sci. 25(5):ieaf078. https://doi.org/10.1093/jisesa/ieaf078

Smith CA, O'Maille G, Want EJ, et al. 2005. METLIN: a metabolite mass spectral database. Ther. Drug Monit. 27(6):747–751. https://doi.org/10.1097/01.ftd.0000179845.53213.39

Snart CJP, Hardy ICW, Barrett DA. 2015. Entometabolomics: applications of modern analytical techniques to insect studies. Entomol. Exp. Appl. 155(1):1–17. https://doi.org/10.1111/eea.12281

Stuetz W, Schlörmann W, Glei M. 2017. B-vitamins, carotenoids and α-/γ-tocopherol in raw and roasted nuts. Food Chem. 221:222–227. https://doi.org/10.1016/j.foodchem.2016.10.065

Su Z, Xu W, Fu L, et al. 2026. Untargeted metabolomics reveals divergent metabolic profiles between the predatory Arma chinensis and the Phytophagous Halyomorpha halys. J. Insect Sci. 26(1):ieag005. https://doi.org/10.1093/jisesa/ieag005

Sud M, Fahy E, Cotter D, et al. 2007. LMSD: LIPID MAPS structure database. Nucleic Acids Res. 35(Database issue):D527-532. https://doi.org/10.1093/nar/gkl838

Teets NM, Kawarasaki Y, Lee RE, et al. 2012. Energetic consequences of repeated and prolonged dehydration in the Antarctic midge, *Belgica antarctica*. J. Insect Physiol. 58(4):498–505. https://doi.org/10.1016/j.jinsphys.2011.11.011

Tsafack N, Alignier A, Head GP, et al. 2016. Landscape effects on the abundance and larval diet of the polyphagous pest Helicoverpa armigera in cotton fields in North Benin. Pest Manag. Sci. 72(8):1613–1626.

Vreysen MJB, Robinson AS, Hendrichs J, et al. 2007. Area-wide integrated pest management (AW-IPM): principles, practice and prospects. Area-Wide Control Insect Pests.:3–33.

Walter T, Degen J, Pfeiffer K, et al. 2021. A new innovative real-time tracking method for flying insects applicable under natural conditions. BMC Zool. 6(1):35. https://doi.org/10.1186/s40850-021-00097-3

Wang X, Zhong Z, Chen X, et al. 2021. High-Fat Diets with Differential Fatty Acids Induce Obesity and Perturb Gut Microbiota in Honey Bee. Int. J. Mol. Sci. 22(2). https://doi.org/10.3390/ijms22020834

Warbrick-Smith J, Behmer ST, Lee KP, et al. 2006. Evolving resistance to obesity in an insect. Proc. Natl. Acad. Sci. 103(38):14045–14049. https://doi.org/10.1073/pnas.0605225103

Wat LW, Chao C, Bartlett R, et al. 2020. A role for triglyceride lipase brummer in the regulation of sex differences in Drosophila fat storage and breakdown. PLOS Biol. 18(1):e3000595. https://doi.org/10.1371/journal.pbio.3000595

Wilson H, Gress B. 2022. Organic pesticide use in almond: 2015-2019. https://www.cdfa.ca.gov/oars/opca/docs/organic_pesticide_use_in_california_almond.pdf

Wilson H, Burks CS, Reger JE, et al. 2020. Biology and Management of Navel Orangeworm (Lepidoptera: Pyralidae) in California. J. Integr. Pest Manag. 11(1):25.

Wishart DS, Tzur D, Knox C, et al. 2007. HMDB: the Human Metabolome Database. Nucleic Acids Res. 35(Database issue):D521-526. https://doi.org/10.1093/nar/gkl923

Zhou J, Chen X, Yan J, et al. 2018. Brummer-dependent lipid mobilization regulates starvation resistance in Nilaparvata lugens. Arch. Insect Biochem. Physiol. 99(2):e21481. https://doi.org/10.1002/arch.21481

Zhou Y, Qin D-Q, Zhang P-W, et al. 2020. The comparative metabolic response of *Bactrocera dorsalis* larvae to azadirachtin, pyriproxyfen and tebufenozide. Ecotoxicol. Environ. Saf. 189:110020. https://doi.org/10.1016/j.ecoenv.2019.110020

Zhu Y, Wilkinson KL, Wirthensohn M. 2017. Changes in fatty acid and tocopherol content during almond (*Prunus dulcis*, cv. Nonpareil) kernel development. Sci. Hortic. 225:150–155. https://doi.org/10.1016/j.scienta.2017.07.008

Ziegler R. 1991. Changes in lipid and carbohydrate metabolism during starvation in adult Manduca sexta. J. Comp. Physiol. B. 161(2):125–131. https://doi.org/10.1007/BF00262874

Chapter 3: Influence of Larval Diet on Macronutrient Composition and Flight Performance of *Amyelois transitella* (Lepidoptera: Pyralidae)

Abstract

For many Lepidoptera, the nutritional composition of the larval diet can strongly influence the resources available to the adult stage for flight, reproduction, and other activities. The navel orangeworm, *Amyelois transitella* (Lepidoptera: Pyralidae), is a major pest of almonds, pistachios, and walnuts in California. In addition to tree nuts, this moth is known to reproduce on many other agricultural (e.g., figs and pomegranates) and non-crop hosts (e.g., yucca and Mexican buckeye). *Amyelois transitella* does not feed as an adult. Although the species is quite polyphagous, any given individual develops on only a single host, which may have implications for adult flight ability. In this study, *A. transitella* larvae were reared on eight contrasting diets that included wheat bran, figs, almonds, pistachios, and walnuts, as well as degraded remnant almonds, pistachios, and walnuts. Flight mills were then used to compare the performance of adult moths reared on these diets. Macronutrient profiles were quantified for each dietary material, as well as for the adult moths reared from them, both before and after flight. Fresh tree nut diets contained higher concentrations of proteins and lipids than wheat bran and fig, whereas fig tended to have higher concentrations of carbohydrates, and the degraded remnant nuts contained lower moisture levels. These differences between the diets did not appear to influence adult macronutrient composition. Males reared on an artificial diet dispersed the farthest, and larger female moths tended to disperse farther in the longest single flight. Following flight activity, adults tended to have lower lipid and carbohydrate concentrations, while proteins were elevated. The ability of *A. transitella* to compensate

for differences in larval diet quality in a way that avoids negative impacts on flight capacity is not surprising for a polyphagous insect. These findings provide new insight into the nutritional ecology of this pest and will inform future studies better to characterize *A. transitella* dispersal across heterogeneous agricultural landscapes.

Introduction

In California, the navel orangeworm, *Amyelois transitella* Walker (Lepidoptera: Pyralidae), is the most important economic insect pest of almonds, *Prunus dulcis* (Mill.) D.A.Webb (Rosales: Rosaceae) and pistachios, *Pistacia vera* L. (Sapindales: Anacardiaceae), as well as a serious pest of walnuts, *Juglans regia* L. (Fagales: Juglandaceae). California is the leading producer of almonds, pistachios, and walnuts in the United States. Together, these crops account for 2.2 million cultivated acres and generate a combined annual farm value of $7.5 billion (CDFA 2024). *Amyelois transitella* larvae damage reduces crop yield and quality by feeding directly on nut kernels, which also increases processing costs to remove infested nuts and lowers payments to growers (Wilson et al. 2020). Infestation of *A. transitella* is also associated with the presence of *Aspergillus flavus* Link (Eurotiales: Aspergillaceae) (Palumbo et al. 2014), which produces aflatoxin, a known human carcinogen that is heavily regulated in key markets. As such, growers and processors have a very low tolerance for *A. transitella* populations and typically strive for <2% crop infestation.

In California, *A. transitella* produces 3-4 generations annually. *These moths* overwinter as larvae or pupae within remnant degraded “mummy” nuts left behind in orchards after harvest (Wade 1961). In spring, around April, increased temperatures and photoperiod catalyze the emergence of the first flight of *A. transitella* adults. This flight relies heavily on remnant nuts as reproductive sites, since the new crop nuts are not yet available at that time of year. The emergence of the second flight, typically in late June, coincides closely with hull-split, when new crop almonds become vulnerable to *A.*

transitella. Subsequent flights later in the year can make use of new crop pistachios and walnuts as well, which have a slightly later phenology than almonds, along with other host plants found in the landscape, such as figs and pomegranates, along with some natural and ornamental plants (Lara-Villalón et al. 2017).

Aside from water, *A. transitella* does not feed as an adult (Burks 2014), so all energy for somatic and non-somatic tissues is accumulated during larval development. In this way, the dietary diversity of this polyphagous species, and differences in larval diet quality specifically, may have implications for the resources available to adult *A. transitella*, which could impact their flight ability. While differences in larval diet have been shown to influence their developmental rate (Kuenen and Siegel 2010, Kuenen and Siegel 2011), no studies have evaluated impacts on flight ability. This is an important aspect of their ecology to understand, since *A. transitella* is a strong flyer (Sappington and Burks 2014) that is known to move between orchards (Meals and Caltagirone 1971, Andrews et al. 1980, Andrews and Barnes 1982, Bayes et al. 2014).

Not all larval diets are equal, and differences in diet quality translate to distinct nutritional profiles with various ratios of proteins, carbohydrates, and lipids in adult moths. Insect flight is powered through the metabolism of these macronutrients (Beenakkers 1969, Kammer and Heinrich 1978), some of which are used for short flights (carbohydrates) and others for long flights (lipids). Amino acids like proline are utilized less often as flight fuel, although they are important in the dispersal of some beetle species like *Pachylomerus femoralis* Kirby (Coleoptera: Scarabaeidae) (Auerswald et al. 2000) and *Dendroctonus ponderosae* Hopkins (Coleoptera: Curculionidae, Scolytinae)

(Wijerathna and Evenden 2019). Diptera and Hymenoptera rely heavily on carbohydrates for flight compared to Lepidoptera and Orthoptera, which can use both carbohydrates and lipids (Beenakkers et al. 1984). Carbohydrates are commonly found in the forms of glycogen and trehalose in insect bodies. These substances are metabolized to power short flights (Elliott et al. 1984) and earlier stages of long flights before insects switch over to lipids (Van Handel and Nayar 1972, Van der Horst et al. 1980, Sappington et al. 1995). Lipids, specifically neutral lipids like triglycerides, are widely known as the preferred flight fuel for Lepidoptera (Van Handel 1974, Ziegler and Schulz 1986, Murata and Tojo 2002, Matveev et al. 2017). Patterns of lipid mobilization during flight can differ between individual moths, depending on whether they are dispersing locally or over long distances (Sappington et al. 1995).

The nutritional status of adult moths can be determined with colorimetric assays that target proteins, carbohydrates, and lipids (Lee 2019). Proteins are routinely quantified with the long-standing Bradford assay (Bradford 1976). First colorimetric detection of carbohydrates and lipids in insects used anthrone and sulfo-phospho-vanillin assay (Van Handel 1985). The phenol-sulfuric assay can be used instead of anthrone as a faster alternative (Masuko et al. 2005). Modifications have been developed to run assays sequentially on individual small insects (Foray et al. 2012). These lipid, protein, and carbohydrate assays have previously been used to address a variety of research questions in insect ecology, such as characterizing nectar-feeding by parasitoids (Straser et al. 2023), allocation of resources towards reproduction (Muller et al. 2017), and sequential utilization of macronutrients over short- and long-flight periods (Sappington et al. 1995).

Almonds, pistachios, and walnuts, the preferred hosts of *A. transitella*, have relatively high lipid levels compared with other crops (Robbins et al. 2011) and, in this way, may be ideal hosts that support *A. transitella's* ability to make long-distance flights compared with some alternate hosts, such as figs, *Ficus carica* L. (Rosales: Moraceae) (Burks and Brandl 2004, 2005). Diets lacking proper nutrition, specifically C18 fatty acids, have been documented to negatively affect the flight capacity of some moths, such as *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae) (Sakamoto et al. 2004). As such, the dispersal capacity of an individual *A. transitella* moth is likely determined by nutritional status, which itself is the product of the larval diet. While *A. transitella* larvae can make use of both low-quality remnant degraded mummy nuts as well as high-quality new crop nuts, previous studies have documented marked differences in larval development rates and adult size between new and old nuts of the same crop (Kuenen and Siegel 2010, Kuenen and Siegel 2011) as well as between different crop host types (Siegel et al. 2010). In this way, the nutritional status and subsequent dispersal range of adult moths may be more limited in the spring, when *A. transitella* primarily rely on mummy nuts (poorer host material), as opposed to the summer, when they can develop on new crop nuts (better host material).

Here, we characterized the nutritional composition of adult *A. transitella* reared on different contrasting diets and then measured how that influences their dispersal capacity using flight mill assays. Understanding this relationship will further refine our understanding of *A. transitella* flight capacity and ability to move between orchards, as host availability and quality shift over the course of the growing season.

Materials and Methods

Field Sites

All field sites were research orchards located at the UC Kearney Agricultural Research and Extension Center (UC KARE) in Parlier, CA. The tree nut varieties used in this study were “Nonpareil” (almond), “Kerman” (pistachio), and “Chandler” (walnut). The fig variety used was “Black Mission”. The almond plot was maintained similarly to a commercial orchard with applications of synthetic pesticides and fertilizer. The walnut, pistachio, and fig blocks were largely unmaintained, except for pruning and irrigation. In total, 90 nuts/figs (30 per crop) across 15 trees (2 per tree) were inoculated for this study.

A. transitella Source and Rearing Procedures

For this study, *A. transitella* were sourced from a colony at the USDA Agricultural Research Service (ARS) in Parlier, CA. The colony consisted of a single strain, known as “Mendota,” which was collected in 2012 in Fresno County and has since been maintained on a standard wheat bran diet (Finney and Brinkman 1967) in 1-gallon jars.

Diet Treatments - Tree Nut Inoculations

Egg pads, made from paper towels, containing 3–5-day-old fertile eggs, were cut into strips containing ~50 eggs per strip. Each strip was glued (Elmer’s Glue Stick, Newell Brands, Westerville, OH) to a Tyvek® wristband (Uline, Pleasant Prairie, WI). Then, egg strips were attached to tree nuts and figs in the field to encourage neonate

larvae to infest the targeted host crop. Egg strips were attached to the nut by wrapping the wristband around the circumference of the nut hull/husk. Egg strips were deployed in 2024 and 2025 across fresh almonds (7/18/2024 & 7/15/2025), fresh figs (8/22/24 & 8/5/25), fresh pistachios (8/30/24 & 9/8/25), and fresh walnuts (9/10/24 & 9/8/2025), at times that correlated to peak nut kernel vulnerability (e.g., almond hull-split). Plant material (i.e., nut kernels and fig fruits) was collected two weeks after inoculation of fresh diets and at the time of collection for remnant nuts. Remnant nuts were collected from the same orchards on 1/28/25 & 2/3/26 and inoculated with the same method.

Inoculated fresh nuts were covered with a fine mesh bag (1,000 microns) to protect them from further damage. Nuts were harvested after 4 weeks, when larvae had reached the pupal stage. Fresh nuts were moved to a climate-controlled greenhouse (26.0°C ± 3.4°C, 64.2% ± 8.8%) and a crop-specific BugDorm-4F4590 (MegaView Science Co., Ltd, Taiwan). Inoculated remnant nuts were placed in an incubator (I-36NL, Percival Scientific, Perry, IA) set to 26 °C, 60% RH, and 14:10 (L:D) photoperiod in crop-specific cages. All cages were monitored daily for emergence. For emerging adults, their sex was recorded, and they were either flown on a flight mill or stored immediately at -80 °C for nutritional assays.

Diet Treatments – Artificial Diets

The artificial diet used in this study is based on Finney and Brinkman (1967). The main ingredient is red flaky wheat bran. The wheat bran is mixed with honey, glycerine, distilled water, debittered Brewer's yeast, and 1% solution of Vanderzant vitamins

(Sigma-Aldrich, Darmstadt, Germany). Wheat bran was sterilized (20 min at 121 °C) before mixing a batch. Once prepared, the diet was stored at -20 °C until needed.

Quantification of Macronutrient Content of the Diets

Wheat bran diet, fig fruit, and tree nut kernel samples were submitted to the University of California - Davis Analytical lab (UCD Analytical lab) for macronutrient analysis. Fresh fig and nut kernels were collected two weeks after *A. transitella* inoculation, while mummy nuts and wheat bran were collected at the time of inoculation. All samples were dried for 72 h at 55 °C and ground before submission. In total, 24 samples (3 samples per diet) were submitted for analysis. To ensure there was enough diet material for all analyses, 20 g of diet was set aside for each sample (60 g per crop). Individual samples were comprised of 3-5 nuts/figs to meet weight requirements. The fresh walnut samples were compromised in 2025, and so the material was only submitted for analysis in 2026.

The UCD Analytical lab quantified macronutrients according to standard operating procedures (https://anlaborders.ucdavis.edu/methods-of-analysis) for protein (SOP 625), crude fat (SOP 615), and soluble carbohydrates (SOP 680), outlined on their website under plant and feed materials. Crude protein was calculated from the nitrogen content of the diet material using a protein factor of 6.25. Crude fat was determined using Soxhlet extraction with the Randell modification. Soluble carbohydrates (fructose, glucose, and sucrose) were extracted in hot DI water and measured by HPLC with mass selective detection (Johansen et al. 1996). The analysis uses a Phenomenex Luna NH2

(250 mm x 4.6 mm) HPLC column at a flow rate of 2.75 ml/min with acetonitrile: water (78: 22).

Quantification of Macronutrient Content of A. transitella

To examine the effect of diet on nutrient sequestration, protein, total carbohydrate (free sugar + glycogen), and total lipids were quantified both before and after flight. In addition to *A. transitella* flown on the flight mills, 40 moths of equal sexes from each of the 8 diets tested were analyzed to determine a baseline macronutrient level sequestered from each diet**.** In total, 703 moths (320 before flight, 383 after flight) were analyzed. Colorimetric methods were modified from Woodard et al. (2019). Samples were run in triplicate on microplates and needed to have a coefficient of variation (CV) ≤10. If a single cell significantly deviated (CV >15 from the mean) for a sample, it was removed, and the remaining two were used. Samples were run a maximum of three times to meet the CV cutoff.

All insects were dried at 55 °C for 24 h, and dry weights were recorded. Dried *A. transitella* was placed in 2 ml microfuge tubes and pulverized with a steel bead for 1 min at 30 Hz with a TissueLyser LT (Qiagen, Hilden, Germany) in 1,500 µl of DI water. Then the homogenized samples were centrifuged using a Thermo Scientific™ Sorvall™ Legend™ Micro 21RT (Thermo Fisher Scientific, Waltham, MA) at 12,700 × g for 5 min at 4 °C. The individual samples were divided into two portions. The first 1,000 µl was collected, transferred to a fresh microfuge tube, and stored at -20 °C until protein analysis. A white, gooey lipid layer formed at the top of the tube and was left intact

during this step. The remaining 500 µl with the lipid "goo" raft was mixed with 1000 µl of 2: 1 chloroform: methanol. The lipid mixture was shaken for 30 s at 30 Hz with a TissueLyser LT and then immediately centrifuged at 12,700 x g for 5 min at 4 °C. The mixture separated into a nonpolar bottom layer and a polar top layer. Both layers were pipetted into a fresh 2 mL glass vial (DWK Life Sciences Wheaton™, Wertheim, Germany) with a PTFE/14B rubber-lined cap, which contained an additional 1,000 µl of 2:1 chloroform: methanol. Vials sat at room temperature for 6 h to allow layers to separate and were then stored at -20 °C until further analysis.

Protein concentrations were quantified with Bradford reagent (Pierce™ Bradford Plus Protein Assay Reagent, Thermo Fisher Scientific, Waltham, MA (Foray et al. 2012). Samples were thawed at room temperature before the assay. A 5 µl aliquot was plated in triplicate in a polystyrene microplate. To each well, 250 µl of Bradford reagent was added, and the plate was incubated for 5 min at room temperature. The plate was shaken for 10 s before being read at 495 nm on a SpectraMax ABS Plus paired with SoftMax Pro (version 7.2) (Molecular Devices, San Jose, CA). Samples were blank-subtracted and compared to 150, 125, 100, 75, 50, 25, and 12.5 µg/mL bovine serum albumin (Millipore Sigma, Darmstadt, Germany) standards.

Total carbohydrates (free sugars + glycogen) were quantified using phenol-sulfuric acid methods (Masuko et al. 2005). Carbohydrates were read from the top polar layer of the biphasic sample vial containing lipids. 50 µl was plated in triplicate in a polypropylene microplate. To each well, 150 µl of concentrated sulfuric acid was added, followed immediately by 5% phenol (aqueous solution). The plate was incubated for 5

min at 90 °C on a Fisherbrand™ Isotemp™ Digital Dry Bath (Thermo Fisher Scientific) and cooled for 2 min at -20 °C before being read at 490 nm on the spectrometer. Samples were blank-subtracted and compared to 80, 65, 50, 35, 20, 10, and 5 µg/mL dextrose (Millipore Sigma) standards. Standard solution was prepared in the same ratio of methanol: water (7: 5) as the sample.

Whole-body lipid concentrations were quantified using a sulfo-phospho-vanillin assay similar to methods reported by Woodard et al. (2019) and Cheng et al. (2011). From the bottom chloroform layer of each sample in the glass vial, 11 µl was plated in triplicate in a polypropylene microplate. Chloroform was evaporated completely after ~ 1 min at 90 °C on the dry bath. Then 100 µl of concentrated sulfuric acid was added to each well. The plate was shaken for 5 s in the spectrometer and incubated again at 90◦C for 20 min. Plates were then cooled for 2 min at -20 °C, and 50 µl of vanillin (0.2 mg/ ml in 17% phosphoric acid) (Millipore Sigma) was added to each well. Color was allowed to develop for 10 min before the plate was read at 540 nm. Samples were compared to a soybean oil standard (5 µg/µl) dissolved in 2: 1 chloroform: methanol. The standard dilutions were 35, 30, 25, 20, 15, 10, 5 µg, which translated to 7, 6, 5, 4, 3, 2, and 1 µl of standard directly plated.

A. transitella Flight Mill Assays

Two separate rooms were set up with flight mills so male and female *A. transitella* could be flown simultaneously on a single night. Sexes were rotated between rooms every night. All flight mill experiments were performed on an 'Insect FlyteMill'

(Crist Instrument, Hagerstown, MD) or an identical locally fabricated mill (Better World Manufacturing, INC., Fresno, CA). This design is an adaptation of a previous model developed by Naranjo (2019). Each mill is comprised of a hypodermic tubing arm measuring 29.21 cm in length suspended between two opposing magnets in a Teflon bearing to reduce friction. At this length, the distance covered by the tip of the arm per complete revolution is 91.72 cm.

In one room, up to 8 computer-linked flight mills were used in each trial. The flight mills were housed in a room (31.67 ± 0.01 °C, 35.23 ± 0.02 RH, 14:10 L:D), and each mill was held within a separate 60 × 60 × 60 cm cage (BugDorm-6m610, MegaView Science CO, Taiwan) to reduce interference from air movement. Cages were placed on two levels (i.e., top and bottom) on metal racks with up to 4 cages per rack. The floor of the cage was lined with 5.08 cm diameter green circles at a density of eight circles per square foot to provide non-directional visual cues. All mills were run simultaneously, with data relayed to a central computer running the LabVIEW 18.0 software package (National Instruments, Austin, TX). A custom LabVIEW program recorded the number of revolutions and estimated the distance flown by each insect. The total number of flights, distance, duration, maximum velocity, and velocity for the longest flights in a single night were later calculated using the R statistical program version 4.5.1 (R Core Team, 2026). A flight ended and was counted after 1 min of inactivity (i.e., triggering the sensor). Insects that did not engage in at least 2 min of continuous flight were considered non-fliers and excluded from the final analysis. Before tethering, each moth was weighed, and a counterweight comprised of a #1 insect pin

(#1208S1, Bioquip, Rancho Dominguez, CA) and Play-Doh (Hasbro, Pawtucket, RI) was constructed. All counterweights were ± 0.001 g of the tethered insect weight, accounting for both insect and pin. Before tethering, each moth was held at −5°C for 10 min to subdue it. Once chilled, the moth was laid on a foam dissecting block, dorsal side up. The wings were gently spread using flat round-tip forceps (#4522, Bioquip, Rancho Dominguez, CA) to expose the abdomen. Superglue (Loctite, Dusseldorf, Germany) mixed with a small amount of baking soda (Arm & Hammer, Dwight and Church Co., Inc, Ewing, NJ) (Wong et al. 2018) was applied for 30 s to the first abdominal segment beyond the metathorax (Sappington and Burks 2014) with a #1 insect pin (#1208S1, Bioquip, Rancho Dominguez, CA). Moths were stimulated with a stream of breath to verify that the wings were unobstructed by the pin and then mounted onto the flight mill arm opposite the counterweight. Each moth was provided with a small square piece of tissue paper, which provided tarsal contact to reduce trivial flights before the simulated night (Sappington and Showers 1991).

In the other room (27.55 ± 0.09 °C, 48.38 ± 0.27 RH, 14:10 L:D), up to 10 computer-linked flight mills were used in each trial. The mill design was identical to the mills described above. However, they were arranged in an open layout, unhoused in any cage, on a metal rack on levels (5 mills per level). The floor of each level was lined with an identical green circle design. All mills were run simultaneously, with data relayed to a central Arduino (Arduino, Truin, Italy) computer running a custom script (Casey et al. 2023). The total number of flights, distance, duration, maximum velocity, and velocity

for the longest flights in a single night were later calculated in R with code found within Casey et al. (2023).

Moths were flown for a 10.5 h period, of which 10 h was in complete darkness. A dusk period was simulated with three 4 W bulbs during the first 30 min following the automatic shutoff of the main lights controlled by outlet timers. After each moth had been tethered, the programs (i.e., LabVIEW and Arduino) were set to begin recording at the start of dusk and end at the start of dawn (lights on). No flight behavior was recorded outside of the 10.5 h period, as *A. transitella* is not known to be active during the day. The following morning, moths were removed from the flight mills within 30 min after dawn and placed immediately in a -80 °C freezer. Typically, a single diet was flown at a time, but some overlap occurred with remnant nut diets. Groups of moths were flown in this way over multiple nights, for a total of 65 nights of measured flight.

Statistical Analysis

Diet Material – Macronutrient Data

Macronutrient levels were compared within groups between diets, non-flown, and flown *A. transitella*. Diet macronutrient levels, expressed as percentages of dry weight, for crude protein, total carbohydrate (i.e., fructose, glucose, and sucrose), crude fat, and dry matter were evaluated with a generalized linear model (*glm* function) with a gamma distribution and log link. The diet was a categorical fixed effect.

A. transitella – Macronutrient Data

The quantities of macronutrients are reported in relation to the dry weight of the insect (μg/mg). Again, a logistic regression was used to separately evaluate the levels of protein, total carbohydrates, and total lipids for non-flown and flown groups with a gamma distribution and log link. The diet is a categorical fixed effect, and the plate runs as a random effect. For both plant and insect data, the effects of diet and weight (insect only) were evaluated with likelihood ratio tests using the *drop1* function, which generated the χ^2 and *P*-values reported here. Letters indicating significant differences via Tukey HSD post hoc test were calculated from the estimated marginal means for each macronutrient.

A Wilcoxon rank-sum test (*wilcox.test* function) was used to compare mean macronutrient levels between non-flown and flown groups by sex across all diets. The relationship between the lipid-to-carbohydrate ratio and total distance flown was examined using Spearman's rank correlation coefficient (*cor.test* function) for each sex separately. The R^2 and rho (ρ) values are reported.

Principal Component Analysis (PCA) was used to estimate the influence of the nutritional profile of diets on the nutritional profile and flight behavior of *A. transitella.* The PCA was conducted with the *prcomp* function, and a biplot was generated (*fviz_pca_biplot* function in 'factoextra' package). A PCA was run on the non-flown and flown moths separately, but sexes were combined.

A. transitella – Flight Data

Individual logistic regressions were used to evaluate the total distance, total duration, distance of the longest single flight, duration of the longest single flight, velocity of the longest single flight, and maximum velocity with a gamma distribution and log link (*glmer* function in the 'lme4' package) in the R statistical program, version 4.5.1 (https://www.r-project.org/). The number of flights, treated as count data, was also evaluated using a negative binomial distribution. Models were selected through a hierarchical simplification process.

Full models initially included all biologically relevant fixed effects and interactions. Non-significant interaction terms were sequentially removed using the *drop1* function to obtain the minimal adequate model while maintaining model hierarchy. Ultimately, diet type (host crop), diet age (i.e., fresh or remnant), and moth fresh weight were included as fixed effects with no interaction term. Random effects were included as the room nested within the date. Sexes were evaluated separately.

The influence of fixed effects flight parameters was evaluated with likelihood ratio tests using the *drop1* function, which generated the χ^2 and *P*-values reported here. Letters indicating significant differences via Tukey HSD post hoc test (*cld* function in 'multcomp' package) were calculated from the estimated marginal means (*emmeans* function in 'emmeans' package) for each flight parameter.

Results

Macronutrient Composition of Diet Material

The macronutrient profiles of each diet varied significantly. All tree nuts had more protein ($\chi^2 = 5.79$, df = 7, $P < 0.001$) compared to wheat bran and figs (Figure 3.1). Within the tree nut diets, pistachios had the most protein, followed by almonds and then walnuts. Tree nut diets also contained the highest lipid content ($\chi^2 = 27.51$, df = 7, $P < 0.001$) compared to wheat bran and figs (Figure 3.2). For both proteins and lipids, figs had the lowest concentration of all the diets tested. In contrast, figs had the highest levels of total sugars ($\chi^2 = 37.649$, df = 7, $P < 0.001$), followed by wheat bran (Figure 3.3). Fresh pistachio, almond, and remnant pistachio were equivalent in total sugar content. Similarly, all remnant nut types had roughly equal sugar levels. Walnuts contained the least sugar of the different diets.

All remnant tree nuts had the highest percentage of dry matter ($\chi^2 = 0.04$, df = 7, $P < 0.001$), indicating that they had a lower moisture content relative to the other diets (Figure 3.4). Wheat bran and figs had more water (i.e., the lower percent dry matter), but figs had the lowest percent dry matter. Fresh tree nuts were in the middle, with almonds and walnuts having similar dry matter percentages but significantly more than fresh pistachios

Macronutrient Composition of A. transitella

Average protein (µg/mg) differed significantly among non-flown male *A. transitella* across diets ($\chi^2 = 21.37$, df = 7, $P = 0.003$), with individuals reared on remnant

almonds and figs exhibiting the highest values. In contrast, fresh walnuts and remnant pistachios produced the lowest. Intermediate responses were observed for wheat bran, pistachio, fresh almond, and remnant walnut, which did not differ consistently from either extreme group. However, 50% of the fig and remnant pistachio samples did not make the CV cutoff and are excluded from the results (Table 3.1). For flown male *A. transitella*, average protein content differed among diets ($\chi^2 = 21.79$, df = 7, P = 0.003), with moths reared on remnant almonds and bran exhibiting the highest values based on dry body weight. Remnant pistachio mummy and fig diet moths were intermediate and not significantly different from the remnant almonds and bran. However, fresh almond, pistachio, walnut, and remnant walnut diet moths had lower protein relative to remnant almonds and bran but did not differ from each other (Table 3.2). With females, the average protein content did not differ across the diets, regardless of flight status (Table 3.1-3.2).

Among non-flown male *A. transitella*, diet had an overall significant effect ($\chi^2 = 14.31$, df = 7, P = 0.05) on the mean carbohydrate content. However, post hoc comparisons did not detect significant differences among diet treatments after adjustment for multiple pairwise comparisons (Table 3.3). For flown male *A. transitella*, carbohydrate content did not differ between treatment groups (Table 3.4). Average carbohydrate content (μg/mg) differed significantly among non-flown female *A. transitella* across diets ($\chi^2 = 14.49$, df = 7, P = 0.04), with individuals reared on walnuts (fresh and remnant), figs, remnant almonds, and fresh pistachios displaying the highest values. In contrast, remnant pistachio moths produced the lowest. Intermediate responses

were observed for wheat bran and fresh almond (Table 3.3). Mean carbohydrate content differed among flown female *A. transitella* with almond (fresh and remnant), remnant pistachio, and fig, producing the highest values. Fresh walnuts resulted in the lowest carbohydrate levels. Fresh pistachios and wheat bran were intermediate and not significantly different from either extreme due to overlapping groupings. Walnut mummy also exhibited intermediate values and did not differ from the higher carbohydrate groups (Table 3.4).

Diet did not have a significant influence on the mean lipid content of non-flown male *A. transitella* (Table 3.5). In contrast, mean lipid content among flown male moths differed between diet groups ($\chi^2 = 21.51$, df = 7, P = 0.003). Moths reared on almond (fresh and remnant) and pistachio maintained the highest mean lipid levels, while those from wheat bran held the lowest. Intermediate responses were observed for figs, remnant pistachios, and walnuts (fresh and remnant), and were like each other. However, mean lipid levels in *A. transitella* from remnant pistachios and fresh walnuts were significantly higher compared to wheat bran (Table 3.6). Diet had a significant influence on mean lipid levels in non-flown ($\chi^2 = 15.16$, df = 7, P = 0.03) and flown female *A. transitella* ($\chi^2 = 14.97$, df = 7, P = 0.04). Non-flown moths reared on fresh pistachios displayed the highest mean lipid content and figs the lowest (Table 3.5). The remaining diets were intermediate. For flown female moths, those reared on wheat bran had significantly lower mean lipid levels compared to all other diets (Table 3.6).

The PCA on non-flown samples explained 72.5% of the total variation, with PC1 accounting for 52.2% and PC2 accounting for 20.3% (Figure 3.5). Fig and wheat bran

separated strongly from the nut crops along PC1, indicating distinct nutritional profiles. Walnuts and remnant pistachios clustered together in the lower-left quadrant, suggesting similar profiles, whereas almonds and fresh pistachios grouped in the upper-left quadrant and were associated with *A. transitella* lipid content. The loading vectors showed that diet protein and diet lipids were strongly positively correlated, while the dry weight of insects was negatively associated with *A. transitella* protein and carbohydrate content.

Flight Performance of A. transitella

Of the 514 *A. transitella* (254 female & 260 male) that were subjected to the flight mill assay, only 383 (196 female & 187 male) met the qualifications to be considered flyers. The influence of diet varied across flight parameters in male *A. transitella* (Table 3.7). Diet type impacted fresh weight, with the largest males emerging from walnuts and the smallest from pistachios and figs. Additionally, diet age was important (Table 3.8) with fresh diets producing larger males (13.70 ± 0.58 mg) than remnant nuts (10.70 ± 0.59 mg). Only the diet type was significant for total distance and duration. *Amyelois transitella* reared on wheat bran tended to fly similarly to walnut-reared males but further than those reared on almond, pistachio, and figs. For total flight duration, male *A. transitella* reared on wheat bran only flew significantly longer than fig-reared moths. Diet age was significant for the distance ($\chi^2 = 9.32$, df = 1, P = 0.002) and duration ($\chi^2 = 13.77$, df = 1, P < 0.001) of the single longest flight with remnant diets greater than fresh. In contrast, grouped by crop, the longest single flight distance was greater for male *A. transitella* reared on wheat bran and almonds relative to those reared on pistachios.

Similarly, the longest single flight duration was greater for males reared on wheat bran relative to walnut and pistachios, with almonds and figs in between. The velocity (m/s) of the longest single flight was only impacted by diet type. Males reared on walnuts flew faster than males from figs. No differences were found in maximum flight velocity or bouts of flight across the various diet treatments.

Overall, there was less variation across flight parameters between female *A. transitella* reared on the different diets (Table 3.9). Like males, the largest moths emerged from walnuts and the smallest from figs. Diet age (Table 3.10) was a significant factor, with fresh diets producing larger females (19.60 ± 1.16 mg) compared to remnant nuts (16.20 ± 1.22 mg). Diet type did not influence any other flight parameters except the distance of the longest single flight. However, diet only had an overall significant effect ($\chi^2 = 9.37$, df = 4, P = 0.05). Post hoc comparisons did not detect differences among the diet treatments after adjusting for multiple pairwise comparisons. Weight was an important effect among females. Heavier females displayed a greater distance and duration for the longest flight. Diet age was significant in both the velocity of the longest single flight ($\chi^2 = 5.02$, df = 1, P = 0.03) and maximum velocity ($\chi^2 = 3.96$, df = 1, P = 0.05), with females from fresh diets flying faster. No differences were found for total distance, duration, and bouts of flight.

Influence of Flight on Macronutrient Composition of A. transitella

Protein concentration was higher in flown compared to non-flown moths in female *A. transitella* reared on all remnant tree nuts and male moths reared on wheat

bran, remnant almond, remnant pistachio, and fresh walnut (Table 3.11, Figure 3.6). In contrast, carbohydrates were generally decreased in flown moths compared to non-flown moths in female moths reared on fresh pistachios and walnuts, as well as males reared on wheat bran, remnant almonds, fresh pistachios, and all walnuts (Table 3.12). However, the level of carbohydrates in the flown (n = 21) female *A. transitella* from remnant pistachios was significantly higher relative to the non-flown (n = 14) group (Figure 3.7). Similarly, lipid concentrations were also lower *a*mong females; mean lipid levels were lower for fresh almond and walnut moths (Table 3.13, Figure 3.8). For males, depletion of lipids is noted in wheat bran and remnant walnut-reared moths. However, for moths reared on remnant almonds and fresh pistachios, the flown group displayed significantly higher mean lipid levels (Figure 3.8).

The PCA explained 73.6% of the total variation, with PC1 accounting for 45.5% and PC2 accounting for 28.1% (Figure 3.9). Wheat bran and fig separated strongly from the nut crops, indicating distinct nutritional and/or flight profiles. Wheat bran was associated with positive PC1 values and variables related to flight performance, while fig separated primarily along positive PC2 values and was associated with diet carbohydrates and *A. transitella* protein content.

Pistachios and almonds clustered together on the negative side of PC1, suggesting similar profiles and associations with *A. transitella* lipid content, diet protein, diet lipids, and percent dry matter of diet. Fresh walnut separated strongly in the lower-right quadrant and was associated with variables related to velocity and distance measures. The remnant walnut occupied a more intermediate position between the major groupings.

The loading vectors showed strong positive relationships among total distance, max velocity, velocity of the longest single flight, and moth fresh weight. The bouts of flight and *A. transitella* protein content were negatively correlated with other flight parameters like total distance and duration.

Influence of Macronutrient Composition on Flight Performance

Out of 524 moths subjected to the flight mill assay, 324 moths (173 females and 151 males) had both lipid and carbohydrate signatures that met the CV cutoff that could be used to evaluate the influence of these macronutrients on flight. Overall, no consistent relationship between the lipid-to-carbohydrate ratio and total distance flown was found among females (Figure 3.10) or males (Figure 3.11). Generally, the ratio decreased or remained unchanged, but for *A. transitella* reared on almond (females) and pistachio mummies (males), the ratio increased.

Discussion

In this study, the effects of larval diet on *A. transitella* macronutrient profiles and flight ability were evaluated. The flight performance was largely consistent with findings of previous studies (Sappington and Burks 2014, Rovnyak et al. 2018), which have demonstrated that polyphagous insects like *A. transitella* can make use of highly variable larval diets with little impact on flight performance. Our data, however, provide a unique and more detailed look at the impacts of the potential host plant material available to *A.*

transitella across the wide range of agroecosystems that exist in California's Central Valley region.

Macronutrient Composition of the Diets

Tree nuts are a dynamic resource for *A. transitella,* which have varying levels of quality throughout the year as they develop and mature. Development of *A. transitella* often takes longer on remnant nuts compared to fresh new crop nuts and artificial diets (Kuenen and Siegel 2010). A combination of factors is likely the cause of this, but the degradation of nutrients is often cited (Kuenen and Siegel 2010, Siegel et al. 2010, Kuenen and Siegel 2011). There were differences in protein, carbohydrates, and lipids between the various tree nuts evaluated. In contrast with previous studies, there were no differences between fresh and remnant samples of the same host crop. The macronutrient analysis reported crude fat and protein instead of specific quantities of amino and fatty acids. The nutrient profile of tree nuts changes rapidly as the nuts mature (Jin et al. 2023, Adaskaveg et al. 2025). The subtle differences in specific molecules may have large impacts on *A. transitella* development. This might partially account for the reported slower development of remnant nuts. The potentially large influence of subtle differences is further supported by studies showing that even crop variety can differentially affect *A. transitella* development (Siegel et al. 2010, Kuenen and Siegel 2011). Similarly, different varieties of date palm, *Phoenix dactylifera* L. (Arecales: Arecaceae), were shown to influence the development of *Ectomyelois ceratoniae* Zeller (Lepidoptera: Pyralidae) (Nay and Perring 2008). All that said, fresh and old nuts were quite different in terms of

moisture content, with all remnant nuts having less water (i.e., higher percent dry mass) than fresh nuts, and this may be the key limiting factor for *A. transitella* development across different hosts. For example, baked pistachios increased *A. transitella* development time, likely due to water loss (Kuenen and Siegel 2011). The larval diet is the only source of water for *A. transitella* before adulthood, so its presence very likely plays an important role in host crop quality. Yet, countering this argument, the balance of macronutrients remains vital because figs and bran had the most water of the diets tested and exerted opposing effects on flight behavior.

Baseline Composition of Macronutrients in A. transitella

Diets varied in their macronutrient ratios, with tree nuts higher in lipids and proteins and figs rich in carbohydrates. Generally, *A. transitella* was seemingly able to overcome these deficiencies and surpluses in terms of nutrient accumulation, and few consistent differences were seen in the macronutrient levels of the moths reared on the different diets. This occurs in other polyphagous species. No differences were found in lipid or protein content in female *Malacosoma disstria* Hübner (Lepidoptera: Lasiocampidae) (Colasurdo et al. 2009) or in carbohydrate and protein stores of *Schistocera americana* (Orthoptera: Acridiae) reared in diets varying in protein levels (Hahn 2005). However, life history traits like fecundity and larval survivorship of *Plodia interpunctella* (Lepidoptera: Pyralidae) were negatively affected when reared on figs compared to pistachios (Borzoui et al. 2018, Vukajlović et al. 2019). Unfortunately, these traits were not recorded in this study. When insects are feeding on unbalanced diets, they

may consume more food in order to meet their nutritional requirements, known as compensatory feeding (Simpson and Simpson 2017). Additionally, post-ingestion strategies such as enzyme regulation to deal with excessive nitrogen (Truzi et al. 2021) and carbohydrates (Borzoui et al. 2018) can alter the effect of larval diets. Alternatively, in this study, the larger *A. transitella* may have significantly reduced the concentration of some nutrients, such as protein, due to increased lipid stores. This is supported by the negative correlation of moth size and macronutrient content (Figure 3.5).

Flight Performance of A. transitella

The measurement of *A. transitella*'s flight potential with computerized flight mills has been the focus of several studies in recent years. *Amyelois transitella* is considered a strong flyer, with flight ability unaffected by age (Sappington and Burks 2014), and mated females tend to disperse farther than when unmated (Rovnyak et al. 2018). However, the flight ability of *A. transitella* is adversely affected by mass-rearing, shipping, and irradiation (Reger et al. 2021). In all previous studies, *A. transitella* were reared on a wheat bran diet (Finney and Brinkman 1967) akin to the one used in this study, and moths reared on this type of wheat bran performed similarly (Figure 3.1) to previous reports (Sappington and Burks 2014, Rovnyak et al. 2018). Artificial diets are engineered to provide insects with ideal ratios and quantities of nutrients and minerals. Wheat bran-reared male *A. transitella* significantly outperformed fig-reared moths in terms of total distance and duration flown, and pistachio-reared moths in terms of the longest single flight (Table 3.7). There are a few studies that explore the effect of larval

diet on adult behaviors like dispersal. Reduction of sugar content led to decreased cumulative flight distances among male *Grapholita molesta* Busck (Lepidoptera: Tortricidae) (Su et al. 2021). The balance of nutrients appears to be important for flight. Honey resulted in better flight performance of *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae) relative to less balanced sugar diets (Yang et al. 2025). While fresh diets produced larger insects, the longest single flight was significantly increased in males reared from remnant diets, and like wheat bran (Table 3.7). However, this trend does not appear in any other parameter, and its cause is unclear.

For females, diet had little effect on flight behavior. Larger females tended to engage in a longer single flight, but this trend was not seen overall (Table 3.8). Females from fresh diets were larger and flew faster. Female *A. transitella* are more robust flyers compared to males (Sappington and Burks 2014, Reger et al. 2021) and have no apparent trade-off between dispersal and reproduction (Rovnyak et al. 2018). These attributes may have contributed to dispersal strength among female *A. transitella* in this study.

Effects of Flight Activity on A. transitella Macronutrient Composition

The trends in macronutrient metabolism for flight by *A. transitella* were in line with what is reported for other Lepidoptera species. Proteins were not used and appeared to increase in flown groups relative to non-flown groups (Figure 3.7). This is likely due to the metabolism of carbohydrates and lipids, which decreased the overall dry mass of flown individuals. Carbohydrates are typically the first to be used in take-off and short flights before switching to lipids as the primary flight fuel across multiple orders,

including Lepidoptera, Orthoptera (Van der Horst et al. 1980), and Diptera (Kaufmann and Brown 2008). In this study, there were more incidences of depleted carbohydrate levels (Figure 3.8) than lipids (Figure 3.9) between non-flown and flown moths. This supports the idea that carbohydrates were primarily used, leading to more reductions even in groups that flew shorter distances. In a few cases for carbohydrates and lipids, flown moths maintained significantly higher levels compared to non-flown groups (Figure 3.8-3.9). This could be due to a limitation of the study, in that direct comparison of energy use was not done for the same insects pre- and post-flight. In other studies, the macronutrient concentrations in the hemolymph were measured and were nondestructive, so multiple measures could be taken (Sappington et al. 1995).

Weak and inconsistent relationships between distance and the ratio of lipids to carbohydrates offer limited information (Figure 3.10-3.11). However, most *A. transitella* had reserves of both carbohydrates and lipids remaining after a single night of flight. As such, it might be worth focusing on multiple nights of flight with *A. transitella* to further parse the influence of the quality of larval diet on dispersal potential. For example, *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae) developed faster, had a greater chance of survival, fecundity, and fertility when reared on pistachios compared to figs (Borzoui et al. 2018).

It is also worth noting that eggs were not removed from females before any colorimetric assays. Females emerged with fully developed eggs and often mate on the

first night after eclosion (Wilson et al. 2020). Eggs are a source of proteins, carbohydrates, and lipids that can be reabsorbed for dispersal in some migratory insects (Sappington and Showers 1992). This is not known to occur in *A. transitella*. However, the net reproductive cost to migration is not universally clear, and may be present in other Pyralid species (Shirai 1998, Zhang et al. 2015). Eggs locked resources away from females that they could not use for flight, but they would have been quantified in colorimetric assays. The reduction in macronutrients is likely more prominent in females, but egg macronutrients obscure it.

Conclusions

Amyelois transitella is a polyphagous insect that develops on a variety of host crops across California agroecosystems. Tree nuts appear to be a preferred host and have extreme variation in their availability and quantity, with remnant nuts being the primary source of food and reproductive substrate for the overwintering and first generations. As a capital breeder that only develops on a single nut/fruit as a larva, host crop selection is a critical behavior for *A. transitella* success. While there is evidence that remnant nuts and low-quality alternative hosts slow development, this study found that *A. transitella* tended to be generally unaffected by larval diet.

Limitations and Future Directions

The studies presented here are important for managing *A. transitella,* but they had limitations due to physical space and objectives. The two rooms where the flight mills

were set up were not equally temperature-controlled, with the LabVIEW system warmer by 5 °C on average. This was addressed by rotating groups of moths, but a more unified setup would be preferred. Dispersal is a key behavior, but in this study, it was not as strongly affected by larval diet. Extending the flight period over multiple nights might have provided a more complete representation of the flight potential of *A. transitella* across different diets. Finally, direct comparisons of pre- and post-flight insects may have been a cleaner approach. However, the small size of *A. transitella* likely makes collecting hemolymph without damaging the insect a challenge.

Tables and Figures

Table 3.1. Protein content per dry weight (μg/mg) (mean ± SEM) of non-flown *A. transitella*

	Female		Male	
	n	**Protein**	**n**	**Protein**
Alm. Mum	19	10.94 ± 0.73 **a**	15	14.26 ± 1.35 **b**
Almond	12	11.35 ± 2.00 **a**	15	12.28 ± 0.84 **ab**
Wheat Bran	17	11.09 ± 1.27 **a**	20	9.74 ± 0.45 **ab**
Fig	14	11.43 ± 1.45 **a**	15	15.09 ± 2.01 **b**
Pist. Mum	19	8.27 ± 0.61 **a**	10	3.84 ± 1.05 **ab**
Pistachio	15	10.53 ± 1.04 **a**	13	9.42 ± 1.03 **ab**
Wal. Mum	19	8.04 ± 0.68 **a**	15	8.47 ± 1.25 **ab**
Walnut	18	7.16 ± 1.15 **a**	13	5.67 ± 0.45 **a**

Columns with different letters indicate significant differences in pairwise comparison via Tukey's HSD post hoc test. If no letters are present, no significant differences were detected. Letters are derived from comparisons of means from the generalized linear mixed model, but raw values are reported above.

Table 3.2. Protein content per dry weight (μg/mg) (mean ± SEM) of flown *A. transitella*

	Female		Male	
	n	**Protein**	**n**	**Protein**
Alm. Mum	25	13.12 ± 1.13 **a**	25	22.25 ± 2.51 **a**
Almond	20	11.39 ± 1.13 **a**	12	11.84 ± 1.69 **a**
Wheat Bran	25	16.24 ± 1.26 **a**	24	19.25 ± 1.67 **a**
Fig	22	14.60 ± 1.60 **a**	23	17.52 ± 1.41 **a**
Pist. Mum	17	9.15 ± 1.34 **a**	18	12.95 ± 2.15 **a**
Pistachio	22	10.91 ± 1.07 **a**	25	12.03 ± 0.74 **a**
Wal. Mum	25	11.66 ± 1.33 **a**	25	12.18 ± 1.35 **a**
Walnut	25	9.06 ± 0.59 **a**	25	13.03 ± 0.82 **a**

Columns with different letters indicate significant differences in pairwise comparison via Tukey's HSD post hoc test. If no letters are present, no significant differences were detected. Letters are derived from comparisons of means from the generalized linear mixed model, but raw values are reported above.

Table 3.3. Carbohydrate content per dry weight (μg/mg) (mean ± SEM) of non-flown *A. transitella*

	Female		Male	
	n	**Carbs**	**n**	**Carbs**
Alm. Mum	16	5.47 ± 0.68 **b**	19	6.43 ± 0.52 **a**
Almond	20	5.83 ± 0.39 **ab**	17	5.79 ± 0.45 **a**
Bran	17	4.42 ± 0.47 **ab**	15	3.74 ± 0.54 **a**
Fig	16	6.68 ± 0.36 **b**	15	6.38 ± 0.62 **a**
Pist. Mum	14	3.16 ± 0.54 **a**	8	1.82 ± 0.57 **a**
Pistachio	18	6.33 ± 0.40 **b**	15	6.75 ± 0.69 **a**
Wal. Mum	17	5.46 ± 0.65 **b**	16	4.45 ± 0.66 **a**
Walnut	15	6.70 ± 0.64 **b**	16	5.17 ± 0.31 **a**

Columns with different letters indicate significant differences in pairwise comparison via Tukey's HSD post hoc test. If no letters are present, no significant differences were detected. Letters are derived from comparisons of means from the generalized linear mixed model, but raw values are reported above.

Table 3.4. Carbohydrate content per dry weight (μg/mg) (mean ± SEM) of flown *A. transitella*

	Female		Male	
	n	**Carbs**	**n**	**Carbs**
Alm. Mum	24	5.72 ± 0.79 **c**	20	4.46 ± 0.90 **a**
Almond	22	6.47 ± 0.67 **c**	20	4.69 ± 0.38 **a**
Bran	22	4.27 ± 0.41 **abc**	20	2.87 ± 0.40 **a**
Fig	21	6.47 ± 0.51 **c**	20	4.93 ± 0.46 **a**
Pist. Mum	21	5.62 ± 0.57 **c**	22	4.18 ± 0.49 **a**
Pistachio	22	4.45 ± 0.26 **ab**	23	4.59 ± 0.40 **a**
Wal. Mum	19	4.31 ± 0.64 **bc**	22	2.99 ± 0.40 **a**
Walnut	25	3.56 ± 0.32 **a**	23	3.05 ± 0.41 **a**

Columns with different letters indicate significant differences in pairwise comparison via Tukey's HSD post hoc test. If no letters are present, no significant differences were detected. Letters are derived from comparisons of means from the generalized linear mixed model, but raw values are reported above.

Table 3.5. Lipid content per dry weight (μg/mg) (mean ± SEM) of non-flown *A. transitella*

	Female		Male	
	n	**Lipid**	**n**	**Lipid**
Alm. Mum	20	373.26 ± 22.71 **ab**	20	319.10 ± 9.72 **a**
Almond	20	408.90 ± 35.17 **ab**	20	429.86 ± 43.89 **a**
Bran	20	237.87 ± 16.77 **ab**	20	296.86 ± 12.49 **a**
Fig	20	331.13 ± 21.35 **a**	19	344.63 ± 29.29 **a**
Pist. Mum	20	348.96 ± 23.43 **ab**	20	418.35 ± 26.02 **a**
Pistachio	19	348.10 ± 15.69 **b**	17	357.82 ± 19.90 **a**
Wal. Mum	20	332.52 ± 28.51 **ab**	20	342.66 ± 23.79 **a**
Walnut	17	406.97 ± 26.26 **ab**	18	366.66 ± 25.04 **a**

Columns with different letters indicate significant differences in pairwise comparison via Tukey's HSD post hoc test. If no letters are present, no significant differences were detected. Letters are derived from comparisons of means from the generalized linear mixed model, but raw values are reported above.

Table 3.6. Lipid content per dry weight (μg/mg) (mean ± SEM) of flown *A. transitella*

	Female		Male	
	n	**Lipid**	**n**	**Lipid**
Alm. Mum	25	351.82 ± 14.82 **b**	23	376.90 ± 35.63 **c**
Almond	25	289.46 ± 29.92 **b**	22	378.29 ± 21.91 **c**
Bran	25	205.46 ± 11.50 **a**	25	233.06 ± 12.80 **a**
Fig	23	316.38 ± 19.79 **b**	25	282.58 ± 13.80 **ab**
Pist. Mum	24	297.55 ± 20.50 **b**	25	357.75 ± 21.80 **bc**
Pistachio	23	321.23 ± 12.75 **b**	25	395.40 ± 17.74 **c**
Wal. Mum	25	311.06 ± 20.17 **b**	25	267.61 ± 20.77 **ab**
Walnut	25	297.03 ± 14.59 **b**	25	320.06 ± 22.77 **bc**

Columns with different letters indicate significant differences in pairwise comparison via Tukey's HSD post hoc test. If no letters are present, no significant differences were detected. Letters are derived from comparisons of means from the generalized linear mixed model, but raw values are reported above.

Table 3.7. Distance, duration, velocity, and number of flights (mean ± SEM) for male *A. transitella* for each treatment group

Parameter	Wheat Bran n = 25	Walnut n = 50	Almond n = 39	Pistachio n = 50	Fig n = 23
Fresh Weight (mg)	16.1 ± 0.4 **b**	15.0 ± 0.7 **ab**	11.1 ± 0.3 **a**	11.0 ± 0.4 **a**	11.1 ± 0.5 **a**
Total Distance (km)	9.3 ± 1.6 **b**	5.5 ± 0.9 **ab**	4.1 ± 0.8 **ab**	4.2 ± 0.6 **ab**	2.4 ± 0.9 **a**
Total Duration (min)	265.0 ± 40.0 **b**	151.9 ± 23.6 **ab**	174.2 ± 31.0 **ab**	160.8 ± 23.9 **ab**	96.7 ± 29.9 **a**
Longest Distance (km)	4.6 ± 1.1 **b**	2.5 ± 0.6 **ab**	2.8 ± 0.7 **ab**	2.1 ± 0.6 **a**	1.7 ± 0.8 **ab**
Longest Duration (min)	123.4 ± 30.7 **b**	64.7 ± 16.0 **a**	99.6 ± 22.2 **ab**	65.2 ± 16.2 **a**	56.2 ± 23.6 **ab**
Longest Velocity (m/s)	0.6 ± 0.1 **ab**	0.6 ± 0.0 **b**	0.4 ± 0.0 **ab**	0.5 ± 0.1 **ab**	0.4 ± 0.0 **a**
Max Velocity (m/s)	0.8 ± 0.1 **a**	0.8 ± 0.1 **a**	0.8 ± 0.1 **a**	0.8 ± 0.1 **a**	0.8 ± 0.1 **a**
No. of Flights	24.0 ± 3.1 **a**	19.4 ± 1.6 **a**	22.7 ± 3.0 **a**	25.1 ± 2.7 **a**	21.1 ± 4.2 **a**

Table 3.8. Summary of Generalized Linear Mixed Model on flight parameters for male *A. transitella* for each treatment group

Parameter	Crop χ^2	Age χ^2	Weight χ^2
Fresh Weight **(mg)**	23.14***	10.13**	-
Total Distance **(km)**	11.78*	2.63	0.67
Total Duration **(min)**	10.85*	3.14	0.01
Longest Distance **(km)**	12.83*	9.32**	0.33
Longest Duration **(min)**	16.26**	13.77***	0.44
Longest Velocity **(m/s)**	9.99*	1.01	1.29
Max Velocity **(m/s)**	3.55	0.01	0.7
No. of Flights	3.22	1.11	0.08

$P < 0.05$*, $P < 0.01$**, $P < 0.001$***

Table 3.9. Distance, duration, velocity, and number of flights (mean ± SEM) for female *A. transitella* for each treatment group

Parameter	Wheat Bran n = 25	Walnut n = 50	Almond n = 50	Pistachio n = 49	Fig n = 22
Fresh Weight **(mg)**	21.6 ± 0.6 **b**	22.1 ± 0.9 **ab**	17.9 ± 0.9 **ab**	15.9 ± 0.6 **ab**	16.3 ± 0.7 **a**
Total Distance **(km)**	11.8 ± 1.9 **a**	10.2 ± 1.4 **a**	5.8 ± 1.0 **a**	7.3 ± 1.2 **a**	7.3 ± 1.7 **a**
Total Duration **(min)**	285.6 ± 38.1 **a**	267.3 ± 27.9 **a**	187.5 ± 24.1 **a**	174.7 ± 22.4 **a**	209.4 ± 40.5 **a**
Longest Distance **(km)**	5.8 ± 1.3 **a**	5.7 ± 1.0 **a**	2.8 ± 0.6 **a**	4.4 ± 1.0 **a**	5.0 ± 1.6 **a**
Longest Duration **(min)**	128.8 ± 27.5 **a**	140.6 ± 21.8 **a**	81.1 ± 15.0 **a**	102.3 ± 18.3 **a**	137.6 ± 40.7 **a**
Longest Velocity **(m/s)**	0.7 ± 0.0 **a**	0.6 ± 0.0 **a**	0.5 ± 0.0 **a**	0.6 ± 0.1 **a**	0.6 ± 0.0 **a**
Max Velocity **(m/s)**	0.9 ± 0.1 **a**	0.8 ± 0.1 **a**	0.8 ± 0.0 **a**	0.7 ± 0.1 **a**	0.7 ± 0.0 **a**
No. of Flights	19.9 ± 1.9 **a**	19.0 ± 1.9 **a**	23.5 ± 2.5 **a**	19.2 ± 2.2 **a**	18.2 ± 2.5 **a**

Rows with different letters indicate significant differences in pairwise comparison via Tukey's HSD post hoc test. $P < 0.05$*, $P < 0.01$**, $P < 0.001$***

Table 3.10. Summary of Generalized Linear Mixed Model on flight parameters for female *A. transitella* for each treatment group

Parameter	Crop χ^2	Age χ^2	Weight χ^2
Fresh Weight **(mg)**	12.44*	3.97*	-
Total Distance **(km)**	7.17*	1.04	2.79
Total Duration **(min)**	4.68	0.15	1.85
Longest Distance **(km)**	9.73	0.02	6.64**
Longest Duration **(min)**	7.9	1.27	5.18*
Longest Velocity **(m/s)**	4.31	5.02*	0.31
Max Velocity **(m/s)**	2.96	3.96*	0
No. of Flights	4.18	0.58	3.66

$P < 0.05$*, $P < 0.01$**, $P < 0.001$***

Table 3.11. Summary of the Wilcoxon rank sum test for the mean protein content in non-flown and flown *A. transitella*

Sex	Crop	Non-flown (n)	Flown (n)	statistic	P
Female	Bran	17	25	145	0.09
	Almond	12	20	102	0.50
	Alm. Mum	19	25	139	0.02
	Pistachio	15	22	154	0.75
	Pist. Mum	19	17	47	<0.001
	Walnut	18	25	146	0.05
	Wal. Mum	19	25	131	0.01
	Fig	14	22	117	0.24
Male	Bran	20	24	8	<0.001
	Almond	15	12	106	0.46
	Alm. Mum	15	25	96	0.01
	Pistachio	13	25	108	0.10
	Pist. Mum	10	18	12	<0.001
	Walnut	13	25	16	<0.001
	Wal. Mum	15	25	165	0.54
	Fig	15	23	108	0.06

Table 3.12. Summary of the Wilcoxon rank sum test for the mean carbohydrate content in non-flown and flown *A. transitella*

Sex	Crop	Non-flown (n)	Flown (n)	statistic	P
Female	Bran	17	22	220	0.36
	Almond	20	22	194	0.53
	Alm. Mum	16	24	239	0.20
	Pistachio	18	22	327	<0.001
	Pist. Mum	14	21	49	<0.001
	Walnut	15	25	319	<0.001
	Wal. Mum	17	19	188	0.42
	Fig	16	21	182	0.68
Male	Bran	15	20	246	<0.001
	Almond	17	20	232	0.06
	Alm. Mum	19	20	268	0.03
	Pistachio	15	23	275	0.002
	Pist. Mum	8	22	80	0.73
	Walnut	16	23	294	0.001
	Wal. Mum	16	22	264	0.01
	Fig	15	20	202	0.09

Table 3.13. Summary of the Wilcoxon rank sum test for the mean lipid content in non-flown and flown *A. transitella*

Sex	Crop	Non-flown (n)	Flown (n)	statistic	P
Female	Bran	20	25	323	0.10
	Almond	23	25	399	0.02
	Alm. Mum	20	25	286	0.42
	Pistachio	19	23	269	0.21
	Pist. Mum	20	24	304	0.14
	Walnut	17	25	315	0.01
	Wal. Mum	20	25	264	0.76
	Fig	20	23	267	0.38
Male	Bran	20	25	391	<0.001
	Almond	20	22	248	0.49
	Alm. Mum	20	23	144	0.04
	Pistachio	17	25	134	0.04
	Pist. Mum	20	25	323	0.10
	Walnut	18	25	255	0.47
	Wal. Mum	20	25	342	0.04
	Fig	19	25	308	0.10

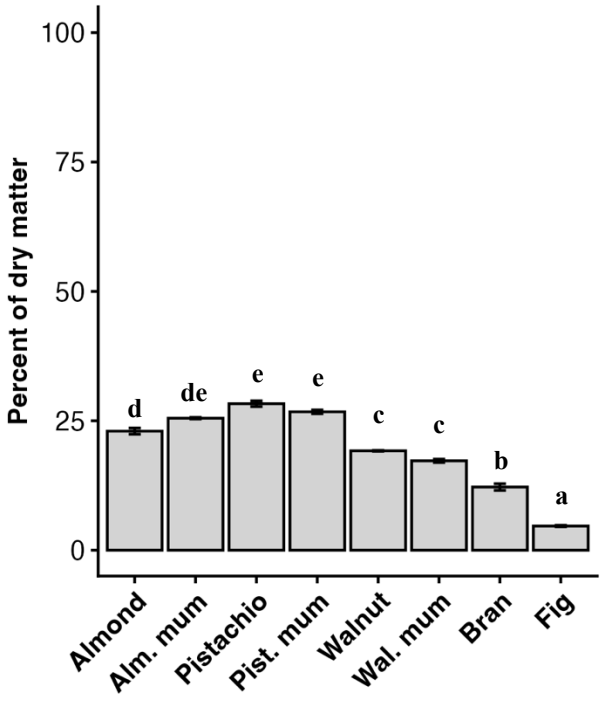

Figure 3.1. Mean crude protein percentage of the diet dry mass. Columns with different letters indicate significant differences, $P < 0.05$ in pairwise comparisons via Tukey's HSD post hoc test.

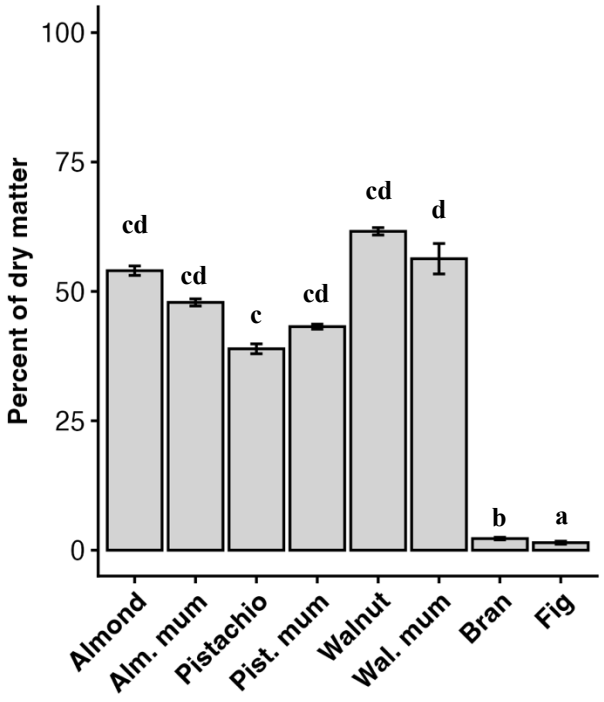

Figure 3.2. Mean crude lipid percentage of the diet dry mass. Columns with different letters indicate significant differences, $P < 0.05$ in pairwise comparisons via Tukey's HSD post hoc test.

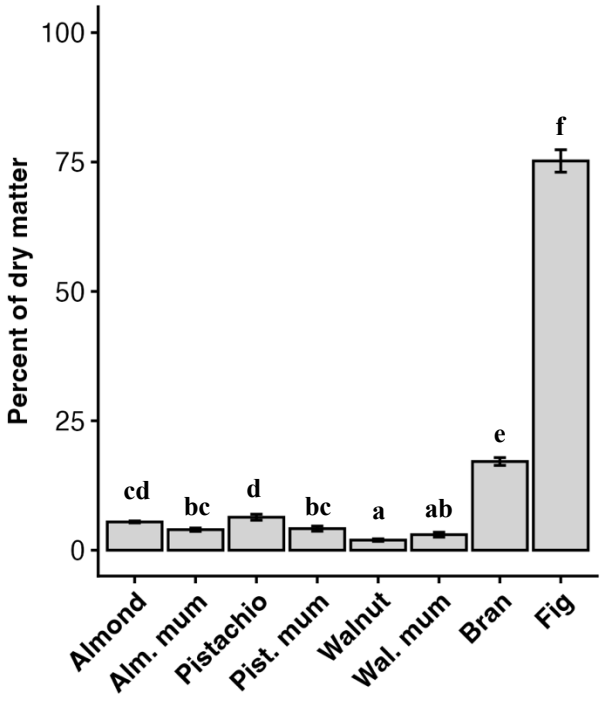

Figure 3.3. Mean total sugar percentage of the diet dry mass. Columns with different letters indicate significant differences, $P < 0.05$ in pairwise comparisons via Tukey's HSD post hoc test.

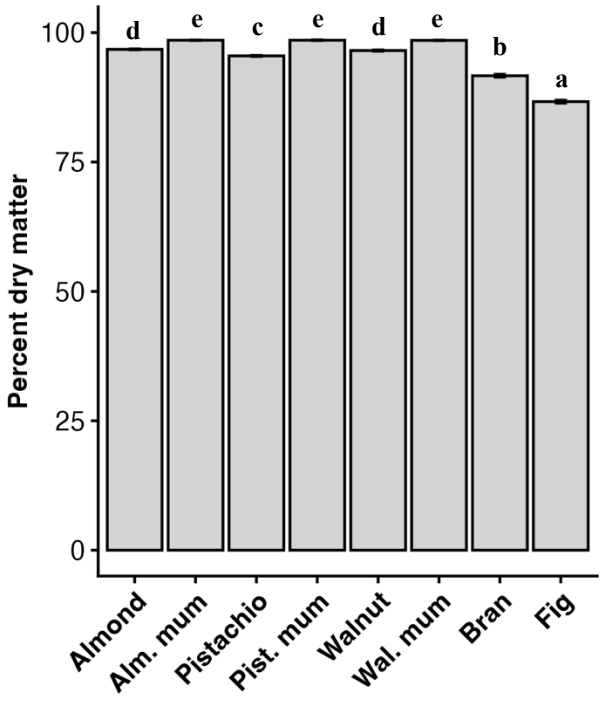

Figure 3.4. Mean dry mass percentage of the diets. Columns with different letters indicate significant differences, $P < 0.05$ in pairwise comparisons via Tukey's HSD post hoc test.

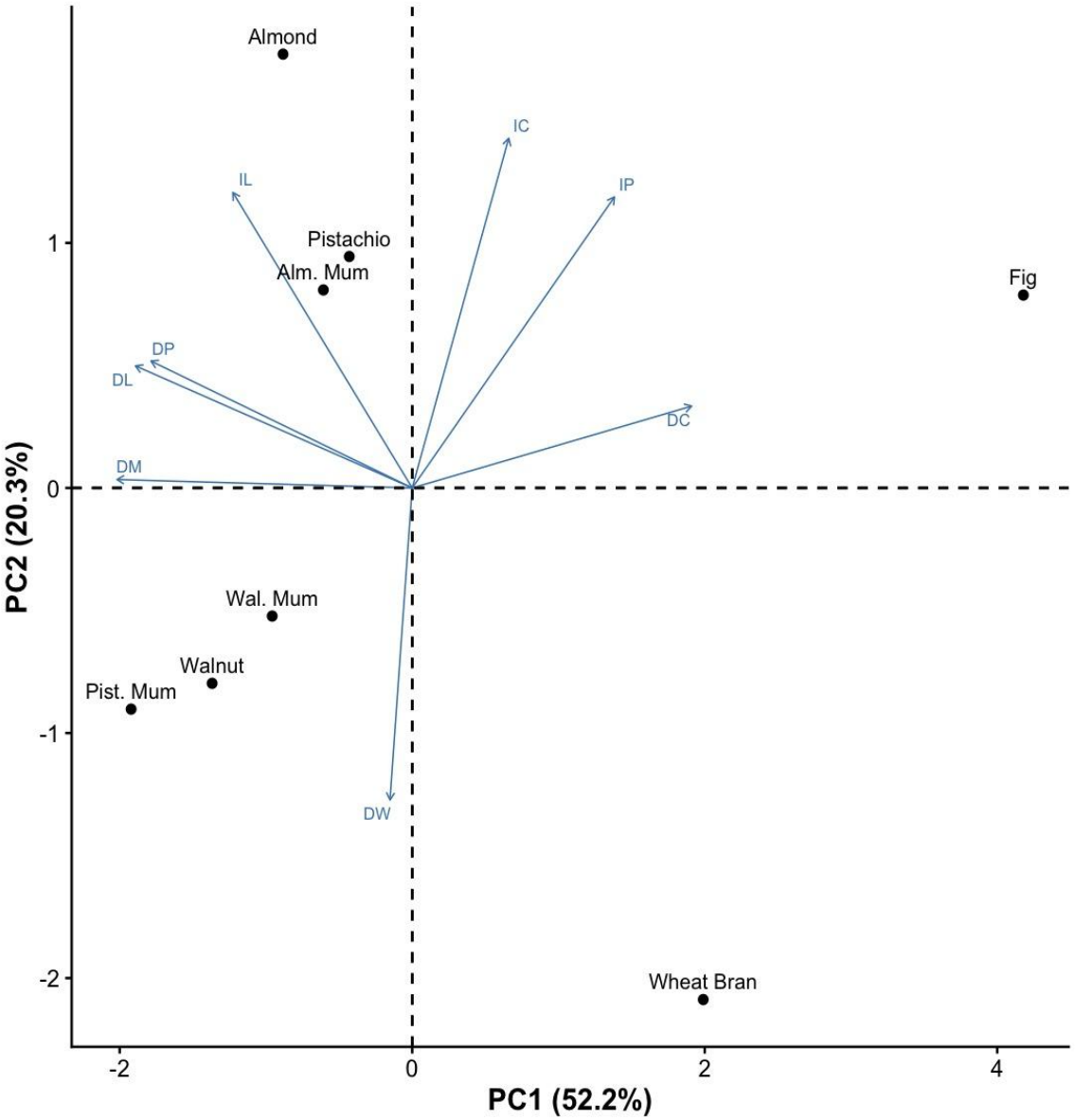

Figure 3.5. PCA biplot of nutritional profiles of diets and non-flown *A. transitella*. Calculated means of each variable were used in the analysis. A small vector angle indicates the variables are positively correlated. An angle of 90° indicates that the variables are uncorrelated. The angle close to 180° indicates the variables are negatively correlated. DP – dietary proteins, DC – dietary carbohydrates, DL – dietary lipids, DM – diet dry matter, IP – insect proteins, IC – insect carbohydrates, IL – insect lipids, and DW – insect dry weight.

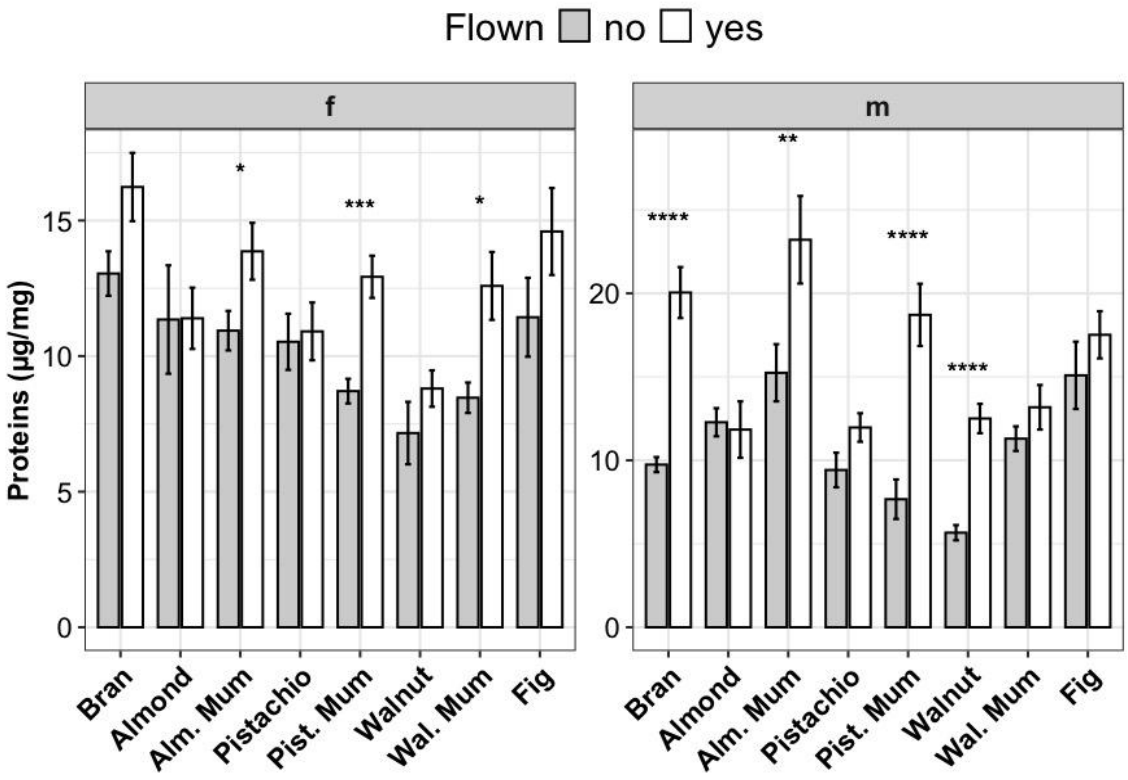

Figure 3.6. Bar plots depicting the difference in mean protein content with SEM bars between non-flown and flown *A. transitella*. Significant differences were determined via the Wilcoxon rank sum test. $P < 0.05$*, $P < 0.01$**, $P < 0.001$***, $P < 0.0001$****

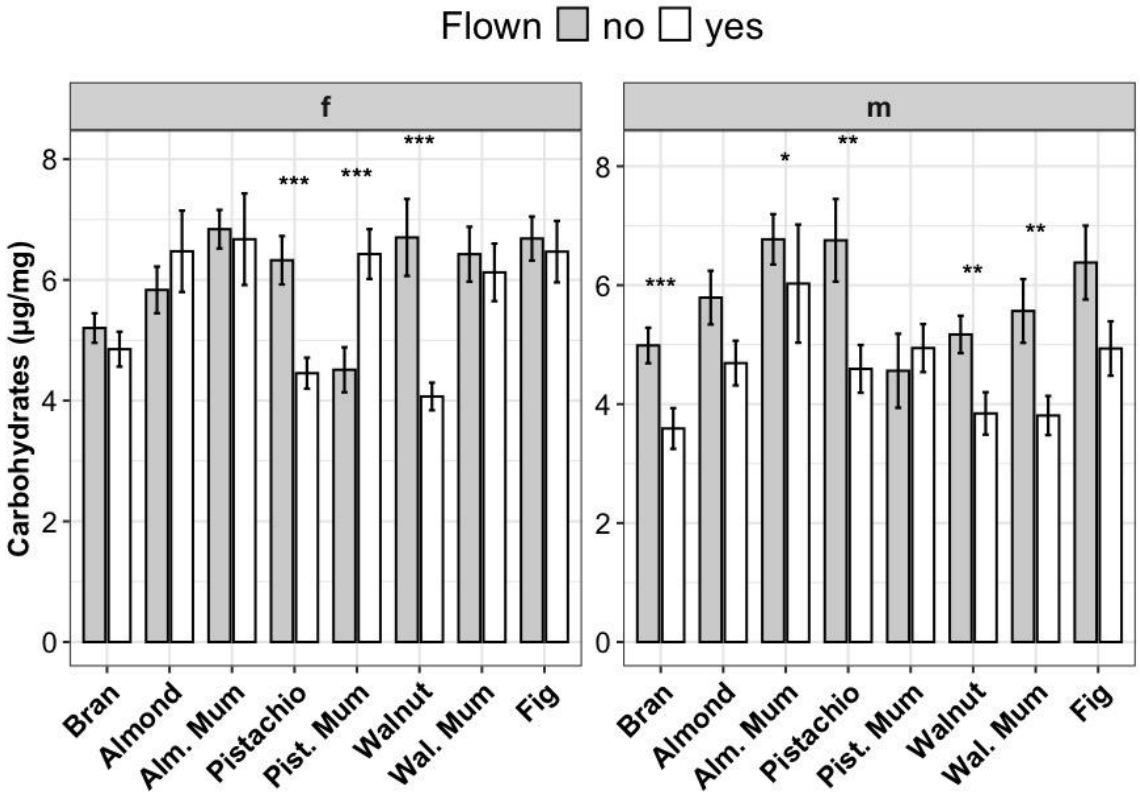

Figure 3.7. Bar plots depicting the difference in mean carbohydrate content with SEM bars between non-flown and flown *A. transitella*. Significant differences were determined via the Wilcoxon rank sum test. P < 0.05*, P < 0.01**, P < 0.001***, P < 0.0001****

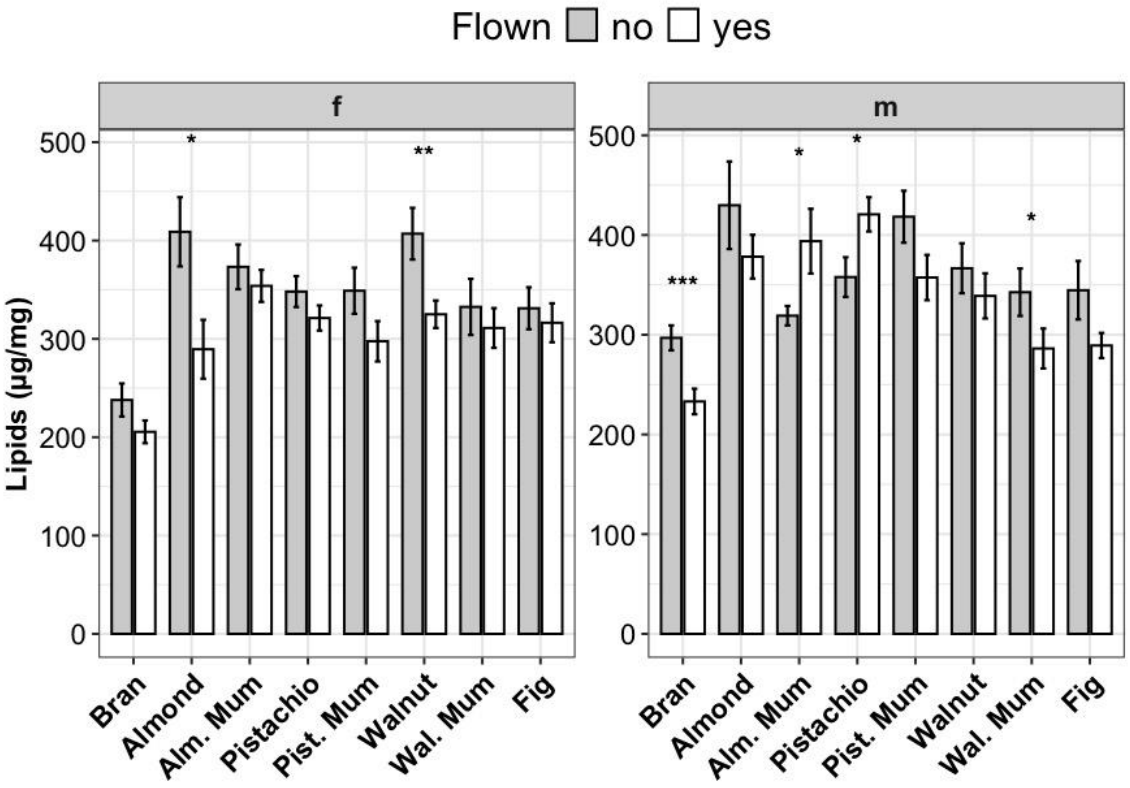

Figure 3.8. Bar plots depicting the difference in mean lipid content with SEM bars between non-flown and flown *A. transitella*. Significant differences were determined via the Wilcoxon rank sum test. $P < 0.05$*, $P < 0.01$**, $P < 0.001$***, $P < 0.0001$****

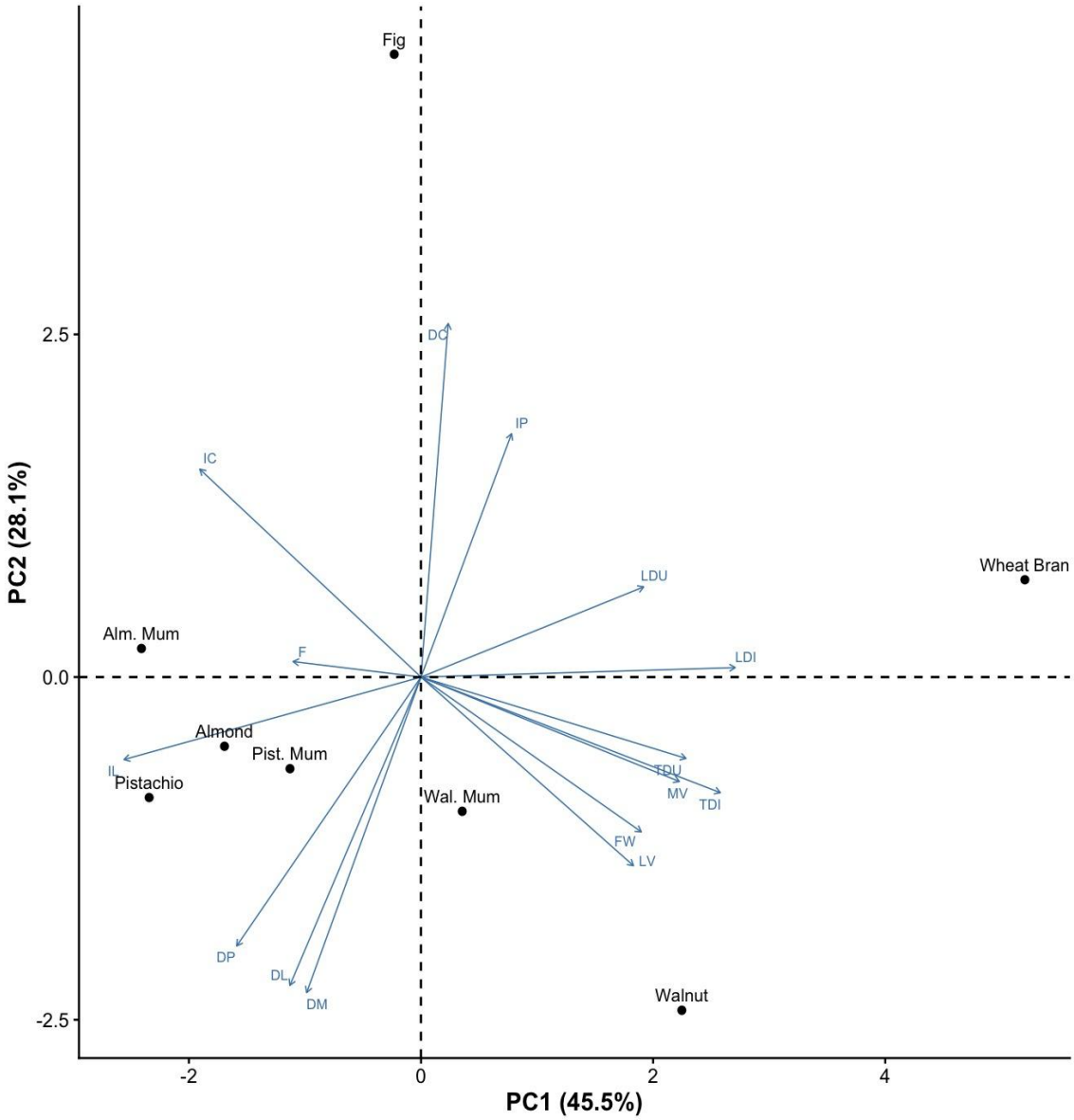

Figure 3.9. PCA biplot of nutritional profiles of diets and flown *A. transitella*. Calculated means of each variable were used in the analysis. A small vector angle indicates the variables are positively correlated. An angle of 90° indicates that the variables are uncorrelated. The angle close to 180° indicates the variables are negatively correlated. DP – dietary proteins, DC – dietary carbohydrates, DL – dietary lipids, DM – diet dry matter, IP – insect proteins, IC – insect carbohydrates, IL – insect lipids, FW – insect fresh weight, TDI – total distance, TDU – total duration, LDI – longest distance, LDU – longest duration, LV – longest velocity, MV – max velocity, and F – bouts of flight.

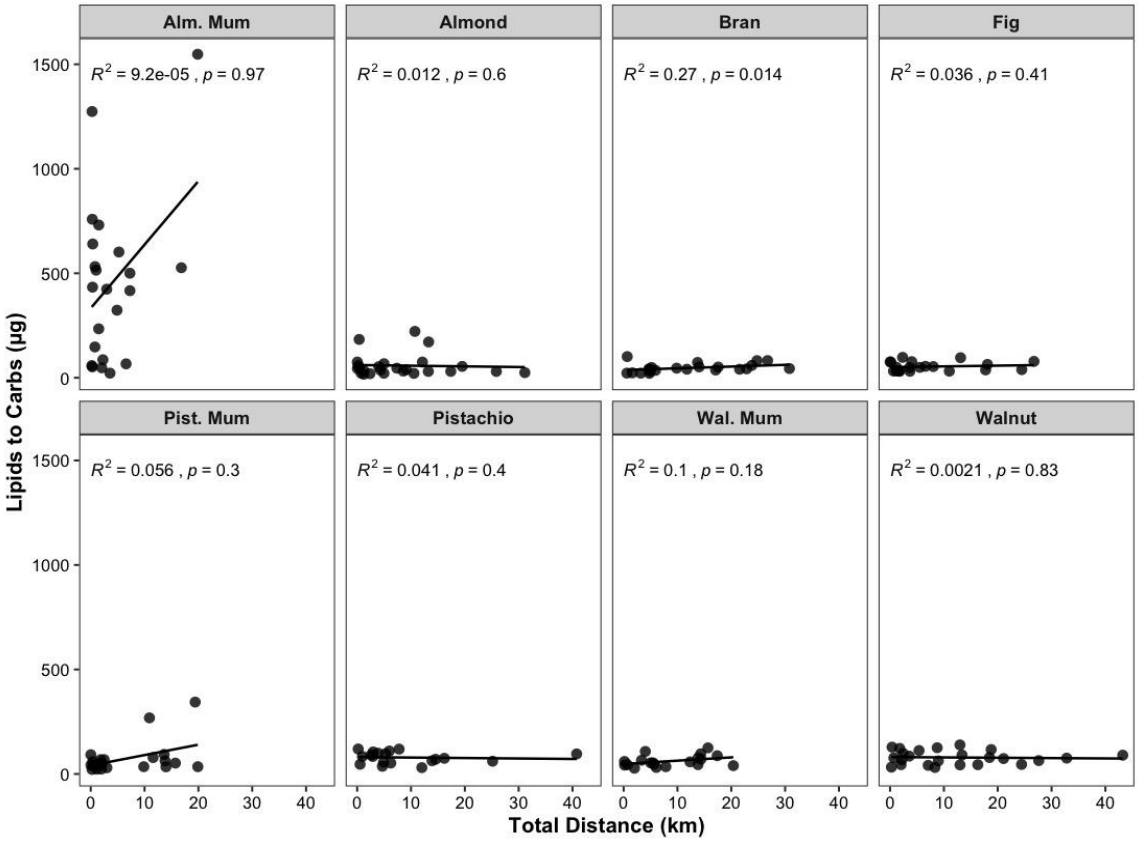

Figure 3.10. Relationship between total flight distance (km) and lipid-to-carbohydrate ratio (µg) in female *A. transitella via* Spearman's rank correlation coefficient. Each panel represents a diet, with points showing individual moths and lines indicating linear regressions. Coefficient of determination (R^2) and rho values are shown for each regression

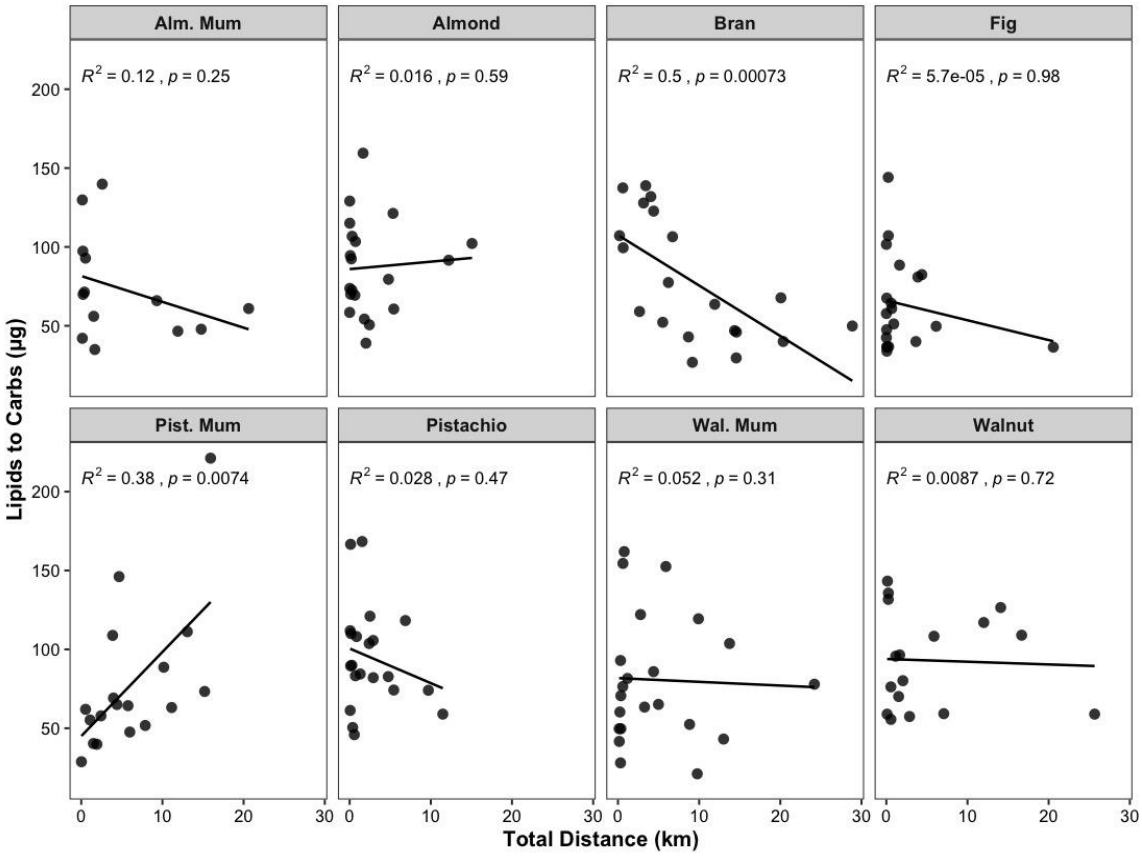

Figure 3.11. Relationship between total flight distance (km) and lipid-to-carbohydrate ratio (µg) in male *A. transitella via* Spearman's rank correlation coefficient. Each panel represents a diet, with points showing individual moths and lines indicating linear regressions. Coefficient of determination (R^2) and rho values are shown for each regression

References

Adaskaveg JA, Lee C, Wei Y, et al. 2025. In a nutshell: pistachio genome and kernel development. New Phytol. 246(3):1032–1048. https://doi.org/10.1111/nph.70060

Andrews KL, Barnes MM. 1982. Invasion of Pistachio Orchards by Navel Orangeworm Moths from Almond Orchards1. Environ. Entomol. 11(2):278–279. https://doi.org/10.1093/ee/11.2.278

Andrews KL, Barnes MM, Josserand SA. 1980. Dispersal and Oviposition by Navel Orangeworm Moths 1. Environ. Entomol. 9(5):525–529. https://doi.org/10.1093/ee/9.5.525

Auerswald L, Marco HG, Gade G. 2000. Metabolic aspects of flight in the dung beetle Pachylomerus femoralis (Kirby) (Coleoptera : Scarabaeidae). Afr. Entomol. 8(2):243–249.

Bayes SK, Hellerstein MK, Fitch M, et al. 2014. You are what you eat: fatty acid profiles as a method to track the habitat movement of an insect. Oecologia. 175(4):1073–1080. https://doi.org/10.1007/s00442-014-2976-9

Beenakkers AMTh, Van der Horst DJ, Van Marrewijk WJA. 1984. Insect flight muscle metabolism. Insect Biochem. 14(3):243–260. https://doi.org/10.1016/0020-1790(84)90057-X

Beenakkers AT. 1969. Carbohydrate and fat as a fuel for insect flight. A comparative study. J. Insect Physiol. 15(3):353–361.

Borzoui E, Bandani AR, Goldansaz SH, et al. 2018. Dietary protein and carbohydrate levels affect performance and digestive physiology of Plodia interpunctella (Lepidoptera: Pyralidae). J. Econ. Entomol. 111(2):942–949.

Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72(1–2):248–254.

Burks CS, Brandl DG. 2004. Seasonal abundance of the navel orangeworm, Amyelois transitella, in figs and the effect of peripheral aerosol dispensers on sexual communication. J. Insect Sci. 4(1):40.

Burks CS, Brandl DG. 2005. Quantitative Assessment of Insect Pest Damage to Figs. Crop Manag. 4(1):1–7. https://doi.org/10.1094/CM-2005-0510-01-RS

Casey MT, Machtley SA, Merten PV, et al. 2023. A simple computerized Arduino-based control system for insect rotary flight mills. J. Insect Sci. 23(4):5. https://doi.org/10.1093/jisesa/iead053

Cheng Y-S, Zheng Y, VanderGheynst JS. 2011. Rapid quantitative analysis of lipids using a colorimetric method in a microplate format. Lipids. 46(1):95–103.

Colasurdo N, Gélinas Y, Despland E. 2009. Larval nutrition affects life history traits in a capital breeding moth. J. Exp. Biol. 212(12):1794–1800.

Elliott J, Hill L, Bailey E. 1984. Changes in tissue carbohydrate content during flight of the fed and starved cockroach, Periplaneta americana L. Comp. Biochem. Physiol. A Physiol. 78(1):163–165.

Finney GL, Brinkman D. 1967. Rearing the navel orangeworm in the laboratory. J. Econ. Entomol. 60(4):1109–1111.

Foray V, Pelisson, PF, Bel-Venner, MC, et al. 2012. A handbook for uncovering the complete energetic budget in insects: the van Handel's method (1985) revisited. Physiol. Entomol. 37(3):295–302.

Hahn DA. 2005. Larval nutrition affects lipid storage and growth, but not protein or carbohydrate storage in newly eclosed adults of the grasshopper Schistocerca americana. J. Insect Physiol. 51(11):1210–1219.

Jin F, Zhou Y, Zhang P, et al. 2023. Identification of Key Lipogenesis Stages and Proteins Involved in Walnut Kernel Development. J. Agric. Food Chem. 71(10):4306–4318. https://doi.org/10.1021/acs.jafc.2c08680

Johansen HN, Glitsø V, Bach Knudsen KE. 1996. Influence of Extraction Solvent and Temperature on the Quantitative Determination of Oligosaccharides from Plant Materials by High-Performance Liquid Chromatography. J. Agric. Food Chem. 44(6):1470–1474. https://doi.org/10.1021/jf950482b

Kammer AE, Heinrich B. 1978. Insect flight metabolism. In: Advances in insect physiology. Vol. 13. Elsevier. p. 133–228.

Kaufmann C, Brown MR. 2008. Regulation of carbohydrate metabolism and flight performance by a hypertrehalosaemic hormone in the mosquito Anopheles gambiae. J. Insect Physiol. 54(2):367–377.

Kuenen L. P. S., Siegel JP. 2010. Protracted emergence of overwintering Amyelois transitella (Lepidoptera: Pyralidae) from pistachios and almonds in California. Environ. Entomol. 39(4):1059–1067.

Kuenen L.P.S., Siegel JP. 2010. Protracted Emergence of Overwintering Amyelois transitella (Lepidoptera: Pyralidae) From Pistachios and Almonds in California. Environ. Entomol. 39(4):1059–1067. https://doi.org/10.1603/EN09277

Kuenen LPS, Siegel JP. 2011. Variable Developmental Rate and Survival of Navel Orangeworm (Lepidoptera: Pyralidae) on Pistachio. J. Econ. Entomol. 104(2):532–539. https://doi.org/10.1603/EC10177

Lara-Villalón M, Vanoye-Eligio V, Solís MA, et al. 2017. The Navel Orangeworm, Amyelois transitella (Walker) (Lepidoptera: Pyralidae), Discovered in Northeastern Mexico Feeding on Sapindaceae. Proc. Entomol. Soc. Wash. 119(4):601–605. https://doi.org/10.4289/0013-8797.119.4.601

Lee JC. 2019. What we can learn from the energetic levels of insects: a guide and review. Ann. Entomol. Soc. Am. 112(3):220–226.

Masuko T, Minami A, Iwasaki N, et al. 2005. Carbohydrate analysis by a phenol–sulfuric acid method in microplate format. Anal. Biochem. 339(1):69–72.

Matveev E, Kwon JJ, Judd GJR, et al. 2017. The effect of cold storage of mass-reared codling moths (Lepidoptera: Tortricidae) on subsequent flight capacity. Can. Entomol. 149(3):391.

Meals DW, Caltagirone LE. 1971. Invasion Rate and Pattern of Infestation of an Almond Orchard by the Navel Orangeworm 1. J. Econ. Entomol. 64(1):90–92. https://doi.org/10.1093/jee/64.1.90

Muller D, Giron D, Desouhant E, et al. 2017. Maternal age affects offspring nutrient dynamics. J. Insect Physiol. 101:123–131.

Murata M, Tojo S. 2002. Utilization of lipid for flight and reproduction in Spodoptera litura (Lepidoptera: Noctuidae). Eur. J. Entomol. 99(2):221–224.

Naranjo SE. 2019. Assessing insect flight behavior in the laboratory: a primer on flight mill methodology and what can be learned. Ann. Entomol. Soc. Am. 112(3):182–199.

Nay JE, Perring TM. 2008. Influence of Host Plant Stages on Carob Moth (Lepidoptera: Pyralidae) Development and Fitness. Environ. Entomol. 37(2):568–574. https://doi.org/10.1093/ee/37.2.568

Palumbo JD, Mahoney NE, Light DM, et al. 2014. Spread of Aspergillus flavus by navel orangeworm (Amyelois transitella) on almond. Plant Dis. 98(9):1194–1199.

Reger JE, Wenger JA, Brar G, et al. 2021. Evaluating flight performance of mass-reared and irradiated navel orangeworm (Lepidoptera: Pyralidae) for sterile insect technique. J. Econ. Entomol. 114(4):1542–1548.

Robbins KS, Shin E-C, Shewfelt RL, et al. 2011. Update on the healthful lipid constituents of commercially important tree nuts. J. Agric. Food Chem. 59(22):12083–12092.

Rovnyak AM, Burks CS, Gassmann AJ, et al. 2018. Interrelation of mating, flight, and fecundity in navel orangeworm females. Entomol. Exp. Appl. 166(4):304–315.

Sakamoto R, Murata M, Tojo S. 2004. Effects of larval diets on flight capacity and flight fuel in adults of the common cutworm, Spodoptera litura (Lepidoptera: Noctuidae). Appl. Entomol. Zool. 39(1):133–138.

Sappington TW, Burks CS. 2014. Patterns of flight behavior and capacity of unmated navel orangeworm (Lepidoptera: Pyralidae) adults related to age, gender, and wing size. Environ. Entomol. 43(3):696–705.

Sappington TW, Fescemyer HW, Showers WB. 1995. Lipid and carbohydrate utilization during flight of the migratory moth, Agrotis ipsilon (Lepidoptera: Noctuidae). Arch. Insect Biochem. Physiol. 29(4):397–414.

Sappington TW, Showers WB. 1992. Reproductive maturity, mating status, and long-duration flight behavior of Agrotis ipsilon(Lepidoptera: Noctuidae) and the conceptual misuse of the oogenesis-flight syndrome by entomologists. Environ. Entomol. 21(4):677–688.

Shirai Y. 1998. Laboratory evaluation of flight ability of the Oriental corn borer, Ostrinia furnacalis (Lepidoptera: Pyralidae). Bull. Entomol. Res. 88(3):327–333. https://doi.org/10.1017/S0007485300025943

Siegel JP, Bas Kuenen LPS, Ledbetter C. 2010. Variable development rate and survival of navel orangeworm (Lepidoptera: Pyralidae) on wheat bran diet and almonds. J. Econ. Entomol. 103(4):1250–1257.

Simpson SJ, Simpson CL. 2017. The mechanisms of nutritional compensation by phytophagous insects. Insect-Plant Interact. 1990.:111–160.

Straser RK, Reger JE, Wilson H. 2023. Diet quality influences nutrient retention and reproductive fitness of the biocontrol agent Hadronotus pennsylvanicus (Hymenoptera: Scelionidae). J. Insect Physiol.:104522.

Su S, Wang X, Jian C, et al. 2021. Life-History Traits and Flight Capacity of *Grapholita molesta* (Lepidoptera: Tortricidae) Using Artificial Diets With Varying Sugar Content. O'Donnell C, editor. J. Econ. Entomol. 114(1):112–121. https://doi.org/10.1093/jee/toaa256

Truzi CC, Vieira NF, de Souza JM, et al. 2021. Artificial Diets With Different Protein Levels for Rearing Spodoptera frugiperda (Lepidoptera: Noctuidae). J. Insect Sci. 21(4):2. https://doi.org/10.1093/jisesa/ieab041

Van der Horst DJ, Houben NMD, Beenakkers AT. 1980. Dynamics of energy substrates in the haemolymph of Locusta migratoria during flight. J. Insect Physiol. 26(7):441–448.

Van Handel E. 1974. Lipid utilization during sustained flight of moths. J. Insect Physiol. 20(12):2329–2332.

Van Handel E. 1985. Rapid determination of glycogen and sugars in mosquitoes. J Am Mosq Control Assoc. 1(3):299–301.

Van Handel E, Nayar JK. 1972. Direct use of carbohydrates during sustained flight in the moth, Spodoptera frugiperda. Insect Biochem. 2(6):203–208.

Vukajlović FN, Predojević DZ, Miljković KO, et al. 2019. Life history of *Plodia interpunctella* (Lepidoptera: Pyralidae) on dried fruits and nuts: Effects of macronutrients and secondary metabolites on immature stages. J. Stored Prod. Res. 83:243–253. https://doi.org/10.1016/j.jspr.2019.07.007

Wade W. 1961. Biology of the navel orangeworm, Paramyelois transitella (Walker), on almonds and walnuts in northern California. Hilgardia. 31(6):129–171.

Wijerathna A, Evenden M. 2019. Energy use by the mountain pine beetle (Coleoptera: Curculionidae: Scolytinae) for dispersal by flight. Physiol. Entomol. 44(3–4):200–208.

Wilson H, Burks CS, Reger JE, et al. 2020. Biology and Management of Navel Orangeworm (Lepidoptera: Pyralidae) in California. J. Integr. Pest Manag. 11(1):25.

Woodard SH, Duennes MA, Watrous KM, et al. 2019. Diet and nutritional status during early adult life have immediate and persistent effects on queen bumble bees. Conserv. Physiol. 7(1):coz048.

Yang H, Guo S, Sun Y, et al. 2025. Adult feeding on different carbohydrates affects the reproduction and flight performance of Spodoptera frugiperda (Lepidoptera: Noctuidae). J. Econ. Entomol.:toaf075.

Zhang L, Pan P, Sappington TW, et al. 2015. Accelerated and Synchronized Oviposition Induced by Flight of Young Females May Intensify Larval Outbreaks of the Rice Leaf Roller. PLOS ONE. 10(3):e0121821. https://doi.org/10.1371/journal.pone.0121821

Ziegler R, Schulz M. 1986. Regulation of lipid metabolism during flight in Manduca sexta. J. Insect Physiol. 32(10):903–908.

Conclusion

Main Findings

The research presented in this dissertation sought to better define the influence of the larval host crop on adult *A. transitella* dispersal and to explore techniques for tracing movement at the landscape level. Findings from this work suggest that tracking moth dispersal in the field remains challenging, despite the use of mark-release-recapture methods. The larval host crop has a detectable influence on adult behavior and the metabolome. The findings for each of the studies included in this dissertation are summarized to address the key questions proposed in the Introduction.

What is the field dispersal range of *A. transitella* in pistachio orchards?

Blacklight traps appeared to have a more localized trapping radius than combo lures, suggesting that combo lures are more effective for detecting dispersing *A. transitella* from a greater distance. Despite the improved attractiveness of combo lures, overall recapture rates remained low and were substantially below the flight potential previously reported from laboratory flight mill studies. This discrepancy highlights challenges in tracking insect movement in the field and the need to evaluate insect dispersal in multiple settings. Additionally, direct comparisons between mass-reared and locally-reared moths demonstrated lower recovery of mass-reared moths in both field recapture studies and laboratory flight cylinder assays, indicating reduced field performance and flight capability in mass-reared individuals.

What other intrinsic markers from tree nuts are present in *A. transitella*?

Larval diet was shown to transfer distinct chemical signatures to adult *A. transitella*, demonstrating the potential to identify larval host origin using intrinsic biomarkers. Several untargeted approaches were evaluated, and while all methods showed some capacity to classify moths according to host crop, each method also had important limitations. Compound-specific isotope analysis (CSIA) was influenced by environmental and management variation among orchards, reducing its practicality for field applications. Untargeted metabolomics using GC/MS provided moderate classification accuracy, although performance was generally lower than that of LC/MS-based approaches. Similarly, untargeted lipidomics showed promising results under controlled conditions, but classification accuracy declined when applied to field-collected samples, highlighting the challenges of environmental variability and the need for more targeted biomarkers. Lutein emerged as a particularly promising marker because it was highly concentrated in both pistachio kernels and pistachio-reared *A. transitella,* allowing for discrimination between moths from pistachio vs almond/walnut. High-performance liquid chromatography (HPLC) provided greater sensitivity and specificity than spectrophotometric methods, although spectrophotometry offered advantages in speed and cost-effectiveness. Overall, no single analytical method consistently determined larval host origin under all conditions. However, combining targeted biomarkers such as lutein with broader molecular approaches, including fatty-acid profiling, may provide a more robust framework for tracing pest origin and movement in agricultural systems.

What is the influence of *A. transitella* larval diet on adult dispersal?

Host crops differed substantially in their macronutrient composition, with tree nuts generally containing higher levels of proteins and lipids, whereas wheat bran and figs contained greater concentrations of carbohydrates. Comparisons between fresh and remnant nuts revealed relatively few nutritional differences, aside from reduced water content in remnant nuts. Despite these differences among host crops, adult *A. transitella* responses were highly variable and did not always correspond consistently with host nutritional profiles. Larval diet influenced both size and dispersal, although the effects varied among traits and between sexes. The largest moths generally emerged from walnut and wheat bran diets, particularly among males. Moths reared on wheat bran also exhibited the greatest dispersal distances in both sexes, although the difference was only marginal compared with some other diets. Colorimetric assays indicated that carbohydrates and lipids were reduced in flown moths, suggesting that these macronutrients serve as important energy sources for flight. In contrast, proteins appeared more concentrated in flown individuals. Overall, the results indicate that larval host crop can influence adult dispersal capacity, but *A. transitella* remains highly adaptable and capable of developing successfully across a broad range of diets. Consequently, first-night dispersal capacity was generally maintained regardless of larval host origin.

Future Directions

Future studies should integrate the methods presented in this dissertation with established approaches for tracing *A. transitella* movement and further evaluate the

influence of larval diet on additional life-history parameters. Rather than tracking movement with passive methods, active tracking tools like harmonic radar provide insight into moth movement in real-time. This approach may reveal if low recapture rates are due to mass emigration from the release site. Alternatively, production of a higher quality sterile moth with incentives for more grower participation due to the low risk of increased damage, and similar MRR studies can be conducted on a broader scale. Tracking the movement of *A. transitella* lutein shows promise and may be combined with the FAME analysis. A single extraction and analysis would be the ideal protocol. Fatty acids and lutein are both nonpolar and can be extracted in similar solvents, so developing a protocol to detect both with an HPLC is an appropriate place to start. The influence of larval diet on dispersal and other adult behaviors is still unclear. Future studies should focus on evaluating the dispersal of *A. transitella* over multiple nights and the impact of larval diet on other life history parameters, like fecundity.

Implications of the Research

This dissertation is a mix of basic and applied research that ultimately benefits tree nut growers of California and *A. transitella* IPM. Improved understanding of A. transitella movement may support the development of more effective management strategies. Although many of the findings were incremental, they provide important advances toward understanding the ecology and management of *A. transitella*. The navel orangeworm, *Amyelois transitella,* is a very adaptive polyphagous pest, persistent in tree nut orchards. As California growers contend with reduced insecticide efficacy, shifting

markets, water limitations, and emerging pests, area-wide integrated pest management strategies may provide a sustainable path forward. By bridging the biological, molecular, and human dimensions through interdisciplinary collaboration, this work provides foundational knowledge and practical tools for future efforts to strengthen IPM in sustainable ways.