NELSON, PETER NICHOLAS. Conservation Biological Control in North Carolina Flue-cured Tobacco to Promote the Predator Jalysus wickhami Van Duzee (Hemiptera: Berytidae). (Under the direction of Drs. Hannah Burrack and Clyde Sorenson).

Flue-cured tobacco (Nicotiana tabacum L.) is the most valuable crop produced in North Carolina (NC). Insecticide applications for arthropod pest control represent a significant cost for producers and can reduce the abundance or activity of predatory and parasitic arthropods. Conservation biological control is an approach to promoting the activity of such natural enemies within integrated pest management programs, utilizing a variety of approaches to employ this natural control for pest management.

Jalysus wickhami Van Duzee (Hemiptera: Berytidae) is the most abundant predatory arthropod in NC flue-cured tobacco, consuming the tobacco-feeding Lepidopteran pests Heliothis virescens (Fabricis, 1777) (Lepidoptera: Noctuidae) and Manduca spp. (Lepidoptera: Sphingidae): Manduca sexta (Linnaeus, 1763) and Manduca quinquemaculata (Haworth, 1803). The goal of my dissertation research was to conserve J. wickhami and promote the biological control it provides by assessing the influence of insecticide use and evaluating arthropod-plant interactions that may benefit the predators and reduce plant damage.

Imidacloprid is widely used in NC flue-cured tobacco production and is applied as a plants systemic prophylactic against early season pests. J. wickhami are obligate plant feeders, and plant feeding may be a route of exposure to the xylem-mobile imidacloprid which could reduce biological control by J. wickhami. In the first chapter of my dissertation, I determined in laboratory experiments that exposure to tobacco tissues treated with imidacloprid via systemic or foliar applications was equally toxic to J.
wickhami, and that fifth instars were more tolerant of exposure to the insecticide than adults. In my second chapter, I performed field studies to assess the influence of imidaclprid on J. wickhami, H. virescens, and Manduca spp. population dynamics. Imidaclorpid applications did not reduce J. wickhami abundance nor increase pest abundance, indicating that the insecticide is compatible with the predators.

In my third and fourth chapters, I investigated how interactions between arthropods and tobacco plants might influence J. wickhami. Tobacco plants, which are covered in glandular trichomes, trap arthropods (carrion) on their surfaces that J. wickhami will readily feed upon. Scavenging by predators on this underappreciated form of plant-provided food reduces plant damage in wild flowers, and my goal was to assess whether a similar mutualism occurs in a domesticated plant under agronomic conditions. In my third chapter, I performed several field experiments in which I manipulated the abundance of arthropod carrion by adding frozen Drosophila spp. adults to tobacco plants. Results indicate that augmenting plants with carrion increased J. wickhami abundance and reduced damage to plants, although it does not reduce herbivore abundance.

The goal of my fourth chapter was to investigate potential mechanisms by which arthropod carrion on tobacco plants increases J. wickhami abundance. Through greenhouse and laboratory experiments using frozen Drosophila spp. adults as arthropod carrion, I determined that J. wickhami prefer to lay eggs on plants with carrion over plants without carrion. Arthropod carrion reduced egg cannibalism by J. wickhami adults, but not nymphs, and only under high densities.
Conservation Biological Control in North Carolina Flue-cured Tobacco, Focused on the Predator *Jalysus wickhami* Van Duzee (Hemiptera: Berytidae).

by

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DEDICATION

To Aloysius Krajnik.
BIOGRAPHY

Pete Nelson was born and raised in Alpena, Michigan, spending much of his free time outdoors. He received a B.S. of Entomology from Michigan State University in 2009 and subsequently began graduate studies directed by Dr. Mark Whalon, earning his M.S. in Entomology in 2014. Pete moved in Raleigh, North Carolina, in 2014 to study under Drs. Clyde Sorenson and Hannah Burrack.
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Chapter 1: Toxicity of imidacloprid to natural enemies of tobacco (*Nicotiana tabacum* L.) feeding caterpillar pests

1.1 Introduction

Neonicotinoid insecticides, predominantly imidacloprid, have been adopted for pest management in tobacco production over the last 20 years (Toennisson and Burrack, 2017). Greenhouse applications of imidacloprid provide prophylactic protection against early season pests including thrips (*Frankliniella fusca* Hinds and *Frankliniella occidentalis* Pergande) (Thysanoptera: Thripidae) (Groves et al., 2001), green peach aphid (*Myzus persicae* Sulzer) (Hemiptera: Aphididae), and flea beetles (*Epitrix hirtipennis* Melsheimer) (Coleoptera: Chrysomelidae) (Burrack and Toennisson, 2018; Semtner et al., 1999). Imidacloprid does not control tobacco-feeding caterpillar pests, including *Heliothis virescens* (Fabricus, 1777) (Lepidoptera: Noctuidae) and *Manduca* spp. (Lepidoptera: Sphingidae) (Lagadic et al., 1993) but can have deleterious effects on natural enemies (Mizell and Sconyers, 1992; Stapel et al., 2000).

*Campoletis sonorensis* (Cameron) (Hymenoptera: Ichneumonidae) and *Toxoneuron nigriceps* (Viereck) (Hymenoptera: Braconidae) are solitary endoparasitoids of *H. virescens* (Danks et al., 1979; Rabb et al., 1955). *C. sonorensis* oviposits primarily in young *H. virescens* (first through third instars) and prefers third instars (Gunasena et al., 1990). *T. nigriceps* parasitizes fifth and younger instars (Lewis and Vinson, 1971). Both species may provide significant control of *H. virescens* and together can parasitize up to 90% of larvae in tobacco fields (Chamberlin and Tenhet, 1926; Neunzig, 1969; Vinson, 1972; Vinson and Barras, 1970).
Jalysus wickhami Van Duzee (Hemiptera: Berytidae) is a voracious predator of caterpillar eggs, consuming up to 25 H. virescens eggs per day during their ~95 d lifespan (Elsey and Stinner, 1971; Jackson and Kester, 1996). In addition to animal prey, plant feeding is essential to the long-term survival of J. wickhami (Jackson and Kester, 1996) and represents a potential route of exposure to systemically applied insecticides (Jackson and Lam, 1989).

Insecticide use, including systemically-applied imidacloprid, has been linked to reductions in H. virescens parasitoid and predator abundance. Immature C. sonorensis and T. nigriceps may be exposed to systemically applied imidacloprid and its metabolites in the hemocoel of H. virescens that have fed on treated plants, with exposure causing lethal and sublethal effects on T. nigriceps (Taylor et al., 2015). Commercial tobacco fields receiving more insecticide applications, including imidacloprid, contained fewer J. wickhami (Slone and Burrack, 2016). The magnitude of exposure of J. wickhami to imidacloprid through plant feeding and toxicity has not been established.

Natural enemy susceptibility to insecticides may differ between life stages (Bartlett, 1964), sexes (Croft and Brown, 1975), geographic location (Croft and Jeppson, 1970), and route of exposure (Smith and Krischik, 1999; Stapel et al., 2000). We sought to determine the toxicity of imidacloprid to C. sonorensis, T. nigriceps, and J. wickhami through a series of laboratory assays. Potential sex-based differences in imidacloprid toxicity to C. sonorensis and T. nigriceps were examined through topical exposure. Because J. wickhami may be exposed topically through foliar treatments or via plant feeding, we simulated both these exposure routes in experiments on field
collected adults from two presumably isolated populations in North Carolina, and on colony-reared nymphs.

1.2 Materials and Methods

1.2.1 Insect collection and culture

Insects used in these studies were originally collected from tobacco fields at the North Carolina State University Lower Coastal Plain Research Station (Lenoir County, North Carolina, 35.297404, -77.574259) and Upper Coastal Plain Research Station (Edgecombe County, North Carolina, 35.894264, -77.680346). Colonies of *C. sonorensis* and *T. nigriceps* were established from field-collected females and were cultured in laboratory-reared *H. virescens*. We held subsequent parasitoids at a ratio of 1:1 female: male in 30x30x30cm BugDorm-1 containers (Megaview Science, Taichung, Taiwan), where they were fed honey, provided water ad libitum, and maintained at 25.0°C, 70%RH, and 14:10 L:D. We used parasitoids within five days of adult emergence in all experiments. We held adult *J. wickhami* from each location in separate cages under similar temperature and light conditions with a potted tobacco plant, var. K326. Nymphs were generated from a single colony established from adults collected from both locations. *J. wickhami* were fed *M. sexta* and *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae) eggs and dead *Drosophila* spp. (Diptera: Drosophilidae) flies. We held field-collected adults for five days prior to experimental use and used fifth instars that had molted within 24 hrs.
1.2.2 *Campoletis sonorensis* and *Toxoneuron nigriceps* Bioassays

Technical grade ($\geq 98.0\%$ purity) imidacloprid (Bayer Crop Science, Monheim, Germany) was used in wasp bioassays to precisely administer small doses of the material. We prepared serial dilutions of imidacloprid in acetone immediately prior to use. Using a Hamilton repeating syringe (Hamilton Company, Reno, Nevada), we applied 0.5 μl (*C. sonorensis*) and 1.0 μl (*T. nigriceps*) doses of four serial dilutions of imidacloprid and an acetone control to the pronotum of adult wasps. Prior to treatment, we anesthetized parasitoids with CO$_2$ and measured their mass. After allowing pesticide solutions to evaporate, we transferred individual wasps to 30 ml clear portion containers (Dart Container Corporation, Mason, MI) and assessed mortality at 48 hr. We replicated assays at least four times using separate generations and a minimum of 10 insects per dose.

1.2.3 *Jalysus wickhami* Bioassays

1.2.3.1 Contact Exposure

Admire Pro (42.8% imidacloprid) (Bayer Crop Science, Monheim, Germany) was used in *J. wickhami* assays to simulate field exposure via plant systemic uptake and foliar application. We prepared serial dilutions of Admire Pro in DI H$_2$O immediately prior to use. We dipped 2.7 cm discs excised from untreated leaves of greenhouse grown tobacco var. K326 into four serial dilutions of Admire Pro and a water control. After they were dry, we placed the treated discs in 30 ml portion containers on a 2.0 mm layer of water agar (Alfa Aesar, Ward Hill, MA) to maintain leaf turgidity. We added a single *J. wickhami* to each arena, testing equal numbers of male and female adults collected
from both locations, or colony-reared nymphs. We held arenas under the previously
described environmental conditions and assessed *J. wickhami* mortality at 96 hr. We
replicated the assay five times with a minimum of eight insects per dose.

1.2.3.2 Plant-Systemic Exposure

Following methods described by Prabhaker *et al.* (2006), we treated 31 cm long leaves
of greenhouse grown tobacco var. K326 systemically in serial dilutions of Admire Pro in
DI H$_2$O prepared immediately prior to use. We suspended leaf petioles in 250 ml of
three serial dilutions and a water control for 24 hr and subsequently excised 2.7 cm
diameter discs from the leaves. We placed the discs in the previously described *J.
wickhami* arenas and singly added equal numbers of field collected adult males and
females or nymphs. We held arenas under the same environmental conditions,
assessed mortality at the same interval followed in the contact assay, and replicated the
assay five times with a minimum of eight insects per dose.

1.2.4 Statistical Analyses

We performed all statistical analyses in RStudio Version 10.1.136 (RStudio Team
2016). Dose and concentration-response curves were generated using a two-parameter
log-logistic model to estimate effective doses (LC$_{50}$ or LD$_{50}$) and 95% confidence
intervals using the DRC package (Ritz et al., 2005). Effective doses and confidence
intervals were estimated separately by sex for parasitoids, and by route of exposure per
life stage and population for *J. wickhami*. Significant differences between LC$_{50}$ and LD$_{50}$
estimates were determined by comparing 95% confidence interval ranges.
1.3 Results

1.3.1 *Campoletis sonorensis* and *Toxoneuron nigriceps* Bioassays

The LD$_{50}$ values for imidacloprid were estimated to be 0.000992 (95% confidence intervals: 0.000742 - 0.00134) μg/mg insect and 0.000832 (95% CI: 0.000697 - 0.000994) μg/mg insect for female and male *C. sonorensis*, respectively, and these were not significantly different (Table 1.1). The LD$_{50}$ values for female and male *T. nigriceps* were estimated to be 0.0506 (95% CI 0.0383 - 0.0669) μg/mg insect and 0.0508 (95% CI 0.0421 - 0.0614) μg/mg insect, respectively, and these were not significantly different (Table 1.1). *C. sonorensis* had significantly lower LD$_{50}$ values than *T. nigriceps*, as confidence intervals did not overlap (Table 1.1).

1.3.2 *Jalysus wickhami* Bioassays

Estimated LC$_{50}$ values for *J. wickhami* exposed to imidacloprid via contact was 0.231 (95% CI: 0.169 – 0.315) μL/mL and 0.226 (95% CI: 0.168 – 0.304) μL/mL for populations from Kinston and Rocky Mount, respectively (Table 1.2). Estimated LC$_{50}$ values for plant-systemically exposed imidacloprid for populations from Kinston and Rocky Mount were 0.150 (95% CI: 0.0955 – 0.235) μL/mL and 0.0999 (95% CI: 0.047 – 0.211) μL/mL, respectively (Table 1.2). Adult LC$_{50}$ estimates did not significantly differ between populations or routes of exposure. *J. wickhami* nymphs were far more tolerant to imidacloprid than adults. Nymph mortality following both contact and plant systemic exposure to imidacloprid did not reach 50% at rates a full order of magnitude greater than tested against adults, therefore LC$_{50}$ values were not estimated (Table 1.2).
1.4 Discussion

Our results indicate that imidacloprid is toxic to *Campeletis sonorensis*, *Toxoneuron nigriceps*, and *Jalysus wickhami* adults, but is relatively benign to *J. wickhami* nymphs. *C. sonorensis* were less tolerant to imidacloprid exposure than *T. nigriceps*, and susceptibility did not differ between sexes of either species (Table 1.1). Imidacloprid toxicity to *J. wickhami* adults did not differ between geographically distinct populations or routes of exposure (Table 1.2).

Toxicity of imidacloprid towards several ichneumonid parasitoids has been documented through multiple routes of exposure including contact (Hill and Foster, 2000), residual exposure (Morales et al., 2005), topical, and host diet (Medina et al., 2007). Our results indicate that imidacloprid is toxic to adult *C. sonorensis* (Table 1.1) although previous work by our group indicated that larvae are not affected when exposed to imidacloprid in host bodies (Taylor et al., 2015). We also found that *T. nigriceps* is susceptible to topical imidacloprid applications (Table 1.1); imidacloprid toxicity has also been documented in other Braconid parasitoids of economically important pests (Adán et al., 2011; Liburd et al., 2004; Liu et al., 2015; Preetha et al., 2010). We documented acute toxicity to adults of both parasitoid species but did not assess sublethal effects. Sublethal effects, including host searching ability (Tran et al., 2004), longevity (Sohrabi et al., 2012), and reproductive fitness (Paine et al., 2011) of Hymenopteran parasitoids can be adversely impacted by imidacloprid, and future study of these organisms should focus on these additional effects.

The estimated LD$_{50}$ values for male and female *C. sonorensis* were significantly lower than estimated LD$_{50}$ values for *T. nigriceps* (Table 1.1). Hymenopteran parasitoid
susceptibility to insecticides may vary due to differences in mass, with increased mass associated with higher tolerance to insecticides (Lasota and Kok, 1986; Rathman et al., 1992; Scott and Rutz, 1988). *C. sonorensis* females and males are 51 and 61 times more susceptible to imidacloprid than *T. nigriceps* and on average weigh 5.5 and 6.9 times less than *T. nigriceps*, respectively (Table 1.1).

*J. wickhami* adults died when exposed to imidacloprid through contact (foliar dips) or systemically through plants (Table 1.2). Importantly, our study confirms that adult *J. wickhami* can be exposed to, and may be negatively affected by, imidacloprid when plants are systemically treated with the insecticide. Exposure to other systemic insecticides through plant feeding has been established for *J. wickhami* (Jackson and Lam, 1989) and other important Hemipteran predators including *Orius insidiosus* Say, 1832 (Hemiptera: Anthocoridae), *Geocoris punctipes* (Say) (Hemiptera: Geocoridae) (Prabhaker et al., 2011), and *Podisus nigriceps* (Dallas) (Hemiptera: Pentatomidae) (Torres and Ruberson, 2004).

Arthropod susceptibility to insecticides may differ between geographically distinct populations due to a range of biotic and abiotic factors, including history of insecticide use and local pest management (Croft and Brown, 1975). However, *J. wickhami* susceptibility to imidacloprid was not significantly different for contact and systemic exposure between populations from Kinston and Rocky Mount, NC (Table 1.2). Both populations were collected from North Carolina State University research stations, and it is plausible to expect that management practices, including pest management, may have been relative similar.
Nymph mortality was never greater than 50% when we exposed nymphs to imidacloprid through contact or systemic routes (Table 1.2) despite increasing rates a full order of magnitude higher than those used against adult *J. wickhami*. Immature stages of other hemipteran species have been reported to be more tolerant to insecticides than adults, including *Blissus occiduus* Barber (Hemiptera: Blissidae) (Stamm et al., 2011), *Lygus lineolaris* (Palisot de Beauvois) (Heteroptera: Miridae) (Hollingsworth et al., 1997), and *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae) (Allen et al., 2010). The mechanism(s) for this tolerance is unknown and may include increased activity of detoxifying enzymes compared to adults, including gluthathion transferase (Kostaropoulos et al., 1996; Tiwari et al., 2011) and cytochrome P450 (Ranasinghe et al., 1997; Tiwari et al., 2011). Regardless of mechanism, nymphal tolerance to imidacloprid may aid in the persistence of *J. wickhami* in crop fields after application of the material.

In this study, we have documented that imidacloprid is harmful to a complex of natural enemies of caterpillar pests and that toxicity varies between species, by route of exposure, and by life stage. Determining the direct, lethal activity of insecticides is the first step in developing conservation biological control strategies, and our results inform such efforts in tobacco as well as crops such as cotton, soy, and peanut. We also provide evidence that susceptibility to imidacloprid may not be straight-forward. Altering application methods and refinements to the timing of insecticides can minimize non-target effects, reduce natural enemy disruption, and improve pest control (Bartlett, 1964; Gentz et al., 2010; Mizell and Sconyers, 1992; Ripper et al., 1951; Ruberson et al., 1998).
1.5 Tables

Table 1.1 Summary data of toxicity (48 h) data computed from log-logistic models for *Campoletis sonorensis* and *Toxoneuron nigriceps* exposure to imidacloprid. LD values followed by different letters within a column are significantly different based on the criterion of non-overlap of 95% confidence intervals.

<table>
<thead>
<tr>
<th>Species</th>
<th>Sex</th>
<th>n</th>
<th>Mean weight (mg)</th>
<th>LD₅₀ (μg/mg insect)</th>
<th>95% Confidence Interval (μg/mg insect)</th>
<th>Slope (± SE)</th>
<th>χ² (df)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Campoletis sonorensis</em></td>
<td>Female</td>
<td>299</td>
<td>3.169</td>
<td>0.000992b</td>
<td>0.000742 – 0.00133</td>
<td>1.12 (± 0.201)</td>
<td>1.53 (2)</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>443</td>
<td>2.190</td>
<td>0.00083b</td>
<td>0.000697 – 0.000994</td>
<td>1.32 (± 0.167)</td>
<td>1.53 (2)</td>
</tr>
<tr>
<td><em>Toxoneuron nigriceps</em></td>
<td>Female</td>
<td>87</td>
<td>17.459</td>
<td>0.0506a</td>
<td>0.0383 – 0.0669</td>
<td>2.45 (± 0.920)</td>
<td>0.0722 (2)</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>68</td>
<td>15.216</td>
<td>0.0508a</td>
<td>0.0421 – 0.0614</td>
<td>3.35 (± 1.04)</td>
<td>0.864 (2)</td>
</tr>
</tbody>
</table>
Table 1.2 Summary data of toxicity (96 h) data computed from log-logistic models for *Jalysus wickhami* exposure to imidacloprid. Brackets contain μL imidacloprid/mL conversion based on 42.8% imidacloprid in Admire Pro.

<table>
<thead>
<tr>
<th>Route of Exposure</th>
<th>Lifestage</th>
<th>Source</th>
<th>n</th>
<th>LC$_{50}$ (μL Admire Pro/mL)*</th>
<th>95% Confidence Interval (μL Admire Pro/mL)*</th>
<th>Slope (SE)</th>
<th>χ² (df)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contact</td>
<td>Adult</td>
<td>Kinston</td>
<td>200</td>
<td>0.231 [0.0989]</td>
<td>0.169 – 0.315 [0.0723 – 0.135]</td>
<td>1.10 (0.235)</td>
<td>1.69 (2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rocky Mount</td>
<td>200</td>
<td>0.226 [0.0967]</td>
<td>0.168 – 0.304 [0.719 – 0.130]</td>
<td>1.16 (0.239)</td>
<td>1.25 (2)</td>
</tr>
<tr>
<td>Nymph</td>
<td></td>
<td>Lab Colony</td>
<td>125</td>
<td>&gt; 3.00 [1.28]</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Systemic</td>
<td>Adult</td>
<td>Kinston</td>
<td>160</td>
<td>0.150 [0.0642]</td>
<td>0.0955 – 0.235 [0.0408 – 0.101]</td>
<td>0.814 (0.336)</td>
<td>1.38 (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rocky Mount</td>
<td>160</td>
<td>0.0999 [0.0428]</td>
<td>0.0474 – 0.211 [0.0203 – 0.0903]</td>
<td>0.593 (0.332)</td>
<td>0.302 (1)</td>
</tr>
<tr>
<td>Nymph</td>
<td></td>
<td>Lab Colony</td>
<td>125</td>
<td>&gt; 4.50 [1.93]</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Chapter 2: Assessing the compatibility of imidacloprid with conservation of the predator Jalysus wickhami Van Duzee (Hemiptera: Berytidae) in flue-cured tobacco (Nicotiana tabacum L.)

2.1 Introduction

Flue-cured tobacco (Nicotiana tabacum L.) is the most valuable crop produced in North Carolina (NC), grown on over 67,000 ha with a total value of ca. $647 million USD in 2016 (USDA NASS, 2018). Insecticides represent a significant cost for tobacco growers (Bullen and Fisher, 2018), and are applied to the soil as prophylactic systemic treatments targeting early-season pests and foliar treatments targeting mid and late season pests (Burrack and Toennisson, 2018). Recent research has revealed that unnecessary insecticide applications are often made by tobacco growers when treatment thresholds are not observed which increases pest management costs and reduces the abundance of natural enemies (Slone and Burrack, 2016). These unnecessary applications may, therefore, potentially increase tobacco pest populations.

Tobacco has a relatively small group of associated herbivores (Burrack and Toennisson, 2018) and natural enemies (Jackson et al., 1989). Jalysus wickhami Van Duzee (Hemiptera: Berytidae) is the most abundant predatory arthropod in NC flue-cured tobacco fields and feeds on the pests Myzus persicae (Sulzer, 1776) (Hemiptera: Aphididae), Heliothis virescens (Fabricius, 1777) (Lepidoptera: Noctuidae), and Manduca spp. (Lepidoptera: Sphingidae): Manduca sexta (Linnaeus, 1763) and Manduca quinquemaculata (Haworth, 1803) (Elsey and Stinner, 1971; Lawson, 1959). J. wickhami can consume up to 1000 Manduca spp. eggs and 2000 H. virescens eggs during an 80-day lifetime (Elsey and Stinner, 1971; Jackson and Kester, 1996),
potentially providing a massive amount of biological control. The basic biology of *J. wickhami* has been studied (Elsey, 1973; Kent D Elsey, 1974a, 1974b; Elsey and Stinner, 1971), but there is a paucity of information regarding its compatibility with contemporary tobacco pest management practices and interactions with other arthropods.

Conservation biological control, the practice of enhancing reproduction, survival, and efficacy of natural enemies (DeBach, 1964), has the potential to contribute to management of arthropod pests within the framework integrated pest management programs (Stern et al., 1959). Conservation biological control (CBC) can be implemented by modifying pesticide use patterns or manipulating the environment to favor natural enemies (Eilenberg et al., 2001). For CBC to be effective in improving pest management, knowledge of the life-histories of natural enemies of interest is required, including biology and ecology of the organisms within a select agro-ecosystem (Letourneau, 1998). Similarly, information detailing the effects of crop management tactics, including insecticide use, on natural enemies is required for management decisions promoting biological control (Barbosa, 1998; Croft, 1990).

Systemic applications of neonicotinoid insecticides have been widely adopted by flue-cured tobacco growers for early-season pest management over the last 20 years (Toennisson and Burrack, 2017). Applied as a greenhouse tray drench prior to transplant, imidacloprid is frequently used as a prophylactic against *M. persicae* (Merchán and Burrack, 2017), *Epitrix hirtipennis* (Melsheimer) (Coleoptera: Chrysomelidae) (Burrack and Toennison, 2018), and *Frankliniella fusca* (Hinds) (Thysanoptera: Thripidae), the primary vector of tomato spotted wilt virus in NC (Groves
et al., 2001). The xylem-mobile insecticide is translocated throughout the plant, and target pests are exposed when they consume the treated plant tissue and sap. *J. wickhami* also facultatively feed on tobacco plants in addition to insect prey, taking drinks of sap. *J. wickhami* may be exposed to carbamate (aldicarb, aldoxycarb) and organophosphate (disulfoton, fostheitane) insecticides when applied systemically (Semtner, 1979), but these are no longer used in tobacco production and similar exposure to imidacloprid has not been confirmed.

Understanding the ecology and biology of natural enemies, as well as their interaction and compatibility with pest management tactics, is key when incorporating conservation biological control strategies into integrated pest management programs. We conducted small plot experiments over two years in two locations with the goal of determining whether a common pest management tactic in North Carolina flue-cured tobacco production, systemically applied imidacloprid, was compatible with *J. wickhami*. We measured the abundance of *J. wickhami* and its prey, *H. virescens*, and *Manduca* spp., to assess possible effects of imidacloprid on *J. wickhami* biological control. We also evaluated the potential for other arthropods to explain *J. wickhami* abundance.

### 2.2 Materials and methods

**2.2.1 Experimental design**

We conducted field experiments in 2015 and 2016 at the North Carolina Department of Agriculture and Consumer Services Lower Coastal Plain Research Station (Lenoir County, North Carolina 35.297404, -77.574259) and Upper Coastal Plain Research Station (Edgecombe County, North Carolina, 35.894264, -77.680346). Organically
produced tobacco seedlings (var. NC 196) were used to ensure plant material was free of imidacloprid residues. Plants were left untreated or treated with Admire Pro (42.8% imidacloprid) (Bayer CropScience, Research Triangle Park, North Carolina) at a rate of 17.76 ml/1000 plants using one of two systemic application methods: (1) a greenhouse tray drench less than two days prior to transplant, immediately rinsed into growing media; and (2) a soil water drench applied at transplant. Plots consisted of eight 15.24 m long rows with 25 plants per row, spaced 1.22 m apart, arranged in a randomized complete block design with four replicates per treatment.

We transplanted tobacco seedlings on 4 May 2015 (Lenoir County), 8 May 2015 (Edgecombe County), 25 April 2016 (Lenoir County), and 27 April 2016 (Edgecombe County) and followed standard agronomic practices for flue-cured tobacco production in North Carolina (Brown et al. 2016), with the exception that no insecticides, aside from systemic imidacloprid treatments, were applied.

2.2.2 Insect assessment

We surveyed plots weekly for focal insects (J. wickhami adults and nymphs, H. virescens larvae, and Manduca spp. larvae), and any other predatory arthropods by inspecting entire tobacco plants. We inspected all plants in rows four and five early in the season (15 May to 27 June 2015, 23 May to 22 June 2016), when plants were small and insect abundance was low. Thereafter, all plants in row five were inspected through the first week of August (1 August 2015, 1 August 2016), at which point North Carolina flue-cured tobacco harvest is underway. We deployed yellow sticky traps in 2015 to monitor J. wickhami, but they proved to be ineffective (see Appendix A).
2.2.3 Statistical analyses

We standardized all insect counts (focal insects and other predatory arthropods) from plant inspections by row to account for the different number of rows inspected earlier (two) and later (one) in the season. Season-long abundance and weekly means were log-transformed (log+1) for analysis, but non-transformed data are presented for clarity. All statistical analyses were performed in SAS v. 9.4 (SAS Institute, Cary NC). Degrees of freedom for all analysis of variance tests were calculated using the procedure described by Kenward and Roger (1997).

The influence of imidacloprid treatments (tray drench, transplant drench, untreated control) on focal insect season-long abundance was analyzed independently using a linear mixed model (PROC MIXED). For each focal species, a full model was developed with the season-long abundance of the insect as the response variable, insecticide treatment as the fixed variable, and with the following random effects: year, location, the interaction of year and location, and block nested within location.

To assess the influence of insecticide treatment on focal insect abundance throughout the growing season, we used a linear mixed model (PROC MIXED) with repeated measures. We fit the following model for each focal species: weekly mean insect abundance as the response variable, insecticide treatment, week after treatment, and their interactions as fixed variables, and the following random variables: year, location, the interaction of year and location, and block nested within location. The repeated statement utilized compound symmetry structure and the subject was the interaction of the plot by location by year.
We performed stepwise regression (PROC GLMSELECT) to determine the influence of other arthropods on *J. wickhami* season long abundance. We included the following predictor variables: “chewing” predators, the sum of Chrysopidae (Neuroptera) and Coccinellidae (Coleoptera); “piercing-sucking” predators, the sum of hemipteran families Anthocoridae, Geocoridae, Nabidae, and Reduviidae; Oxyopidae; *H. virescens*; *Manduca* spp.. The variables were assessed with full interactions and *J. wickhami* season long abundance was log-transformed. Stepwise model selection was use and effect inclusion in the model ended when none of the effects outside of the model had significant *F*-tests (Cohen, 2006). The default select and stop criterion, based on Schwarz Bayesian information criterion, was used for the model, and the model with the lowest Akaike’s information criterion values was chosen (SAS Institute, 2012). We quantified the relationship between predictor variables and *J. wickhami* abundance chosen by stepwise regression through linear regression using PROC REG. We calculated variance inflation factor (VIF) scores for each term to ensure our model was not confounded by multicollinearity.

### 2.3 Results

During 2015 and 2016, we counted a total of 3885 *J. wickhami*, which was 80.4% and 82.8%, respectively, of all predatory arthropods surveyed (Table 2.1). Other predatory taxa surveyed included members of the families Chrysopidae (Neuroptera), Coccinellidae (Coleoptera), Anthocoridae (Hemiptera), Geocoridae (Hemiptera), Nabidae (Hemiptera), Reduviidae (Hemiptera) and Oxyopidae (Aranae), (Table 2.1).
We counted a total of 1599 *H. virescens* and 1469 *Manduca* spp. larvae during 2015 and 2016.

There were no differences between imidacloprid treatments in the season-long abundance of *J. wickhami* \( (F=1.19; \text{df}= 2,35.8; P=0.3156) \), *H. virescens* \( (F=0.13; \text{df}= 2, 35.7; P=0.8798) \), and *Manduca* spp. \( (F=2.03; \text{df}= 2,36; P=0.1464) \) (Table 2.2). Abundance of all focal insects varied significantly over time (*J. wickhami*: \( F=146.96; \text{df}=11, 531; P<0.0001 \), *H. virescens*: \( F=63.07; \text{df}= 11, 531; P<0.0001 \), *Manduca* spp.: \( F=99.34; \text{df}= 11, 531; P<0.0001 \)). *J. wickhami* abundance was highest 10 through 12 weeks after treatment (WAT), while *H. virescens* abundance peaked eight WAT and *Manduca* spp. abundance was greatest 11 WAT (Figure 2.1).

The model selection process for stepwise regression was stopped at the third step with the final model including *H. virescens*, *Manduca* spp., and “chewing” predators \( (\text{AIC} = -73.63994) \). The linear regression model including *H. virescens*, *Manduca* spp., and “chewing” predators was significant \( (F=14.77, \text{df}= 3,44; P < 0.0001) \), explaining 50.2% of *J. wickhami* season long abundance variance. All predictor variables were positively correlated *J. wickhami*, with *Manduca* spp. explaining the greatest amount of variability, followed by “chewing” predators and *H. virescens* explaining the least amount (Table 2.3).

### 2.4 Discussion

Modifying insecticide use to conserve natural enemies is one approach to conservation biological control (Newsom et al., 1976), and thus it is necessary to understand how insecticide use affects natural enemies. Systemic insecticide applications generally
reduce the risk of exposure to non-target organisms compared to other application methods, but natural enemies feeding on pollen and nectar may ingest insecticides translocated to those tissues (Cloyd and Bethke, 2011; Smith and Krischik, 1999). *J. wickhami* must consume plant sap for long-term survival (Jackson and Kester, 1996) and therefore may be exposed to xylem-mobile imidacloprid (Jeschke et al., 2011).

Laboratory research demonstrates that exposure to tobacco leaves treated systemically with imidacloprid reduces *J. wickhami* survival (P. N. Nelson, 2018). This study incorporated the two most commonly used application methods for imidacloprid in tobacco: a greenhouse tray drench prior to transplant (most common) and an in furrow application (less common). Foliar imidacloprid applications in tobacco are extremely rare. *J. wickhami* abundance in either imidacloprid treatment did not differ from untreated controls (Table 2.2) nor did *H. virescens* and *Manduca* spp. larval abundance, indicating that the method of systemic application does not influence the potential for exposure to those insects.

*J. wickhami* populations did not increase appreciably until seven weeks after transplant, at which point imidacloprid titers in plants may be reduced to concentrations incapable of producing acute toxicity. Likewise, imidacloprid efficacy against *Myzus persicae* eventually decreases post-transplant (Semtner and Srigiriraju, 2005), as titers of imidacloprid probably decrease throughout the season as observed in studies on other crops (Huseth et al., 2014).

Early-season imidacloprid applications are not used to control lepidopteran tobacco pests due to limited caterpillars toxicity (Lagadic et al., 1993; Wink and Theile, 2002). Therefore, any differences in *H. virescens* or *Manduca* spp. abundance between
treatments is unlikely to be the direct result of systemically-applied imidacloprid. We found the abundance of *H. virescens* and *Manduca* spp. did not differ between insecticide treatments, indicating that other factors regulating pest caterpillar populations were neither unaffected by imidacloprid. As no other pest management tactics were used in our experiments, it is reasonable to conclude that biological control provided by natural enemies was not affected. Visual inspections of plants in 2015 and 2016 confirmed that *J. wickhami* is the most abundant predator in North Carolina tobacco agro-ecosystems, therefore we infer that the predator’s role in regulating *H. virescens* and *Manduca* spp. was not influenced by imidacloprid.

Predator populations may respond to variations in prey abundance, typically exhibiting positive numerical responses through population growth as prey abundance increases (Solomon, 1949). Season long abundance of *H. virescens* and *Manduca* spp. were two effects retained in a model resulting from stepwise multivariate regression, and our results indicate that *J. wickhami* abundance was positively correlated with the abundance of *H. virescens* and *Manduca* spp. larvae (Table 2.3). It is important to note that both variables were derived from larval counts, while *J. wickhami* typically consumes eggs (Elsey and Stinner, 1971). However, we can assume that most lepidopteran larva surveyed originated from eggs laid within the same plots, thus increasing caterpillar abundance should correlate with increased *J. wickhami* prey abundance.

Generalist predators (Holling, 1966; Luck, 1984) are thought to exhibit a weak functional response to prey densities, but alternative food resources can help overcome this by causing a positive numerical response and increasing the number of predators
consuming prey (Eubanks et al., 2000; Eubanks and Denno, 1999). Numerical responses are driven by predator aggregation (Döbel and Denno, 1994) and reproduction (Dixon and Guo, 1993). Our results indicate that *J. wickhami* respond positively to the presence of prey and that *Manduca* spp. abundance explained more variance than *H. virescens* abundance (Table 2.3). This may be due to the fact that peak *J. wickhami* abundance coincided with peak *Manduca* spp. abundance (Figure 2.1). Future research should investigate the potential of providing alternative foods earlier in the season to increase *J. wickhami* abundance.

Interactions between predators may be antagonistic, additive, or synergistic, and their outcomes can have significant biological control implications. *J. wickhami* abundance was also positively correlated with “chewing” predators (Table 2.3). Investigating the interaction between *J. wickhami* and “chewing” predators was not one of our explicit research goals and future efforts could clarify this by assessing whether the predators respond to prey abundance in manner similar to *J. wickhami*, the potential for intra-guild predation (Polis et al., 1989) or synergistic interactions between the predators (Losey and Denno, 1998a, 1998b).

Our efforts presented herein were partially focused on determining the effects of early-season systemic imidacloprid applications on *J. wickhami* and predation on *Heliotis virescens* and *Manduca* spp. To accomplish this, systemic imidacloprid treatments were the only pest management tactic employed and thus our results are not representative of North Carolina commercial flue-cured tobacco production. Preventing tobacco caterpillar pests from surpassing economic thresholds typically requires 1-3 foliar insecticide applications per year which are linked to declines in *J. wickhami*
abundance (Slone and Burrack, 2016). Our plots received no foliar insecticide applications and therefore the abundance of caterpillar and *J. wickhami* were higher than those typical in commercial tobacco fields. Future efforts assessing the toxicity of commonly used foliar insecticides to *J. wickhami* could be utilized in developing recommendations for insecticides compatible with the predators.

Plant-feeding can enhance predator arthropod life-history traits or may be required for development and survival, as in the case of *J. wickhami*. Systemically applied insecticides have the potential to cause effects detrimental to the predator, but our results indicate that imidacloprid applications are compatible with *J. wickhami*. As such, this research should function as a starting point for improving conservation biological control with *J. wickhami* in contemporary flue-cured tobacco production, as detailed knowledge of the life systems of natural enemies is required for successful implementation.
### 2.6 Tables

Table 2.1 Summary of North Carolina flue-cured tobacco predatory arthropods from plant inspections in 2015 and 2016.

<table>
<thead>
<tr>
<th>Predator Taxa</th>
<th>2015</th>
<th>2016</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anthocoridae (Hemiptera)</td>
<td>3 (0.0026)</td>
<td>3 (0.00084)</td>
</tr>
<tr>
<td><em>Jalysus wickhami</em> (Berytidae: Hemiptera)</td>
<td>939 (0.80)</td>
<td>2946 (0.83)</td>
</tr>
<tr>
<td>Chrsyopidae (Neuroptera)</td>
<td>3 (0.0026)</td>
<td>18 (0.0051)</td>
</tr>
<tr>
<td>Coccinellidae (Coleoptera)</td>
<td>20 (0.017)</td>
<td>357 (0.10)</td>
</tr>
<tr>
<td>Geocoridae (Hemiptera)</td>
<td>169 (0.14)</td>
<td>171 (0.048)</td>
</tr>
<tr>
<td>Nabidae (Hemiptera)</td>
<td>7 (0.0060)</td>
<td>27 (0.0076)</td>
</tr>
<tr>
<td>Oxyopidae (Aranae)</td>
<td>9 (0.0077)</td>
<td>25 (0.0070)</td>
</tr>
<tr>
<td>Reduviidae (Hemiptera)</td>
<td>15 (0.013)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><strong>Sum</strong></td>
<td><strong>1168</strong></td>
<td><strong>3557</strong></td>
</tr>
</tbody>
</table>
Table 2.2 Combined 2015 and 2016 season-long focal insect abundance in response to imidacloprid treatments. Means within rows were not significantly different from each other (P > 0.05).

<table>
<thead>
<tr>
<th>Insect</th>
<th>Imidacloprid Treatment</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untreated Control</td>
<td>Tray Drench</td>
<td>Transplant Water</td>
<td></td>
</tr>
<tr>
<td>Jalysus wickhami</td>
<td>107.8 ± 28.1</td>
<td>61.1 ± 9.1</td>
<td>69.2 ± 10.6</td>
<td></td>
</tr>
<tr>
<td>Heliothis virescens</td>
<td>30.3 ± 3.5</td>
<td>30.0 ± 3.1</td>
<td>31.1 ± 4.1</td>
<td></td>
</tr>
<tr>
<td>Manduca spp.</td>
<td>33.1 ± 9.3</td>
<td>22.4 ± 4.5</td>
<td>34.0 ± 11.3</td>
<td></td>
</tr>
</tbody>
</table>
Table 2.3 Results of linear regression evaluating arthropod influence on *Jalysus wickhami* abundance. VIF: variance inflation factor.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Partial R²</th>
<th>Estimate (SE)</th>
<th>t</th>
<th>P</th>
<th>VIF</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Manduca</em> spp. season-long abundance</td>
<td>0.319</td>
<td>0.0051 (0.0012)</td>
<td>4.41</td>
<td>&lt;0.0001</td>
<td>1.04727</td>
</tr>
<tr>
<td>“chewing” predators season-long abundance</td>
<td>0.132</td>
<td>0.0053 (0.0020)</td>
<td>2.62</td>
<td>0.0119</td>
<td>1.14952</td>
</tr>
<tr>
<td><em>H. virescens</em> season-long abundance</td>
<td>0.051</td>
<td>0.062 (0.030)</td>
<td>2.11</td>
<td>0.0403</td>
<td>1.10742</td>
</tr>
</tbody>
</table>
2.7 Figures

Figure 2.1 Combined 2015 and 2016 Jalysus wickhami (A), Heliothis virescens (B), and Manduca spp. (C) weekly abundance.
Chapter 3: Arthropod entrapment increases specialist predators on a sticky crop and reduces damage

3.1 Introduction

Plant defenses against herbivores fall into broad two categories: direct or indirect. Both defensive strategies have been exploited for agricultural pest management; direct defense is the foundation for host plant resistance (Painter, 1951), while indirect defense is intrinsic to biological control (Price et al., 1980). Host plant resistance and biological control are not exclusive in their effects on pests, and their interactions may be disruptive, complementary, or synergistic (Bottrell et al., 1998; Cortesero et al., 2000). For example, manipulating *Pisum sativum* L. epicuticular waxes increases resistance against *Acyrthosiphon pisum* (Harris) (Hemiptera: Aphididae) (Eigenbrode et al., 1997), improves predator effectiveness (Eigenbrode et al., 1998), but also reduces predator oviposition (Rutledge et al., 2003). Understanding interactions between plant resistance and biological control is crucial to developing integrated pest management programs, since both strategies are fundamental to the broad management approach (Cortesero et al., 2000; Peterson et al., 2016; Stern et al., 1959).

Trichomes are plant morphological features that exhibit a variety of shapes and sizes that can help regulate abiotic stress and are considered a primary defense against herbivores attacking plants (Levin, 1973). Glandular trichomes produce exudates that are repellent or toxic (Avé et al., 1987; Kauffman and Kennedy, 1989), aid natural enemies in locating herbivores (Weinhold and Baldwin, 2011), and entrap arthropods (LoPresti et al., 2015). Glandular trichomes may be targeted in the development of arthropod resistant crop varieties through selection for morphological and chemical
attributes that inhibit pest activity (Glas et al., 2012; Kennedy, 2003). Predators and parasitoids may be affected by glandular trichomes in the same manner as target pests, potentially reducing protection provided by natural enemies (Riddick and Simmons, 2014a). Interactions with natural enemies may not always be antagonistic; in some cases, predation of herbivores is not affected by the presence of trichomes (Björkman and Ahrné, 2005; Obrycki and Tauber, 1984) or may be improved (Styrsky et al., 2006).

Recent research investigating arthropod-plant interactions on wildflowers with glandular trichomes has revealed that trichomes can provide alternative food for natural enemies (Krimmel and Pearse, 2013; LoPresti et al., 2018, 2015). A group of predatory arthropods adapted to maneuvering on such “sticky” plants exists, utilizing a variety of adaptations to avoid entrapment on surfaces generally treacherous to arthropods (Voigt et al., 2007; Voigt and Gorb, 2010, 2008). These predators take advantage of arthropods entrapped on the sticky surfaces, frequently scavenging on the carrion. Arthropod carrion on sticky plants functions similarly to plant provided foods: carrion increases predator abundance, reduces herbivory, and improves plant fitness (Krimmel and Pearse, 2013; LoPresti et al., 2018, 2015). As arthropod carrion is found on the surface of many sticky plants (LoPresti et al., 2015), including economically important plants, such a protective mutualism could improve biological control in economically important plants.

Grown worldwide (FAO, 2016), *Nicotiana tabacum* L., cultivated tobacco, is an economically valuable crop (USDA NASS, 2016) and, along with its wild relatives, an important system for studying plant biology, genetic engineering, and arthropod-plant interactions (Baldwin, 2001; Ganapathi et al., 2004; Zhang et al., 2011). Tobacco
exhibits multiple trichome types that decrease in density as leaves grow (Bentley and Wolf, 1945), including glandular trichomes that do or do not produce exudates (Johnson et al., 1985). Trichomes play a critical role in insect resistance in tobacco, with glandular trichomes impairing movement and limiting the establishment of lepidopteran pests and other small insects (Jackson et al., 1989; Severson et al., 1985). Likewise, glandular trichomes may entrap lepidopteran egg parasitoids (Marcovitch and Stanley, 1937; Rabb and Bradley, 1968) and reduce the mobility of several generalist predators (Belcher and Thurston, 1982; Elsey, 1974; Shah, 1982).

*Jalysus wickhami*, the most abundant predatory arthropod in tobacco, prefers stickier tobacco varieties (Jackson et al., 1989, 1988), which it can maneuver on easily due to leg morphology (Henry, 1997; Southwood, 1986). *J. wickhami* preys on eggs and small instars of *Heliothis virescens*, *Manduca sexta*, and *Manduca quinquemaculatua*, the primary lepidopteran pests of tobacco in the southeastern U.S. (Elsey, 1972; Elsey and Stinner, 1971). In addition to predation, *J. wickhami* has been reported to scavenge on dead arthropods trapped in tobacco glandular trichomes (Elsey, 1972; Elsey and Stinner, 1971; Lawson, 1959; Wheeler and Schaefer, 1982).

LoPresti and Toll (2017) established criteria for observational data to indicate that a protective mutualism exists between arthropod predators and sticky plants, and observations in tobacco satisfy two of these: (1) the most abundant predator, *J. wickhami*, readily scavenges on entrapped carrion and (2) *J. wickhami* can reduce populations of *H. virescens* and *Manduca* spp. (*M. sexta* and *M. quinquemaculatua*). Thus, our goal was to assess whether such a mutualism exists in tobacco, which could be generalized to other economically-important plants covered in glandular trichomes.
Utilizing arthropod carrion as an alternative food source for predatory arthropods could improve biological control on sticky plants, taking advantage of plant traits typically involved in direct defense. We performed field experiments under agronomically relevant conditions in which tobacco plants were augmented with carrion, and evaluated *J. wickhami* and pest abundance, plant damage, yield, and carrion entrapment on insecticide-treated plants. Finally, we reviewed the literature to survey economically important plants that trap arthropods on their surface to illustrate the potential of this tactic.

### 3.2 Materials and methods

#### 3.2.1 Experimental design

We performed research in 2016 and 2017 at the North Carolina Department of Agriculture and Consumer Services Lower Coastal Plain Research Station (Lenoir County, North Carolina, 35.297404, -77.574259) and Upper Coastal Plain Research Station (Edgecombe County, North Carolina, 35.894264, -77.680346). Tobacco variety NC 196 was used in all experiments.

In 2016, we manipulated carrion abundance on individual plants in plots treated with systemic imidacloprid treatments (Admire Pro, 42.8% imidacloprid, Bayer Crop Sciences, Research Triangle Park, NC, USA) in a split-plot, randomized complete block design. Main plots consisted of eight rows spaced 1.22 m apart, 15.24 m long, with 25 tobacco plants per row. Plants in main plots were left untreated or treated with Admire Pro (42.8% imidacloprid, Bayer Crop Sciences, Research Triangle Park, NC, USA) at 17.76 ml/1000 plants via a greenhouse tray drench or in-furrow application and were
replicated four times. Split-plots were randomly assigned to two plants near both ends of rows three and six of main plots. We manipulated arthropod carrion by sprinkling 0.05 g of frozen *Drosophila* spp. adults (~30 individuals, sourced form NCSU genetics laboratory colonies) from 0.5 m above plants assigned to carrion augmentation treatments; plant assigned to ambient treatments received no *Drosophila* spp. carrion. Colony-reared *Drosophila* spp. carrion has been used in other manipulative experiments (Krimmel and Pearse, 2013; LoPresti et al., 2018), and we had previously observed *Drosophila* spp. naturally on tobacco plants (Nelson observation). Carrion was applied weekly from 15 June to 23 August.

In 2017, we repeated individual plant experiments and performed whole plot experiments in which two carrion densities were manipulated on a larger scale. All plants in both experiments were treated with imidacloprid in a greenhouse tray drench (17.76 ml/1000 plants) to reduced interference of non-lepidopteran pests with treatments, and replicate plots consisted of three 6.5 m long rows, spaced 1.22 m apart with 10 plants per row. Individual plant experiments received the same carrion treatments applied in 2016, replicated eight times, in a randomized complete block design. Carrion treatments were assigned to two plants near either end of row two and were applied weekly from 15 June to 8 August. Whole plot experiments received the following carrion treatments replicated eight times in a randomized complete block design: ambient carrion (no addition), low carrion (0.05 g frozen *Drosophila* spp. adults), and high carrion (0.20 g frozen *Drosophila* spp. adults). We applied carrion treatments to all plants in plots weekly from 15 June to 8 August.
3.2.2 Arthropod carrion entrapment survey

In 2016, we surveyed dead arthropods trapped on tobacco plants to evaluate the effect of systemic imidacloprid applications on arthropod entrapment. We performed weekly visual inspections of plants in main plots of the individual plant experiment to compare entrapment between untreated, greenhouse tray drench, and transplant water drench treated plots. We performed four 60-second surveys per plot, counting the number of dead arthropods greater than 1 mm in length trapped on the surface of tobacco plants, excluding those typically associated with tobacco (M. persicae, H. virescens, Manduca spp., J. wickhami, etc). Carrion entrapment was assessed weekly from 30 May, when J. wickhami first appear in tobacco fields (Elsey and Stinner, 1971; P. Nelson, 2018), to 4 July, at which point imidacloprid efficacy against early season pests is reduced (Semtner and Srigiriraju, 2005). We did not survey carrion on plants assigned to carrion manipulation treatments.

3.2.3 Arthropod surveys

We surveyed arthropods in individual plant and whole plot experiments by inspecting entire plants for J. wickhami, H. virescens, and Manduca spp. Plants assigned carrion manipulation treatments were surveyed in individual plant experiments, while all plants in row two were surveyed in whole plot experiments. Surveys were performed weekly, beginning at the start of carrion augmentation, and concluded one week after augmentation ended.
3.2.4 Plant damage assessment

We evaluated the effect of carrion manipulation on plant injury by assessing reproductive structure (flower and seed capsule) damage and green leaf weight. Reproductive structures were harvested from individual plant experiments on 1 September 2016 (Edgecombe Co), 23 August 2016 (Lenoir Co.), 9 August 2017 (Edgecombe Co), and 15 August (Lenoir Co.). Damage was estimated in the laboratory by inspecting structures for evidence of caterpillar feeding and assigning one of two responses: damaged or undamaged. In 2017, we measured green leaf weight in whole plot experiments. Six tobacco leaves were collected from lower, mid, and upper stalk positions from seven plants in row three of plots, and their combined mass was measured by stalk position on 8 August (Edgecombe Co.) and 15 August (Lenoir Co.).

3.2.5 Statistical analysis

We performed all statistical analyses in SAS v 9.4 (SAS Institute, Cary NC). Carrion survey, season-long arthropod counts, and plant damage for all experiments were analyzed with independent linear mixed models (PROC MIXED), using the Kenward Roger (1997) procedure to calculate degrees of freedom. We performed post-hoc mean separations using Tukey’s test at $\alpha < 0.05$. All means and standard errors reported are untransformed data; data was back transformed if necessary.

We log-transformed (log+1) weekly arthropod carrion counts for analysis of variance with repeated measures. The transformed data was the response variable and imidacloprid treatment, week after treatment, and their interactions were fixed effects. The model included location and block nested within location as random effects and the
repeated measures statement utilized compound symmetry with the interaction of replicate plots and location as the subject.

We divided arthropod counts and reproductive structure damage assessments in 2016 individual plant experiments by the number of plants per split-plot (two) to account for pseudo-replication. Season-long counts of *J. wickhami*, *H. virescens*, and *Manduca* spp. were square-root transformed for analysis of variance in separate models. Imidacloprid treatment (main plot), carrion augmentation treatment (split-plot), and their interactions were fixed effects in the models, and random effects included location, block nested within location, and the interaction of imidacloprid treatment and block. The proportion of damaged reproductive structures was calculated and was analyzed using the same model structure as season-long arthropod counts.

We accounted for pseudo-replication in 2017 individual plant experiments in the same manner described for 2016 experiments and square-root transformed season-long arthropod counts. Independent models for each arthropod had the same structure: carrion treatment as the fixed effect and location and block nested within location as random effects. The proportion of damage to reproductive structures was arcsine transformed and analyzed using the same model structure. Season-long arthropod counts in whole plot experiments were log-transformed, green leaf weight was square root transformed, and both were analyzed using the same model structure described for 2017 individual plant analyses.
3.3 Results

3.3.1 Arthropod entrapment literature review

A variety of economically important plant entrap arthropods on their surface, including flowers, herbs, and annual and perennial crops (Table 3.1). Our review drew heavily from and expanded upon the survey by LoPresti et al. (2015), identifying 26 species or varieties that trap insects typically with glandular or hooked trichomes. Citations and are included in the Appendix B.

3.3.2 Arthropod carrion entrapment survey

Arthropod carrion, entrapped on the surface of tobacco plants, was not influenced by imidacloprid treatments ($F=0.72; \text{df}=2, 98; P=0.4893$) but did vary significantly by week ($F=30.77; \text{df}=4, 98; P<0.0001$). Carrion abundance initially decreased, peaked in week eight and decreased again (Fig. 3.1). The interaction between imidacloprid treatment and week was not significant ($F=0.51, \text{df}=8, 98; P=0.8491$).

3.3.3 Individual plant experiment: 2016

*Jalysus wickhami* abundance was influenced by imidacloprid ($F=9.51; \text{df}=2, 8.64; P=0.0066$) and carrion ($F=29.19; \text{df}=1, 26.5; P<0.0001$) treatments but the interaction between the two effects was not significant ($F=0.13; \text{df}=2, 26.5; P=0.8772$) (Fig. 3.2a). *Jalysus wickhami* counts were greatest in main plots not treated with imidacloprid and were greater on plants augmented with arthropod carrion. As expected, caterpillar densities were not influenced by imidacloprid (*Heliothis virescens* $F=2.82; \text{df}=2, 21; P=0.0842$; *Manduca* spp. $F=3.23; \text{df}=2, 35; P=0.0515$), but carrion treatments (*Heliothis
virescens $F=0.86$; $df=1,21; P=0.3635$; Manduca spp. $F=0.48$; $df=1,35; P=0.4943$), and their interaction with imidacloprid were not significant ($Heliothis virescens F=0.17$; $df=2,21; P=0.8446$; Manduca spp. $F=0.04$; $df=2,35; P=0.9585$) (Fig. 3.2).

Plants receiving carrion augmentation had a lower proportion of damaged reproductive structures than ambient treatments ($F=14.95$; $df=2,27.7; P=0.0006$), but plant damage was not affected by imidacloprid treatment ($F=2.24$; $df=2,8.5; P=0.1658$) or the interaction of the two effects ($F=2.01$; $df=2,27.7; P=0.1534$) (Fig. 3.3).

3.3.4 Individual plant experiment: 2017

Plants augmented with carrion had higher season long $J. wickhami$ densities than ambient treatments ($F=46.30$; $df=1,29; P<0.0001$) (Fig. 3.4a). Both $H. virescens$ and Manduca spp. season-long counts were not influenced by carrion treatments ($F=0.55$; $df=1,15; P=0.4681$; $F=3.37$; $df=1,15; P=0.0863$, respectively) (Figs. 3.4a & 3.4b), while the proportion of damaged reproductive structures was significantly reduced on plants augmented with carrion ($F=7.43$; $df=1,29; P=0.0107$) (Fig. 3.5).

3.3.5 Whole plot experiment: 2017

Low and high carrion augmentation treatments had significantly higher $J. wickhami$ abundance than the ambient treatment but the two augmentation rates were not significantly different from each other ($F=45.52$; $df=2,26.6; P<0.0001$; Fig. 3.4a). Season-long means of $H. virescens$ did not differ significantly due to carrion treatments ($F=1.38$; $df=2,30; P=0.2671$) (Fig. 3.4b), nor did Manduca spp. ($F=0.69$; $df=2,32.5; P$
Green leaf weight was significantly influenced by both carrion treatment \((F = 12.26; \text{df} = 2, 122; P < 0.0001)\) and stalk position \((F = 52.71; \text{df} = 2, 199; P < 0.0001)\) but the interaction of the two factors was not significant \((F = 1.59; \text{df} = 4, 119; P = 0.1813)\). Overall, green leaf weight was significantly greater on plants augmented with arthropod carrion compared to ambient treatments, and there was no difference between low and high carrion augmentation rates \((\text{Fig. 3.6})\). Green leaf weight was greatest at low stalk positions, followed by mid and upper stalk positions \((\text{Fig. 3.6})\).

### 3.4 Discussion

Arthropod carrion trapped by trichomes is an underappreciated form of plant-provided food that influences trophic interactions. Trichome entrapment is typically considered a direct defense against herbivores, but research indicates trichomes are also involved in indirect defense. Augmenting carrion on tobacco plants increased densities of \textit{J. wickhami}, reduced damage to reproductive structures, and increased green leaf weight. This is the first report of a carrion-mediated protective mutualism with a member of Solanaceae and with an economically important plant grown under agronomically-relevant conditions. Our review of the literature revealed that numerous economically important plants trap arthropods on their surface, suggesting this phenomenon has potential to enhance biological control in a variety of cropping systems.

Provisioning natural enemies with alternative food is predicted to increase their abundance \((\text{van Baalen et al., 2001; van Rijn et al., 2002})\) and carrion augmentation in individual plant and whole plot experiments boosted \textit{J. wickhami} populations compared to ambient carrion treatments. In whole plot experiments, \textit{J. wickhami} numbers did not
differ between low and high carrion augmentation treatments. Natural enemy populations grow partly in response to food supplementation via increased reproductive rates (van Rijn and Sabelis, 2005) however, this mechanism may be more readily observed in organisms with shorter generation times (Sabelis, 1992; van Rijn et al., 2002). *J. wickhami* have two generations per growing season (Elsey and Stinner, 1971) which may not provide enough time for raised reproductive rates to increase abundance. Plots assigned to high augmentation treatments received four times the amount of carrion as low treatment plots; this difference may not have been great enough to increase *J. wickhami* numbers. Testing a wider range of augmentation rates could identify the limits at which this mutualism increases predator abundance.

Plants benefit from provisioning carrion to predators by increasing their own fitness, assessed by measuring damage to reproductive structures (Krimmel and Pearse, 2013; LoPresti et al., 2018, 2015). In our study, carrion augmentation reduced damage to tobacco flowers and seed capsules, indicating tobacco benefits in the same manner. In addition to improving plant fitness, reductions in damage to these structures has economic implications for tobacco grown for seed in nursery production. Tobacco, like many domesticated plants, is grown to produce large amounts of leaf mass (Brown et al., 2018). By measuring green leaf weight, a proxy for yield, we determined that carrion augmentation increases leaf mass. This is the first report of this protective mutualism reducing damage to vegetative plant structures, indicating carrion augmentation could improve biological control of foliar pests as well as flower and fruit pests.
Despite the reduction in plant damage, lepidopteran pest (*H. virescens* and *Manduca* spp.) numbers did not differ in response to carrion augmentation. Over shorter periods of time, supplying alternative food may reduce prey consumption, especially if that food is substitutable (Tillman, 1982; van Rijn and Sabelis, 2005). Alternately, a reduction in damage without a change in pest numbers indicates that the effect of *J. wickhami* on pests may be non-consumptive (Thaler and Griffin, 2008; Werner and Peacor, 2003). Non-consumptive effects of natural enemies are becoming increasingly valued for their role in pest management (Eubanks and Finke, 2014; Hermann and Landis, 2017) and may contribute to reductions in herbivory in other sticky plant mutualisms (LoPresti et al., 2015). Evaluating the relative importance of predator non-consumptive effects would clarify trophic interactions on sticky plants. Determining the potential for substitutability of arthropod carrion is necessary for further development into a conservation biological control tactic.

This is the first report of a carrion-mediated protective mutualism assessed in an agroecosystem. Excessive pesticide use in agroecosystems can simplify food webs and destabilize predator dynamics (Croft and Brown, 1975; Ripper, 1956). Systemic imidacloprid applications are frequently used in North Carolina flue-cured tobacco production for early season pest management (Burack and Toennisson, 2018); these applications are compatible with *J. wickhami* (P. Nelson, 2018), but reduce the availability of the prey *Myzus persicae* (Merchán and Burack, 2017). Arthropod carrion on tobacco plant surfaces did not differ between imidacloprid treatments, suggesting carrion may be available for *J. wickhami* when prey is absent due to imidacloprid use. LoPresti et al. (2015) reported that volatile cues produced by *Aquilegia eximia* Van
Houtte ex Planch. attract arthropods, leading them to their demise (but see Appendix C). Systemically applied insecticides may translocate to exudates of glandular trichomes and impair arthropods (Cherry and Pless, 1969; Reddy et al., 1970), thus evaluating the effect of insecticide use on carrion entrapment in other crops is warranted.

Inhibiting insect movement is one mechanism of antixenosis in host plant resistance (Kogan and Ortman, 1978; Painter, 1951). Insect entrapment by plants has been reported in over 110 genera in 49 families (LoPresti et al., 2015); our species-specific review includes flowers, herbs, agronomic, and horticultural crops (Table 1). Selecting or breeding for varieties with trichomes could benefit integrated pest management programs, as insect entrapment may contribute to both direct (resistance) and indirect plant defenses (biological control). Domestication may reduce plant defenses (Chen et al., 2015) and assessing wild relatives of crops for arthropod entrapment could initiate development of this feature into an efficacious conservation biological control tactic. For instance, wild tomato (Gentile et al., 1968; Simmons et al., 2004) and potato (Gibson and Turner, 1977; Obrycki and Tauber, 1984) relatives are “stickier” (increased trichome density) than their domesticated counterparts and trap arthropods on their surfaces.

Trichomes may produce harmful effects against natural enemies and reduce their efficacy in controlling herbivores (Eisner et al., 1998; Kennedy, 2003; Riddick and Simmons, 2014a) but this narrative of morphology mediating arthropod-plant interactions is incomplete. Predatory arthropods able to maneuver on sticky plant surfaces without ill effects have been observed on multiple wild hosts (Krimmel and
Pearse, 2013; LoPresti et al., 2018, 2015; Lopresti and Toll, 2017); we have found members of this group (berytids, reduvids, oxyopids) on tobacco plants as well (P. Nelson, 2018). Natural enemy interaction with glandular trichomes may be nuanced and requires ecologically-relevant studies to determine if this direct defense impedes predators and parasitoids. Coccinellid movement may be impaired by glandular trichomes (Cottrell and Yeargan, 1999; Shah, 1982), however, their efficacy in reducing aphid abundance was increased on sticky versus non-sticky races of the same plant (Krimmel and Pearse, 2014).

Provisioning natural enemies with food is a conservation biological control tactic; utilizing non-crop plants to provide extra-floral nectar or pollen is one approach (Berndt et al., 2002; Hansen, 1983; Lee and Heimpel, 2003; Wong and Frank, 2013). Arthropod carrion could be developed into a similar resource via exploitation of plant morphology or application of artificially reared carrion to augment alternative food for predatory arthropods. Evaluating a range of augmentation rates and varietal differences in carrion entrapment could advance the development of this tactic. Trichomes are not a dead end for biological control, but considering appropriate natural enemies and their interactions with plants in crop-specific context is necessary to prevent the failure of such efforts (Davidson et al., 1992; Krimmel, 2014; Riddick and Simmons, 2014b).
### 3.6 Tables

Table 3.1 Economically important plants reported to entrap arthropods on their surface. Data presented is derived partially from the survey of carrion entrapping families and genera by LoPresti et al. (2015). References are presented in Appendix B. GT: glandular trichomes, HT: hooked trichomes, NGT: non-glandular trichomes.

<table>
<thead>
<tr>
<th>Binomial name</th>
<th>Common name</th>
<th>Family</th>
<th>Genus</th>
<th>Entrapment mechanism</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Abelmoschus esculentus</em> (L.) Moench</td>
<td>Okra</td>
<td>Malvaceae</td>
<td>Abelmoschus</td>
<td>GT</td>
<td>(Duraimurugan and Regupathy, 2005)</td>
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<td><em>Cajanus cajan</em> (L.) Millsp.</td>
<td>Pigeon pea</td>
<td>Fabaceae</td>
<td>Cajanus</td>
<td>GT</td>
<td>(Romeis et al., 1998)</td>
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<td><em>Cannabis sativa</em> L.</td>
<td>Marijuana</td>
<td>Cannabaceae</td>
<td>Cannabis</td>
<td>GT</td>
<td>(Potter, 2009)</td>
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<td><em>Cicer arietinum</em> L.</td>
<td>Chickpea</td>
<td>Fabaceae</td>
<td>Cicer</td>
<td>GT</td>
<td>(Romeis et al., 1999)</td>
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<tr>
<td><em>Cucumis sativus</em> L.</td>
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<td>Curcurbitaceae</td>
<td>Cucumis</td>
<td>HT</td>
<td>(Ricci and Ceppelletti, 1988)</td>
</tr>
<tr>
<td><em>Pelargonium x hortorum</em> L.H.Bailey</td>
<td>Garden geranium</td>
<td>Geraniales</td>
<td>Geraniaceae</td>
<td>GT</td>
<td>(Walters, 1988)</td>
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<td><em>Lagenaria siceraria</em> (Molina) Standl.</td>
<td>White-flowered gourd</td>
<td>Curcurbitaceae</td>
<td>Lagenaria</td>
<td>NGT</td>
<td>(LoPresti et al., 2018)</td>
</tr>
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<td>Fabaceae</td>
<td>Medicago</td>
<td>GT</td>
<td>(Kishaba et al., 1992)</td>
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<td>Oregano</td>
<td>Lamiaceae</td>
<td>Origanum</td>
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<td>(Small, 1985), (Shade et al., 1979)</td>
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<td>Scarlett runner bean</td>
<td>Fabaceae</td>
<td>Phaseolus</td>
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<td>(Davidson et al., 1992), (Ricci and Ceppelletti, 1988)</td>
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Table 3.1 (continued)

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<tr>
<th>Binomial name</th>
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<th>Genus</th>
<th>Entrapment mechanism</th>
<th>References</th>
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<td><em>Phaseolus lunatus</em> L.</td>
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<td>(Riddick and Wu, 2011)</td>
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<td>Phaseolus</td>
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<td>(Quiring et al., 1992), (Pillemer and Tingey, 1978)</td>
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<td>Fabaceae</td>
<td>Phaseolus</td>
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<td>(Shah, 1982)</td>
</tr>
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<td>Ericaceae</td>
<td>Rhododendron</td>
<td>GT</td>
<td>(Sugiura and Yamazaki, 2006)</td>
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<td>Rosaceae</td>
<td>Rosa</td>
<td>NGT</td>
<td>(Yamazaki et al., 2014)</td>
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<td>Lamiaceae</td>
<td>Salvia</td>
<td>GT</td>
<td>(Corsi and Bottega, 1999)</td>
</tr>
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<td><em>Salvia sclarea</em> L.</td>
<td>Clary sage</td>
<td>Lamiaceae</td>
<td>Salvia</td>
<td>GT</td>
<td>PN Nelson observation (Taneja and Woodhead, 1987)</td>
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<td>Curcurbitaceae</td>
<td>Sicana</td>
<td>GT</td>
<td>(Kellogg et al., 2002)</td>
</tr>
<tr>
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<td>Potato</td>
<td>Solanaceae</td>
<td>Solanum</td>
<td>GT</td>
<td>(Obrycki and Tauber, 1984)</td>
</tr>
<tr>
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<td>Vitaceae</td>
<td>Vitis</td>
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</tr>
</tbody>
</table>
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Chapter 4: Arthropod carrion influences plant choice, oviposition, and cannibalism by a specialist predator on a sticky plant

4.1 Introduction

Plants may defend themselves from herbivore attack by providing alternative food resources for natural enemies. This indirect defensive strategy is utilized by numerous species and relies on the fact that many carnivorous arthropods are facultatively or obligately zoo-phytophagous, feeding on plant tissues at some point during their lives (Bugg et al., 1991; Jervis et al., 1996; Limburg and Rosenheim, 2001; Porter, 2018). By providing food, plants increase the abundance or efficacy of natural enemies which can lead to a reduction in herbivore density and damage (Bakker and Klein, 1992; Stapel et al., 1997; van Rijn et al., 2002; Wäckers, 2003). Some plant species have specialized food body structures to facilitate these interactions (Heil et al., 1998; Rickson, 1980). More common types of plant-provided foods (PPFs) utilized by natural enemies include pollen, floral, and extra-floral nectar (Wackers, 2005).

Plants covered in trichomes can entrap arthropods on their surface (LoPresti et al., 2015; P. Nelson, 2018). These “tourists” (Moran and Southwood, 1982), unaccustomed to maneuvering on the complex plant surfaces, are trapped by the outgrowths either via impalement on hooked trichomes (Pillemer and Tingey, 1978; Ricci and Ceppelletti, 1988; Riddick and Wu, 2011) or by capture in the sticky exudates of glandular trichomes (McKinney, 1938; Romeis et al., 1998; Shade et al., 1979). A guild of specialist predators able to maneuver on the plant surfaces without becoming entrapped facultatively feed on the dead arthropods, increasing their abundance and
reducing damage to plants (Krimmel and Pearse, 2013; LoPresti et al., 2018, 2015; P. Nelson, 2018).

PPFs may increase natural enemies on plants via multiple mechanisms. Volatiles produced by flowers can attract parasitoids to nectar (Wäckers, 2004), and alternative foods may arrest or retain natural enemies after reaching plants (McMurtry et al., 1991; Stapel et al., 1997). Pollen can maintain predators during periods of low prey density, reducing emigration (van Rijn and Sabelis, 1990), and increase fecundity or shorten development time (Cocuzza et al., 1997; Salas-Aguilar and Ehler, 1977; Vandekerkhove and De Clercq, 2010). Whether arthropod carrion, entrapped on plants, functions similarly to any of the previously-mentioned mechanisms is unknown; thus far authors have postulated that carrion either attracts or retains predators on the plants (Krimmel and Pearse, 2013; LoPresti et al., 2015).

*Nicotiana tabacum* L. is an economically important annual crop covered in glandular trichomes (Bentley and Wolf, 1945) that trap numerous insect species (Jackson et al., 1989; Marcovitch and Stanley, 1937; Rabb and Bradley, 1968; Severson et al., 1985). The predator *Jalysus wickhami* Van Duzee (Hemiptera: Berytidae) is associated primarily with “glandular-hairy” hosts, including *N. tabacum* (Wheeler and Henry, 1981), and readily scavenges on arthropod carrion entrapped on plant surfaces (Elsey, 1972; Elsey and Stinner, 1971; Lawson, 1959; P. Nelson, 2018; Wheeler and Schaefer, 1982). *J. wickhami* respond positively to increased availability of arthropod carrion and are involved in defending plants against herbivore attack (Krimmel and Pearse, 2013; Lopresti and Toll, 2017; P. Nelson, 2018).
Our goal was to assess how arthropod carrion influences predator behaviors potentially contributing to increased abundance on plants provisioning the resource. We hypothesized that arthropod carrion may influence multiple arthropod behaviors that can increase their abundance on plants (Table 1). Through a series of experiments we assessed the effect of arthropod carrion on predator plant preference, oviposition preference, and egg cannibalism by different life stages.

4.2 Materials and methods

We performed experiments with *J. wickhami* and *N. tabacum* due to their ease of collection from fields and cultivation in the greenhouse, respectively. *Drosophila* spp. cadavers were selected as a surrogate for naturally occurring arthropod carrion and have been utilized in other manipulative experiments (Krimmel and Pearse, 2013; LoPresti et al., 2018; P. Nelson, 2018). Although we measured cannibalism in greenhouse experiments, they were designed to assess the effect of carrion on aggregation and egg laying while microcosm experiments were designed to the effect of carrion on directly on cannibalism.

4.2.1 Insect material

We collected *J. wickhami* from *N. tabacum* (var. NC 196) fields at the North Carolina Department of Agriculture and Consumer Services Lower Coastal Plain Research Station (Lenoir County, North Carolina 35.297404, -77.574259) and Upper Coastal Plain Research Station (Edgecombe County, North Carolina, 35.894264, -77.680346). We maintained a colony of *J. wickhami* in a 1.2x1.2x1.2 m (LxWxH) cage covered in
overwintering fabric (Dewitt, Sikeson, MO) and providing the insects with four *N. tabacum* plants (var. K326) potted in 20 cm tall, 11 L plastic pots. Plants were infested with *Myzus persicae* (Sulzer, 1776) (Hemiptera: Aphididae) and we added frozen *M. sexta* eggs, *H. virescens* eggs, and *Drosophila* spp. adults to plant leaves every one to two weeks. We kept the colony in a greenhouse with temperatures fluctuating from 35°C during the day to 22°C at night and a photoperiod of 16:8 light: dark.

We facilitated *J. wickhami* egg production by holding two females and one male in 30 ml plastic cups (Dart Container Corporation, Mason, MI) with a 2.5 cm diameter disc of *N. tabacum* leaf placed adaxial side up on a 0.5 cm layer of 2% water agar (Alfa Aesar, Ward Hill, MA). Adults were provided ca. six *Drosophila* spp. adult cadavers; cups were covered with a paper lid and were held in a growth chamber at 25°C, 14:10 light: dark, and 70% relative humidity. We removed eggs daily for four days and stored them at 25°C in *N. tabacum*-lined cups for no longer than three days prior to experimental use.

We collected *Drosophila* spp. adults from North Carolina State University genetics laboratory cultures and froze them until use.

4.2.2 Plant material

We used *N. tabacum* var. K326 in all experiments. *N. tabacum* was planted in 16 cm diameter clay pots filled with 50:50 Fafard 4p mix (Sun Gro Horticulture, Agawarm, MA): play sand and were fertilized with Osmocote 14-14-14 (The Scotts Company, Marysville, OH). Plants were grown for six weeks in the greenhouse and were ca. 20 cm
tall and 20 cm in diameter with eight to ten leaves per plant at the time of use in experiments.

4.2.3 Greenhouse experiments

We performed both greenhouse experiments under the previously described environmental conditions, using 1.0x0.5x0.5 m cages covered in overwintering fabric.

4.2.3.1 Plant and egg laying choice

We placed two potted *N. tabacum* plants 15 cm from longitudinal cage edges and randomly assigned the plants one of the following treatments: no carrion or carrion addition (four *Drosophila* spp. cadavers added to each leaf). We introduced six adult *J. wickhami* at a ratio of 50:50 female: male to the center of cages and assessed the number of insects on each plant at 2, 4, 8, 12, 24, and 48 hr post introduction. At 48 hr we removed all *J. wickhami* and destructively sampled the plants to count the number of eggs laid on the plants. We used a randomized complete block design with five blocks and replicated the experiment on five dates.

4.2.3.2 Egg cannibalism

We placed a single potted *N. tabacum* plant in the center of a cage and added three *J. wickhami* eggs to the adaxial leaf surface along the mid rib each of two haphazardly selected leaves (observations made during the prior experiment indicate that *J. wickhami* egg laying is clustered, predominantly on adaxial leaf surfaces, and frequently along leaf midribs). Plants were assigned to one of two treatments: no carrion or carrion
addition (three *Drosophila* spp. cadavers added per leaves with *J. wickhami* eggs). We introduced six *J. wickhami* adults at 50:50 female: male to the center of cages and assessed egg cannibalism after 48 hr. We used a randomized complete block design with four blocks and replicated the experiment on five dates.

4.2.4 Microcosm egg cannibalism

We performed microcosm experiments using the previously-described cups lined with agar and *N. tabacum* leaves. We assessed egg cannibalism by adult *J. wickhami* by adding five colony-reared eggs to cups and one of two carrion treatments: no carrion or carrion addition (five *Drosophila* spp. cadavers). A single adult *J. wickhami* was introduced was introduced to the cups, which were then covered with paper lids. We assessed egg cannibalism at 24, 48, and 72 hrs and replicated the experiment on five dates with at least four repetitions per treatment. We assessed egg cannibalism by fifth instar *J. wickhami* by adding three colony-reared eggs to cups and the same carrion treatments (carrion addition: three *Drosophila* spp. cadavers). A single fifth instar *J. wickhami* was introduced to the cups which were covered with paper lids. We assessed egg cannibalism at 24, 48, and 72 hrs and replicated the experiment on four dates, blocking by date, with at least four repetitions per treatment.

4.2.5 Statistical analyses

We performed all statistical analyses using SAS v9.4 (SAS Institute, Cary, NC) and used Tukey’s test ($\alpha < 0.05$) for post-hoc mean separations. Response variables were
transformed to meet assumptions of normality as needed, but non-transformed data are presented for clarity.

We analyzed adult log-transformed (log10(x+1)) *J. wickhami* counts with a linear mixed model (PROC MIXED) with repeated measures, using the following structure: carrion treatment, assessment time, and their interactions as fixed effects, and replicate and block nested within replicate as random effects. The interaction between block, replicate, and assessment time was the subject of repeated measures, using compound symmetry. Mean *J. wickhami* eggs per plant were square root transformed and analyzed with the following model: carrion treatment as the fixed effect and replicate, block nested within replicate, and the interaction between treatment and block as random effects.

Egg cannibalism by *J. wickhami* in the greenhouse experiments was evaluated using a linear mixed model. We log transformed the number of eggs cannibalized and used the following model structure: carrion treatment as the fixed effect and replicate and block nested within replicate as random effects. Egg cannibalism by nymphs and adults in microcosm experiments was analyzed using separate linear mixed models with repeated measures. The number of eggs cannibalized was log transformed and carrion treatment and assessment date and their interactions were fixed effects. We assigned replicate as the random effect and utilized a repeated statement with compound symmetry structure, with the interaction of repetition and replicate as the subject.
4.3 Results

4.3.1 Greenhouse experiments

*J. wickhami* abundance was significantly greater on plants with added carrion 
\((F=132.14, 1, 216; P<0.0001)\), and abundance on plants regardless of carrion treatment was greatest at 24 hr \((F=22.95, 1,4; P<0.0001, \text{Fig. 1})\). Further, plants receiving carrion additions had four times the number of eggs as control plants \((F = 27.42, 1, 4; P = 0.0064, \text{Fig. 2})\). Egg cannibalism by *J. wickhami* adults in caged experiments was infrequent and did not differ in the presence of carrion \((F=0.21, 1,19, P=0.6526) (\text{Fig. 3})\).

4.3.2 Microcosm experiments

Egg cannibalism was significantly lower in the presence of arthropod carrion \((F = 53.71, 1, 128; P <0.0001)\) and was highest during the last assessment period (72 hr) \((F=9.18, 2,128; P= 0.0002, \text{Fig. 4})\). The presence of carrion did not alter egg cannibalism by *J. wickhami* nymphs \((F=0.95, 1, 105; P =0.3315)\), but cannibalism did increase over time \((F =13.05,2, 105; P <0.0001)\) and was highest at 48 and 72 hr (Fig. 5).

4.4 Discussion

PPFs exist in a variety forms (floral and extra-floral nectar, pollen, food bodies, elaiosomes, etc.) (Wackers, 2005) and supplement the diet of natural enemies, thereby enlisting them in defense against herbivores. PPFs influence natural enemy behavior, including interactions with plants and other arthropods (Eubanks et al., 2000; Jamont et al., 2014; Stapel et al., 1997), and can enhance performance (Fouly et al., 1995; Kiman and Yeargar, 1985). Dead arthropods, entrapped on plant surfaces by trichomes, are
another form of plant-provided food utilized by predatory arthropods able to navigate the plant surfaces without becoming ensnared (Krimmel and Pearse, 2013; LoPresti et al., 2018, 2015; Lopresti and Toll, 2017; P. Nelson, 2018).

Our results demonstrate that the food source influences the behavior of the predator *J. wickhami*. In greenhouse experiments performed to assess the effect of carrion on arthropod-plant interactions, *J. wickhami* adults were observed on *N. tabacum* plants augmented with *Drosophila* spp. carrion at greater frequencies, and *J. wickhami* oviposited more eggs on these plants as well. *Drosophila* spp. carrion reduced egg cannibalism by *J. wickhami* adults, but only at the higher density assessed in microcosm experiments, and did not reduce egg cannibalism by nymphs.

Natural enemy abundance typically increases with the availability of PPF. This numerical response is due in part to the aggregation of natural enemies to food resources (Solomon, 1949) and a variety of predators are attracted to and feed on nectar and pollen, increasing their abundance (Bentley, 1977; Nomikou et al., 2010; Sutherland et al., 1999; Wong and Frank, 2013). Our results indicate that *J. wickhami* respond similarly to arthropod carrion, as more adults were observed on *N. tabacum* plants augmented with *Drosophila* spp. cadavers than those without. Aggregation is the result of attraction (Wäckers, 2004) and retention (Stapel et al., 1997). Although we observed greater frequencies of *J. wickhami* adults on plants with carrion, we did not attempt to parse out sensory modalities contributing to attraction or assess retention rates. Olfactory cues are involved in the attraction of scavenging grasshoppers to carrion (Bomar, 1993); assessing whether *J. wickhami* respond positively to volatiles
produced by carrion or the interface of plants and carrion could help elucidate aggregation mechanisms.

In addition to aggregation, egg laying by natural enemies may contribute to numerical responses (Solomon, 1949) and can increase with the availability of alternative foods (van Rijn and Sabelis, 2005). PPF consumption by natural enemies may overcome dietary deficiencies and increase fecundity (Cocuzza et al., 1997; Eubanks and Styrsky, 2005; Jacometti et al., 2010; Kiman and Yeargan, 1985). We observed a significant increase in the number of eggs laid on plants with arthropod carrion which is likely due to adults aggregating on the same plants. Similar short-term effects of PPF availability on Coccinellidae (Coleoptera) predator oviposition has been observed in the field (Cottrell & Yeargan, 1999) and may be explained in part by PPF functioning as an oviposition stimulant (Evans and Dixon, 1986). Our experimental design precluded assessing the influence of arthropod carrion on fecundity, as we did not rear *J. wickhami* on different diets; further research investigating this is warranted.

Cannibalism is pervasive amongst arthropods, including carnivorous (Elgar, 1992) and non-carnivorous taxa (Richardson et al., 2010), and it can be the result of a variety of density-dependent and independent factors (Fox, 1975; Polis, 1981; Richardson et al., 2010). Cannibalism by natural enemies may be increased when prey abundance is reduced or low in quality (Denno et al., 2004; Hironori and Katsuhiro, 1997; Moreno-Ripoll et al., 2012; Snyder et al., 2000) and is ameliorated by alternative foods, including PPFs (Cottrell and Yeargan, 1998; Frank et al., 2010; Leon-Beck and Coll, 2007). Arthropod carrion had mixed effects on egg cannibalism by *J. wickhami*: at low adult densities, egg cannibalism was infrequent and did not decrease when carrion
was present. At higher densities, egg cannibalism by adults was reduced in the presence of carrion, but nymph cannibalism did not differ. Increased population density is frequently linked to cannibalism (Fox, 1975; Polis, 1981; Richardson et al., 2010) and is a plausible explanation for differences observed in egg cannibalism by *J. wickhami* in our experiments. Microcosm cannibalism experiments had substantially higher *J. wickhami* densities (one per 30 cm³) compared to greenhouse experiments (one per 41,666 cm³). We have counted as many as 22 *J. wickhami* per *N. tabacum* plant and observed cannibalism in the field. Assessing cannibalism was not our main goal in carrying out greenhouse experiments, and relatively lower densities of carrion were adequate to observe effects on egg laying.

Predation is frequently an asymmetrical interaction, as size hierarchies (interspecific, due to ontogeny, or intraspecific) can drive cannibalism and intra-guild predation (Polis, 1981; Polis et al., 1989). Similarly, scavenging by *J. wickhami* could be driven by differences in size between the predators and arthropod carrion. Egg cannibalism by *J. wickhami* nymphs was not reduced in the presence of *Drosophila* spp. cadavers, which were approximately the same length as the fifth instar predators. Scavenging by *Loxosceles reculsa* Gertsch & Mulaik, 1940 (Aranae: Sicariidae) is dependent on the size of carrion relative to live prey (Vetter, 2011) and assessing similar interactions with *J. wickhami* nymphs is warranted. Diet-mixing can differ between arthropod life stages and life-history omnivory amongst predators and parasitoids is not uncommon (Coll and Guershon, 2002). *J. wickhami* nymphs may have different nutritional requirements than adults that prevent them from scavenging as readily.
Carrion consumption, or scavenging, is a predominantly facultative predatory behavior and can have significant effects on trophic interactions (Barton et al., 2013; Beasley et al., 2012; Wilson and Wolkovich, 2011). While scavenging on vertebrate carrion by arthropod decomposers (Benbow et al., 2015) and conspecifics by social arthropods (Sun and Zhou, 2013) is well studied, facultative scavenging by arthropods on arthropod carrion has been neglected. Facultative scavenging by a diverse group of arthropods has been reported (Coelho and Hoagland, 1995; Foltan et al., 2005; Lavigne and Pfadt, 1964; Pierce, 1995; Vetter, 2011; Wheeler, 1974) but few studies have assessed the effects of scavenging on individuals and communities (LoPresti et al., 2018; LoPresti, 2018; P. Nelson, 2018; Peng et al., 2013). We hope that our research will help highlight the importance of this underappreciated trophic interaction and inspire similar research in other systems.

Plant trichomes are typically considered to be detrimental to herbivorous and carnivorous arthropods (Levin, 1973; Riddick and Simmons, 2014a), but recent research has begun untangling the intricacies of arthropod-plant interactions on these complex plant surfaces. Our efforts contribute to the growing body of literature demonstrating that specialist predators thrive on these typically treacherous plants, taking advantage of alternative food resources on the phylloplane. While the importance of alternative food for predators is understood, empirical studies with such resources are predominantly limited to plant tissues (van Baalen et al., 2001; Wackers et al., 2005). Arthropod carrion is a type of PPF made available due to plant morphology, similar to pollen and fungus (Pozzebon and Duso, 2008; Roda et al., 2003). Future
efforts recognizing and assessing the importance of different forms of alternative foods and their interactions with plants could improve the study of predator-prey dynamics.
4.5 Tables

Table 4.1 Hypothesized effects of arthropod carrion contributing to predator abundance based on reported effects of plant provided food on mechanism influencing natural enemy abundance.

<table>
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<th>Hypothesized effect of carrion on mechanism</th>
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<td><strong>Predator-plant interactions</strong></td>
<td></td>
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<tr>
<td>Attraction</td>
<td>+ (nectar, Wäckers, 2004)</td>
<td>+</td>
</tr>
<tr>
<td>Retention</td>
<td>+ (pollen, McMurtry et al., 1991)</td>
<td>+</td>
</tr>
<tr>
<td>Oviposition</td>
<td>+ (pollen, Cottrell &amp; Yeargan, 1999)</td>
<td>+</td>
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<tr>
<td><strong>Predator performance</strong></td>
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<tr>
<td>Survival</td>
<td>+ (extra-floral nectar, Limburg &amp; Rosenheim, 2001)</td>
<td>+</td>
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<tr>
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<td>+ (pollen, Salas-Aguilar &amp; Ehler, 1977)</td>
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<td>Fecundity</td>
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<tr>
<td>Cannibalism</td>
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<td>-</td>
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<td>Intra-guild predation</td>
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<td>Carrion increases IGP (LoPresti et al., 2018)</td>
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4.6 Figures

Figure 4.1 Mean (SEM) *J. wickhami* adults per plants in greenhouse cage experiments. Differences in *J. wickhami* between treatments were statistically significant, different upper case letters indicate statistically significant differences in assessment time in the carrion addition treatment, different lower case letters indicate statistically significant differences in assessment time in the no carrion treatment (Tukey’s HSD, \( \alpha \leq 0.05 \)).
Figure 4.2 Mean (SEM) *J. wickhami* eggs oviposited on plants in greenhouse cage experiments. Different upper case letters indicate statistically significant differences in carrion treatments (Tukey’s HSD, $\alpha \leq 0.05$).
Figure 4.3 Mean (SEM) *J. wickhami* eggs cannibalized by adults in greenhouse cage experiments.
Figure 4.4 Mean (SEM) *J. wickhami* eggs cannibalized by adults in microcosm experiments. Differences in egg cannibalism by adults were statistically significant between treatments, different upper case letters indicate statistically significant differences in assessment time in the carrion addition treatment, different lower case letters indicate statistically significant differences in assessment time in the no carrion treatment (Tukey’s HSD, $\alpha \leq 0.05$).
Figure 4.5 Mean (SEM) *J. wickhami* eggs cannibalized by fifth instars in microcosm experiments. Egg cannibalism did not differ significantly between carrion treatments, different upper case letters indicate statistically significant differences in assessment time (Tukey’s HSD, \( \alpha \leq 0.05 \)).
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Appendix A: *J. wickhami* movement on yellow sticky traps

During 2015 we deployed Pherocon® Unbaited AM yellow sticky traps (Trece, Inc., Adair, OK, USA) to monitor predatory arthropods including *J. wickhami*. Two traps were placed equidistant from each other between rows four and five of replicate plots and were suspended directly above the canopy of tobacco plants by attaching them to wooden stakes with binder clips, adjusting their height throughout the season. We deployed and changed traps weekly from 15 May to 5 August. Yellow sticky traps captured a total of seven *J. wickhami* during 2015. Other predatory taxa captured included Chrysopidae (4) (Neuroptera), Coccinellidae (66) (Coleoptera), Anthocoridae (4) (Hemiptera), Geocoridae (18) (Hemiptera), Nabidae (5) (Hemiptera), and Oxyopidae (5) (Aranae). While retrieving yellow sticky traps used to monitor arthropods in tobacco fields for another project in 2016, we observed multiple *J. wickhami* walking across the surface of the sticky traps without becoming entrapped (see Fig. A.1). This may help explain the ineffectiveness of yellow sticky traps for monitoring *J. wickhami* in 2015, although similar monitoring methods have been effective in monitoring the predators in tomatoes (Pease and Zalom, 2010).

Reported hosts of *J. wickhami* are predominantly characterized as “glandular-hairy” (Wheeler and Henry, 1981), and their propensity for association with such plants may be partially explained by their anatomy. *Jalysus wickhami* have two morphological features which have been postulated to be involved in their ability to travel across “sticky plant” surfaces without becoming entrapped. In his monograph of Berytidae of the Western hemisphere, Henry (1997) postulated that the dentate claws may allow berytids to “tip-toe” on sticky plant surfaces or grip trichome stems, facilitating their
radiation to plants covered in trichomes. Similarly, Southwood (1986) speculated that the elongate legs and swollen femoral tips provide “enlarged tibia-femoral articulation”, causing an “increase in the leverage to swing the apex of the leg.” Given that the surface of a yellow sticky trap is considerably more uniform than the surface of a tobacco leaf or other plant covered and glandular trichomes, we speculate that elongated legs and enlarged tibia-femoral articulation provide *J. wickhami* the ability to maneuver on “sticky plants”. Assessing the mechanics of *J. wickhami* locomotion on sticky plant surfaces may help elucidate other potential mechanisms used by insects for movement, like those reported by Voigt et al. (2007) and Voigt and Gorb (2010, 2008).
Figure A.1 *Jalysus wickhami* adult walking across yellow sticky trap. Photo by P.N. Nelson.
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Appendix B: Carrion entrapment review bibliography


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Appendix C: Olfactory attraction assays

(LoPresti et al., 2015) reported *Aquilegia eximia* Van Houtte ex Planch. attracts arthropods passing by, luring them to their death when they are trapped by glandular trichomes. Chemical and morphological features of glandular trichomes on *Nicotiana tabacum* L. and its relatives are involved in host acceptance, ovipositional behavior, and prey location by natural enemies (Elsey & Chaplin, 1978; Jackson et al., 1989; Weinhold & Baldwin, 2011). We performed field experiment to determine if *Nicotiana tabacum* produced olfactory cues that also attract arthropods on sticky plant surfaces to enlist predators for defense.

Experiments were performed in 2016, at the Upper and Lower Coastal Plain Research Stations. We used methodology similar to that described by LoPresti et al. (2015), testing the attractiveness of the following plants treatments: *N. tabacum* (glandular trichomes), *Gossypium arboreum* L. (aglandular trichomes), *Ipomoea batatas* L. (glabrous), and an empty control. Briefly, we placed plant material (five 10 mm discs excised haphazardly from leaf centers) in 90 mm diameter plastic petri dishes and covered them with plastic tulle mesh coated in Tanglefoot (Contech; Victoria, British Columbia, Canada). Petri dishes were placed on the ground one meter apart and at least 10 meters from any plant treatment crops. The petri dishes were deployed for 24 hrs after which the number of arthropods entrapped in the coated mesh were assessed in a lab. The entire experiment was repeated on six different dates at each location with five replicate petri dishes per plant treatment. Data was analyzed in SAS v9.4 using PROC MIXED. Carrion entrapment data was log transformed and was analyzed using
the following model: plant tissue treatment as fixed effect and date and location as random effects.

No significant difference was detected between glabborous, aglandular, and glandular plant tissues and the empty controls \((F=0.68, 3, 230; P = 0.5679; \text{Fig. B.1})\). Our results do not reflect those of LoPresti et al. (2015). \textit{N. tabacum} and \textit{A. eximia} are unrelated and likely produce exudates with different chemicals that may not result in the same arthropod-plant interactions. Assessing whether carrion entrapment happens randomly in \textit{N. tabacum} or is the result of other sensory modalities is warranted in future studies.
Figure B.1 Arthropod entrapment on sticky petri dish traps.
Bibliography


