Comparing the development and fitness of navel orangeworm, *Amyellois transitella*, raised on agricultural and non-agricultural host plants

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ABSTRACT

Navel orangeworm (NOW), *Amyellois transitella,* is an invasive moth and agricultural pest in California. NOW is an herbivore generalist that uses a variety of foods as a strategy to increase their fitness. Recent work has shown that NOW appears to move in between different nut crops quite readily, implying that NOW might also be mobile enough to move from non-agricultural plants to agricultural crops. I collected non-agricultural plants from the Central Valley and reared moths on each plant independently to determine survivorship, development time, and adult weight. I used jars containing each plant type and NOW eggs. The jars were kept in a walk-in chamber and examined from eggs to adulthood. Black walnuts and acorns had the only non-agricultural survivorship and moths raised on acorns took the longest time to develop into adults. Pistachios and wheat bran showed significant changes in weight ($p < .001$). Significant differences in adult weight suggested that diet type instead of sex had a larger effect on weight. It is possible that NOW is acting as a source population for native habitats. NOW could possibly be feeding on non-agricultural plants in nature and should be further examined. Having a broader understanding of the biology of NOW can help implement more efficient management systems.

KEYWORDS

integrative pest management (IPM), landscape ecology, host range, generalist, dispersal

INTRODUCTION

Invasions by plants, animals, pathogens, and insects into non-native environments pose one of the most significant threats to biodiversity both within natural ecosystems and agricultural settings (Perrault et al. 2003). For agriculture, one study estimates that 40% of all insect damage to crops in the U.S. is attributable to alien species (Pimentel et al. 2000). Not only are these alien species harmful to the ecosystem and biodiversity, but also economically harmful. Moth species alone cause millions of dollars of damage every year to agricultural fields (USDA 2011). Navel orangeworm (NOW), *Amyellois transitella,* is an invasive moth and agricultural pest to California. Larvae bore into fruits and nuts and can consume most of the item, producing large amounts of webbing and frass (insect feces). NOW larval damage can also lead to fungal infections, such as the mold that produces aflatoxin (Almond Board 2012). NOW eggs are opaque white when first laid. After about a day, they turn pink, then reddish orange (Pickel et al. 2012). Once hatched, the larvae feed and grow through five different instars (or stages). Once the larvae are at the 5th instar, they will wrap themselves in a pupa for a week, than emerge as adults. NOWs' origin is not completely known, although it was first described in Mexico in the early 1900s (Wade 1961). The first reported sighting in southern California occurred in 1942, and has since rapidly spread northward (Wade 1961). The generalist feeding strategy of NOW has allowed for its population explosion (Niu et al. 2009). NOW also tends to overwinter in the shells of nuts, allowing them to survive until spring. As a consequence, NOW causes millions of dollars in damage each year, predominantly in the Central Valley, feeding on a variety of nut trees (Kuenen et al. 2008, Siegel et al. 2008, Burks 2010). It is the primary pest of almonds, pistachios, and walnuts, and attacks a number of other agricultural crops such as figs and pomegranates (Meals and Caltagirone 1995). Management of this pest is important because the U.S. tree nut industry in the last decade has generated, on average, nearly $4 billion in annual farm cash receipts, with almonds, walnuts, pistachios and pecans accounting for most of the sales (USDA 2008).

Researchers have been working to improve the efficacy of NOW management practices. One management tactic includes removing "mummy nuts", which are left over nuts (common in pistachios, almonds, and walnuts) that were not picked up during harvest and remained in the trees or on the ground with their shell still intact (Almond Board 2012). NOW use these mummy nuts to overwinter and survive until spring (Siegel et al. 2008). For example, in pistachio orchards there may be more than 30,000 mummy nuts left behind per acre after harvest (Siegel et al. 2008). Other management practices include: harvesting before new NOW generations emerge, insecticides, mating disruption, and biological control (Kuenen et al. 2008, Pickel et al. 2011, Almond Board 2012). However, all of these practices have been implemented with no research done on NOW movement from non-agricultural space to agricultural. Moreover, research on the generalist diet and current management practices have only focused on the agricultural crops.

Herbivore generalists will use a variety of foods as a strategy to increase their fitness. As a consequence, generalists require more robust management protocols (Tikannen et al. 2000). To understand how the generalist strategy of insect herbivores has evolved, it is necessary to understand the role of the host plants in their life cycles and population dynamics, in addition to host plant phylogeny (Tikannen et al. 2000). Although generalist herbivores are able to complete their life cycles on several species of host plants, there is usually wide variation in the fitness of individuals grown on different hosts (Tikannen et al. 2000, Lavoie et al. 2004, Moreau et al. 2006). The host plant influences the growth and survival of larvae with direct implications for adult fitness (Tikkanen et al. 2000). For example, NOW uses mummy nuts to survive the winter by burrowing in the shells (Siegel et al. 2008). Females of the overwintered generation lay their eggs singly on mummy nuts. The first generation, and most of the second, is completed in these nuts. Some of the second generation larvae infest the new crop in the summer when the husk begins to split. Females emerging at this time prefer to lay eggs on the opened husk or on the exposed nutshell (Pickel et al. 2011, USDA 2011). Nuts or seedpods that can have split husks are susceptible to NOW invasion and will be taken into account for the plants chosen in this study. Recent work in the StephenWelter lab at University of California, Berkeley has shown that NOW appears to move in between different nut crops quite readily, implying that NOW might also be mobile enough to move from non-agricultural plants to crops (Bayes Personal Communication 2012). The plants could be harboring large numbers and supplementing the pest population to nearby fields. The role of non-agricultural plants as a source population of NOW for nearby growers has not been examined, and could be drastically affecting the nut tree industry.

In this study I examine the comparative biology of NOW larvae and adults. I will investigate if NOW is found on non-agricultural plant collections. Then, I will rear NOW colonies, starting from eggs to adults, in a lab setting to compare NOW's survivability on non-agricultural and agricultural plants. I will test agricultural plants that are most commonly attacked by NOW, and other agricultural crops that have the potential to harbor NOW populations that could affect neighboring nut crops. I hypothesize that NOW will survive on a variety of non-agricultural plants, especially if they have seed pods with shells, or produce stone fruits that could provide shelter for overwintering (Siegel et al. 2008). I further hypothesize that development time (eggs to adults) will vary greatly among the different plants. A longer development period has negative implications for performance since the exposure time of larvae to natural enemies is effectively extended and a lower body weight is typically attained (Price 1997). An alternative hypothesis is that survivorship is low possibly due to the following factors: plant availability in the field, different nitrogen and nutrition levels amongst plants (Lavoie and Oberhauser 2004), or the age of the seeds or fruit (Gibbs et al. 2006). It is also possible that NOW in the field has adapted to resist pesticides, while the ones in lab have not developed such an adaptation, and any trace amounts on plants may negatively affect survivorship.

METHODS

Study Site

I reared a NOW colony in the lab of Environmental Science, Policy, and Management Professor Stephen Welter, at the University of Berkeley, California. Moths were kept in a walk-in environmental chamber maintained at 26.5° C with a 16:8 hour light:dark photoperiod (Burks et al. 2011). I collected agricultural fruits from produce stores and orchards and non-agricultural plants from several locations in the Central Valley of California (Table 1).

Table 1: Plant types and their collection site. Sites varied between stores and growers for agricultural plants. Sites were in the Central Valley of California for non-agricultural plants.

Common name	Scientific Name	Location of collection
Organic Oranges	*Citrus Sinensis*	The Produce Center, Berkeley, CA
Raw Pistachios	*Pistacia vera*	Berkeley Bowl, Berkeley, CA
English Walnuts	*Juglans regia*	Grower in Escalon, CA
Almonds	*Prunus dulcis*	Grower in Escalon, CA
Black Walnuts	*Juglans nigra*	Just South of Escalon, CA
Toyon	*Heteromeles arbutifolia*	San Luis Reservoir State Recreation Area Gustine, CA
Arizona Cypress	*Cupressus arizonica*	San Luis Reservoir State Recreation Area, Gustine, CA
Coast Live Oak Acorns	*Quercus agrifolia*	UC Berkeley Campus, Berkeley, CA

Since my research question involves the possible movement of NOW between non-agricultural sites to agricultural sites, I collected all non-agricultural plants adjacent to agricultural orchards and within a five mile radius of agricultural sites (Fig. 1 and Fig. 2). Since this study has not been done before, I looked for non-agricultural plants that seemed suitable for NOW diet. I looked for plants with seed pods, shells or fruiting bodies.

Figure 1: Collection site of black walnuts. Located 2.9 miles southwest of Escalon, California. The distance between the surrounding orchards and collection site is .54 miles. (Google Earth 2008).

Figure 2: Collection site of toyon and Arizona cypress. Located in Gustine, California. Distance between surrounding orchards and collection site is 3.9 miles. (Google Earth 2008)

Research Design

In order to determine the development and fitness of NOW, moths were reared on each plant independently. Eggs were collected from a NOW colony kept in the Welter lab. The "lids" of the colony jars are paper towels secured with rubber bands. After adults mate, females will lay their eggs in the grooves of the paper towels. I could visually see the eggs in the paper towel and counted one hundred eggs, ripped off the part of the paper towel that contained the counted eggs, and placed this ripped-off section into another one gallon jar containing a weighed amount of one of the plant types (Table 2). I secured a "lid" on these new jars with paper towels and rubber bands. The paper towels also ensured air flow within the jar.

Table 2. Plant type and weight. Weight of plant types were taken with a Mettler PM 4600 DeltaRange Scale.

Plant Type	Weight (grams)
Organic Oranges	~200 depending on individual orange
Raw Pistachios	150
English Walnuts	150
Almonds	150
Black Walnuts	200 including parts of outer shell
Toyon	90, including some branches and foliage
Arizona Cypress	100 including conifer seeds and foliage
Coast Live Oak Acorns	200
Flakey Wheat Bran (Control)	200

The control diet was a mixture of wheat bran, honey, glycerol, and water (Kuenen et al. 2008). I washed the oranges to reduce the risk of pesticide residue before placing them in jars (Bayes Personal Communication 2012). With a pilot study, all weight measurements were under the assumption that the determined amount is an efficient amount of food for 100 larvae. I made a total of 8 replicate jars for each plant type for a total of 800 eggs with the exception of coast live oak acorns. I was only able to collect enough to make 5 replicate jars for a total of 500 eggs. I kept jars for 60 days and discarded the jar if I saw no development (See **Data Collection** for details on development). Some jars took much longer than 60 days to develop. If I saw development, but larvae were not adults by 60 days, I kept the jar to continue larvae development until adulthood. I observed each jar for two minutes every three days for larval development and emerging adult NOW (Bayes Personal Communication 2012). Once adults emerged form a particular jar, I then checked that jar every day to collect adults. This ensured that I collected adults before males and females could mate which would thus affect weight results. I kept count of individual adult moths as I collected them; I collected adult moths separately with plastic soufflé cups and stored them in a freezer. Around 7-10 days later, I weighed them using a Mettler Toledo MT5 scale, and then dried them in an oven at 40̊ C for 2 hours (Handa et al. 2012) to exclude water weight in the final weight results. I then reweighed the moths to record a final dry weight.

Data Collection

I recorded survivorship, development time, and adult weight. In nature, pupation can occur before winter or after, however survivorship tends to be higher for overwintering larvae (Siegel et al. 2008). I recorded survivorship as the percent of eggs to become adults. I observed and recorded development time in days from eggs to adults. When observing I looked for instar level and pupation. NOW larvae have 5 instars (1 being the smallest and youngest, and 5 being the largest and oldest) before they pupate. I visually estimated the instar level when I observed for development time. I recorded wet and dry adult weight to the nearest milligram.

Data Analysis

The independent variable throughout the study was plant species. Survivorship will be calculated as the percentage of eggs that became adults. The standard error was included to account for the possibility of miscounted eggs. To determine significant differences between the averages of development time, I compared average number of days on a graph. While oranges, toyon, and Arizona cypress, had 0% survivorship, I was able to track some development time through instars. I used analysis of variance (ANOVA) to determine if there were differences between the change in weight of moths from each plant type. This helped me determine if there was significance in water weight increase by each plant type. Since my dry weight data had high variation, I used a Kruskal-Wallis test to compare the adult dry weight by sex and diet type. I used R Studio (RStudio Team 2012) and R Commander (Fox 2005) packages for my statistical analysis.

RESULTS

Survivorship

The control wheat bran had 73% survivorship. For the agricultural plants, I found that pistachios had the highest survival rate of 42% and almonds had the lowest non-zero survival rate at 2% (Fig. 3). Black walnuts (17%) and acorns (3%) had the only non-agricultural survivorship. Oranges, toyon and Arizona cypress had 0% survivorship. Overall survivorship was higher in agricultural plants than in non-agricultural plants.

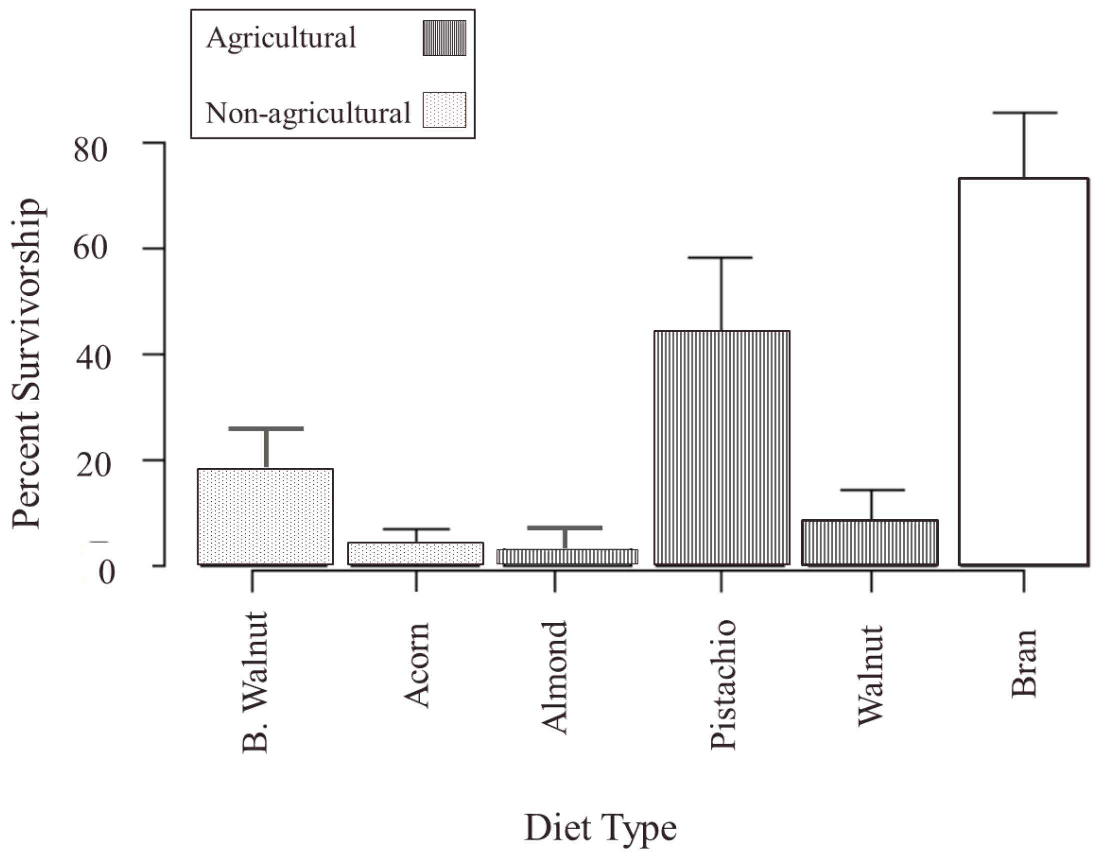

Figure 3: Survivorship of NOW on diet types. I determined survivorship by the percentage of adults that emerged from a known amount of eggs. Means ±1 standard error.

Development Time

I observed a wide range of development time for NOW on each plant. I found that NOW feeding on acorns had the longest time to adulthood, averaging 94 days (Fig 4). NOW feeding on oranges and acorns never developed into adulthood, however I still observed and recorded the development of each instar (Fig. 4). Toyon, and Arizona cypress showed no development.

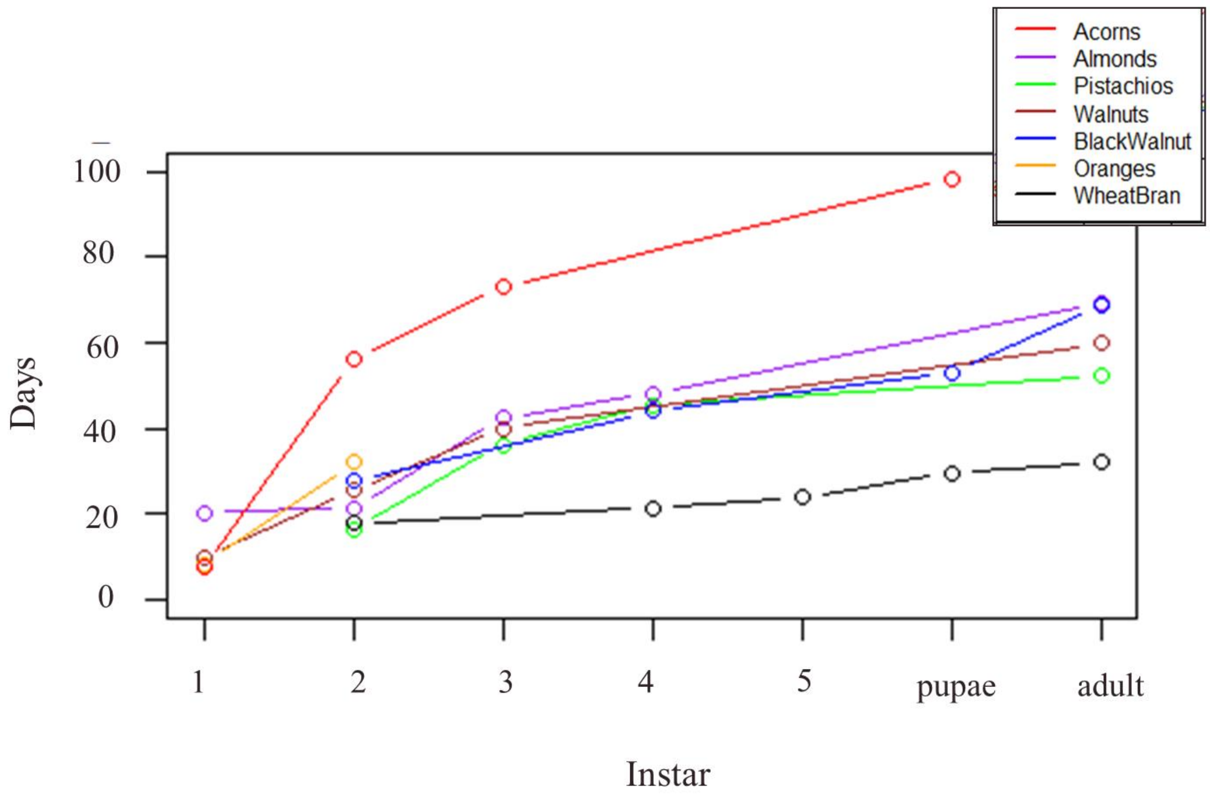

Figure 4: Development of instars of each plant type. I determined instars by a visual estimation of body length and width across all replicates. Replicates varied and some averages did not follow an upward trend and were omitted to show a logical upward trend. Development times were similar across diets with the exceptions of wheat bran and acorns.

Adult Weight

Adult change in weight

I compared the change in weight amongst diet using ANOVA which yielded a significant difference, $F(5, 911)= 29.66$, $p < .001$. A post hoc Tukey test showed that the wheat bran and pistachio moths had the greatest differences in change in weight compared to the other diets. Table 3 gives a list of the significant p values.

Table 3: Significant plant pairs when comparing change in weight of moths. The tukey test compared the change in weight of moths raised all the plant types. Wheat bran and pistachios showed significant differences with other plant types.

Plant pairs compared	Significant P values
Wheat bran : Acorn	.00278
Pistachio : Almond	.00183
Wheat Bran : Almond	< .001
Pistachio : Black Walnut	< .001
Wheat Bran : Black Walnut	< .001
Pistachio : Walnut	< .001
Wheat bran : Pistachio	< .001
Wheat Bran : Walnut	< .001

Adult dry weight

Since I had high variance for the dry weights of the moths, I used a Kruskal-Wallis test to determine significance across my data. I first compared male and female moths across all diet types (Fig. 5). The Kruskal-Wallis test gave results of H=206, 1 d.f., $p < .001$. I then did another Kruscal-Wallis test to compare weight by sex and diet (Fig. 6). This comparison gave me results of H=396, 11 d.f., $p < .001$. I then did a Kruscal-Wallis post hoc test to determine where difference was. Wheat bran and pistachio adults were again significantly different, regardless of sex ($p < .01$). There was a pattern of wheat bran and black walnut adults having significant differences, regardless of sex; while also a pattern of pistachio and walnut adults having significant differences, regardless of sex (all p values < .01).

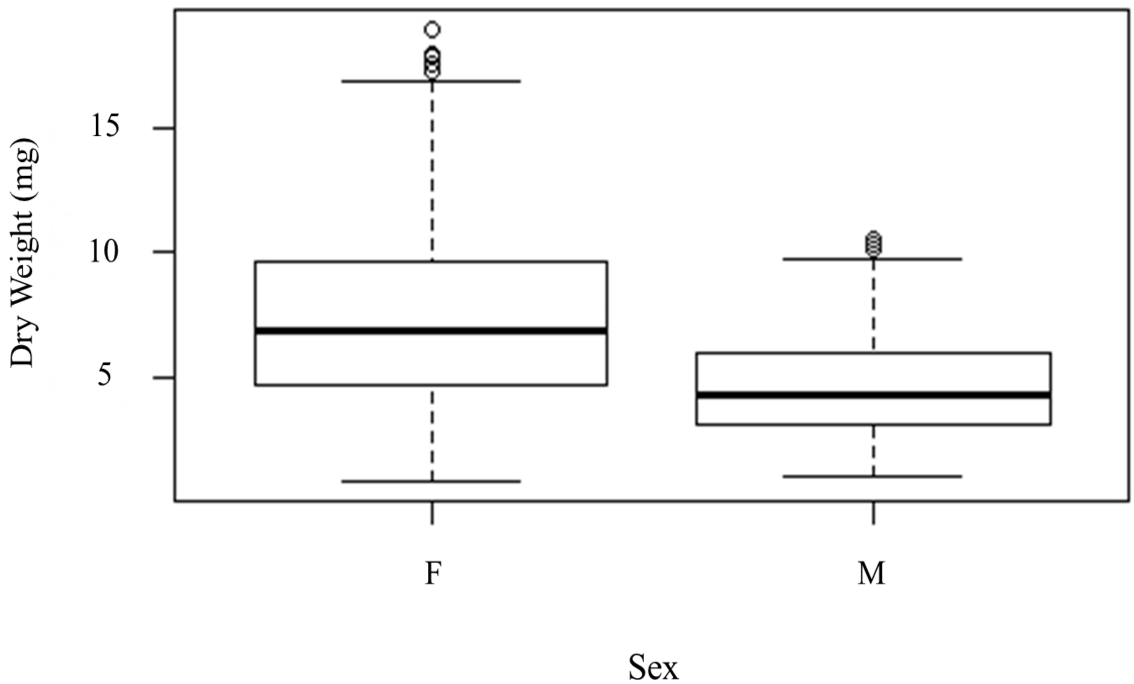

Figure 5: Weight Comparison by Sex. Females weighed an average of 7.3 milligrams (SD= 3.4) and males averaged 4.6 milligrams (SD=2).

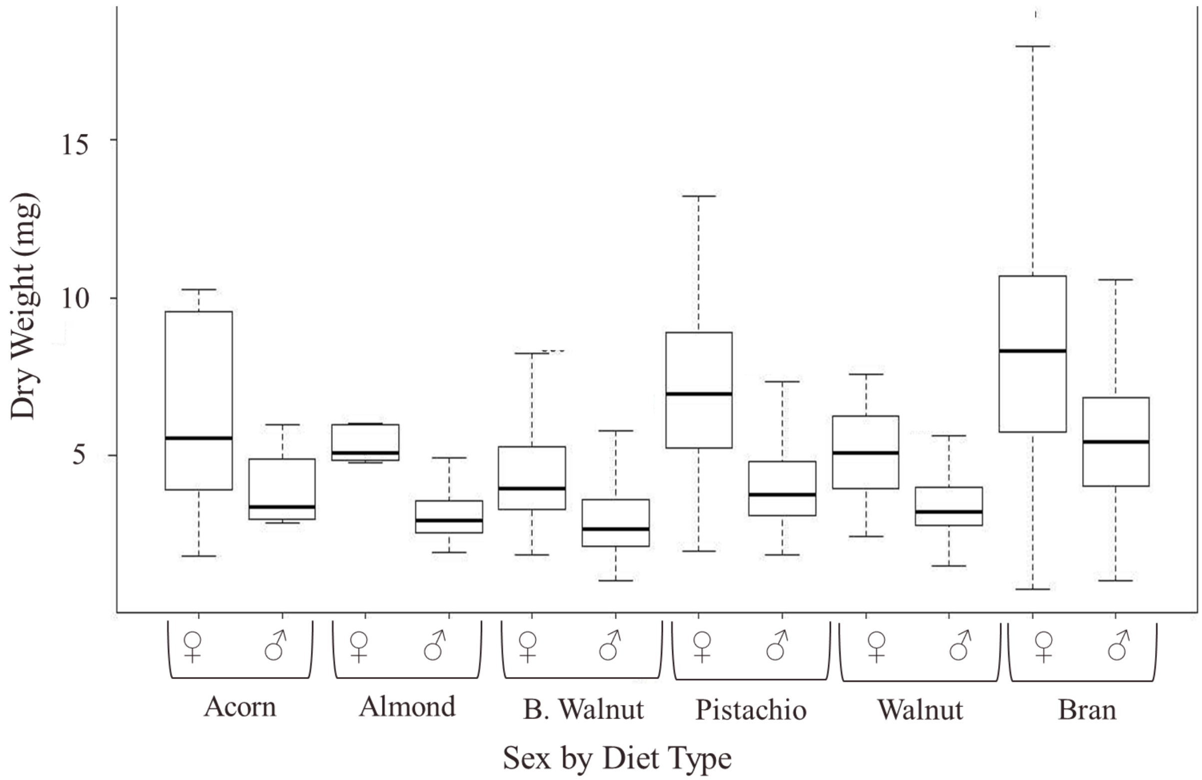

Figure 6: Weight Comaprison by Sex and Diet. In all diet types, females weighed more than males. While there were many differences between a certain sex and a certain diet, the most significant differences appeared when adults from one diet, regardless of their sex, were significantly different from another diet.

DISCUSSION

I compared the development and fitness of NOW raised on agricultural and non-agricultural host plants while also contributing to the knowledge gap of which previously unknown plant types NOW is able to develop into adulthood. I have shown that NOW is able to develop and survive on non-agricultural plants such as black walnuts and coast live oak acorns. Development time was similar in all plant types, with the exception of acorns, which took 20 days longer than all other plant types to develop. I hypothesized that development time would have more variance amongst plant types. Wheat bran and pistachio adults had the most significant change in weight. Dry weight significance was greatest in pairs of: walnut to pistachio, wheat bran to black walnut, and pistachio to wheat bran adults, regardless of sex. These findings do not prove that NOW are surviving on black walnut and coast live oak in nature. Moreover, during non-agricultural plant collections, I found no evidence of NOW, so further analysis on the threat of neighboring orchards from the collection sites was excluded in this study. However, it is possible that NOW is feeding on non-agricultural plants that neighbor orchards and should be further investigated. If NOW is to be found on non-agricultural plants and harboring populations to agricultural sites, there would need to be drastic changes to current management tactics.

Survivorship

Survivorship was highest for black walnuts in the non-agricultural plants, and highest in pistachios for the agricultural plants. I was unable to do a correlation with survivorship of plants that had shells, because the almonds, walnuts, and pistachios that I received were already de-shelled. The only plants that had shells were acorns and black walnuts; in which survivorship was so different that another factor was more significant in survivorship. Regardless, there seemed to be little significance for survivorship with shell containing plants. Almonds had significantly less survivorship than pistachios, both shell-containing nuts in nature. Black walnuts had a larger survivorship than acorns; both contain shells. Although shells may help in the overwintering process (Siegel et al. 2008, Almond Board 2012) it does not appear to be essential to NOW survival. NOW also overwinters in oranges, (Siegel et al. 2008); however in my experiment there was no survivorship from oranges. The larvae only reached the second instar before the oranges would mold over. This is an indication that survivorship depends on the nutritional value of the plant, despite the very generalist diet of NOW (Gibbs et al 2010). While NOW may be able to survive feeding off one plant, it may not be the most nutritionally desirable for fitness and fecundity as other plants. However, it is still important to take shells and stone fruits into account. One study reported that many stone fruits and shelled nuts are harvested early, stored in bins, and then transported (Higbee et al. 2001). It is possible that NOW is surviving in shells while the population is being spread by traveling through storage bins. While shells may not be essential to survival, they may offer protection from predators and pesticides and therefore are highly beneficial to NOW.

This is a clear indication that there are other factors affecting survivorship. The plants' water content could have had an effect on survivorship. (see subtitle *" Adult change in* *weight*”) The 0% survivorship in toyon, oranges, and Arizona cypress could be from several factors as stated in my hypothesis: plant availability in the field, different nitrogen and nutritional value, plant age, or specifically for oranges, the problem of mold. NOW in nature is known to feed on oranges (Kuenen et al. 2008, Siegel et al. 2008, Burks 2010), however in my controlled chamber I could not stop the oranges from molding over and killing the larvae. The enclosed chamber, constant temperature, or other lab conditions could have caused the oranges to mold over before larvae could develop. Further analyses could be done to determine how much of my hypotheses are a factor to NOW survivorship.

Development Time

This data is very limited since many times I could not see larvae for weeks at a time. I saw evidence of larvae such as holes in the nuts or frass. However some replicates I did not see until they were adults. The limited data did not show any clear trends in development time. From Figure 4, most plants developed around the same time. The two outliers were the control wheat bran and acorns. The wheat bran developed moths the quickest; NOW reached adulthood in an average of 30 days. The acorns had a large variety of adult emergence. One replicate had a few adults within 40 days, but then no adults were seen until 30 days later.

There is a possibility that the pesticide residue was large enough to hinder development, since the growers I received the nuts from use pesticides. One study determined that evolutionary resistance to insecticides is highly possible (Niu et al. 2011), however, the colony of NOW I used from the Welter lab have been feeding from wheat bran for over 120 generations and could have lost its ability to resist pesticides. This has likely selected for individuals that have high fitness from eating wheat bran, in which case this population may not be an accurate representation of what NOW is able to eat in nature. If I had made more replicates for this study, and tested fecundity, it is possible development time could have decreased in the number of days, as NOW could have evolved and adapted over the course of my study. There is also the question of evolution amongst plant types. Comparing the relatedness of the plant types that NOW is known to develop on, could help determine closely related non-agricultural plant types that NOW could also adapt to. NOW could enjoy more plant types that are closely related, or feed on a select few from every evolutionary branch. In conclusion the differences in the average length of development within each plant type, combined with the wide range of values under uniform conditions for each plant, lead me to conclude that variable development is an inherent characteristic of NOW in the population I tested, and could also be true for the species in general.

Adult Weight

Adult change in weight

I compared the change of weight of adult moths to determine which diets were contributing the most to water weight. Since weight is a good indicator of fitness (Tikkanen et al. 2000), I wanted to know how water weight applied to fitness. Wheat bran and pistachio adults had the greatest significance in change in weight, which means these diets had the most water. This most likely has correspondence with the highest survivorship from these diets. Wheat bran larvae had a faster development time than pistachio larvae, however, both of these diets were the quickest to develop out of all the plant types. While weight is an important factor for fat storage and longevity, water weight also seems to play an important role in the survivorship and development time of NOW. Toyon and Arizona cypress are both drought resistant plants and probably have very little water content. This is important since I had 0% survivorship on these plants. Therefore, non-agricultural plants that have higher water content are more susceptible to being eaten by NOW.

Adult dry weight

Sex and diet both had an effect on adult dry weight; however the most significant comparisons included both sexes from a certain plant type. Since there are biological differences between sexes (Molleman et al. 2011), these differences played an important role in adult weight. However diet type seemed to be the more prominent factor effecting weight. Wheat bran and pistachio adults, wheat bran and black walnut adults, and pistachio and walnut adults all differed significantly regardless of sex. From Figure 6, walnut and black walnut averages were much lower than wheat bran and pistachio weight averages. These results may also correspond with the adult change in weight results and survivorship results. After water was removed, pistachio and wheat bran still produced the heaviest moths, which also had the greatest amount of survivors. Since walnuts and black walnuts are closely related, this may also have some correspondence for the trends seen. However there is much room for error in both the change in weight data and dry weight data. I had to change weighing scales sometime during my study because the prior scale used (in grams) was less precise. The latter scale measured in milligrams, which was more appropriate and precise for NOW weight. When running my analysis I excluded data collected that were in grams, however that was data lost in the analysis.

Study limitations

In order to draw results from my data, more replicates would have been necessary. I was able to prepare 8 replicates, however at least 10 would have been ideal for more significant results (Bayes Personal Communication 2012). Time permitting, once I knew if NOW were to survive on the plant types or not, I would have addressed the question of fecundity. Results on fecundity and fitness together would provide a clearer possibility of non-agricultural plants harboring pest populations. Furthermore, the similar trends seen in development time amongst most plants were not anticipated. This made it challenging to contrast agricultural and non-agricultural plants; due to the survivorship results, it is clear that these plants have different nutritional value but it was difficult to infer that with similar development times.

Future directions

This particular study on expanding the knowledge of the diet of NOW has not been addressed. My results have indicated two more plants that are capable of sustaining NOW; it is now essential to address adult fecundity and NOW reproduction. Vegetation distribution information is limited, however farmers should be aware of what plant types surround their orchards and expand management if surrounding invasive populations of NOW put their orchard at risk. Another future study would be to indicate a population of NOW in neighboring plants. Efforts have been made in creating an insect counter that could be used to monitor pests outside of agricultural lands (Hoffman et al. 2010). Setting up an established plane with sensors and monitoring which insects fly through gives spatial and temporal information.

Conclusion

There is only one published source, which by no means is extensive, that lists all of the plant species that NOW has been seen on (Wade 1961). This source is also 50 years old and is unlikely to reflect current trends. This study was also purely observational and there was no attempt to look at the survivorship on these different plant species. Thus understanding more about NOW host range will provide growers and pest management programs with crucial information about this pest. Furthermore, understanding the host range of NOW will aid in the development of the current mating disruption program. If populations of NOW in agricultural crops are being harbored by nearby non-agricultural plants, this would allow for further research possibilities on migrating NOW. Understanding where NOW is populating and the movement of NOW beyond agricultural areas would increase our ability to implement the current management programs efficiently and effectively. On a broader scale, this research could greatly improve our understanding of how invasive species both utilize both agricultural and native plants and shed light on the interface between agricultural and native habitats. Current insect pest management is not addressing the root of the invasive pest problem by focusing on the agricultural sector.

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REFERENCES

Bayes, Stephen. 2012. Personal Communication. ESPM Graduate Student.

Burks, C. 2010. Mating disruption for navel orangeworm in central California: year 3. 2010. Annual International Research Conference on Methyl Bromide Alternatives and Emissions Reductions. Orlando, FL. USA.

C. Pickel, C., Grant, J.A., Bentley, W.J., Hasey, J.K., Coates, W.W., and Van Steenwyk, R.A. 2011. *UC IPM Pest Management Guidelines: Walnut*. UC ANR Publication 3471. Insects and Mites

California Almonds: The Almond Board of California. 2012. Navel Orangeworm. <http://www.almondboard.com/Growers/orchardmanagement/PestManagement/InsectsMites/NavelOrangeworm/Pages/Default.aspx>

Fox, J. 2005. The R Commander: A basic statistics graphical user interface to R. Journal of Statistical Software, 14(9): 1--42.

Gibbs, M., L.A. Lace, M.J. Jones, and A.J. Moore. 2006. Multiple host-plant use may arise from gender-specific effects. Journal of Insect Science **6**:1-8.

Google Earth 6.0. 2008. <http://www.google.com/earth/index.html>. Accessed 1 December 2012.

Handa,C., T. Okubo, A. Yoneyama, M. Nakamura, M. Sakaguchi, N. Takahashi, M. Okamoto, A. Tanaka-Oda, T. Kenzo, T. Ichie, T. Itioka. 2013. Change in biomass of symbiotic ants throughout the ontogeny of a myrmecophyte, Macaranga beccariana (Euphorbiaceae). Journal of Plant Research **126:** 73-79.

Higbee, B.S., C.O. Calkins, and C.A. Temple. 2001. Overwintering of codling moth(Lepidoptera: Tortricidae) larvae in apple harvest bins and subsequent moth emergence. Journal of Economic Entomology **94:**1511-1517.

Hoffman, W.C., P.C. Jank, J.A. Klun, and B.K. Fritz. 2010. Quantifying the movement of multiple insects using an optical insect counter. Journal of American Mosquito Control Association **26:** 167-171.

Kuenen, L.P.S, W. Bentley, H. C. Rowe, and B. Ribeiro. 2008. Bait formulations and longevity of navel orangeworm (NOW) egg traps tested. California Agriculture **62**: 36-39.

Lavoie, B. and K.S. Oberhauser. 2004. Compensatory feeding in *Danaus plexippus* (Lepidoptera: Nymphalidae) in response to variation in host plant quality. Environmental Entomology **33**: 1062-1069.

Meals D.W., and L.E. Caltagirone. 1995. Navel Orangeworm. In: Nechols J.R. (ed.) Biological control in the western United States. Regents of the University of California, division of agriculture and natural resources publication. San Pablo, California, pp. 196-198.

Molleman, F., J. Javois, T. Esperk, T. Teder, R.B. Davis, and T. Tammaru. 2011. Sexual differences in weight loss upon eclosion are related to life history strategy in Lepidoptera. Journal of Insect Physiology **57**: 712-722.

Moreau, J., B. Benrey, and D. Thiery. 2006. Assessing larval food quality for phytophagous insects: are facts as simple as they appear? Functional Ecology **20**: 592-600.

Niu, G., J.P. Siegel, M.A. Schuler, and M.R. Berenbaum. 2009. Comparative toxicity of mycotoxins to navel orangeworm (Amyelois transitella) and corn earworm (Helicoverpa zea). Journal of Chemical Ecology. **35**(8):951-957.ew

Perrault, A., M. Bennet, S. Burgiel, A. Delach, and C. Muffett. 2003. Invasive species, agriculture and trade: Case studies from the NAFTA context. Second North American Symposium on Assessing the Environmental Effects of Trade. Mexico City, USA.

Pimentel, D. 1993. Habitat factors in new pest invasions. Pages 148-162 *in* K.C. Kim and B.A. McPheron,editors. Evolution of Insect Pests: Patterns of Variation. John Wiley & Sons, New York, New York, USA.

Price, P.W., 1997. Insect Ecology. John Wiley & Sons, New York. 874

RStudio Team. 2012. Rstudio: Integrated development environment for R. RStudio, Inc., Boston, MA. <http://www.rstudio.com/.>

Siegel, J.P., L.P.S Kuenen, B.S. Higbee, P. Noble, R. Gill, G.Y. Yokota, R. Krugner, and K.M. Daane. 2008. Postharvest survival of navel orangeworm assessed in pistachios. California Agriculture **62:** 30-35

Tikkanen, O.P., P. Niemelä, and J. Keränen. 2000. Growth and development of a generalist insect herbivore, *Operophtera brumata*, on original and alternative host plants. Oecologia **122**:529–536.

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

United States Department of Agriculture. 2008. Fruit and Nut Crop Production. National Agricultural Statistics Services. <http://www.nass.usda.gov/Statistics_by_State/California/Publications/California_Ag_Statistics/2008cas-frt.pdf>

United States Department of Agriculture. 2011. Sterile Insect Control of Invasive Pests, with a Focus on Moths. <http://www.ars.usda.gov/research/projects/projects.htm?accn_no=421179>