Estimating the impact of the European wool-carder bee, *Anthidium manicatum*, in the northeastern United States

A dissertation

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ABSTRACT

Invasive species can have devastating impacts on invaded communities - displacement of native species, disruption of ecosystem processes, and large economic costs. But many invasive species do not have significant impacts. It is therefore important to established direction (positive/negative) and magnitude of impact when assessing invasive species. Unfortunately, for most invasive species there are no studies on impact. This is particularly true for invasive, unmanaged bees. Therefore, this dissertation aims to fill this gap for the most widespread unmanaged bee in the world, *Anthidium manicatum* (the European wool-carder bee). Concerns have been raised about this bee in North America. Male *A. manicatum* defend flowers, and aggressive interactions can result in severe injury or death to native pollinators. Female *A. manicatum* are polylectic with low floral constancy, making them poor pollinators. They also remove plant trichomes for nesting material. Due to these concerns, we measured impact in five ways: (1) impact of female *A. manicatum* nesting behavior on the plant *Stachys byzantina*, (2) role of naïveté in interactions between a native pollinator, *Bombus impatiens*, and *A. manicatum*, (3) impact of *A. manicatum* on *B. impatiens* foraging behavior and fitness, (4) impact of *A. manicatum* on fitness of a forage crop, *Vicia villosa*, and (5) habitat suitability of *A. manicatum*. We provide evidence that female *A. manicatum* cause chemical changes in *S. byzantina* when they remove trichomes, which attracts other *A. manicatum,* leading to additional carding damage. We also provide evidence that *A. manicatum* may benefit from interspecific naïveté, and that male *A. manicatum* impact *B. impatiens* through interference competition and female *A. manicatum* impact *B. impatiens* through exploitative competition. Additionally, we provide evidence of a decrease in fitness components for *Vicia villosa* associated with *A. manicatum*. Finally, we modeled habitat suitability for *A. manicatum* and found an association between *A. manicatum* and developed habitat. Taken together, these results provide strong evidence that invasive *A. manicatum* are having a negative impact on the native community; however, the magnitude of impact is likely low, as we found no fitness effects in *B. impatiens*, and *A. manicatum* were rarer across invaded habitat than predicted.

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Lastly, I would like to thank the bees. Many of you have sacrificed your life for this dissertation, and I hope your sacrifice will provide valuable knowledge to help other pollinators down the road.

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CHAPTER 1

Introduction

With increasing globalization, the world is vastly more connected. This allows the transfer of people and goods to flow across borders and oceans at unprecedented rates (Ricciardi et al. 2013). But with this connectivity comes the unintended introduction of non-native species. The most troubling of these introductions are those of exotic invasive species. Exotic invasive species (hereafter referred to just as invasive) are defined here as introduced, non-native species that are established, are increasing their invaded range, and are having an impact on the native community (Kolar and Lodge 2001, Keller et al. 2011).

Many introduced species never make it to invasive species status (Williamson and Brown 1986). At each stage along the invasion pathway many species will fail to progress to the next stage (reviewed in Keller et al. 2011). The invasion process starts when an individual of a species or a population enter a pathway of introduction (cargo shipments, ballast water, etc.). This is often human-mediated, and can be through accidental or purposeful transport. Once the individual or population arrives in non-native territory, the species is considered introduced. Many introduced individuals or populations will perish before or at this stage. However, some species will become established. Finally, a species becomes invasive if it moves beyond establishment and expands its range while having measurable environmental, economic, or human health impacts (Keller et al. 2011).

Some species invasions have resulted in devastating consequences. On the International Union for Conservation of Nature's (IUCN) red list of threatened species, over 50% of species extinctions are considered at least in part due to invasive species, with 20% of species extinctions blamed exclusively on invasive species (Clavero and García-Berthou 2005). But many introduced species do not become invasive, and do not have negative impacts. It is therefore unsurprising that the field of invasion ecology has attempted to find common factors among successful invasions that might help predict when invasions will occur, and what impact invasions will have.

Predicting invasion

Since Elton formalized much of invasion ecology theory in 1958 with his book "The Ecology of Invasions by Animals and Plants", the field has focused on finding common traits that correlate with invasion - either traits of invasive species, or traits of invaded habitat. For instance, there is evidence that behavioral flexibility of non-native species increases the chances of successful invasion. In a study of non-native birds, brain size (associated with behavioral flexibility) was a positive predictor of invasiveness (Sol et al. 2005). Generalist behavior for food or habitat selection have also been correlated with invasive potential (Holway et al. 2002, Jeschke and Strayer 2006, Blackburn et al. 2009, Schweiger et al. 2010, Beggs et al. 2011). These findings are largely unsurprising, as they provide the invader with the ability to take advantage of novel resources and habitats.

Efforts have also focused on defining characteristics of highly invaded habitats. One popular hypothesis is that less diverse habitats and/or those with high disturbance are more vulnerable to invasions – the biotic resistance hypothesis (Elton 1958). It is believed that these habitats will have more vacant niches for invasive species to inhabit. However, there is no substantial empirical evidence to support this hypothesis, other than perhaps at small scales (Keller et al. 2011, Henriksson et al. 2016). Alternative habitat correlates of invasibility have also been tested, such as abiotic conditions of the habitat (habitat matching hypothesis), community saturation (niche opportunity hypothesis), and presence of key species and their relationship to the invader (species identity hypothesis); however, none have garnered substantial support (Henriksson et al. 2016). The weighted species richness hypothesis, which considers both species richness and presence of key species in a habitat, may provide a more promising avenue of research in predicting habitat vulnerability, though further testing of this hypothesis is needed (Henriksson et al. 2016).

Ultimately, most hypotheses involving the use of species or habitat traits for predicting invasions have had low or varying support (Simberloff 2009). Alternatively, the use of propagule pressure for predicting invasions has garnered substantial support (Lockwood et al. 2005, Simberloff 2009). A propagule is one introduction event. Propagule size indicates how many individuals are in each propagule. Together, propagule number and propagule size make up propagule pressure – the number of individuals introduced and how many discrete introductions there are (Lockwood et al. 2005). The theory of propagule pressure essentially proposes that many introductions with many individuals involved in each introduction will aid in invasion success, and this theory has largely been supported (Sakai et al. 2001). This makes logical sense, as more introductions increases the chances of success, and more individuals will decrease the chances of failure due to the natural vulnerabilities of small populations (e.g. Allee effects) and their susceptibility to demographic and environmental stochasticity (Simberloff 2009).

Estimating impact

Invasive species impacts can be economic and/or ecological. Economic impacts include degradation of ecosystem functioning, and impaired aesthetics of natural habitats. There is an entire body of literature devoted to assigning monetary value to invasive species impacts. For instance, invasive species are estimated to cost the United States between 131-185 billion dollars per year (reviewed in Marbuah et al. 2014). While these economic consequences are important when considering the threat of non-native species, the focus here will be on ecological impacts.

Ecological impacts include measurable changes in properties of native species (behavior, physiology, etc.), populations, communities, or ecosystems (Ricciardi et al. 2011). All introduced species interact with their environment, and therefore have some ecological impact (hereafter only referred to as ‘impacts’). But impacts can vary greatly in magnitude and whether they are positive or negative (Blackburn et al. 2014). Unfortunately, determining impacts of invasive species has been a significant challenge for the field of invasion ecology (see Jeschke et al. 2014). For most invasive species, there exists no quantitative information on impact. This is particularly troubling, as some more well studied invasive species have significant negative impacts, such as native species extirpation or extinction, or ecosystem and community transformations (Simberloff et al. 2013, Blackburn et al. 2014). Predicting which invasive species will have high magnitude, negative impacts is therefore essential for conservation purposes.

Arguably, the invaded range of an invasive species is one of the best correlates of impact magnitude. Species with large ranges will interact with a greater number of native species across a greater proportion of native species' habitat (Ricciardi et al. 2011). One of the problems with using invaded range to estimate overall impact (magnitude and directionality), however, is that an invader's impact can be variable across its invaded habitat. This variability can be due to differences in the invader's abundance, its functional role across changing abiotic environments, and/or interactions with local species (Ricciardi et al. 2011). It is therefore important to measure impact on different levels of ecological complexity (individuals, populations, communities, and ecosystems) and at different spatial and temporal scales to fully evaluate the impact of an invasive species (Blackburn et al. 2014). However, this can often be very resource and time consuming.

Given limited resources available to evaluate the vast number of exotic species introductions, anything that increases our ability to predict impacts is very valuable. Some species traits do appear to correlate with directionality of impact. For instance, novel species that use resources differently from native species are more likely to be high impact invaders (Saul and Jeschke 2015). An example of this comes from aquatic systems, where invaders entering a community without congeners were more likely to displace native species (Ricciardi and Atkinson 2004). These findings suggest that highly novel invaders should therefore be prioritized for evaluation.

Gaps in knowledge

Invasive insects have received disproportionately less attention in the literature than invasive plants, vertebrates, or aquatic organisms (Kenis et al. 2009). Exotic bees and their impacts have been particularly under studied (Goulson 2003). Nonetheless, significant concerns have been raised about the impact of exotic bees as competitors with native bees, their role in spreading weedy plants, spread of parasites and pathogens to native species, and hybridization (reviewed in Russo 2016).

The majority of studies related to exotic bees have focused on exotic managed pollinators, namely honey bees (*Apis mellifera*) and bumble bees (*Bombus* spp.), though even this body of research is relatively limited, as there remain few studies that quantitatively determine impact. Whereas a few studies have experimentally tested the impact of managed pollinators (Thomson 2004, 2006, Kenta et al. 2007), the majority of studies have been correlational (*Apis mellifera*: Kato et al. 1999; Dupont et al. 2004; Paini and Roberts 2005; Thomson

2016. *Bombus terrestris*: McQuillan and Hingston 1999; Nagamitsu et al. 2010). Most correlational studies compare diversity or abundance of native bees before and after arrival of an exotic bee. Though correlational studies alone should be used with caution when estimating impact of an invasive species, as other factors may have led to a shift in community composition. Manipulative studies, which specifically measure the impact of invasive species presence on native species, therefore provide much stronger evidence of direct impacts. However, absence of quantitative evidence does not mean absence of impact. Goulson (2003) notes the gap in literature on estimating impact of invasive bees is likely due to challenges with executing interspecific competition studies. Interspecific competition is the most commonly cited hypothetical impact of exotic bees. Therefore, manipulative, interspecific competition studies represent an area of research that needs to be expanded.

For more than two thirds of exotic bees there have been no empirical studies to determine impact (for a review of exotic bees see Russo 2016). For non-*Apis/Bombus* species, research on impact is even scarcer, with impact studies on only 8 species out of the 67 known non-*Apis/Bombus* exotic bees (Russo 2016). For those that have been studied, there is evidence of nesting site and floral resource competition, spread of pathogens and parasites, and assisted spread of invasive weeds (Russo 2016). But much is still left unknown about the impact of exotic, unmanaged bees, which is unfortunate, as these represent the majority of exotic bee introductions (Russo 2016).

Scope of this dissertation

The next four chapters of this dissertation begin to illuminate the impact of one unmanaged invasive bee, *Anthidium manicatum* (the European wool-carder bee). *A. manicatum* is the most widespread unmanaged bee in the world (Strange et al. 2011). Native to Europe, western Asia and northern Africa, *A. manicatum* is now nearing worldwide distribution with invasive populations in North America, South America, east Asia, New Zealand and the Azores (Strange et al. 2011, Soper and Beggs 2013, Weissmann et al. 2017). *A. manicatum* are solitary, cavity nesting bees in the family Megachilidae.

Unlike most bees, male *A. manicatum* are generally larger than female *A. manicatum* (Severinghaus et al. 1981). Males display resource defense, with the largest males defending floral territories. Smaller males typically do not defend territories, but instead wander between them. Females visit floral territories for nectar, pollen, and nesting material, so larger male territory owners gain access to significantly more receptive females compared to smaller males (Starks and Reeve 1999). Mating is promiscuous, with both males and females mating multiply, with no extended associations (Lampert et al. 2014). However, the last male to mate with a female before she lays eggs obtains a significant fertilization advantage (Lampert et al. 2014).

A. manicatum are generalist foragers, but are most commonly found foraging at plants in the families Lamiaceae, Scrophulariaceae, and Leguminosae (Severinghaus et al. 1981, Payette 2001), with a strong association with exotic ornamental plants common to residential gardens in North America (Miller et al.

2002, Maier 2009). *A. manicatum* are bivoltine, with 80 days of activity on average in temperate regions (Wirtz et al. 1992, Payne et al. 2011).

Though hypothetical impact of *A. manicatum* has been widely suggested (Strange et al. 2011, Colla 2016, Russo 2016) there have been no empirical studies addressing impact in North America. For instance, there are concern over male *A. manicatum* territorial defense. Male *A. manicatum* use spines at the base of their abdomen to ram into heterospecific pollinators attempting to gain access to defended flowers (Haas 1960, Pechuman 1967, Severinghaus et al. 1981, Wirtz et al. 1988, Starks and Reeve 1999). These aggressive interactions can lead to severe injury or death to attacked bees in their native range (Wirtz et al. 1988).

Concerns over female *A. manicatum* have also been raised. Female *A. manicatum* are generally considered poor pollinators as they are polylectic and show low floral constancy. There is evidence that they will transfer heterospecific pollen at higher rates than do many native pollinators, potentially leading to fitness consequences for plants (Soper and Beggs 2013). Additionally, female *A. manicatum* may serve as significant resource competitors with native pollinators. Within *A. manicatum*'s native range, *Bombus* spp. have been shown to avoid flowers previously visited by *A. manicatum* females at higher rates than flowers previously visited by conspecifics or congeners (Gawleta et al. 2005), though the mechanism behind this behavior is unknown.

Given concerns over *A. manicatum* invasion, my dissertation serves as the most complete assessment of the impact of *A. manicatum* in North America, to date. In the following chapters, I investigate impact of *A. manicatum* on two plants (*Stachys byzantina*, and *Vicia villosa*), and a native pollinator (*Bombus impatiens*). In the final chapter, I model habitat suitability for *A. manicatum* in the northeastern United States to better estimate *A. manicatum*'s current and future range.

CHAPTER 2

Title:

The European wool-carder bee (*Anthidium manicatum*) eavesdrops on plant volatile organic compounds (VOCs) during trichome collection.

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Abstract

The plant-pollinator relationship is generally considered mutualistic. This relationship is less clear, however, when pollinators also cause tissue damage. Some Megachilidae bees collect plant material for nests from the plants they pollinate. In this study, we examined the relationship between *Anthidium manicatum*, the European wool-carder bee, and the source of its preferred nesting material – *Stachys byzantina,* lamb's ear. Female *A. manicatum* use their mandibles to trim trichomes from plants for nesting material (a behavior dubbed "carding"). Using volatile organic compound (VOC) headspace analysis and behavioral observations, we explored (a) how carding effects *S. byzantina* and (b) how *A. manicatum* may choose specific *S. byzantina* plants. We found that removal of trichomes leads to a dissimilar VOC bouquet compared to intact leaves, with a significant increase in VOC detection following damage. *A. manicatum* also visit *S. byzantina* plants with trichomes removed at a greater frequency compared to plants with trichomes intact. Our data suggest that *A. manicatum* eavesdrop on VOCs produced by damaged plants, leading to more carding damage for individual plants due to increased detectability by *A. manicatum*. Accordingly, visitation by *A. manicatum* to *S. byzantina* may incur both a benefit (pollination) and cost (tissue damage) to the plant.

Keywords:

Communication; cue; herbivore; pollinator; recognition; signal.

Introduction

Insect damage can cause cascading chemical changes within plants that help with healing and protect against further herbivore damage (Karban and Myers 1989). However, these chemical changes are not just within the plant. Plants can change the volatile organic compounds (VOCs) they emit in response to damage, which can affect the surrounding community of plants, herbivores, and beneficial plant visitors such as predatory and parasitic wasps (Pare and Tumlinson 1999, Holopainen and Gershenzon 2010). Here, we investigate a relatively understudied plant-insect relationship - flowering plants and Hymenopteran visitors that can act as both mutualists and antagonists.

Many Hymenoptera have evolved to use changes in plant VOCs for their benefit. For example, predatory and parasitic wasps can use changes in VOCs as a way to locate lepidopteran hosts (reviewed in Pare and Tumlinson 1999). Herbivore damage induces changes in plant VOCs, either causing an increase in production of VOCs, or a change in compounds emitted. Wasps have evolved to use this plant response as a signal for presence of potential prey (predatory wasps) or hosts (parasitic wasps) (Turlings et al. 1990). Following herbivore damage, plants such as corn and cotton have been shown to produce VOCs unique to herbivore damage that are distinguishable from background odors. The release of these VOCs coincides with periods when parasitoids are most likely to be foraging, contributing to recruitment of beneficial parasitoids by the plant (Turlings et al. 1995).

Bees foraging for nectar and pollen have also evolved the ability to use plant VOCs as a signal of resource quality. However, unlike predatory and parasitic wasps, foraging bees generally associate these cues with a lower quality resource (pollen and nectar), and avoid these herbivore damaged plants (Mothershead and Marquis 2000, Kessler and Halitschke 2007, 2009). For instance, wild tomato flowers (*Solanum peruvianum*) were visited less frequently by pollinators following both real herbivore damage and a jasmonic acid treatment to induce plant response to herbivore damage (Kessler et al. 2011).

Accordingly, changes in plant VOCs may be repellent or attractive to Hymenoptera, largely based on what they are searching for. Generally, we expect changes in plant VOCs following damage to be repellent to Hymenoptera seeking floral resources (typical plant-pollinator relationship), but attractive to Hymenoptera in search of prey or hosts (typical plant-parasitoid relationship). Furthermore, we would expect VOCs produced by damaged plants to be an evolved signal to attract beneficial parasitoids that can lower herbivore loads. Conversely, we would expect a change in plant VOCs to be a cue for pollinators (non-directed passive transfer of information) since changes in VOCs are often repellent to beneficial pollinators (Bradbury and Vehrencamp 2001, Smith and David 2003).

Plant-pollinator interactions are often thought of as mutually beneficial, but the relationship can be complicated when the pollinator also causes damage to the plant. These opposing roles can occur during different life stages of the pollinator; caterpillars might consume plant tissues but later pollinate the same plant as adults (Pettersson 1991, Thompson and Pellmyr 1992, Kessler et al. 2010). Conflicts can also occur during the same life stage. Mason bees, leaf-cutter bees, and other members of the family Megachilidae serve as pollinators, but their collection of nesting material can damage the plants they visit.

Here, we focus on one member in the family Megachilidae, *Anthidium manicatum*, that collects plant trichomes for nest construction. Trichomes are small glandular hairs used in plant water regulation (Fahn 1986), herbivore deterrence, and storage of VOCs (Levin 1973). *A. manicatum*, the European wool-carder bee, is a solitary bee species whose common name derives from the females' "carding" behavior – female bees cut plant trichomes with their mandibles, ball them up (a behavior reminiscent of carding wool), and fly them back to a cavity where they will use the trichomes to line their nest (Müller et al. 1996, Payne et al. 2011). The relationship between *A. manicatum* and their trichome sources is complicated. A local population of *A. manicatum* can both fill the role of pollinator and cause damage to leaf tissue through collection of trichomes from the same plants they visit for floral resources.

We chose *Stachys byzantina*, lamb's ear, as our focal plant for this study because *S. byzantina* has dense glandular trichomes (Salmaki et al. 2009). It is also the most commonly cited source of trichomes for *A. manicatum* nesting material (Severinghaus et al. 1981, Wirtz et al. 1988, Payette 2001, Miller et al. 2002, Gibbs and Sheffield 2009, Hicks 2011, Payne et al. 2011, Strange et al. 2011, Garbuzov and Ratnieks 2014). *S. byzantina* are also commonly visited by *A. manicatum* for food resources, possibly providing a significant contribution to the plant's reproductive fitness (Severinghaus et al. 1981, Payette 2001). Predicting how *S. byzantina* may have evolved to respond to *A. manicatum* damage is therefore complicated, as attraction of *A. manicatum* could yield both a benefit and a cost to the plant. Likewise, understanding how *A. manicatum* may have evolved to use VOCs produced by *S. byzantina* is also of interest, as the upregulation of VOCs may be either attractive or repellent.

Here, we explore this complicated plant-pollinator relationship, by specifically looking at the relationship between *A. manicatum* and *S. byzantina* as a source of nesting material. First, we investigated if mechanical carding damage changes the VOC output of *S. byzantina*. We performed headspace VOC collection and used GC-MS and GC-FID for identification and relative quantification of VOCs. Second, we determined how trichome damage impacted *A. manicatum* visitation to *S. byzantina*. We quantified the distribution of bee carding damage in semi-natural *S. byzantina* populations, and compared visitation rates of *A. manicatum* to damaged (trichomes removed) versus undamaged (trichomes intact) *S. byzantina* plants. Through these studies, we aimed to gain a clearer understanding of the plant-pollinator relationship; specifically, we wished to better understand the relationship when the pollinator also serves a role more commonly filled by plant pests.

Methods

Collection and analysis of VOCs released by mechanically carded Stachys byzantina *plants*

We compared differences in VOCs emitted between mechanically carded and uncarded *S. byzantina* leaves. Eleven *S. byzantina* plants of approximately equal size were purchased from a local plant nursery. All plants were checked for general health and lack of previous bee carding damage. We then collected and analyzed headspace VOCs from both mechanically carded and uncarded leaves, as well as from ambient air (negative control), at the University of New England (Biddeford, ME). Volatile headspace collections were taken from a mechanically carded region (one stalk consisting of about five leaves, two of which were mechanically carded) and compared to volatile headspace from an uncarded region (one stalk consisting of about five leaves) on the same plant (see Fig. 2.1). Mechanical carding was performed using a razor blade (Fig. 2.2). Two healthy leaves, one younger and one older, were chosen haphazardly and the trichomes on the top surface of their leaves were removed using the razor blade. We confirmed similarity of mechanical damage to natural carding damage by visual comparison under a dissection microscope; however, mechanical carding can only be assumed as a representation of actual *A. manicatum* carding damage. Additionally, *A. manicatum* carding is found most commonly on the underside of leaves, though also found on the tops of leaves; however, for mechanical carding, only the trichomes on the top of the leaves were removed due to the delicate nature of the underside of the leaf.

To collect headspace volatiles, each stalk was enclosed in a clear PET plastic cup with an open dome lid and a volume of 850 ml. Purified air was allowed to flow into the chamber through the bottom at a rate of 850 ml/min. At the top of the chamber, we inserted a volatile collection trap containing 50 mg of Super-Q adsorbent (Alltech Associates, Inc, Deerfield, IL). We then attached this trap to a vacuum pump pulling air through the trap at a rate of 850 ml/min for three hours. We extracted the filters with 100 µl dichloromethane, and 600 ng of nonyl-acetate was added as an internal standard. Samples were subsequently analyzed by Gas Chromatography-Mass Spectrometry (GC-MS) and quantified using Gas Chromatography-Flame Ionization Detector (GC-FID).

We analyzed all samples quantitatively on an Agilent Technologies model 7890A gas chromatograph equipped with model 7693 auto sampler (Agilent Technologies, Wilmington, DE) and flame ionization detection. One µl of each extracted sample was injected on-column and analyzed on an Agilent J&W HP-5 30m x 320µm x 0.25µm 19091J-413 capillary column (J&W Scientific, Folsom, CA) with helium as a carrier gas at a constant linear velocity of 32 cm·sec-1. The oven temperature was maintained at 40°C for 5 min and then increased at a rate of 5°C·min1- to 280°C and held for 5 min. The injector temperature was set to track the oven and the detector temperature was 300°C. Data were analyzed on MSD ChemStation DS software (Agilent). Estimated abundance was calculated by comparison to the internal standard.

For qualitative analyses, the same GC was connected to an Agilent Technologies MS5975C mass spectrometer operating in electron impact mode. We analyzed one µl of each sample using split less injection at 250°C. An Agilent J&W HP-5MS 30m x 250µm x .25µm column was used with helium as a carrier gas at a constant velocity of 40 cm·sec-1. The oven temperature was maintained at

40°C for 5 min and then increased at a rate of 5°C·min1- to 280°C and held for 5 min. The transfer line temperature was set to at 280°C and the ion source temperature to at 230°C. Data were analyzed on MSD ChemStation DS software. We identified VOCs by comparison of mass spectra with spectra in the Wiley 9th and NIST 11 MS Library, and spectra obtained of authentic compounds. We also compared GC retention times of VOCs with GC retention times of the authentic compounds on the HP-5MS column.

Comparison of the patterns of VOC composition between treatments was performed using multivariate analysis. A data matrix of pairwise Bray-Curtis dissimilarity indices between samples was built, and nonmetric multidimensional scaling (NMDS) (R package vegan) was used to visualize patterns of dissimilarity (see similar methods in Soler et al. 2012). NMDS finds the best two-dimensional representation of the distance matrix, allowing for visualization of grouping between treatments. A Permutational Multivariate Analysis of Variance (PERMANOVA) was then used to test the null hypothesis - no difference between treatments (mechanical carding and no carding). The PERMANOVA was based on 1000 permutations, and is nonparametric (with only one factor). Similarity percentage (SIMPER) was then used to identify which compounds were responsible for differences between treatments. All analyses were performed using R version 3.3.1 (R Core Team 2016).

Within-plant distribution of bee carding damage on Stachys byzantina

We assessed 54 *S. byzantina* plants for within plant distribution of bee carding damage. We visited five plant nurseries in eastern Massachusetts between June and July 2012. All *S. byzantina* plants at each nursery were checked for carding damage; however, only “large” plants with visible carding damage were included in the study (39 plants total) due to the required minimum number of leaves needed in our analysis (18 mature leaves; see Fig. 2.3). We identified three “reference” leaves on each plant: an uncarded leaf, a minimally carded leaf (only one carding track, “singly carded”), and a heavily carded leaf (two or more carding tracks, “multiply carded”; see Fig. 2.3). Selection of references leaves was almost entirely random. Most of the bee carding damage is on the undersides of leaves. Therefore, on approach to the plant, we could not visually assess damage before turning over leaves (at random). Reference leaves were identified as the first leaf we found on the plant to have the level of damage characterized above. The five closest leaves surrounding the reference leaf were then checked for carding damage, without any overlap of leaf groupings, and the damage on these five leaves was recorded.

We used generalized linear mixed models to compare the number of bee carded leaves occurring around each type of reference leaf. The response variable, carding damage on the five leaves surrounding a reference leaf, was considered as a binomial response (0 – uncarded leaf, 1 – carded leaf) for each of the five leaves. We included type of reference leaf as the factor of interest, and individual plant and nursery location were included as random effects. The most parsimonious model was chosen through comparison of AICc scores (Table S2.1). If competing models were within 2.0 ΔAICc, the simplest model was chosen. Both the marginal R^2 (R^2GLMM(m)) and the conditional R^2 (R^2GLMM(c)) are reported, as calculated in (Nakagawa and Schielzeth 2013). The marginal R^2 describes the proportion of variance explained by the fixed factor alone. The conditional R^2 describes the proportion of variance explained by both the fixed and random factors (Nakagawa and Schielzeth 2013). Data had a binomial distribution, and we used a logit link function. All analyses were performed using R version 3.3.1 (R Core Team 2016).

"Small" *S. byzantina* plants, those with fewer than 18 mature leaves, could not be used in the analysis above, but were still checked for bee carding damage. Percent of total leaves with any carding damage was recorded. This gave us an estimate of average carding damage per plant in semi-natural populations.

Anthidium manicatum *attraction to mechanically carded versus uncarded* Stachys byzantina *plants*

To further test if carding damage is attractive to *A. manicatum*, we observed differences in visitation rates to mechanically carded *S. byzantina* plants versus uncarded *S. byzantina* plants. We performed 32 trials at seven locations at or around Tufts University in Medford, MA. Locations were picked based on presence of foraging *A. manicatum* and presence of *Nepeta cataria* (catmint), a preferred plant for pollen and nectar collection (Payette 2001; pers. obs.). These locations were considered optimal observation areas as they were likely to have high traffic of *A. manicatum* to the area.

Trials were carried out between August and September, 2014 between 11:00-16:00h on days with no precipitation when the temperature was between 21-32°C. This ensured that observations were done when the bees were at their most active (Couvillon et al. 2010). For each trial, we placed two non-flowering *S. byzantina* plants of similar size at the testing location. Because we were focused on visits related to nest material gathering (females) or looking for mates (males) only non-flowering plants were used. Before trials, *S. byzantina* plants were housed in the Tufts University greenhouse and had no prior carding damage. Plants ranged in size but typically had approximately 20-25 leaves. One *S. byzantina* plant was haphazardly chosen to be mechanically carded; approximately 25% of its leaves were carded with a razor. Only the tops of the leaves were carded to minimize damage to the delicate backs of the leaves. Both the underside and tops of leaves are naturally carded by *A. manicatum*. Twenty-five percent was chosen as it is the average amount of bee carding damage we found naturally on “small” *S. byzantina* plants in local plant nurseries (see previous methods). The plants were placed about 1.5 meters from each other, and equidistant from the focal flowering plant (catmint). We observed visitation and behavior of *A. manicatum* near the *S. byzantina* plants for thirty minutes from about 1.5 meters away.

During the testing period, we recorded the number of visits by *A. manicatum* to each *S. byzantina* plant. A visit was counted whenever an *A.*

manicatum came in close proximity to the test plant (generally within 15 cm) and hovered over or landed on the plant. We included hovering behavior at the test plant as a visit because male *A. manicatum* spend most of their time in flight (80% of observed time; Wirtz et al., 1988). They generally hover to inspect flowers, conspecifics, heterospecifics, or look for mates (Severinghaus et al. 1981). We would therefore expect both hovering (males) and direct lands (females) to indicate attraction to the plant.

Unfortunately, differentiation between female and male *A. manicatum* was often not possible due to the speed of visitations and relative similarity in appearance between small males and females. Therefore, because it could not be determined every time, sex of the visitor was not included in the analysis. Testing was repeated at each site (four or five times), but at least 24 hours apart. The possible effect of pseudo-replication from the same individual visiting several times could not be completely removed from the study, particularly for territorial males who are likely to visit several times if guarding that area. However, we do not anticipate this significantly effecting the data as trials were done at seven locations far enough apart that an established territorial male would not be guarding multiple locations.

We used generalized linear mixed models (R package lme4) to compare number of visits to mechanically carded *S. byzantina* plants to number of visits to uncarded plants. Model response variable was number of *A. manicatum* visits, the fixed effect was treatment, and location was included as a random effect. Due to the paired design of the experiment, trial ID was also included as a random effect.

Models used a Poisson distribution with a log link function. The same parameters were then used to compare number of bee carding events during the trial period between carded and uncarded plants. The same methods were used to select the most parsimonious model as described previously (Table S2.1). All statistical analyses were performed using R version 3.3.1 (R Core Team 2016).

Results

Measure of VOCs released by mechanically carded Stachys byzantina *plants*

We were able to identify ten compounds detected from *S. byzantina* headspace collection, as well as tentatively identify three more (Table 2.1). Most of the VOCs were green leaf volatiles or terpenes. The NMDS (stress = 0.064) showed that the VOCs detected from mechanically carded leaves were significantly dissimilar compared to those detected from uncarded leaves (PERMANOVA; F=8.96, df=1, p<0.001) (Fig. 2.4). Abundance of five compounds explained most of the dissimilarity between carded and uncarded leaves: β-pinene (72.5% dissimilarity), *(Z)*-3-hexenol (61.1%), homosalate (49.4%), β-cubebene (37.2%), and *(Z)*-3-hexenyl acetate (19.7%). It should also be noted that the NMDS analysis grouped the VOCs from carded leaves, while VOCs from uncarded leaves were much more scattered (Fig. 2.4). Overall, there was an 83.9% dissimilarity between treatments, with greater abundance of VOCs detected in the mechanically carded treatment (Table 2.1). Generally, there were also more unidentified VOCs detected from carded leaves compared to uncarded leaves (Fig. S2.1).

Within plant distribution of bee carding damage on Stachys byzantina *plants*

Analyses showed that the bee carded reference leaves (both singly and multiply carded) had significantly more bee carded leaves around them than the uncarded reference leaves ($R^2GLMM(m) = 0.03$, $R^2GLMM(c) = 0.42$; $X^2 = 18.63$, df = 2, $p < 0.001$; Fig. 2.5). Given the amount of carding damage on each plant, it is unlikely that it resulted from one individual making several return trips. However, extensive damage from one individual cannot be ruled out.

Anthidium manicatum *visits to mechanically carded versus uncarded* Stachys byzantina *plants*

More *A. manicatum* visits were made to plants with mechanical carding compared to uncarded plants ($R^2GLMM(m) = 0.15$, $R^2GLMM(c) = 0.77$; $X^2 = 44.64$, df = 1, $p < 0.001$, Fig. 2.6). The simplest model within 2.0 ΔAIC removed location ID as a random effect. However, due to the territorial behavior of male *A. manicatum,* location is an important effect to control for. We would expect locations with territorial males to have higher visitation rates compared to locations without a territorial male. Therefore, we kept location as a random effect included in the model (as this model also was within the 2.0 ΔAIC threshold) (Table S2.1a).

There was no difference in occurrences of carding by *A. manicatum* between plants that were mechanically carded (7) and those that were uncarded (4) ($R^2GLMM(m) = 0.00$, $R^2GLMM(c) = 0.76$; $X^2 = 0.80$, df = 1, $p = 0.372$).

Discussion

We have shown that *S. byzantina* leaves with their trichomes removed (mechanically carded) released a significantly different bouquet of VOCs compared to leaves with their trichomes intact (uncarded). Four of the five compounds that explained the most dissimilarity have previously been associated with changes in Hymenoptera behavior: *(Z)*-3-Hexenyl acetate (Whitman and Eller 1990, Bruinsma et al. 2009), β-cubebene (Patricio et al. 2004, Belz et al. 2013), *(Z)*-3-hexenol (Whitman and Eller 1990, Turlings et al. 1995), and β-pinene (Hoebeke et al. 2011).

Additionally, we provide evidence that *A. manicatum* carding damage is grouped in distribution among semi-natural populations of *S. byzantina*, with more carding occurring near leaves with carding damage. However, we should make note that the marginal R^2 value for this model was relatively low, indicating that only 3% of the variability is explained by the factor (reference leaf) alone. The conditional R^2 value is higher (0.42) suggesting between plant variation and between location variation was high. However, plant variability is also suggestive of an attractive effect of carding damage. As you might expect damage in one area of the plant to increase attractiveness (and therefore damage) of the entire plant if a change in VOCs is attractive. Furthermore, results of our behavioral trials also support our hypothesis that a change in VOCs is attractive, as more *A. manicatum* visited mechanically carded *S. byzantina* plants than uncarded *S. byzantina* plants.

Together, our results support the hypothesis that *A. manicatum* carding damage induced changes in *S. byzantina* VOCs, and that these chemical changes are likely to account for the increase in attractiveness of carded plants to other *A. manicatum*. Furthermore, the VOC profile of carded treatments was similar across individual plants. This supports the idea that *A. manicatum* can use this consistent cue when searching for nesting material or mates by forming a VOC template for *S. byzantina*.

An alternative hypothesis is that *A. manicatum* are using visual cues to identify plants with or without carding damage. However, we don't think this is likely given the amount of carding damage on the undersides of leaves. Additionally, it's not clear what advantage carded plants would have for *A. manicatum*. Using VOCs to identify plants more easily therefore seems like a more likely explanation for increased visitation at carded plants.

The differences that may exist between mechanical carding damage and bee damage, however, remains unknown. While the mechanical removal of trichomes is visually similar to removal of trichomes by bees, additional effects that bee damage may have on the plant cannot be accounted for. For instance, bees may leave a chemical cue on the plants they visit that can be recognized by other bees (e.g. scent marking; Gawleta et al. 2005), or perhaps leave behind a signal that is changing the chemistry of the plant (e.g. insect saliva's manipulation of plant defensive chemicals; Musser et al. 2006b). However, our data support the attractive role of VOCs in both damage types (mechanical and bee carding); the intricacies of how these two types of damage influence both *S. byzantina* and *A.*

manicatum remains to be explored.

A. manicatum's exploitation of plant VOCs is not a unique trait among Hymenoptera (De Moraes et al. 1998, Kessler and Baldwin 2001, Kessler and Halitschke 2007, Dicke and Baldwin 2010, Kessler et al. 2011). However, the relationship between *A. manicatum* and *S. byzantina* does not fit the typical plant-pollinator model. Individual *A. manicatum* can be both a pollinator and a pest to the same plant. Since *A. manicatum* are attracted to previously carded *S. byzantina,* this system seems to follow trends more similar to plant-parasitoid systems, rather than plant-pollinator systems where changes in VOCs are usually repellent (Mothershead and Marquis 2000, Kessler and Halitschke 2007, 2009).

Attraction of predatory or parasitic wasps often benefit the plant through direct reduction in herbivores. In contrast, attraction of *A. manicatum* following plant tissue damage does not appear to bring any similar benefits; in fact, emitting VOCs that attract *A. manicatum* will likely increase the amount of damage a plant sustains due to additional *A. manicatum* carding. *A. manicatum* visitation is also unlikely to significantly increase the plant's reproductive fitness through pollination services. *A. manicatum* are generally considered poor pollinators (Soper and Beggs 2013) and *S. byzantina* often reproduce clonally (Legkobit and Khadeeva 2004). Therefore, since the benefits (pollination services) to the plant in attracting *A. manicatum* are minimal at best, a change in VOCs by the plant likely did not evolve as a signal in response to *A. manicatum*.

Instead, *A. manicatum* have likely evolved to eavesdrop on plant signals intended for other uses, e.g. tissue damage repair, beneficial parasitoid attraction, or defense priming (Turlings et al. 1995, Heil and Karban 2009). (*Z*)-3-hexenyl acetate is commonly associated with herbivore damage in many systems, and its production is thought to increase plant defense (Loughrin et al. 1994, Röse et al. 1996, 1998, De Moraes et al. 2001, Rodriguez-Saona et al. 2002). This supports the idea that *S. byzantina* is emitting VOCs, such as (*Z*)-3-hexenyl acetate, in response to the damage caused by *A. manicatum*, not due to an evolved benefit of attracting *A. manicatum*. This interaction could therefore be considered eavesdropping, as the receiver (*A. manicatum*) likely gains a benefit while the sender (*S. byzantina*) does not.

The benefit to *A. manicatum* in eavesdropping is relatively straightforward. Due to increased production of VOCs following damage, *A. manicatum* can likely detect VOCs from a damaged plant more easily than those given off by an undamaged plant. Female *A. manicatum* would therefore benefit by using this cue as a way to reduce their search time for nesting material. Male *A. manicatum* would greatly benefit by using this cue in association with female *A. manicatum* presence. Similar to parasitoid wasps, if male *A. manicatum* associate a change in plant VOCs with presence of female *A. manicatum*, they should be able to decrease their search time for potential mates. However, what effect increased visitation by *A. manicatum* (male or female) has on *S. byzantina* can only be speculated.

Trichomes are important for plant water regulation (Fahn 1986) and herbivore deterrence (Levin 1973). Anecdotally, we have noticed that herbivore damage is common on naturally carded sections of *S. byzantina* plants. We therefore hypothesize that removal of trichomes would incur a fitness cost to the plant; however, direct effects of trichome removal on the plant should be examined. A change in VOCs is also likely to decrease flower attractiveness to other pollinators, which would be an additional cost (Mothershead and Marquis 2000, Kessler and Halitschke 2007, 2009).

This study explores the relationship between *A. manicatum*, a world-wide invasive species (Strange et al. 2011, Russo 2016), and *S. byzantina*, its most commonly cited source of nesting material. Much is still left unknown in this system – fitness effect of carding damage to plants, importance of pollination services provided by *A. manicatum*, and importance of specific VOCs in both plant fitness and *A. manicatum* attraction, to name a few. Given the prevalence of *A. manicatum* (Strange et al. 2011) and *S. byzantina* throughout temperate regions, we propose this system as one particularly well suited for further exploration of this type of complicated plant-pollinator relationship.

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Figures

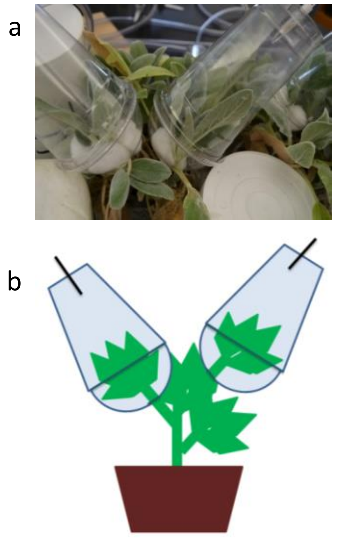

Figure 2.1. Headspace VOC collection on one *S. byzantina* plant. (a) Picture; (b) Schematic. VOCs were collected from eleven plants total. Each plant had both treatments – VOCs collected from a mechanically carded and an uncarded region.

Figure 2.2. Mechanical removal of trichomes on *Stachys byzantina* using a razor blade. Only the trichomes from the tops of the leaves were removed to avoid damage to the leaf tissue on the more irregular undersides of the leaves.

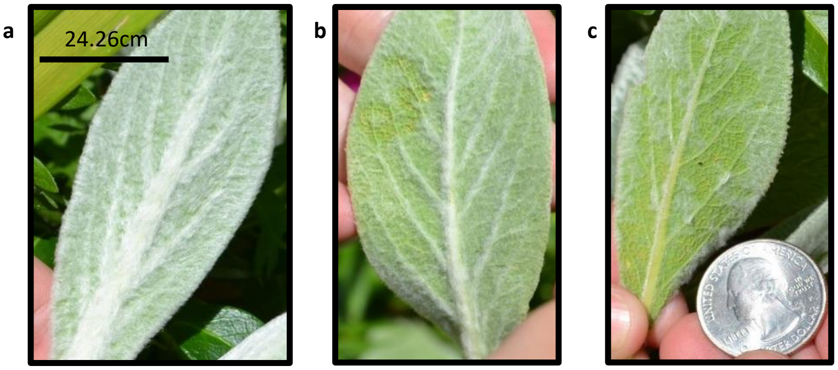

Figure 2.3. Carding distribution methods. Three reference leaves were chosen on each *Stachys byzantina* plant – (a) uncarded, (b) singly bee carded and (c) multiply bee carded. Five surrounding leaves were then checked for damage around each reference leaf, with no overlap between leaf groupings.

Table 2.1. Identified volatile organic compounds (VOCs) detected through headspace collection of *Stachys byzantina* leaves that underwent two treatments – mechanical removal of trichomes (carded) or no manipulation (uncarded).

Collections ran for three hours and VOCs were quantified and qualified through GC-MS and GC-FID. Non-metric multi-dimensional scaling (NMDS) was used to visualize differences between the relative proportions of VOCs detected from the two treatments (carded and uncarded leaves) (Fig. 2.4). Similarity percentage (SIMPER) was then used to determine contribution of each VOC to dissimilarity between treatments.

				NMDS	
VOC	**Classification**	**Carded (ng over 3 hours)**	**Uncarded (ng over 3 hours)**	**Average contribution to dissimilarity**	**SD of contribution**
(Z)-3-Hexenyl acetate	Green leaf volatile	372.2	16	0.165	0.110
β-cubebene*	Sesquiterpene	603.5	33.27	0.146	0.138
Homosalate*	Ester of Salicylic acid	183.4	24.64	0.103	0.112
(Z)-3-Hexenol	Green leaf volatile	202.5	3.91	0.098	0.084
β-pinene	Monoterpene	321.7	43.45	0.095	0.088
(+) valeranone*	Sesquiterpenoid	167.6	24.55	0.053	0.029
α-pinene	Monoterpene	141.7	30	0.044	0.043
(E)-2-Hexenal	Green leaf volatile	100.5	1	0.038	0.039
Limonene	Monoterpene	146	2.45	0.035	0.034
Hexyl acetate	Green leaf volatile	45.7	1.64	0.021	0.017

(Z)-2-Hexenal	Green leaf volatile	36.4	1	0.019	0.018
(E)-2-Hexenol	Green leaf volatile	26.2	1.45	0.012	0.012
(E)-2-Hexenyl acetate	Green leaf volatile	19	1.09	0.008	0.006

*Tentative identification

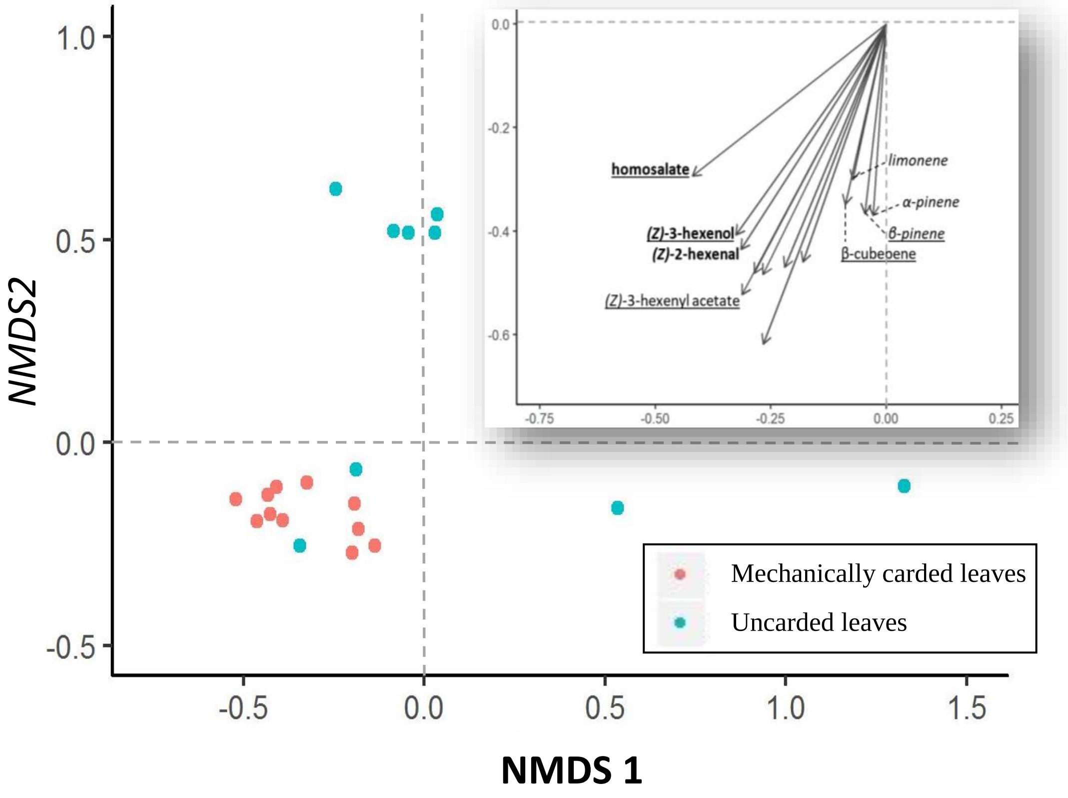

Figure 2.4. Non-metric multi-dimensional scaling (NMDS) of the relative proportions of VOCs detected under two treatments – uncarded and mechanically carded *Stachys byzantina* leaves. NMDS based on Bray-Curtis dissimilarity index (stress = 0.064). Inset graph shows average VOC contribution to dissimilarity (no points under the inset picture). Compounds in bold were the most important

loadings for NMDS1, and compounds in italics were the most important loadings for NMDS2. Underlined compounds were the top five compounds driving dissimilarity between the treatments.

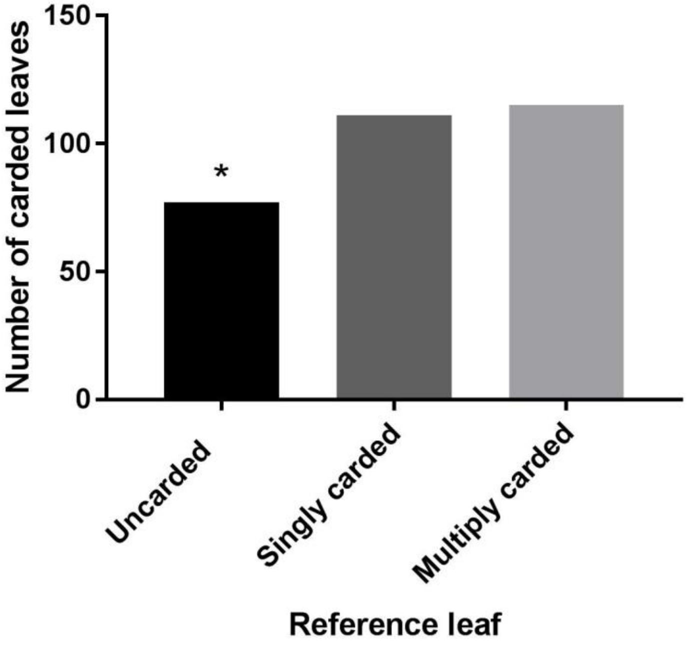

Figure 2.5. Number of carded leaves around each type of reference leaf. There were significantly fewer total carded leaves around an uncarded reference leaf than either type of carded reference leaf (singly or multiply carded) (GLMM; $X^2 = 18.63$, df = 2, *$p < 0.001$, 54 plants).

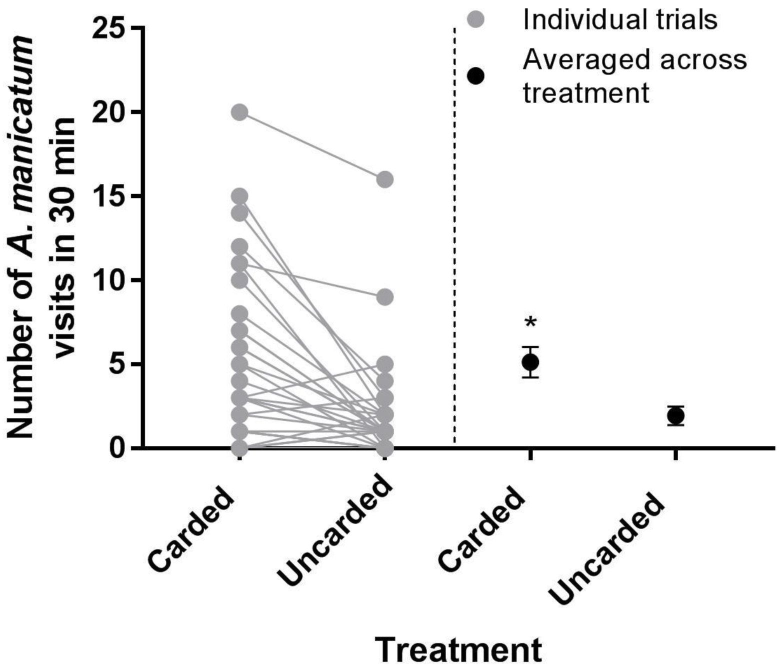

Figure 2.6. Average number of *A. manicatum* visits to mechanically carded *S. byzantina* versus uncarded *S. byzantina*. Each observational trial lasted 30 minutes. Each trial is represented by the grey dots and lines, with almost all trials have more visits at the carded plant than the uncarded plant. Means by treatment are shown by the black dots, with standard error. Overall, there were significantly more *A. manicatum* visits to mechanically carded *S. byzantina* than uncarded *S. byzantina* (GLMM; $X^2 = 44.65$, df = 1, *$p < 0.001$).

Supplementary Material

Table S2.1. Generalized linear mixed models were used to determine effect of fixed factors on response variables. Random effects were included and model fit was determined by comparison of AIC scores. Analyses were performed using R version 3.3.1, with package lme4.

A. Within plant distribution of bee carding damage on *S. byzantina* plants			
Model rank	**Fixed effects**	**Random effects**	**ΔAICc**
1	Reference leaf	Plant ID	0.0
2	Reference leaf	Plant ID, Location	1.1
3	*Null*	Plant ID	15.0
4	*Null*	Plant ID, Location	16.0
5	Reference leaf	Location	99.3
6	*Null*	Location	110.4
7	Reference leaf	-	137.2
8	*Null*	-	147.3
B. *A. manicatum* visits to mechanically carded versus uncarded *S. byzantina* plants			
Model rank	**Fixed effects**	**Random effects**	**ΔAICc**
1	Treatment	Location, Trial	0.0
2	Treatment	Trial	1.0
3	*Null*	Trial	46.6
4	Treatment	Location	63.3
5	Null	Location	108.8
6	Treatment	-	120.2
7	*Null*	-	165.8
C. *A. manicatum* carding events on mechanically carded versus uncarded *S. byzantina* plants			
Model rank	**Fixed effects**	**Random effects**	**ΔAICc**

1	*Null*	Trial	0.0
2	Treatment	Trial	1.4
3	Treatment	Location, Trial	3.7
4	*Null*	Location	11.6
5	Treatment	Location	13.0
6	*Null*	-	13.2
7	Treatment	-	14.5

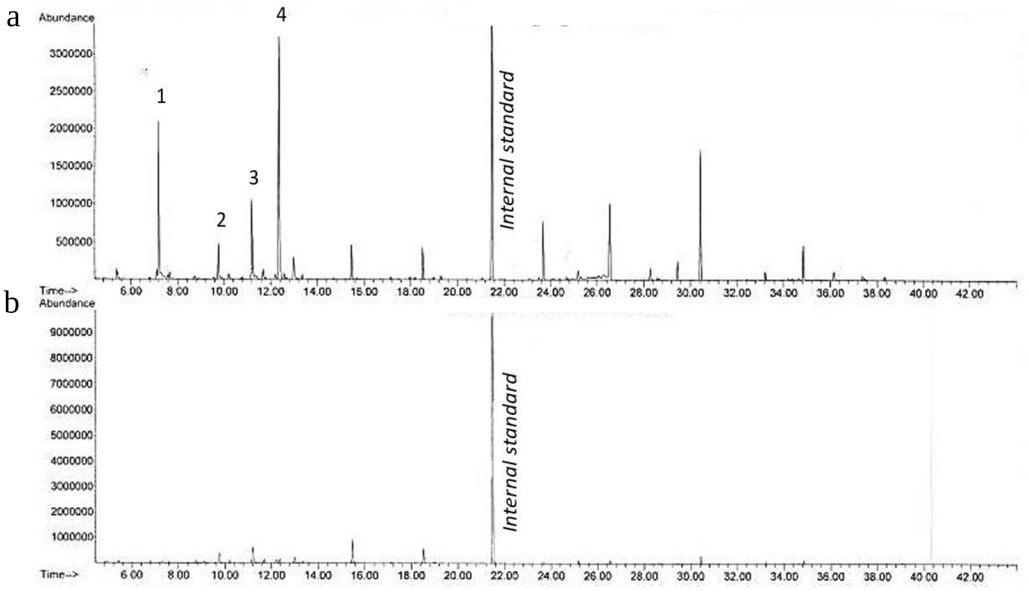

Figure S2.1. Gas chromatography – mass spectrometry (GC-MS) reading for headspace VOCs collected from one *S. byzantina* plant. (a) Mechanically carded leaves; (b) Uncarded leaves. Reading is from one plant, but results are representative of all eleven plants included in the analysis. Identified VOCs include - 1: (*Z*)-3-hexenol; 2: α-pinene; 3: β-pinene; 4: (*Z*)-3-hexenyl acetate.

CHAPTER 3

Title:

Presence of the invasive bee, *Anthidium manicatum*, causes both direct and indirect effects on the common eastern bumble bee, *Bombus impatiens*

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Abstract

Exotic predators can have direct effects (consumptive effects) and indirect effects (non-consumptive effects) on naïve prey. Here, we look at an invasive bee that may similarly cause both fatal and non-fatal effects for native pollinators. *Anthidium manicatum* was first discovered in North America in 1963, and male *A. manicatum* aggressively defend floral territories for mating opportunities. They attack heterospecific pollinators, often resulting in severe injury or death (i.e. fatal effects). Additionally, female *A. manicatum* are likely significant resource competitors to native pollinators (i.e. non-fatal effects). Here, we provide the first manipulative study to determine impact of *A. manicatum* presence on native pollinators. Using screened enclosures, we observed interactions between *A. manicatum* and a native pollinator, *Bombus impatiens.* We found evidence for both fatal effects (*B. impatiens* forager death) and non-fatal effects (avoidance behavior) depending on if male or female *A. manicatum* are present. Our data provide evidence of fatal effects when male *A. manicatum* are present, and evidence of non-fatal effects when either male or female *A. manicatum* are present. *B. impatiens* colonies exposed to male *A. manicatum* had significantly fewer workers than colonies exposed to only female *A. manicatum*, displayed avoidance of *A. manicatum* while foraging, and ultimately had low reproductive output. In colonies exposed to female *A. manicatum*, *B. impatiens* foragers also avoided *A. manicatum*, and had low reproductive output. Our results provide the first quantitative evidence of *A. manicatum* negatively effecting native pollinators in North America.

Keywords: invasive species, pollinator decline, *Bombus impatiens*, behavior, fitness.

Introduction

As global commerce grows, the movement of species outside their historic ranges increases (Lodge 1993, Vitousek et al. 1996). The negative impact of exotic species on native ecosystems has been well documented; competition or predation from exotic species is the primary source of risk for 400 out of 958 species listed as threatened or endangered under the Endangered Species Act (Wilcove et al. 1998, Pimentel et al. 2000). Exotic predators have a disproportionate effect on native communities (Elton 1958, Williamson 1996, Gurevitch and Padilla 2004). They can have both direct effects (consumptive effects [CE]) and indirect effects (non-consumptive effects [NCE]). Here, we define CE as those effects that lead to immediate prey death, and NCE as those effects that might have downstream fitness consequences, such as anti-predator behavior.

Under the naïve prey hypothesis, prey are expected to respond ineffectively to novel predators due to a lack of evolutionary history (Sih et al. 2010). In these cases, they are likely to suffer high CE. However, naïve prey can often respond effectively to a novel predator, either because the novel predator is similar to a native one, or they have a general anti-predator response. This response can therefore decrease CE within the population, but may increase NCE (e.g. hiding, fleeing, etc.). High NCE can still have long term fitness effects on the prey population (e.g. missed foraging or mating opportunities, expenditure of energy, etc.) (Sih et al. 2010). We believe this framework can be useful when considering the effects of an invasive bee, *Anthidium manicatum,* whose behavior may cause similar direct and indirect effects on a naïve, native pollinator (*Bombus impatiens*). We define invasive as an introduced, exotic species which is established, increasing its invaded range, and having an impact on the community (Kolar and Lodge 2001, Keller et al. 2011).

Anthidium manicatum, the European wool-carder bee, is the most widespread unmanaged bee in the world (Strange et al. 2011). Native to Europe, *A. manicatum* was first documented in North America in the early 1960s in Ithaca, NY (Jaycox 1967). Since then, it has rapidly expanded its range across the continent (Gibbs and Sheffield 2009, Strange et al. 2011). This range expansion alone is concerning (Wirtz et al. 1988, Strange et al. 2011, Colla 2016), but it's behavior is what makes this species a particularly noteworthy invader.

A. manicatum is a solitary bee that uses a resource defense mating system (Haas 1960, Pechuman 1967, Severinghaus et al. 1981, Starks and Reeve 1999). Within a defended floral territory, males discourage foraging by heterospecifics through direct attacks that often result in severe injury or death to the encroaching pollinator (similar to CE). Heterospecific injuries are most commonly sustained during aerial altercations where male *A. manicatum* use five spines at the base of their abdomen to fracture the wings of other pollinators (Wirtz et al. 1988). In *A. manicatum*'s native range, the most common heterospecific recipients of attacks are honey bees (*Apis mellifera)* and bumble bees (*Bombus* spp.), and these species will avoid foraging in floral territories that are well defended by male *A. manicatum* (similar to NCE) (Wirtz et al. 1988). So, within this system we would predict effects similar to CE, classified here as fatal effects [FE] (direct attacks leading to pollinator death) and effects similar to NCE, classified here as non-fatal effects [NFE] (avoidance of floral resources due to presence of *A. manicatum*). However, while male *A. manicatum* territorial behavior has been observed in North America (Severinghaus et al. 1981, Starks and Reeve 1999), the effects of these attacks on native pollinators have never been studied.

Pollinators native to northeastern North America do not have a long evolutionary history with *A. manicatum*, or similarly aggressive species. *A. manicatum* were first observed in the early 1960s, but were not considered invasive in the region until 2011 (Strange et al. 2011). We would therefore consider native pollinators relatively naïve, and predict that many native species will not respond appropriately to territorial male *A. manicatum*, and suffer high FE as a result. Alternatively, if native species do respond appropriately and avoid foraging near *A. manicatum*, they could suffer high NFE due to missed foraging opportunities (Sih et al. 2010). Additionally, female *A. manicatum* may also cause NFE for heterospecific pollinators, specifically bumble bees (*Bombus* spp.).

Bumble bees (*Bombus* spp.) and honey bees (*Apis mellifera*) use odor cues deposited on flowers by previous visitors to avoid visiting flowers depleted of resources. Odor cues are not species specific, allowing for bumble bees to avoid flowers recently visited by honey bees, and vice versa (Stout and Goulson 2001).

There is also evidence that a similar cue is used by bumble bees to avoid flowers recently visited by *A. manicatum* females. Within *A. manicatum*'s native range, bumble bees (*Bombus terrestris*) will specifically avoid flowers visited by female *A. manicatum*, more so than flowers visited by conspecifics or congeners (Gawleta et al. 2005). In North America, *A. manicatum* forage at many of the same plants used by native bumble bees (Severinghaus et al. 1981, Payette 2001). We would therefore expect the presence of female *A. manicatum* to elicit a similar avoidance behavior from North American bumble bees. Unlike the response to male *A. manicatum*, we would not expect naïveté to play a role in avoidance of resources visited by female *A. manicatum*, as scent cues appear to be non-species specific, or at least recognizable across species.

We chose to focus on *A. manicatum*'s effect on bumble bees (*Bombus* spp.) due to their predicted high rate of interaction within the natural environment (Wirtz et al. 1988, Soper and Beggs 2013) and the importance of bumble bee pollination services (Cameron et al. 2011, Drummond 2012, Barfield et al. 2015, Wilson et al. 2016). Additionally, bumble bee populations are showing decline worldwide, with several species in North America showing a population decline of up to 96% when compared to historic abundance data (Colla and Packer 2008, Goulson et al. 2008, Grixti et al. 2009, Cameron et al. 2011). One of the greatest contributors to bumble bee decline is habitat loss and resource shortage (Colla and Packer 2008, Grixti et al. 2009). We therefore want to explore if *A. manicatum* invasion could be causing additional resource constraints. Particularly since *A.*

manicatum might therefore cause cryptic habitat fragmentation in otherwise well suited native pollinator habitat.

Resource shortage can have significant fitness consequences for bumble bees (*Bombus* spp.). Colonies will produce significantly fewer reproductives (males and future queens) under resource constraints (Pelletier and McNeil 2003, Thomson 2004, 2006, Colla and Packer 2008, Elbgami et al. 2014). Loss of foragers through interactions with male *A. manicatum* (FE) and/or avoidance of floral resources due to presence of male or female *A. manicatum* (NFE) could therefore have a significant effect on a colony's reproductive fitness.

Loosely following the predator-prey naïveté framework, we made predictions on the effects of male versus female *A. manicatum* on native, naïve bumble bees (*B. impatiens*) (Table 3.1). To test these hypotheses, we used screened enclosures where the exposure of bumble bees to *A. manicatum* could be manipulated. We then tested *B. impatiens* under three different *A. manicatum* treatments: male *A. manicatum* only, female *A. manicatum* only, and both male and female *A. manicatum.* Additionally, to test the effect of naïveté, we tested *B. impatiens* response to *A. manicatum* at an initial exposure, and a second exposure to *A. manicatum*.

Following the naïveté hypothesis, we predicted that *B. impatiens* would not respond appropriately (would not avoid male *A. manicatum*) during the initial exposure to male *A. manicatum*, and suffer high FE (forager death). However, individual bumble bees (*Bombus* spp.) have shown a strong aptitude for learning, and changing their foraging behavior provided sufficient personal experience and/or social transmission of information (Jones et al. 2015, Alem et al. 2016, Dunlap et al. 2016). Therefore, we predicted that *B. impatiens* would change their foraging tactics to avoid interactions with male *A. manicatum* during a second exposure. This would decrease FE but increase NFE. Since we are predicting learning at the individual forager level, we would expect this change in behavior to occur within an individual's lifetime (~36 days in lab reared colonies) (Jandt and Dornhaus 2009).

We would not, however, expect similar effects when exposed to female *A. manicatum*. Due to bumble bees' ability to detect scent marks left by heterospecifics, we predicted that *B. impatiens* would avoid foraging near female *A. manicatum* at the initial exposure (high NFE). This is because flowers in enclosures with female *A. manicatum* are likely to be more heavily depleted of resources compared to when only male *A. manicatum* are present, as females collect substantial amounts of both pollen and nectar. We therefore also expect this effect to remain during the second exposure. It is less clear what response will occur when both male and female *A. manicatum* are present.

NFE were determined by recording use of the enclosures by *B. impatiens* foragers when *A. manicatum* were present and comparing to when *A. manicatum* were absent from enclosures. FE were determined by comparing the number of adult foragers in each colony following exposure to *A. manicatum*, and reproductive fitness was compared between colonies to measure the downstream effects of FE and NFE.

Methods

Study organism

The common Eastern bumble bee (*B. impatiens*) was specifically chosen because it is commercially available. While the complete rearing history of *B. impatiens* populations used for commercial purposes is not known, we expect colonies to be naïve to *A. manicatum*, given commercial housing and short evolutionary history of *B. impatiens* and *A. manicatum* near rearing facilities (Howell, MI). While *B. impatiens* has not shown any population decline, it is used as a proxy for effects on bumble bees more broadly; however, effects may not be universally translatable across the genus. *B. impatiens* colonies were ordered from Koppert Biological Systems Inc. (Howell, MI). Each with approximately 75 workers, a queen, and brood.

Experimental setup

Three screened research enclosures were set up at a field site on Tufts University campus (Medford, MA) to manipulate exposure rate of *B. impatiens* to *A. manicatum*. Previous work has shown normal behavior for *A. manicatum* in similar research enclosures (Payne et al. 2011), and we also saw a full suite of typical behaviors throughout the experiment. Each enclosure was composed of two pop-up tents (Playhut EZ Outdoor portable screen room; measuring 3 m x 3 m across, and 2.1 m tall) side by side (Fig. 3.1). Each enclosure had one bumble bee hive (*B. impatiens*) placed behind it. Bumble bees had access to both sides of the research enclosure through clear polyethylene tubes fixed to the hive entrance.

Tubes allowed access to both sides of the enclosure, as well as to the surrounding environment (a small meadow with wildflowers in an urban residential setting). Each side of the enclosure had flowering plants (*Nepeta* spp., *Lavandula* spp., and *Salvia* spp.) known to be visited by both *B. impatiens* and *A. manicatum*. Artificial nectar was also provided in equal amounts at feeding stations in each side of the enclosure to encourage use of enclosures (artificial nectar provided by Koppert Biological Systems Inc.). After *B. impatiens* colonies were installed at the field site (23 June 2014), they were allowed one week to acclimate during which time artificial nectar was available within the hive as well (supplied by Koppert Biological Systems Inc. at the base of the hive). At the end of the week, *B. impatiens* workers from all three colonies were foraging in the enclosures as well as in the surrounding environment. At that time, we removed the nectar bags from the base of the hive, but nectar was still available at feeding stations in the enclosures.

Comparing B. impatiens *use of enclosures when exposed to* A. manicatum

We began the treatment regime with data collection on 30 June 2014. To establish a baseline assessment of *B. impatiens* foraging levels within each enclosure, no *A. manicatum* treatment occurred on week one (non-treatment week: no *A. manicatum* present). Number of *B. impatiens* present in each enclosure, on each side, was recorded four times a day (9:00, 11:00, 13:00, and 15:00), five days a week. All *B. impatiens* in the enclosure were counted whether they were actively foraging, or resting at the time of the count.

Week two was the initial exposure of *B. impatiens* to *A. manicatum*. Three to five *A. manicatum* were maintained in side A of each enclosure according to applied treatments (Enclosure 1: 3-5 male *A. manicatum*, Enclosure 2: 3-5 female *A. manicatum*, and Enclosure 3: 3-5 (total) male and female *A. manicatum*) (Fig. 3.1). Three to five *A. manicatum* were chosen as an appropriate treatment as this is a typical number of *A. manicatum* observed within a floral territory of similar size to the enclosure (pers. obs.). *A. manicatum* were only added to one side of the enclosure during treatment weeks to mimic a patchy distribution of *A. manicatum* within the environment (as might be expected during early invasions), and to ensure enough floral resources were available to avoid colony failure. Again, during week two, the number of *B. impatiens* in each enclosure was recorded four times a day, which was used as an assessment of avoidance behavior (a decrease in *B. impatiens* use of enclosures would indicate avoidance of *A. manicatum,* or high NFE).

At the end of week two, all *A. manicatum* were removed from enclosures. Week three was another non-treatment week (no *A. manicatum* present) to see if avoidance behavior changed following removal of *A. manicatum*. Week four was the second exposure of *B. impatiens* to *A. manicatum* according to the same treatments as in week two; however, this time *A. manicatum* were only added to side B (Fig. 3.1). Again, avoidance behavior (NFE) was assessed by counting number of *B. impatiens* foragers in the enclosures. We used generalized linear mixed models (GLMM) (R package lme4) to compare number of *B. impatiens* in each enclosure across the four weeks of data collection. From the onset of the experiment there were significant enclosure side preferences by each colony. Colony 1 preferred side B throughout the experiment (with very few foragers entering side A), and colonies 2 and 3 preferred side A (with very few going into side B). Since this preference was unrelated to treatment, data from both sides of each enclosure were grouped for analyses. For example, during week two (first *A. manicatum* exposure), data from side A and B were averaged across the week (even though only side A had *A. manicatum* present). Data from weeks 1 and 3 were designated as non-treatment weeks (no *A. manicatum* present). Week 2 was exposure 1 (first *A. manicatum* exposure), and week 4 was exposure 2 (second *A. manicatum* exposure).

We used GLMMs to compare the effect of each treatment (non-treatment, exposure 1, or exposure 2) on number of *B. impatiens* foraging in the enclosure. Each enclosure was modeled separately. Random effects included data collection time, day, and side of enclosure (Table S3.1). Random effects were included to account for any differences in bee activity level during the day, or between days, as well as the biases *B. impatiens* had between side A and B in the enclosures. For all models (enclosures 1, 2, and 3), we used a Poisson distribution with a log link function. Model selection was done for each enclosure through comparison of AICc scores (Burnham and Anderson 2002) (Table S3.1). If competing models were within 2.0 ΔAICc, the simplest model was chosen. Both the marginal R^2 (R^2GLMM(m)) and the conditional R^2 (R^2GLMM(c)) are reported, as calculated in (Nakagawa and Schielzeth 2013). The marginal R^2 describes the proportion of variance explained by the fixed factor alone. The conditional R^2 describes the proportion of variance explained by both the fixed and random factors (Nakagawa and Schielzeth 2013). These analyses were performed using R version 3.3.1 (R Core Team, 2013).

Bumble bee colony fitness measurements

At the end of the four weeks of testing, bumble bees foraged in the enclosures (no *A. manicatum* present) and in the surrounding environment until reproductives were first observed (2.5 weeks after the end of testing). At that time, all hive entrances were closed at night, as most workers should be in the hive at night. The following day (13 August 2014), colonies were freeze-killed at -20°C and stored at -20°C until Fall 2015. In Fall 2015, colonies were brought to a cold room (5°C) and examined. Fitness components were compared between the three colonies.

To estimate FE (worker death) and downstream effects of NFE and FE (fitness effects), we compared number of adult workers and reproductives (males and queens) between the three colonies. We assumed one of the queens in each colony was the founding queen, so we only included queens beyond one as “new” reproductives. Average weights of males, queens and workers were determined. Males and queens were each weighed individually. Since we could not determine which queen was the original, we included all queens present for average weight. Workers were weighed in groups of ten, and the average weight per grouping was used (weight of 10 workers/10) to determine overall average worker weight per colony. Number of pupal-stage brood cells were also recorded.

We used a chi-square analysis to compare total workers between each colony at the time they were freeze-killed. A one-way ANOVA was used to compare average weight of workers between the three colonies. Number of brood cells per colony was compared using a chi-square analysis. We also used a chi-square analysis to compare number of reproductives between colonies. The null hypotheses for each test was that there would be no difference between colonies. These analyses were performed using GraphPad Prism 7.

Results

Bombus impatiens *avoidance behavior during initial and second exposure to* A. manicatum *treatments*

In Enclosure 1 (male *A. manicatum* treatment), there were significantly fewer *B. impatiens* in the enclosure during week 4 (second exposure) compared to the other weeks (R^2GLMM(m) = 0.06, R^2GLMM(c) = 0.81; $X^2 = 42.20$, df = 2, $p < 0.001$). *B. impatiens* did not avoid the enclosure during the initial exposure (week 2) but then we saw a significant drop in use of the enclosure during the second exposure (week 4) (Fig. 3.2a).

In Enclosure 2 (female *A. manicatum* treatment), there were significantly more *B. impatiens* in the enclosure in week 1 (no *A. manicatum*) compared to the following weeks (GLMM pseudo-$R^2 = 0.07$; $X^2 = 7.36$, df = 2, $p > 0.025$). There was a decrease in *B. impatiens* use of the enclosure during the first exposure to *A. manicatum* (week 2), followed by a further decrease in use in weeks 3 and 4 (Fig. 3.2b).

In Enclosure 3 (male and female *A. manicatum* treatment), there were significantly fewer *B. impatiens* in the enclosure during the second exposure (week 4) compared to non-treatment weeks (weeks 1 and 3 combined) (R^2GLMM(m) = 0.06, R^2GLMM(c) = 0.71; $X^2 = 26.88$, df = 2, $p < 0.001$). There was no decrease in use of the enclosure during the initial exposure (week 2) compared to the first non-treatment week. However, there was an increase in enclosure use by *B. impatiens* during the second non-treatment week (week 3) followed by a decrease in use during the second exposure (week 4) (Fig. 3.2c).

Fitness metrics for B. impatiens *colonies exposed to different treatments of* A. manicatum

At the end of the colony cycle, there were significantly fewer workers in Hive 1 (male *A. manicatum* treatment) compared to Hive 2 (female *A. manicatum* treatment) or Hive 3 (male and female *A. manicatum* treatment) ($X^2 = 29.45$, df = 2, $p < 0.001$), with the greatest number of workers in Hive 2 (Table 3.2). Average individual weight of workers in each colony did not follow the same trend. Workers in Hive 3 (male and female *A. manicatum* treatment), on average, weighed significantly more than did workers in Hive 1 or Hive 2 (F = 6.574, p = 0.004) (Table 3.2).

Hive 1 only had one new adult reproductive at the time it was freeze-killed (1 male), Hive 2 had two (2 males), and Hive 3 had four (2 males, 2 new queens). However, in Hive 2, two queen cells were in the pupal stage. Since they were clearly distinguishable from other brood cells, they were included as new queens, bringing the total number of reproductives in Hive 2 to four (2 males and 2 queens) (Table 3.2). Hive 2 had significantly more non-queen pupal stage brood cells compared to the other colonies ($X^2 = 20.6$, df = 2, $p < 0.001$) (Table 3.2). We were unable to distinguish between workers and males in the pupal stage; however, since all colonies were producing reproductives, it is likely that they were all males (Pelletier and McNeil 2003).

We calculated reproductive success of each colony using the equation RS = M + 3G (M = number of males, G = number of new queens) which accounts for the disproportionate effort of producing queens compared to males (Pelletier and McNeil 2003) (Table 3.2). Non-queen pupal cells were considered males (Pelletier and McNeil 2003). There was a significant difference in reproductive success between colonies, with Hive 2 (treatment: female *A. manicatum*) having a significantly greater RS ($X^2 = 24.585$, df = 2, $p < 0.001$; Table 3.2). We did not run any statistical analyses on average weight of reproductives, given the small numbers and inability to clearly distinguish between the original queen and new queens. However, weights of reproductives were comparable across treatments (Table 3.2).

Discussion

In this study, we measured both fatal effects (FE) and non-fatal effects (NFE) of male and female *A. manicatum* on a naïve heterospecific pollinator (*B. impatiens*). We (1) determined NFE by measuring avoidance behavior, (2) measured FE by counting number of adult workers following exposure to *A.*

manicatum, and (3) estimated downstream fitness effects of NFE and FE by comparing components of colony reproductive fitness. While this study serves largely as preliminary data due to low sample sizes, it is the first manipulative experiment showing the effects of *A. manicatum* on North American pollinators.

When exposed to male *A. manicatum*, our data support the naïve prey hypotheses. During the initial exposure to only males (Enclosure 1), *B. impatiens* did not respond appropriately (did not avoid interactions with *A. manicatum*). Use of the enclosure did not change. This likely led to increased causalities (high FE) from interactions with territorial males, which is supported by a significant decrease in number of adult workers at the end of the experiment compared to the colony only exposed to female *A. manicatum*. But, as predicted, *B. impatiens* did show significant avoidance behavior (high NFE) during the second exposure, providing support that *B. impatiens* can modify their response to male *A. manicatum* in a relatively short period of time (a couple weeks).

As predicted, exposure to female *A. manicatum* had significantly different effects on *B. impatiens*. Presence of female *A. manicatum* led to immediate avoidance of the enclosure by *B. impatiens* during the first exposure (high NFE). Bumble bees have been shown to avoid flowers due to heterospecific scent marking (Stout and Goulson 2001, Gawleta et al. 2005). Therefore, we expected similar avoidance of flowers visited by female *A. manicatum*, and no effect of naïveté. We also predicted no FE as only male *A. manicatum* perform territorial defense. This was supported by our data, as Hive 2 had significantly more adult workers at the end of the experiment compared to colonies exposed to male *A.*

manicatum. What was surprising is that avoidance behavior by *B. impatiens* was also seen during the second non-treatment week. It is possible that scent marking has more long lasting effects than predicting.

However, we did not find evidence of long lasting repellent scent marking when both male and female *A. manicatum* were present (Enclosure 3). There was a dramatic increase in use of the enclosure during the second non-treatment week, which we would not expect if scent marking explained the avoidance of Enclosure 2 during the third week (second non-treatment week). Enclosure 3 did otherwise support our predictions of general avoidance of *A. manicatum*, as evidenced by the significant increase in use of the enclosure during the second non-treatment week, and an intermediate number of adult workers at the end of the experiment (moderate FE predicted due to lower numbers of male *A. manicatum* compared to Enclosure 1). However, the role of naïveté in Enclosure 3 is less clear, as we would expect different responses to male versus female *A. manicatum*, specifically during the first exposure.

Long term effects of exposure to male and/or female *A. manicatum* did support our hypotheses. Though, we must caution again that results are preliminary due to low sample sizes. We predicted that all colonies would have decreased reproductive success due to lack of sufficient resources, either because of avoidance behavior (NFE), or fewer available foragers (FE). Results suggest this might be the case, as production of new queens was lower overall for all three colonies than would be expected (Cnaani et al. 2002). It has been previously reported that similar colonies of *B. impatiens* (also ordered from Koppert

Biological Systems) have around 2.4% of total population as queens at the end of the colony season (Cnaani et al. 2002). Our colonies had between 0-1.5% new queens, suggesting a lack of resources available for production of queens at the end of the colony life cycle. Additionally, our data suggest that FE (from interactions with male *A. manicatum*) pose the greatest constraint on resource acquisition, as reproductive fitness was lowest in the two colonies exposed to male *A. manicatum*.

Our data provide the first evidence that presence of *A. manicatum* in northeastern United States has a negative impact on a native pollinator (*Bombus impatiens*). Results suggest both fatal effects and non-fatal effects. However, future work with longer duration studies with more treatments (no *A. manicatum* treatment) should be completed to definitively conclude negative fitness effects for *B. impatiens* due to *A. manicatum* presence.

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Figures

Table 3.1 Predictions of direct (FE – fatal effects) and indirect (NFE – non-fatal effects) effects of treatment.

Treatment	**Effect on *B. impatiens* colony during initial exposure**	**Effect on *B. impatiens* colony during second exposure**	**Effect on *B. impatiens* colony fitness**
3-5 Male *A. manicatum*	No avoidance behavior - High **FE** (high worker fatality)	Avoidance behavior - High **NFE** (decreased resource acquisition)	Low numbers of adult workers, decreased reproductive fitness
3-5 Female *A. manicatum*	Avoidance behavior - High **NFE** (decreased resource acquisition)	Avoidance behavior - High **NFE** (decreased resource acquisition)	Decreased reproductive fitness
1-2 Male, and 2-3 Female *A. manicatum*	No avoidance behavior of males - Moderate **FE** (moderate worker fatality); Avoidance behavior of females - Moderate **NFE** (decreased resource acquisition)	High avoidance behavior of females and males - High **NFE** (decreased resource acquisition)	Intermediate number of adult workers, decreased reproductive fitness.

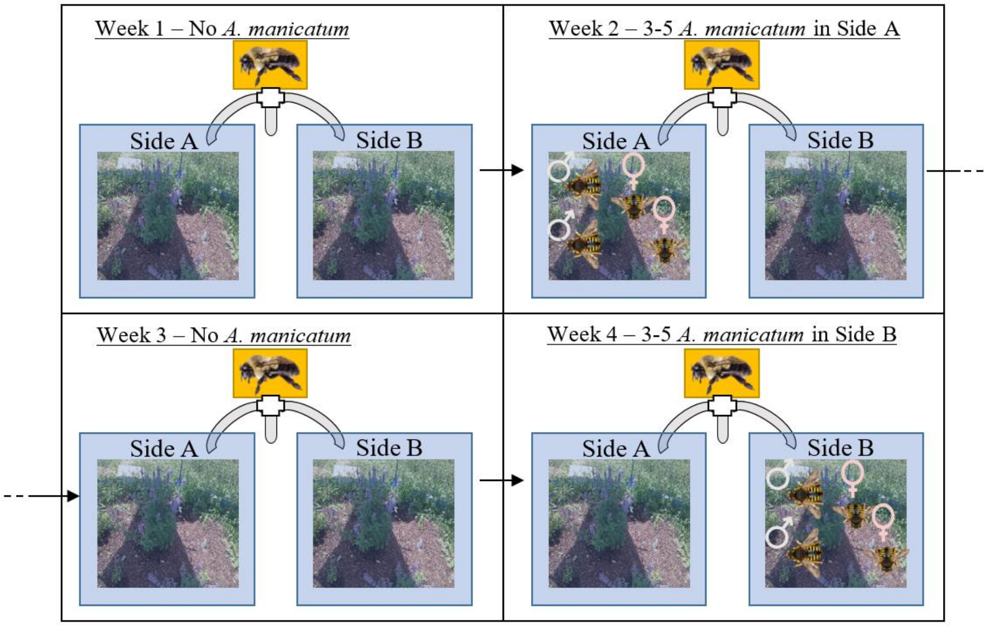

Figure 3.1 Schematic of Enclosure 3, across the four weeks of data collection. On week 1, no *Anthidium manicatum* were in the enclosure. On week 2, 3-5 male and female *A. manicatum* were maintained in side A. On week 3, no *A. manicatum* were in the enclosure. On week 4, 3-5 male and female *A. manicatum* were maintained in side B. Enclosures 1 and 2 followed the same schedule but with different *A. manicatum* treatments (Enclosure 1: 3-5 male *A. manicatum*; Enclosure 2: 3-5 female *A. manicatum*). Each enclosure had flowering plants preferred by both *A. manicatum* and *Bombus impatiens* (*Nepeta* spp., *Lavandula* spp., and *Salvia* spp.). The yellow box indicates placement of one *B. impatiens* hive (Koppert Biologicals, Inc.).

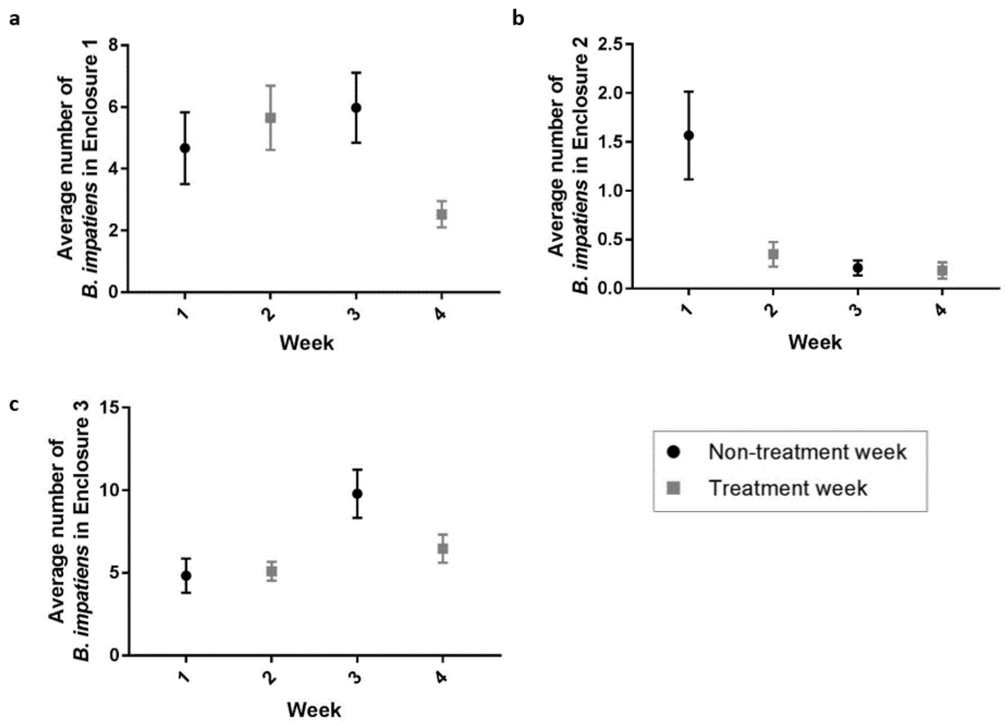

Figure 3.2 Average number of *Bombus impatiens* in enclosures across the four weeks of data collection. Number of *B. impatiens* in each enclosure were counted four times a day, five times a week and then averaged across the week. Black circles indicate non-treatment weeks (no *Anthidium manicatum* present), grey squares indicate a treatment week (**a**: Enclosure 1, male *A. manicatum*; **b**: Enclosure 2, female *A. manicatum*; **c**: Enclosure 3, male and female *A. manicatum*). Error bars are standard error of the mean.

Table 3.2 Colony demographics. Number of workers, males and queens were counted at the end of the colony life cycle. Average weights of workers, and adult reproductives were also measured. Reproductive success was determined based on the equation RS = M + 3G (M = number of males, G = number of new queens). Non-queen brood cells were considered males (Pelletier and McNeil, 2003).

	Hive 1 – male *A. manicatum*	**Hive 2 –** female *A. manicatum*	**Hive 3 –** male and female *A. manicatum*	**P value**
Number of workers	57	165	129	***P* < 0.001**
Average weight of workers (grams ± SEM)	0.036 ± 0.002	0.036 ± 0.001	0.046 ± 0.003	***P* = 0.004**
Pupal stage brood cells*	17	50	23	***P* < 0.001**
Pupal stage brood cells (queens)	0	2	0	-
Number of new adult reproductives	1 (male)	2 (males)	4 (2 males, 2 queens)	-
Average weight of males (grams ± SEM)	0.03	0.042 ± 0.003	0.022 ± 0.013	-
Average weight of queens (grams ± SEM)	0.202	0.189	0.153 ± 0.023	-
Reproductive success* RS = M + 3G	17	58	31	***P* < 0.001**

*RS includes brood cells as males (Pelletier and McNeil, 2003)

Supplementary material

Table S3.1 Generalized linear mixed models were used to determine effect of fixed factors (treatment and week) on response variable (number of *Bombus impatiens* in enclosure). Random effects were included and model fit was determined by comparison of AICc scores. Analyses were performed using R version 3.3.1, with package lme4.

Model Rank	**Fixed effect**	**Random effects**	**ΔAICc**
1	Enclosure 1 - *Treatment* (presence/absence of male *A. manicatum*)	*Time* of data collection, *Side* of enclosure	0.0
2		*Side* of enclosure	1.1
3		*Time* of data collection, *Day* of data collection, *Side* of enclosure	2.1
4		*Day* of data collection, *Side* of enclosure	3.2
5		*Time* of data collection	491.1
6		-	492.7
7		*Time* of data collection, *Day* of data collection	493.2
8		*Day* of data collection	494.8
9	*Null*	-	536.6
1	Enclosure 2 - *Treatment* (presence/absence of female *A. manicatum*)	-	0.0
2		*Side* of enclosure	0.4
3		*Day* of enclosure	1.8
4		*Day* of data collection, *Side* of enclosure	2.0
5		*Time* of data collection	2.1
6		*Time* of data collection, *Side* of enclosure	2.5
7	*Null*	-	3.2
8	Enclosure 2 - *Treatment* (presence/absence of female *A. manicatum*)	*Time* of data collection, *Day* of data collection	4.0
9		*Time* of data collection, *Day* of data collection, *Side* of enclosure	4.2
1	Enclosure 3 - *Treatment*	*Day* of data collection, *Side* of enclosure	0.0

2	(presence/absence of male and female *A. manicatum*)	*Time* of data collection, *Day* of data collection, *Side* of enclosure	2.2
3		*Side* of enclosure	40.8
4		*Time* of data collection, *Side* of enclosure	43.0
5		*Day* of data collection	230.4
6		*Time* of data collection, *Day* of data collection	232.6
7		-	268.8
8		*Time* of data collection	270.9
9	*Null*	-	286.0

CHAPTER 4

Title:

Measuring the impact of an invasive bee, *Anthidium manicatum*, on a native bumble bee, *Bombus impatiens*, and a forage crop, *Vicia villosa*.

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Abstract

Anthidium manicatum is an invasive pollinator reaching worldwide distribution that has the potential to have negative consequences for native pollinators and plants. Male *A. manicatum* aggressively defend floral territories keeping out heterospecific pollinators. Female *A. manicatum* are poor pollinators due to low floral constancy, likely causing high rates of heterospecific pollen deposition. Given the behavior of both male and female *A. manicatum*, we predicted presence of this invasive species to result in direct competition for resources with native pollinators, as well as fitness consequences for *A. manicatum* guarded or visited plants. Despite the potential for significant impacts, no previous studies have looked at fitness consequences in plants or heterospecific pollinators due to season-long exposure to *A. manicatum*. Therefore, across a two-year study, we monitored foraging behavior and measured the fitness of the common eastern bumble bee *(Bombus impatiens)* and the fitness of a forage crop (*Vicia villosa*) in response to *A. manicatum* presence. Using screened enclosures to manipulate the encounter rate of *A. manicatum* and *B. impatiens*, we monitored foraging behavior of *B. impatiens*, *B. impatiens* hive fitness (growth and production of reproductives), and fitness components (seed set and percent germination) of *V. villosa*. We found that *B. impatiens* avoided foraging near *A. manicatum* throughout the testing period (5-6 weeks) in both years. This provides evidence of significant interference competition. Despite this resource exclusion, we found no evidence of fitness consequences in *B. impatiens*. For *V. villosa,* the effect of *A. manicatum* presence is less clear cut. Data suggest reduced production of seeds for plants in association with *A. manicatum* (though not statistically significant, p = 0.067), and reduced germination rates (though this effect disappeared over time). Together, these results suggest *A. manicatum* pose as significant resource competitors, with potential fitness effects for associated plants.

Keywords: plant-pollinator network; exotic species; Bombus; Vicia.

Introduction

With increasing movement of goods and people around the world, introduction of exotic species is increasing at an unprecedented rate (Ricciardi et al. 2013). However, not all exotic species introductions lead to establishment, and even fewer lead to significant ecological impacts (Williamson and Brown 1986). Given limited resources available to combat species invasions, it is important to estimate impact of exotic species; however, determining ecological impact of exotic species has proven a challenge for the field (Ricciardi et al. 2013). This gap in knowledge has been particularly true for exotic pollinators (Goulson 2003). Goulson (2003) notes that this is likely due to challenges with executing interspecific competition studies, not due to lack of ecological impact. Those studies that have attempted to estimate ecological impact have been largely correlational (Kenis et al. 2009). The few studies that have experimentally tested the impact of exotic pollinators have focused on the introduction of managed pollinators such as *Bombus terrestris* and *Apis mellifera* (Thomson 2004, Kenta et al. 2007). The majority of introduced bees are unmanaged and solitary (Russo 2016), however, leaving a large gap in our understanding of how the majority of exotic bees impact invaded ecosystems. Here, we test the impact of the most widespread unmanaged bee in the world, *Anthidium manicatum,* on both a native pollinator (*Bombus impatiens*), and a common forage crop (*Vicia villosa*).

A. manicatum, the European wool-carder bee, is a solitary, cavity nesting bee native to Europe, western Asia, and northern Africa. *A. manicatum* is now nearing worldwide distribution with establishment in northeastern Asia, North America, South America, New Zealand, and the Azores (Strange et al. 2011, Soper and Beggs 2013, Weissmann et al. 2017). *A. manicatum* was first documented in North America in the early 1960s in Ithaca, NY (Jaycox 1967). Since then, it has rapidly expanded its range across the continent (Gibbs and Sheffield 2009, Strange et al. 2011). While this range expansion alone is concerning, its behavior has made it a particularly noteworthy invader (Strange et al. 2011, Colla 2016, Russo 2016).

A. manicatum males use resource defense to secure mating opportunities (Haas 1960, Pechuman 1967, Severinghaus et al. 1981, Starks and Reeve 1999). Within a defended floral territory, males discourage foraging by heterospecific pollinators through direct attacks that often result in severe injury or death to the encroaching pollinator. Well defended territories are more attractive to foraging female *A. manicatum* and allow male *A. manicatum* to secure more mating opportunities (Starks and Reeve 1999). Heterospecific injuries are most commonly sustained during aerial altercations where male *A. manicatum* can fracture the wings of other pollinators (Wirtz et al. 1988). *A. manicatum* attacks are relatively indiscriminate (Severinghaus et al. 1981), but the most commonly attacked heterospecifics are bumble bees (*Bombus* spp.) and honey bees (*Apis mellifera*) (Severinghaus et al. 1981, Wirtz et al. 1988, Soper and Beggs 2013). Here, we focus on the effect of *A. manicatum* interactions on a native bumble bee, *Bombus impatiens* (the common eastern bumble bee).

Bumble bees are some of the most important native pollinators of wild plants and agricultural crops in North America (Cameron et al. 2011, Drummond 2012, Barfield et al. 2015, Wilson et al. 2016). Unfortunately, bumble bee populations are showing decline worldwide, with several species in North America showing a population decline of up to 96% when compared to historic abundance data (Colla and Packer 2008, Goulson et al. 2008, Grixti et al. 2009, Cameron et al. 2011). Following a marked decrease in abundance, the first North American bumble bee, *B. affinis*, was recently designated endangered by the United States government (U.S. Fish & Wildlife Service 2017). Bumble bees (*Bombus* spp.) are particularly susceptible to localized extinction due to their life history traits (Colla and Packer 2008). Reproductives (males and future queens) are generally produced at the end of the colony life cycle, and resource shortage can lead to a significant decrease in number of reproductives produced (Pelletier and McNeil 2003, Thomson 2004, 2006, Colla and Packer 2008, Elbgami et al. 2014). There is substantial evidence that bumble bees will avoid foraging near *A. manicatum* in both *A. manicatum*'s native range (Wirtz et al. 1988) and introduced North American range (Graham et al. 2017c). Avoidance of floral resources due to presence of *A. manicatum* could therefore have a significant effect on a colony's ability to produce reproductives. Any reduction in reproductive output can have long-lasting fitness implications as reproductives are the only chance for colonies to pass on genetic material to next year's population.

In addition to *A. manicatum*'s impact on native pollinators, it is also important to understand *A. manicatum*'s impact on plants. *A. manicatum* are most commonly found foraging at plants in the families Lamiaceae, Scrophulariaceae, and Leguminosae (Severinghaus et al. 1981, Payette 2001), with a strong association with exotic ornamental plants common to residential gardens (Miller et al. 2002, Maier 2009). Female *A. manicatum* are polylectic, and generally considered poor pollinators due to low floral constancy. In a study by Soper and Beggs (2013a), female *A. manicatum* had on average 3.22 plant species represented in each pollen load, while native pollinators averaged 1.36 species.

In contrast, bumble bees have high floral constancy. For blueberry crop pollination, composition of pollen loads was found to average 87.5% blueberry pollen (Stubbs and Drummond 2001), and individual foragers are known to specialize on certain plant species (Heinrich 1976). They also use buzz pollination (a behavior involving high-frequency buzzing to expel pollen from flowers), and are highly efficient at pollinating native plants and crops (Shipp et al. 1994, Morandin et al. 2001). Therefore, both exclusion of bumble bees from flowers, and high rates of heterospecific pollen transfer from *A. manicatum* visitation are expected to lead to significant fitness consequences in plants guarded or visited by

A. manicatum. However, no effect of *A. manicatum* presence on plant fitness has previously been determined.

Here, we investigate the impact of *A. manicatum* presence on the foraging behavior and fitness of a native pollinator, *B. impatiens,* and the fitness effects of *A. manicatum* presence on a common forage crop, *V. villosa* (hairy vetch). This is the first study to look at fitness effects in heterospecific pollinators or plants due to interactions with *A. manicatum.* We predict *B. impatiens* will avoid foraging at resources with *A. manicatum* present throughout the season, and that this resource limitation will lead to significant decreases in colony reproductive fitness. We also predict that *V. villosa* fitness (seed production) will decrease when *A. manicatum* are present. This could either be due to increased transfer of heterospecific pollen from foraging *A. manicatum,* which is known to have significant fitness consequences for plants (reviewed in Morales and Traveset 2008), and/or pollen limitation from decreased visitation from *B. impatiens* (reviewed in Knight et al. 2005).

Methods

Study organisms

The common Eastern bumble bee (*B. impatiens*) was specifically chosen because it is commercially available. While *B. impatiens* has not shown any population decline, it is used as a proxy for effects on bumble bees more broadly; however, effects may not be universally translatable across the genus. In 2015, 12 *B. impatiens* colonies were ordered from Koppert Biological Systems Inc.

(Howell, MI). Each colony arrived around peak production with approximately 75 workers, a queen, and brood. In 2016, 14 *B. impatiens* colonies were ordered from Biobest U.S.A. Inc. (Leamington, Ontario). Each colony had newly emerged workers (~10), a queen, and brood. Smaller colonies were used in 2016 to better estimate how *A. manicatum* presence would affect *B. impatiens* over the colony growth cycle. All hives had queen excluders.

Hairy vetch (*Vicia villosa*) was chosen because it is visited by both *B. impatiens*, and has a quick growing period. It also has low rates of self-fertilization and is considered pollinator dependent (Zhang and Mosjidis 1995, Al-Ghzawi et al. 2008). It only reproduces through seed, and its fruits and seeds are large and easily classified.

Experimental setup 2015 - Monitoring foraging behavior of Bombus impatiens *in response to* Anthidium manicatum *presence*

Twelve screened research enclosures (3 m x 3 m across, and 2.1 m tall) were set up at a field site on the Tufts University campus (Medford, MA) to manipulate exposure rate of *B. impatiens* to *A. manicatum*. Enclosures were set up in two rows, six enclosures per row (Fig. 4.1). Previous work has shown normal behavior for *B. impatiens* and *A. manicatum* in similar research enclosures (Payne et al. 2011, Graham et al. 2017c), and we also saw a full suite of typical behaviors throughout the experiment. Each enclosure had one bumble bee hive (*B. impatiens*) placed just outside the enclosure. *B. impatiens* foragers had access to the research enclosure as well as the surrounding environment (a small meadow with wildflowers in an urban residential setting) through clear polyethylene tubes (3.175 cm inside diameter) fixed to the hive entrance (Fig. 4.2). Each enclosure had flowering plants (*Nepeta* spp., *Salvia* spp., *Monarda citriodora*, and *Agastache foeniculum*) visited by both *B. impatiens* and *A. manicatum*.

After *B. impatiens* colonies were installed at the field site (13 July 2015), they were allowed one week to acclimate during which time artificial nectar (Koppert Biological Systems Inc. (2015), and Biobest U.S.A Inc (2016)) was available at the base of the hive as well as at feeding stations in the enclosures. At the end of the week, *B. impatiens* workers from all colonies were foraging at enclosure plants as well as in the surrounding environment. At that time, we removed nectar access from the hive and from the enclosures.

Following the acclimation week, treatments began. Treatments alternated by enclosure placement (Fig. 4.1). Six enclosures had *A. manicatum* present (1-2 male *A. manicatum* and 2-3 female *A. manicatum*) throughout the experiment and the other six had no *A. manicatum* present. Two data collection methods were used to assess differences in *B. impatiens* foraging behavior between the two conditions (Table S4.1): (1) Number of *B. impatiens* foraging on enclosure plants, and (2) proportion of foragers entering the enclosure compared to the surrounding environment.

(1) Number of *B. impatiens* on enclosure plants was counted 1-3 times per week for six weeks. Observations were made between 09:00-14:00h, on days when the temperature was between 21-32°C with no rain and when both *B. impatiens* and

A. manicatum were observed actively foraging. Enclosures were observed in random order, and order was rerandomized for each data collection.

(2) To monitor forager choice between the enclosure and the field, each hive entrance was monitored for 30 minutes 1-2 times per week over the six-week period. Observations were made between 09:00-12:30h, again only during conditions when both *B. impatiens* and *A. manicatum* were actively foraging. Number of *B. impatiens* exiting the hive were counted as well as where they were traveling to – the research enclosure or surrounding field. Observers sat ~1 meter from the hive with a good view of the hive entrance and clear tubes. Again, order of observations at each enclosure were done randomly.

Experimental setup 2016 – Monitoring foraging behavior and fitness effects in Bombus impatiens *in response to* Anthidium manicatum *presence*

The same research enclosure setup was used in 2016 as in 2015, with the addition of two more enclosures (Fig. 4.1) and two more corresponding *B. impatiens* colonies. Changes to the setup (Fig. 4.2) included the addition of reflective insulation to the board on top of each colony and covering the clear polyethylene tubes to help minimize solar heat as a potential environmental stressor that could impact colony fitness. *B. impatiens* colonies were also much younger than in 2015. This allowed us to determine foraging effects beginning early in the colony life cycle, and determine fitness effects following exposure to *A. manicatum* across the foraging season.

After *B. impatiens* colonies were installed at the field site (14 June 2016), they were allowed the same one-week acclimation period as in 2015. Following the acclimation week, treatments began and continued for nine weeks. The same treatments were used as in 2015 and again, treatments alternated by enclosure placement (Fig. 4.1). The same foraging behavior data were collected as in 2015 - number of *B. impatiens* foragers on enclosures plants and proportion of foragers entering the enclosures. Behavioral data was collected for the first five-weeks of treatment (data collected two times per week).

In addition, metrics for colony fitness were collected. Weight of colonies were measured upon arrival, and then once a week for the duration of the experiment (nine weeks of treatment). Change in weight of *Bombus* spp. colonies is used as a metric for colony growth, and has been shown to be an indicator of resource limitation (Elbgami et al. 2014). Colonies were weighed (Ohaus Ranger 3000, accurate to 0.002 kg) at night (21:00-23:00h) when the majority of individuals were likely to be in the colony. Treatments ended when the first reproductives (males or queens) were seen in the research enclosures or colonies. When reproductives were first spotted (16 August 2016) all colonies were collected at night and freeze-killed at -20°C. The colonies were then stored in a -20°C freezer.

In December 2016, colonies were brought to a cold room (5°C) and colony demographics were recorded - number of workers, pupae, larval clumps, and reproductives (adult males, adult new queens, and queen pupae). We assumed one of the queens in each colony was the founding queen, so we only included queens beyond one as “new” reproductives. All adult workers, queens and males were then lyophilized for 24 hours, and weighed (Mettler Toledo AT261 DeltaRange, accurate to 0.01mg). Workers were weighed in groups of 10, and reproductives were weighed individually.

Bombus impatiens *Statistics*

Average number of *B. impatiens* foraging at enclosure plants over time was compared between treatments using a generalized linear mixed model (R package lme4) in both 2015 and 2016. Given the different initial life stages of the colonies at the start of the experiments, we looked at the effect of treatment each year, instead of combining data across years. Data (number of *B. impatiens* counted at enclosure plants) were log transformed to attain equal variances. Fixed effects of each model were treatment and week, and enclosure was included as a random effect. Log transformed data was also normally distributed.

Proportion of *B. impatiens* foragers exiting the hive and entering the enclosure (out of total foragers exiting the hive) was compared between treatments across time using a generalized linear mixed model (R package lme4) in both 2015 and 2016. Any data points with no foraging activity were removed from the data set, as the reason for inactivity could not be determined. Inactivity was rare and not correlated with hive or treatment. Model response variable was number of *B. impatiens* exiting the hive to the enclosure, the fixed effects were treatment and week, and enclosure number was included as a random effect. Total foragers exiting the hive was included as an offset. Models were created using a Poisson distribution with a log link function.

For the 2016 colony demographics data, we compared the effect of treatment on four demographic variables: number of workers, pupae, larval clumps, and reproductives (adult males, adult new queens, and queen pupae). We used a generalized linear mixed model (R package lme4), with all demographic variables included in the same model. Model response variable was the count of each demographic variable, treatment and demographic variable classification were included as interacting factors, with colony as a random effect. Including an interaction of treatment and demographic variable allowed us to assess whether counts within each demographic variable differed between treatment groups. Model was created using a Poisson distribution with a log link function.

We then compared "reproductive success" (RS) as defined by Pelletier and McNeil (2003) – RS = Males (adult males and pupae) + 3*Queens (adult new queens and queen pupae). Calculation of reproductive success accounts for the disproportionate foraging effort for the colony to produce queens compared to males. Again, we used a generalized linear mixed model (R package lme4), model response variable was RS, with treatment as the factor of interest, and colony as a random effect. Model was created using a Poisson distribution with a log link function.

Average weight of workers, queens, and males, was compared between treatments using a linear mixed model. Data followed the assumptions of a linear model. All demographic variables were included in the same model. Weight was the response variable, treatment and demographic variable were included as interacting factors, and colony as a random effect.

For all models, the most parsimonious model was chosen through comparison of AICc scores (Burnham and Anderson 2002) (Tables S4.2-S4.4). If competing models were within 2.0 ΔAICc, the simplest model was chosen. Both the marginal R^2 (R^2GLMM(m)) and the conditional R^2 (R^2GLMM(c)) are reported, as calculated in (Nakagawa and Schielzeth 2013). The marginal R^2 describes the proportion of variance explained by the fixed factor alone. The conditional R^2 describes the proportion of variance explained by both the fixed and random factors (Nakagawa and Schielzeth 2013).

Change in colony weight was calculated by subtracting the original weight of the colony from the weight each week. Change in weight was then compared between treatments using a repeated measures two-way ANOVA. Data fit the assumptions of a linear model.

All analyses were performed using R version 3.3.1 (R Core Team 2016).

Plant fitness

Vicia villosa (hairy vetch) seeds were purchased from Johnny's Selected Seeds (Fairfield, ME). Seeds were germinated in soil in March 2016, and seedlings were transplanted to 1.5 liter pots, four plants per pot. Plants were kept in a temperature controlled greenhouse until placed in research enclosures in June 2016. At time of enclosure placement, plants were in full bloom. Five pots (20 plants) were placed in each of the 14 research enclosures. Pots were randomly distributed between enclosures.

To compare pollination services between treatments we observed bee visitation rates to *V. villosa*. When number of *B. impatiens* foraging at all enclosure plants was recorded to estimate forager activity (see above), the number of *B. impatiens* and/or *A. manicatum* foraging at *V. villosa* was also recorded. Only female *A. manicatum* were counted, as males do not collect substantial pollen.

To determine plant fitness, mature seed pods (fruits) were collected from plants starting on 16 July 2016 and then once per week until *B. impatiens* were freeze-killed. Pods were grouped by pot in each enclosure and stored in paper envelopes at 5°C. Total pods per pot and number of seeds per pod were recorded. Individual seeds were also classified into three categories – fully developed (black, round), not fully developed (green, tan, or indented seeds), and aborted (very small and indented). All but the aborted seeds were then stored in envelopes at 5°C and both fully developed and not fully developed seeds were used in analyses.

Average weight of seeds was determined by weighing seeds grouped by pot and collection day (Mettler Toledo AT261 DeltaRange, accurate to 0.01mg). Seeds were then grouped by pot, and two seeds per pot (10 seeds per enclosure) were randomly selected and used in the germination trial. Seeds were soaked overnight in tap water. Seeds were then placed in between wetted industrial paper towels, tri-folded, placed in plastic bread bags, knotted shut, and placed in the dark for 10 days at room temperature (23°C). Germination of seeds was checked at day five and day 10.

Vicia villosa *Statistics*

Observed pollinator visits (*B. impatiens* or female *A. manicatum*) to *V. villosa* was compared between treatments using a generalized linear mixed model. Observed visits to *V. villosa* was the response variable, treatment was included as the factor of interest, with enclosure and week as random effects. Model was created using a Poisson distribution with a logit link function. The most parsimonious model was chosen through comparison of AICc scores (Table S4.5a). If competing models were within 2.0 ΔAICc, the simplest model was chosen. Both the marginal R^2 (R^2GLMM(m)) and the conditional R^2 (R^2GLMM(c)) are reported, as calculated in (Nakagawa and Schielzeth 2013).

We then compared average seeds produced by treatment. Total seeds (fully formed and not fully formed) were grouped by enclosure. We then used a one-tailed Students t-test to test our prediction that there would be a higher seed set in enclosures without *A. manicatum*. Shapiro-Wilks test was used to confirm the data met the assumptions of normality, and the F Test was used to confirm equal variances. We similarly compared the average number of aborted seeds between treatments.

Average seed weight, and average seeds per pod were each compared between treatments using Welch's Two Sample t-tests. A Welch's test was used as data did not have equal variances, and both sets of data were log transformed to meet assumptions of normality.

To compare germination rate between treatments, generalized linear mixed models were used. A binomial response of germinated (1) or ungerminated (0) was used for each seed as the response variable, treatment was included as the factor of interest, with enclosure and plant as random effects. Models were created using a binomial distribution with a logit link function. The most parsimonious model was chosen through comparison of AICc scores (Table S4.2b-S4.2c). If competing models were within 2.0 ΔAICc, the simplest model was chosen. Both the marginal R^2 (R^2GLMM(m)) and the conditional R^2 (R^2GLMM(c)) are reported, as calculated in (Nakagawa and Schielzeth 2013).

All analyses were performed using R version 3.3.1 (R Core Team 2016).

Results

Changes in B. impatiens *foraging behavior*

In both 2015 and 2016 there were significantly fewer *B. impatiens* foraging on enclosure plants when *A. manicatum* were present. However, the effect of time differed between years. In 2015, there were significantly fewer *B. impatiens* foraging on enclosure plants when *A. manicatum* were present (R^2GLMM(m) = 0.24, R^2GLMM(c) = 0.74; X^2 = 5.50, df = 1, p = 0.019; Fig. 4.3) with no significant interaction of time and treatment (X^2 = 18.66, df = 14, p = 0.18). In 2016, there was a significant effect of treatment (R^2GLMM(m) = 0.29, R^2GLMM(c) = 0.56; X^2 = 4.01, df = 1, p = 0.045), and a significant interaction of time and treatment ($X^2 = 15.33$, df = 1, $p < 0.001$), with a stronger effect of treatment later in the data collection period (Fig. 4.4).

When observing the proportion of *B. impatiens* foragers entering the enclosure versus the field, we found a significant effect of treatment only in 2015. In 2015, significantly fewer *B. impatiens* foragers went into the enclosure when *A. manicatum* were present compared to *A. manicatum*-free enclosures (R^2GLMM(m) = 0.12, R^2GLMM(c) = 0.38; $X^2 = 4.58$, df = 1, p = 0.032, Fig. 4.5). There was no significant interaction of time and treatment ($X^2 = 0.09$, df = 1, p = 0.763). In 2016, there was no significant effect of treatment or time (R^2GLMM(m) = 0.07, R^2GLMM(c) = 0.21; $X^2 = 0.73$, df = 1, p = 0.392, Fig. 4.6).

Bombus impatiens *colony fitness effects (2016)*

Number of queens or males were not compared individually, as very few were produced (Table 4.1). Instead, they were combined as “reproductives.” There was no significant effect of treatment on number of workers, pupae, larval clumps, or reproductives (R^2GLMM(m) = 0.83, R^2GLMM(c) = 0.96; $X^2 = 0.70$, df = 1, p = 0.403; Table 4.1). Similarly, average weights of queens and males were not compared due to low sample sizes. Average weight of workers was compared between treatments, with no significant effect of treatment (R^2GLMM(m) = 0.00, R^2GLMM(c) = 0.73; $X^2 = 0.08$, df = 1, p = 0.782; Table 4.1).

There was also no significant effect of treatment on average change in colony weight ($F_{1,135} = 0.40$, $p = 0.527$; Fig. 4.7). Colonies from both treatments followed a similar growth trajectory throughout the experiment.

Effect of A. manicatum *presence on plant fitness (2016)*

There was no effect of treatment on observed pollinator visits to *V. villosa* (GLMM; pseudo-$R^2 = 0.01$; $X^2 = 0.34$, df = 1, $p = 0.560$). Number of observed visits was overall relatively low, with 19 pollinator visits (*A. manicatum* females and *B. impatiens*) in *A. manicatum* enclosures, and 20 pollinator visits (*B. impatiens* only) in no *A. manicatum* enclosures (Fig. 4.8).

Vicia villosa (hairy vetch) plants in enclosures with *A. manicatum* produced fewer viable seeds ("fully formed" and "not fully formed"), however, the difference was not significant when alpha is set to 0.05 (t = -1.6, df = 12, $p = 0.066$; Fig. 4.9). There was no significant difference between treatments when comparing average number of aborted seeds ($p = 0.082$), or average number of fully developed seeds only ($p = 0.093$). There was no effect of treatment on average seed weight ($p = 0.125$), or average seeds per pod ($p = 0.943$). At day five of the germination trial, significantly fewer seeds had germinated from plants in the *A. manicatum* present treatment (GLMM; pseudo-$R^2 = 0.4$; $X^2 = 4.04$, df = 1, $p=0.045$); however, this effect went away at day 10 (GLMM; pseudo-$R^2 < 0.0$; $X^2 = 0.29$, df = 1, $p = 0.590$) (Fig. 4.10).

Discussion

We provide strong evidence that presence of the invasive bee *A. manicatum* will discourage foraging by a native pollinator, *B. impatiens*. We also provide support for the prediction that presence of *A. manicatum* may result in fitness effects for *V. villosa*. While effects of invasive *A. manicatum* presence on native pollinators has been speculated for several years (Strange et al. 2011, Colla 2016, Russo 2016) this is the first evidence of long term foraging disruption. Additionally, while previous studies have suggested *A. manicatum* likely cause an increase in heterospecific pollen deposition (Soper and Beggs 2013), this is the first study to quantify fitness consequences for plants due to presence of *A. manicatum*.

We found that *B. impatiens* foraging on enclosure plants was significantly reduced when *A. manicatum* were present across both years of testing. In 2015, there was no effect of time, as this effect was consistent across the season. However, in 2016 there was a significant effect of time, likely following the growth in the colony (as colonies started significantly smaller in 2016, with very few foragers). In 2015, we also saw a significantly lower proportion of total foragers exiting the hive to enclosures when *A. manicatum* were present. This suggests that foragers may be increasing foraging efforts to the surrounding environment to compensate for exclusion from resources in *A. manicatum* enclosures. Contrary to expectations, we did not see the same effect in 2016. One possible explanation for this is that use of smaller colonies in 2016, with a smaller number of foragers, may have limited our ability to detect a significant difference.

Another explanation is that this lack of preference was due to drought in the area during our testing period (June-August 2016) (National Centers for Environmental Information n.d.). Therefore, while we watered enclosure plants regularly, plants may have still been drought stressed, resulting in less attractive plants due to lowered production of floral resources (Alqudah et al. 2011). Therefore, *B. impatiens* foragers may have been seeking out resources elsewhere, regardless of *A. manicatum* treatment. However, our other foraging metric (*B. impatiens* foraging at enclosure plants) does not support this hypothesis, as there were still enough *B. impatiens* foragers using enclosure resources to detect an effect of treatment in 2016. Several more years of data collection would be needed to determine the influence of drought.

Territorial male *A. manicatum* have been of particular concern as their aggressive defense of resources can not only lead to resource exclusion, but also severe injury or death to native pollinators. However, while direct hits were commonly observed between male *A. manicatum* and *B. impatiens*, there was no evidence that this caused a significant decrease in number of foragers within the colony. Our results show that native pollinators will actively avoid foraging near *A. manicatum*, missing out on those floral rewards, but that interactions with *A. manicatum* do not lead to a significant decrease in workers at the end of the season.

Encouragingly, it seems that there are no fitness consequences for *B. impatiens* colonies exposed to *A. manicatum*. However, we caution optimism as exposure to *A. manicatum* in our experiment was limited to the enclosures (though other *A. manicatum* may be within *B. impatiens* foraging radius). Therefore *B. impatiens* colonies may have been able to gain enough resources from unguarded flowers in the surrounding environment to maintain healthy colony growth. However, if *A. manicatum* become more abundant in the environment, as is predicted (Strange et al. 2011), resource shortage for native pollinators is likely to increase. *A. manicatum*'s ability to exclude native pollinators from resources causes cryptic habitat fragmentation. Areas of high floral reward may in fact be largely unusable by native pollinators due to presence of *A. manicatum*. Furthermore, the same caution should be applied to this data as in our foraging data. This period did see a significant drought, so it is possible all colonies were experiencing enough resource shortage to mask any effect of treatment.

It is also worthwhile to point out that these results are only relevant to *B. impatiens*. Therefore, implications for other native bees should be applied with caution. *B. impatiens* have not seen population declines similar to other North American *Bombus* spp., and therefore may not be as vulnerable to stressors. Indeed, the *B. impatiens* range appears stable (Cameron et al. 2011), with local abundances even increasing (Colla and Packer 2008). However, given *A. manicatum*'s indiscriminate attack on heterospecific pollinators, interactions are likely to be costlier for rare or declining species. Additionally, the impact on fitness of native solitary bees is particularly concerning as removal of each individual from the population is the removal of a reproductive. Furthermore, solitary bees tend to have a smaller foraging radius (Gathmann and Tscharntke

2002). Therefore, exclusion from floral resources may have a greater impact on solitary bee resource acquisition compared to *B. impatiens*.

In this study, we also looked at the effect of *A. manicatum* presence on the fitness of a common forage crop, *V. villosa*. We observed no difference in pollinator visits to *V. villosa* between treatments. While *B. impatiens* visits to enclosure plants were lowered when *A. manicatum* were present, total pollinator visits remained about the same due to visits from female *A. manicatum*. However, we found that *V. villosa* plants in enclosures with *A. manicatum* had lower seed production than those without *A. manicatum* present, suggesting that presence of *A. manicatum* decreases *V. villosa* fitness. Though these data only show a strong trend, and not a statistically significant difference between treatments. If the effect is real, lowered seed set could be due to high rates of heterospecific pollen transferred by female *A. manicatum*, as suggested by Soper and Beggs (2011). Additionally, we saw an effect of treatment on seed germination rate at day five post germination initiation. However, this effect went away at day ten, suggesting there are not long term germination effects. Though, the cause of delayed germination rate of *V. villosa* seeds should be investigated further.

This is the first study to show season long disruption of foraging activity in a native pollinator due to presence of *A. manicatum*. This is also the first study providing evidence that suggests fitness effects in plants due to presence of *A. manicatum*. Given the success of *A. manicatum* worldwide, and our results establishing that *A. manicatum* serves as a significant resource competitor with a native pollinator, we recommend action to limit further introduction and spread of

A. manicatum outside its native range. However, implementation of this recommendation may be a challenge given the cryptic nesting behavior of this species. Limiting plantings of exotic ornamental plants, particularly those known to be preferred by *A. manicatum* (Severinghaus et al. 1981, Payette 2001), may help limit the spread and impact of this species. But perhaps more important is limiting the import and movement of *A. manicatum* nests. Further research needs to be done to better understand the vector of introduction for *A. manicatum* in order to decrease the number of introductions and the number of individuals introduced (i.e. propagule pressure). Additionally, increasing availability of native floral resources may help bolster native bee populations to buffer effects of *A. manicatum*.

Acknowledgments

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Figures

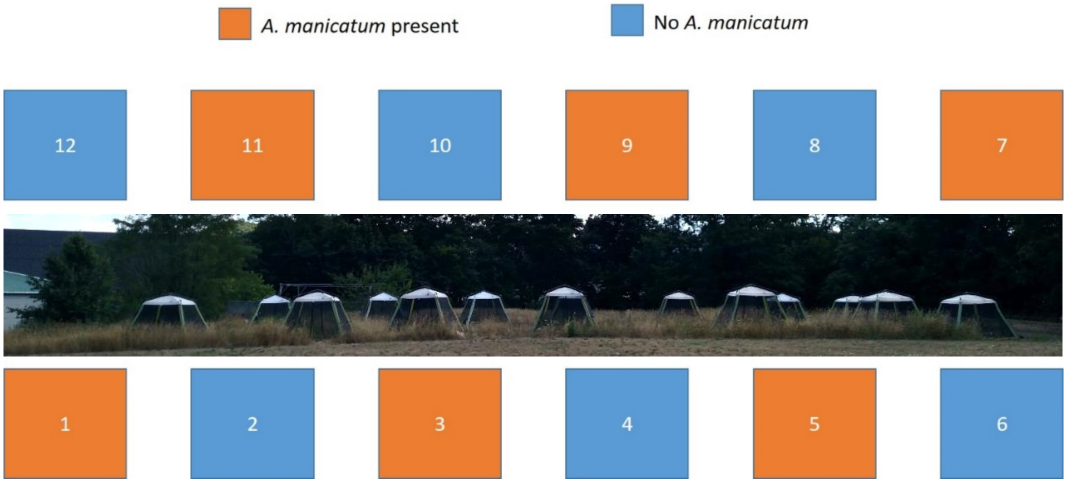

Figure 4.1. Birds-eye diagram of research enclosure setup in 2015. Colored squares represent top views of research enclosures in the field. Orange enclosures house *Anthidium manicatum* throughout the experiment, and blue enclosures never had *A. manicatum*. Inlayed picture is from 2016 when two more enclosures were added (totaling 14).

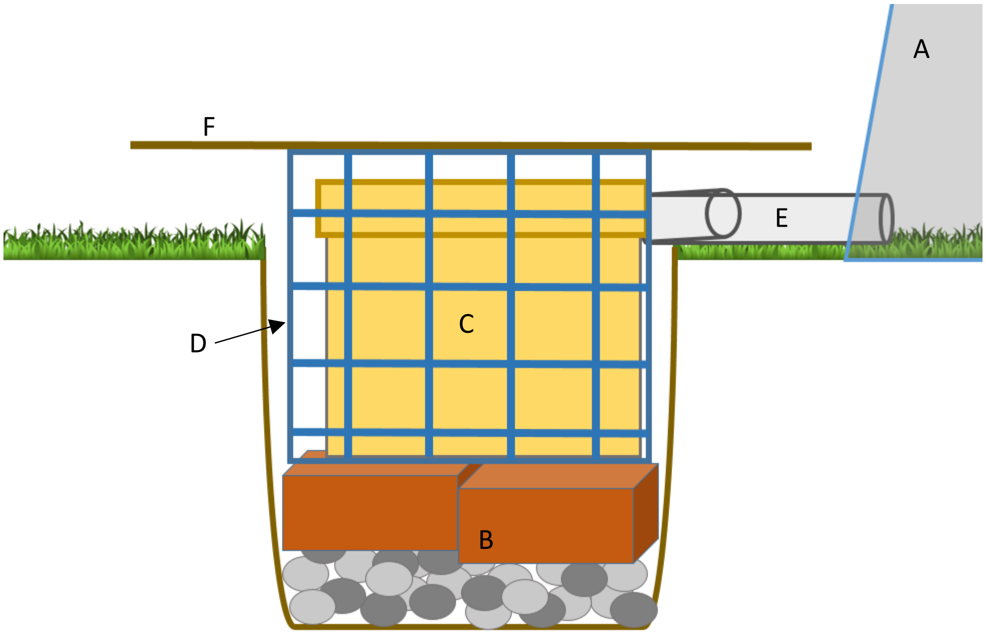

Figure 4.2. Side view of *Bombus impatiens* hive setup. Holes were dug behind screened research enclosures (A). Stone and bricks (B) were placed at the bottom of each hole to allow proper water drainage. Hive boxes (C) were put in plastic milk crates (D) and placed in the holes so that the hive entrance was flush with the surrounding grass. Two polyethylene tubes (E) were affixed to the hive entrance to allow foraging *B. impatiens* to enter the research enclosure or the surrounding environment (small meadow in an urban residential setting). Wood boards (F) (12mm thick) were placed on top of each milk crate and secured with a bungie cord. Boards were to provide protection from solar heat and decrease exposure to rain. In 2016, solar insulation was also added to the top of the board and covering the polyethylene tubes.

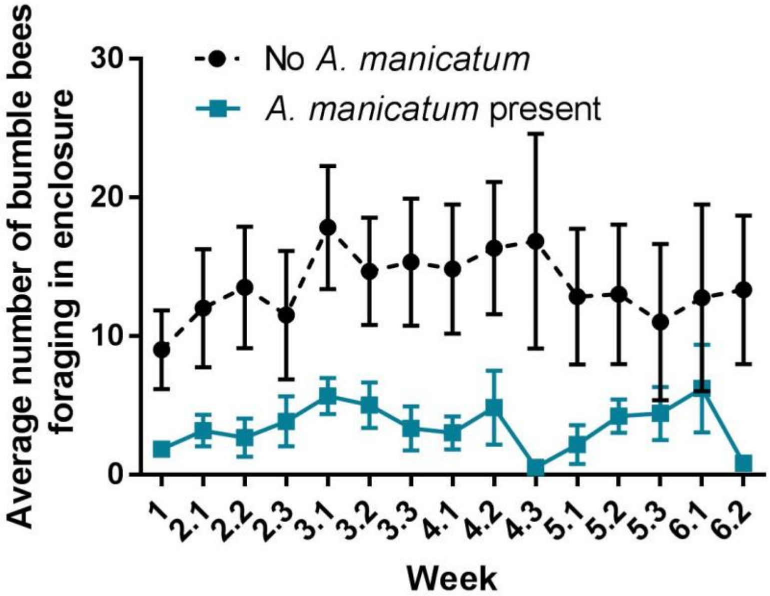

Figure 4.3. Average number of *Bombus impatiens* foraging on enclosure plants in 2015 (July-August). Twelve screened enclosures, each with flowering plants (*Nepeta* spp., *Salvia* spp., *Monarda citriodora*, and *Agastache foeniculum*) known to be preferred by both *B. impatiens* and *Anthidium manicatum*. Half the enclosures had 3-5 male and female *A. manicatum* in them throughout the testing period, the other half remained free of *A. manicatum*. Error bars indicate standard error from the mean.

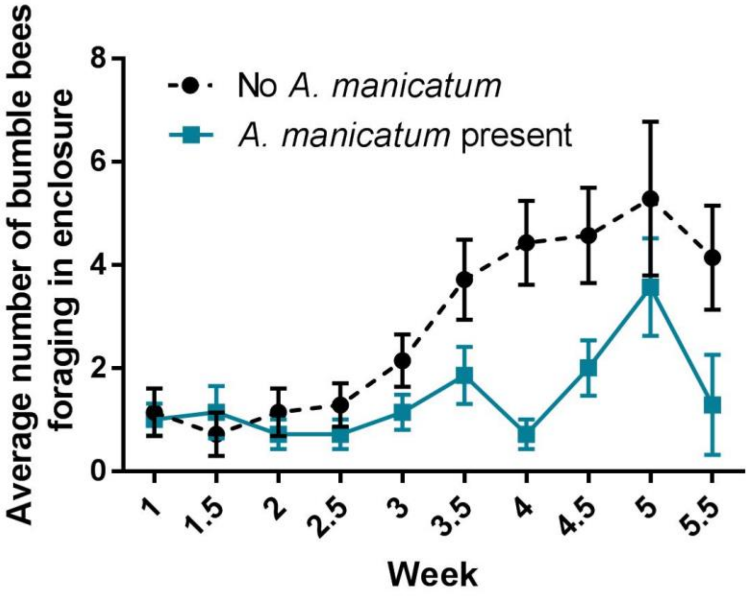

Figure 4.4. Average number of *Bombus impatiens* foraging on enclosure plants in 2016. Fourteen screened enclosures, each with flowering plants (*Nepeta* spp., *Salvia* spp., *Monarda citriodora*, and *Agastache foeniculum*) known to be preferred by both *B. impatiens* and *Anthidium manicatum*. Half the enclosures had 3-5 male and female *A. manicatum* in them throughout the testing period, the other half remained free of *A. manicatum*. Error bars indicate standard error from the mean.

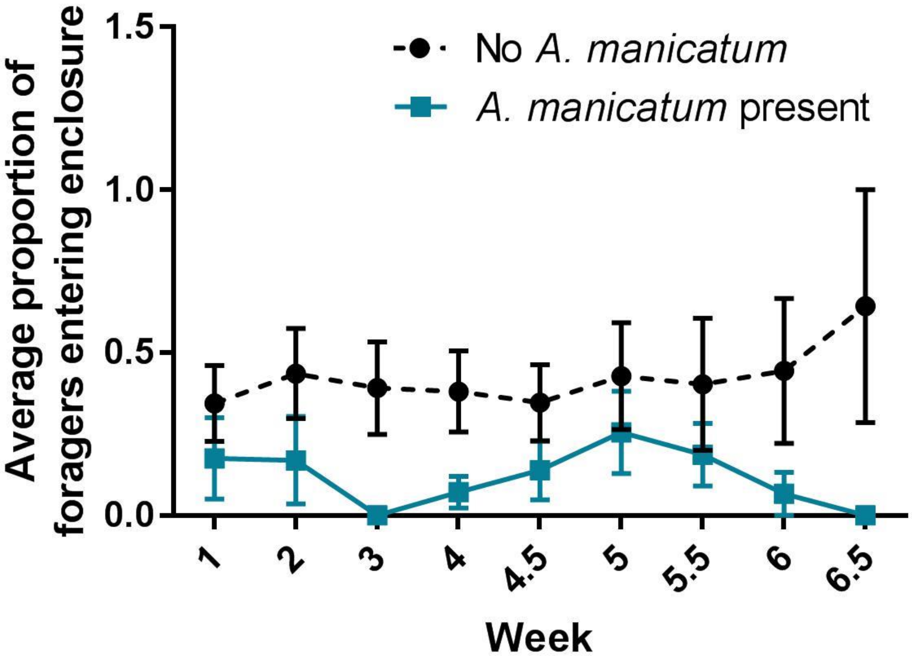

Figure 4.5. Average proportion of total *Bombus impatiens* foragers entering the enclosure compared to the surrounding field in 2015. Twelve screened enclosures, each with flowering plants (*Nepeta* spp., *Salvia* spp., *Monarda citriodora*, and *Agastache foeniculum*) known to be preferred by both *B. impatiens* and *Anthidium manicatum*. Half the enclosures had 3-5 male and female *A. manicatum* in them throughout the testing period, the other half remained free of *A. manicatum*. Error bars indicate standard error from the mean.

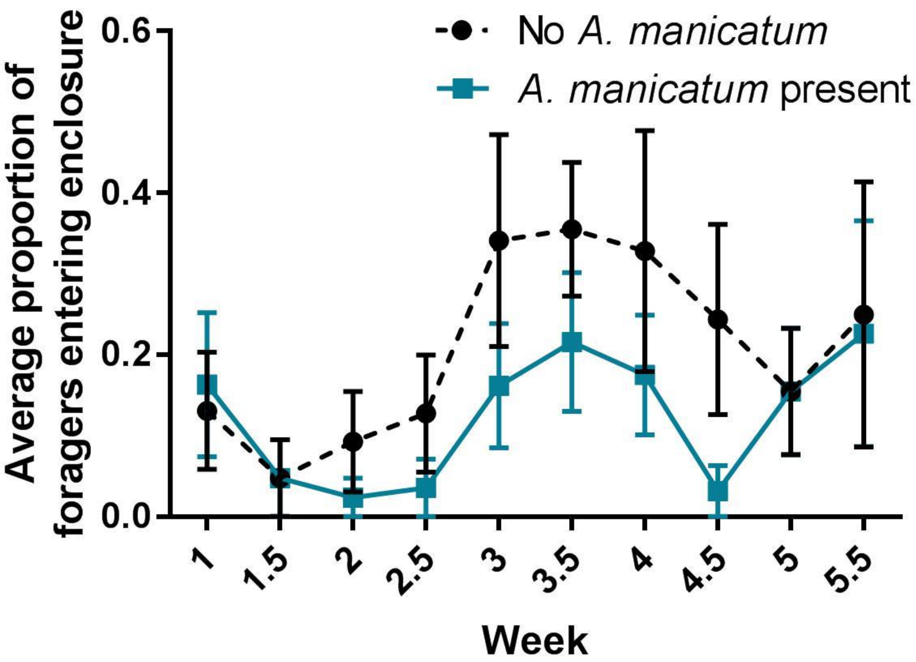

Figure 4.6. Average proportion of *Bombus impatiens* foragers entering the enclosure in 2016. Fourteen screened enclosures, each with flowering plants (*Nepeta* spp., *Salvia* spp., *Monarda citriodora*, and *Agastache foeniculum*) known to be preferred by both *B. impatiens* and *Anthidium manicatum*. Half the enclosures had 3-5 male and female *A. manicatum* in them throughout the testing period, the other half remained free of *A. manicatum*. Error bars indicate standard error from the mean.

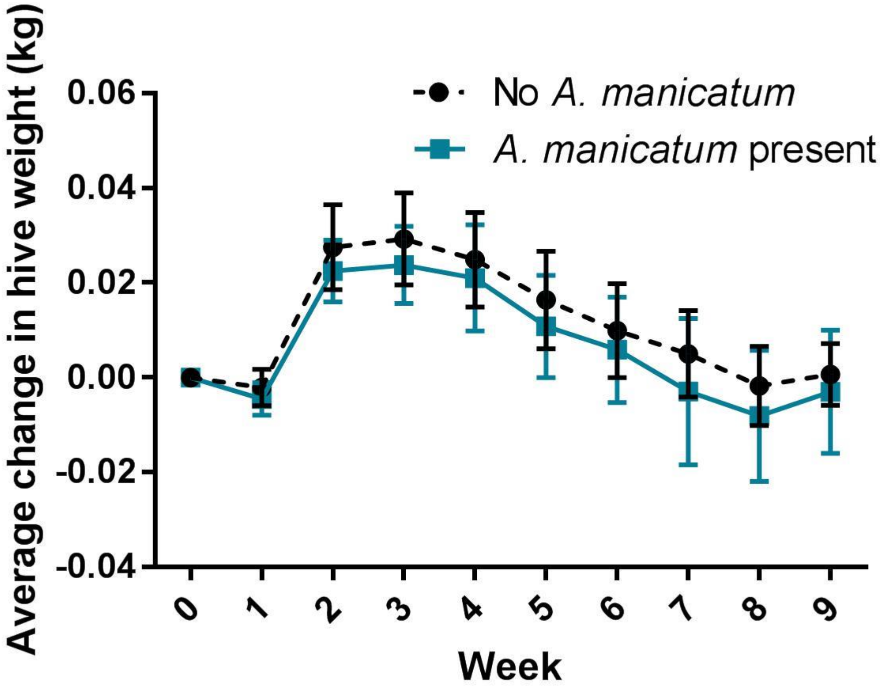

Figure 4.7. Average change in hive weight compared by treatment. Hives were weighed once a week for 9 weeks. Change in weight was compared to their initial weight (Week 0), and compared between treatments - *A. manicatum* present throughout experimental period or No *A. manicatum* present in research enclosures. Error bars indicate standard error from the mean.

Table 4.1. Comparing *Bombus impatiens* colony demographics between treatments. Weights were measured after they were lyophilized for 24 hours. Statistics were not performed for individual reproductive groups (males or queens) due to low sample sizes.

Fitness Metric	***A. manicatum* present (average ± SE)**	**No *A. manicatum* (average +/- SE)**	**Statistical Significance (GLMMs)**
# of Workers	50.4 ± 8.6	61.3 ± 9.9	No, p = 0.27
# of Queens*	2.7 ± 2.6	0.9 ± 0.9	-
# of Males	2.1 ± 1.8	2.7 ± 2.6	-
# of Pupae	9.9 ± 3.0	12.9 ± 3.7	No, p = 0.24
# of Larval Clumps	22.9 ± 6.7	23.0 ± 3.8	No, p = 0.84
# of Reproductives (Males + Queens*)	4.9 ± 2.8	4.6 ± 3.4	No, p = 0.42
Reproductive Success (RS = M[×] + 3Q*)	20.1 ± 6.9	18.1 ± 6.1	No, p = 0.84
Worker weight, Dry (g)	0.046 ± 0.003	0.040 ± 0.001	No, p = 0.78
Queen[†] weight, Dry (g)	0.273 ± 0.090	0.398 ± 0.118	-
Male weight, Dry (g)	0.071 ± 0.008	0.048 ± 0.001	-

*New adult queens and queen pupae

[×] Adult males and non-queen pupae (Pelletier and McNeil 2003)

[†] Original queen with new adult queens

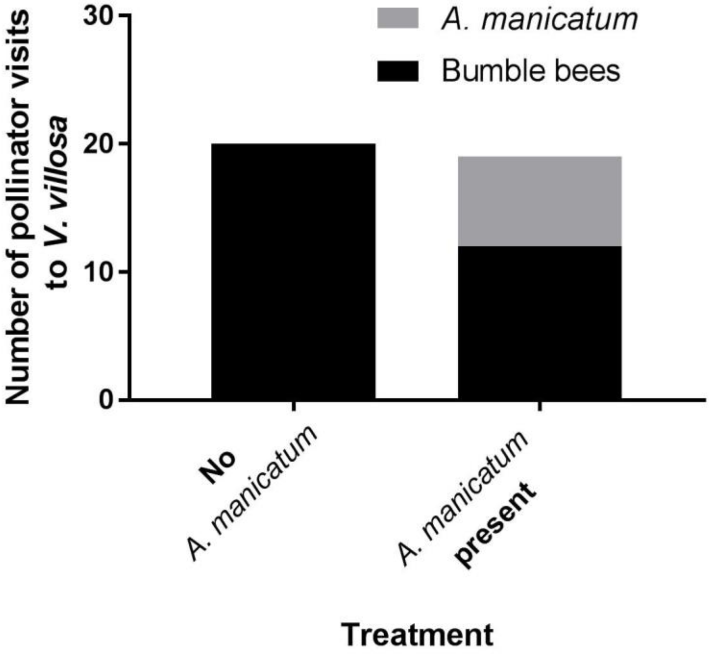

Figure 4.8. Total pollinator visits to *Vicia villosa* across five weeks. The same methods were used to count pollinator visits as described in Figure 4.4, with the inclusion of visits by both *Bombus impatiens* and *Anthidium manicatum*.

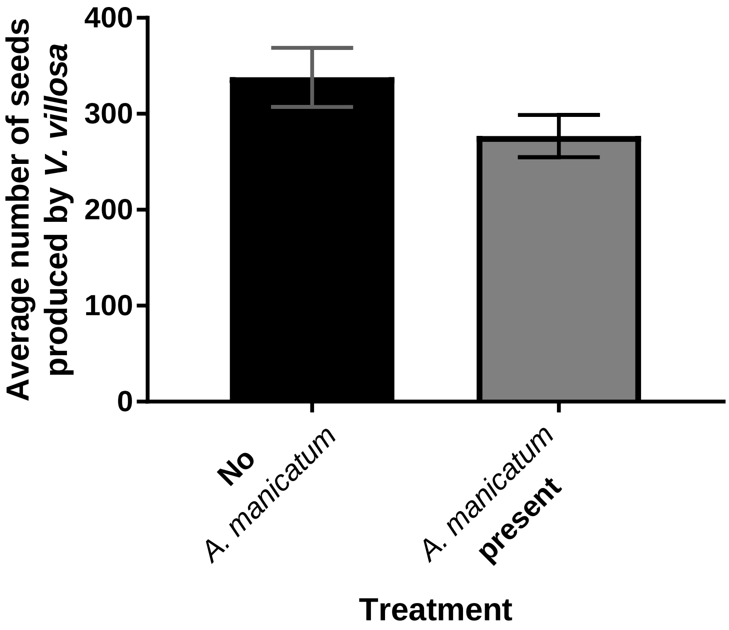

Figure 4.8. Average number of seeds produced by *Vicia villosa* plants under two treatments – *Anthidium manicatum* present or No *A. manicatum*. There were 20 plants per enclosure, and seven enclosures per treatment. Seeds were grouped by enclosure. Aborted seeds were not included in counts.

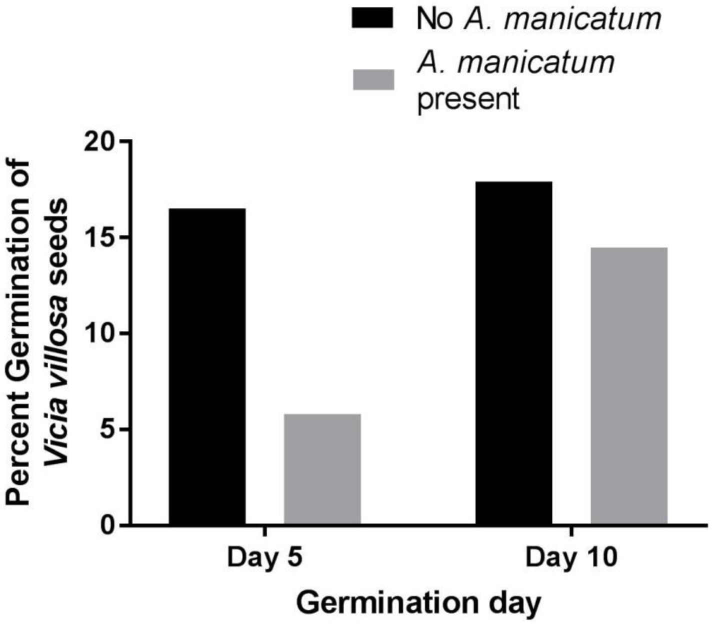

Figure 4.9. Percent germination of *Vicia villosa* seeds from two treatments at two time intervals post initiation of seed germination. There were 20 plants per enclosure, and seven enclosures per treatment. Seeds were group by enclosure and then 10 seeds per enclosure were randomly selected and used in the germination trial.

Supplementary figures

Table S4.1. Outline of data collected during 2015 and 2016 field seasons.

Year	Data collected	Duration of study
2015 – mature *B. impatiens* colonies	– Number of *Bombus impatiens* foraging on enclosure plants. – Proportion of *B. impatiens* foragers entering research enclosure.	Six weeks
2016 – young *B. impatiens* colonies	– Number of *B. impatiens* foraging on enclosure plants. – Proportion of *B. impatiens* foragers entering research enclosure. – Change in *B. impatiens* hive weight over time. – End of season colony demographics. – Number of *B. impatiens* and female *Anthidium manicatum* foraging on *Vicia villosa.* – Plant (*V. villosa*) fitness measurements.	Nine weeks (five weeks of behavioral observations)

Table S4.2. Generalized linear mixed models were used to determine effect of fixed factors on number of *B. impatiens* foragers on enclosure plants. Random effects were included and model fit was determined by comparison of AICc scores. Analyses were performed using R version 3.3.1, with package lme4.

A. 2015 – Number of *B. impatiens* foragers on enclosure plants			
Model rank (top three)	**Fixed effects**	**Random effects**	**ΔAICc**
1	Treatment	Enclosure	0.0
2	*Null* * Week	Enclosure	3.0
3	*Null*	Enclosure	3.0
4	Treatment * Week	Enclosure	32.7
5	Treatment	-	122.2
6	Treatment * Week	-	170.1
7	*Null*	-	170.8
8	Null * Week	-	170.8
B. 2016 – Number of *B. impatiens* foragers on enclosure plants			

Model rank (top three)	Fixed effects	Random effects	∆AICc
1	Treatment * Week	Enclosure	0.0
2	Treatment * Week	-	16.6
3	Treatment	Enclosure	39.0
4	*Null*	Enclosure	39.3
5	*Null* * Week	Enclosure	39.3
6	Treatment	-	50.1
7	*Null*	-	61.8
8	Null* Week	-	61.8

Table S4.3. Generalized linear mixed models were used to determine effect of fixed factors on proportion of *B. impatiens* foragers exiting hive and entering enclosures. Random effects were included and model fit was determined by comparison of AICc scores. Analyses were performed using R version 3.3.1, with package lme4.

A. 2015 - Proportion of *B. impatiens* foragers exiting the hive and entering enclosure			
Model rank (top three)	**Fixed effects**	**Random effects**	**∆AICc**
1	Treatment	Enclosure	0.0
2	*Null* * Week	Enclosure	1.9
3	*Null*	Enclosure	1.9
4	Treatment * Week	Enclosure	4.2
5	Treatment	-	57.6
6	Treatment * Week	-	60.8
7	*Null*	-	98.5
8	*Null* * Week	-	98.5
B. 2016 - Proportion of *B. impatiens* foragers exiting the hive and entering enclosure			
Model rank (top three)	**Fixed effects**	**Random effects**	**∆AICc**
1	Treatment * Week	Enclosure	0.0
2	*Null* * Week	Enclosure	0.2
3	*Null*	Enclosure	0.2
4	Treatment	Enclosure	0.5
5	Treatment * Week	-	13.5
6	Treatment	-	15.2
7	*Null*	-	18.1

8	*Null* * Week	-	18.1

Table S4.4. Generalized linear mixed models were used to determine effect of fixed factors on metrics of *B. impatiens* colony fitness. Random effects were included and model fit was determined by comparison of AICc scores. Analyses were performed using R version 3.3.1, with package lme4.

A. Effect of treatment on number of *B. impatiens* workers, pupae, larval clumps, and reproductives.			
Model rank (top three)	**Fixed effects**	**Random effects**	**ΔAICc**
1	Treatment * Demographic Variable	Colony	0.0
2	Treatment * Demographic Variable	-	121.9
3	*Null* * Demographic Variable	-	882.8
4	*Null*	Colony	882.8
5	Treatment	Colony	884.4
6	Treatment	-	1006.9
7	Null * Demographic Variable	-	1010.7
8	*Null*	-	1010.7
B. Effect of treatment on average weight of *B. impatiens* workers.			
Model rank (top three)	**Fixed effects**	**Random effects**	**ΔAICc**
1	*Null*	Colony	0.0
2	Treatment	Colony	10.1
3	Treatment	-	44.1
4	*Null*	-	44.9

Table S4.5. Generalized linear mixed models were used to determine effect of fixed factors on metrics of *V. villosa* fitness. Random effects were included and model fit was determined by comparison of AICc scores. Analyses were performed using R version 3.3.1, with package lme4.

A. Observed pollinator visits to *Vicia villosa*

Model rank (top three)	**Fixed effects**	**Random effects**	**ΔAICc**
1	*Null* * Week	-	0.0
2	*Null*	-	0.0
3	Treatment	-	1.7
4	*Null* * Week	Enclosure	2.1
5	*Null*	Enclosure	2.1
6	Treatment	Enclosure	3.8
7	Treatment * Week	-	36.1
8	Treatment * Week	Enclosure	38.8

B. Effect of treatment on germination rate of *Vicia villosa* seeds at day 5.

Model rank (top three)	**Fixed effects**	**Random effects**	**ΔAICc**
1	Treatment	-	0.0
2	Treatment	Plant	1.8
3	*Null*	-	2.0
4	Treatment	Enclosure	2.1
5	*Null*	Plant	3.9
6	Treatment	Enclosure, Plant	4.0
7	*Null*	Enclosure	4.0
8	*Null*	Enclosure, Plant	6.0

C. Effect of treatment on germination rate of *Vicia villosa* seeds at day 10.

Model rank (top three)	**Fixed effects**	**Random effects**	**ΔAICc**
1	*Null*	-	0.0
2	*Null*	Plant	1.6
3	Treatment	-	1.8
4	*Null*	Enclosure	2.1
5	Treatment	Plant	3.4
6	*Null*	Enclosure, Plant	3.7
7	Treatment	Enclosure	3.9
8	Treatment	Enclosure, Plant	5.5

CHAPTER 5

Title:

Presence-only modeling is ill-suited for a recent generalist invader, *Anthidium manicatum*.

Authors:

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Abstract

Anthidium manicatum, the European wool-carder bee, is an invasive species of concern given its worldwide invasion and aggressive behavior towards native pollinators. Predicting habitat suitability for this species is imperative for estimating threat to native species, and predicting future spread. Previous habitat suitability models used bioclimatic variables to make predictions across a broad geographic region, but these showed little utility at predicting risk at the local habitat scale. Therefore, we created a model using environmental inputs that vary across a regional scale (land cover type and percent impervious surface) and focused within a more restricted geographic region, the Northeastern (NE) US. Occurrence records were aggregated from open-sourced data and published records, and maximum entropy methods were used to create the model. We created a second model using bioclimatic variables (temperature and precipitation), to compare utility of both model inputs. We then tested the accuracy of both models by performing weighted random sampling and stratified random sampling across the NE to obtain presence and absence data for *A. manicatum*. Given previous predictions of widespread habitat suitability, it was surprising that out of 140 sampled locations, *A. manicatum* were only found at seven. When comparing model accuracy (Cohen's Kappa), both models showed low accuracy (land scape variables KHAT = 0.023; bioclimatic variables KHAT = -0.094). Models were also not significantly different from each other (Z = 0.548). Therefore, presence-only modeling may not be suitable for this system, either because these variables are not capturing factors restricting *A. manicatum*'s range, or it is too early in the invasion process. Additional probability sampling is suggested to refine predictive models.

Key words: Maxent, suitability distribution modeling, invasive species, sampling, GIS

Introduction

Bees provide valuable pollination services to wild plants and crops worldwide (Klein et al. 2007, Brown and Paxton 2009, Potts et al. 2010). Bee abundance and diversity increases ecosystem services and resilience to disturbance (Winfree et al. 2007, Garibaldi et al. 2013, Russo 2016). However, exotic bee introductions have seen a prolific increase within the past 100 years, particularly in North America, and this addition of species generally does not incur the same benefit as an increase in native bee diversity (Russo 2016). We aim to garner a better understanding of the current distribution and habitat suitability of an exotic bee quickly reaching worldwide distribution, *Anthidium manicatum* (the European wool-carder bee) (Miller et al. 2002, Gibbs and Sheffield 2009, Strange et al. 2011, Russo 2016).

The range expansion of *A. manicatum* has been noted as particularly troubling among invasion ecologists and bee researchers due to its rapid rate of spread and the species' potential impact on native species (Strange et al. 2011, Colla 2016, Russo 2016). Native to Europe, western Asia and northern Africa, *A. manicatum* is now established in northeastern Asia, North America, South

America, and New Zealand. *A. manicatum* was first recorded in North America in 1962 near Ithaca, NY (Jaycox 1967). Until 2001, the species seemed restricted to the northeastern United States, but soon after, *A. manicatum* were recorded in Canada and on the western United States coast (Gibbs and Sheffield 2009). In the following years, there was a rapid increase in *A. manicatum* sightings across the United States and southern Canada (Fig. 5.1). While this rapid spread alone is concerning, its behavior makes it a particularly noteworthy invader (Maier 2009, Strange et al. 2011, Colla 2016, Russo 2016).

Anthidium manicatum males aggressively defend patches of floral resources to decrease resource competition with heterospecific pollinators and mate competition with conspecific males (Haas 1960, Pechuman 1967, Severinghaus et al. 1981, Starks and Reeve 1999). They use spines at the base of their abdomen to puncture or fracture the wings of territory intruders. These aerial altercations often result in severe injury or death to attacked bees (Wirtz et al., 1988). While attacks are relatively indiscriminate, honey bees (*Apis mellifera*) and bumble bees (*Bombus* spp.) are the most common recipients (Wirtz et al. 1988, Miller et al. 2002, Maier 2009).

Additionally, female *A. manicatum* are considered poor pollinators (Soper and Beggs 2013), and cause damage to plants through collection of nesting material (Müller et al. 1996, Payne et al. 2011, Graham et al. 2017a). They also compete for floral resources with native pollinators (Payette 2001, Graham et al. 2017b), and are likely to compete with other native Megachilidae for nesting cavities (Barthell et al. 1998, Maier 2009, Griswold et al. 2014).

Given the concerns surrounding *A. manicatum* behavior, estimating current range and predicting its future spread is of high priority. Habitat suitability of *A. manicatum* was modeled previously and suitable habitat was estimated to cover most of the contiguous USA and southern Canada (Strange et al. 2011). However, several factors limited the specificity of this model, including use of presence-only data which is likely influenced by sampling bias, and relatively coarse habitat inputs. Specifically, the previous model used bioclimatic variables, such as mean temperature and precipitation. These variables generally have 1 km^2 pixels and are not variable enough within a region to attain habitat specific predictions of suitability. This very broad scale representation of suitable *A. manicatum* habitat is not very useful for conservation planners and stakeholders that work at a finer scale.

Here, we propose an alternative model using occurrence records in a smaller geographic region, the Northeastern USA, and different predictive correlates - landscape variables that can be used as an indicator of human disturbance. Exotic plants and pollinators have been known to show strong associations with disturbed habitat (Hobbs and Atkins 1988, Burke and Grime 1996, Morales and Aizen 2002). *A. manicatum,* in particular, is known to associate strongly with exotic flowering plants common to urban and residential gardens (Payette 2001, Miller et al. 2002, Maier 2009). Additionally, we expect a strong association of human activity and *A. manicatum* presence due to the predicted route of invasion – accidental human transport of *A. manicatum* nests. *A. manicatum* are cavity nesters, a life history trait highly correlated with invasion success for exotic bees (Gibbs and Sheffield 2009, Russo 2016). Association of *A. manicatum* with disturbed areas in North America has been noted in the past (Miller et al. 2002), but never tested. Through use of habitat suitability modeling, we will test the assumption that suitable *A. manicatum* habitat is highly correlated with human disturbance. We also limited the scope of the study to the region where *A. manicatum* has the longest established invasion history - the Northeastern (NE) USA (ME, NH, VT, MA, RI, CT, and NY), in order to better estimate the full breadth of suitable habitats for this species. Then, to compare the utility of landscape variables as model inputs, we created a second presence-only model using bioclimatic variables. We again restricted the model to the NE and replicated bioclimatic environmental inputs used in a previous habitat suitability model for *A. manicatum* in North America (Strange et al. 2011), both to compare this model to one created with landscape variables and to see if a more restricted geographic focus would increase utility of bioclimatic variables at the habitat scale.

Testing the accuracy of presence-only models with probability based presence-absence data is an important step in determining model utility. This step is often overlooked due to limitations in availability of presence-absence data, and/or limitations in sampling. However, untested presence-only models have several shortcomings that should not go unheeded (Elith et al. 2006, Yackulic et al. 2013, Guillera-Arroita et al. 2014). Effects of sampling bias are common when non-probability sampling methods are used to collect data. Many habitat types are often underrepresented due to low human traffic. To determine the effect of sampling bias on model predictions, independent, well-structured presence-absence data sets should be used to test presence-only model accuracy. However, none such data exist for *A. manicatum* in North America. Therefore, to test the predictive ability of our two models, we generated a probability based presence-absence data set through weighted random sampling and stratified random sampling. We then compared the accuracy of both models using this data.

Methods

First, we created two predictive models for *A. manicatum* presence within the NE US using freely available presence-only data and different sets of environmental variables - landscape variables (Land Cover Model) and bioclimatic variables (Bioclimatic Model). Second, two different sampling efforts were undertaken to collect presence-absence data to test the usefulness of each model in predicting *A. manicatum* presence.

<u>Presence-only data</u>

Data collection

In spring 2013, we aggregated 87 distinct *A. manicatum* occurrence localities in the NE (ME, NH, VT, MA, RI, CT, and NY) available through open access data sources such as Discover Life (Ascher and Pickering 2011) and published records (Maier 2009, Griswold et al. 2014). Data were only included if they had detailed latitude/longitude coordinates and were from peer-reviewed publications or collected/confirmed by a reputable entomologist. This was to decrease the chances of false presences, incorrect identification, or spatial error. However, the largest drawback to using these data is the lack of consistent sampling methods used to collect the samples. For instance, some noted presences were from directed sampling efforts, while others were from convenience sampling. The 87 sample locations in no way exemplify an exhaustive sampling of the NE and do not have equal detection probabilities, but represent the best available data to date.

Land Cover Model

NLCD 2011 land cover type and percent impervious surface (Xian et al. 2011, Homer et al. 2015) were used as environmental metrics. Each layer contained 30 m by 30 m pixels with either discrete land cover classes or continuous estimations of impervious surface for each pixel. Impervious surface, a metric for urbanization and development, was reclassified by deciles instead of a continuous variable to relieve some of the computational stresses of such a large raster, as well as make visualization of the data easier. Both layers were prepared so that extents and grids matched using ArcGIS v10.1 (ESRI 2011). These two layers were chosen because we hypothesized that *A. manicatum* presence would correlate with urbanized areas within the NE due to previous work showing the association of the bee with exotic ornamental plant species (Payette 2001) and high prevalence of observations within urbanized environments. Specifically, we hypothesized that a percent impervious surface level between 50-80% would be most likely to predict *A. manicatum* presence given the high incidents of observations near urbanized areas (medium to high impervious surface level), but also *A. manicatum*'s need for floral resources and nesting cavities (typically associated with some greenspace, or a slightly lower impervious surface level).

Similar to a previous suitability distribution model (SDM) for *A. manicatum* created with presence-only data (Strange et al. 2011) we used the maximum entropy method as employed by the Maxent software (v3.3) (Phillips et al. 2006, Elith et al. 2011). Maxent creates models using presence-only data in combination with user selected environmental variables to estimate relative habitat suitability for each location within a matrix. We included both NLCD land cover type (Homer et al. 2015) and percent impervious surface (Xian et al. 2011) as environmental variables in the same model. The model was fitted using default settings of prevalence, feature type, logistic output and regularization. 20% of the data were randomly withheld to test the model. The area under the curve (AUC) statistic was used to assess model performance, with a score of above 0.5 indicating that the model performed better than random. Maxent uses probability densities to determine the effect of each environmental variable on likelihood of presence, assigning each location within a matrix a relative probability of suitability score from 0 (unlikely) to 1 (very likely). We used 0.5 as the cutoff for suitable habitat. This cutoff was also used when evaluating the suitability of individual environmental variables (land cover type and impervious surface).

Bioclimatic Model

Bioclimatic variables are commonly used in SDMs for predicting invasive species habitat (Jeschke and Strayer 2008). However, we argue that using habitat features as environmental inputs can provide greater utility for conservation planners and stakeholders who are making management decisions at a smaller, habitat specific scale. To compare the utility of using different environmental inputs, we created a second model using ten bioclimatic variables downloaded from the WorldClim database (http://worldclim.org/). The ten variables were chosen based on a previous SDM created for *A. manicatum* in North America (Strange et al. 2011) – mean temperature diurnal range, temperature annual range, mean temperature of wettest quarter, mean temperature of driest quarter, mean temperature of warmest quarter, precipitation seasonality, precipitation of wettest quarter, precipitation of driest quarter, precipitation of warmest quarter, and precipitation of coldest quarter. All variables were downloaded at a spatial resolution of 1 km^2. All layers were prepared so that extents and grids matched using ArcGIS v10.1 (ESRI 2011). We employed the same techniques for building this SDM in Maxent as described above for the Land Cover Model.

Presence-absence data -

General NE Data Collection

In summer 2013, we developed a weighted random sampling scheme to collect presence-absence data on *A. manicatum* within the NE. Since *A. manicatum* are still predicted to be relatively rare across the landscape, we used weighted random sampling to increase the likelihood of acquiring presence locations. Weights were based on the likelihood of presence of *A. manicatum* (40%), likelihood of *Bombus impatiens* (40%), and accessibility (20%) (Fig. S5.1). Likelihood of *A. manicatum* was determined using our Land Cover Model. Likelihood of *B. impatiens* was used as an indicator of “good” pollinator habitat, much of which may be suitable for *A. manicatum*, but underrepresented in our Land Cover Model due to low occurrence records. Estimated habitat suitability for *B. impatiens* was provided by Cameron and colleagues (2011). Accessibility was determined based on distance to a road (10%), and distance from two preselected “home bases” for the authors – Boston, MA and Port Byron, NY (10%). The accessibility weight was created to minimize total costs associated with field efforts.

All samples (pixels, 30 x 30m) within the range of our map were given a number code and a weight. We selected 300 samples based on their weight and without duplicates using the weighted random sample selection in R (R Core Team 2016). This weighting scheme allowed for those samples with a higher weight to have a greater probability of being chosen; however, samples of all weights had the possibility of being chosen. These sampling methods were specifically used to try to optimize our sampling effort due to the anticipated low likelihood of detection for *A. manicatum*. Of the 300 samples chosen, we manually chose the first 100 samples that were within a homogenous set of 9 pixels and looked accessible according to satellite imagery; throwing out locations that were next to pixels with different land cover or impervious surface values, not accessible by car or reasonable hikes, or on inaccessible private property (Table S5.2).

Between June and September 2013, field sampling was performed on days when the temperature was between 70-90 degrees F and with no rain to decrease the likelihood of false absences due to bad weather (Couvillon et al. 2010; pers. obs.). Upon arrival at the sampling location, a hand held GPS (Garmin eTrex 30) was used to confirm location and to get as close as possible to the center of the sample site. We then scanned the site for flowering plants where we were most likely to find bees. The location most likely to be visited by bees within 10 m of the center of each sample site was then monitored closely for a total of 20-30 minutes, as similar methods have been used previously to sample for *A. manicatum* (Maier, 2009). If flowers were present and there was bee activity of any kind, we observed for 30 minutes. If no flowers were available, and/or no bee activity was seen at the location, we stayed only 20 minutes to increase our sampling efficiency. The time of arrival of *A. manicatum* was recorded, and *A. manicatum* were netted whenever possible to confirm identification. Presence of *A. manicatum*, *Bombus* spp., and *Apis mellifera* were recorded, along with weather, time of day, flowers present and any other notable characteristics of the site.

We also took note of the presence of *Anthidium oblongatum*. *A. oblongatum* is another exotic *Anthidium* with a similar distribution in the NE as *A. manicatum*. However, much less is known about its potential impact on native species, so it was not included in any of our analyses.

Targeted Boston-area Data Collection

In summer 2014, a more targeted stratified random sampling scheme was developed within a more geographically constrained region – within the I-495 corridor of Massachusetts. Again, based on the Land Cover Model, locations (pixels, 30m x 30m) within this region were separated into three habitat suitability scores – high (1.0 - 0.67), medium (0.669 – 0.33), and low (0.329 – 0.0). 20 locations were randomly chosen within each suitability score using a random number generator in R (R Core Team 2016).

The same sampling protocol was used in 2014 as in 2013. However, half of the locations (10 within each suitability level) were also randomly chosen for additional sampling methods. At these locations, between 8:00-10:00h three bee bowls were placed at the sampling site, and then collected between 17:00-19:00h. Bee bowls were set up according to recommendations from Sam Droege at the USGS (Droege n.d.). Bowls were purchased from Solo Cup Co (Illinois) (Dart Solo 3.25 oz. Translucent Polystyrene Soufflé Cup (999P325)) and painted fluorescent blue, fluorescent yellow, and white (silica flat) (Guerra Paint and Pigment Corp., New York, NY). One bowl of each color was placed, as a group, at each location. Bowls were filled with soapy water (Dawn Ultra Dishwashing Liquid, Original Scent; diluted 1 tsp/1 liter of water). At pickup, all dishes were checked for bees, and any bees caught were preliminarily identified, placed in plastic bags, and stored in a -20°C freezer. These sites were used to determine if

30 minute observations at sample locations is adequate sampling for *A. manicatum* detection.

Testing Accuracy of Predictive Models

The accuracy of each model in predicting presence/absence of *A. manicatum* during our sampling effort was determined using error matrices. Sampling locations where *A. oblongatum*, but not *A. manicatum*, were collected were removed from analyses, as the correlation between suitable habitat for *A. oblongatum* and *A. manicatum* is unknown. We used 0.5 as the cutoff for expected suitable or unsuitable habitat according to model predictions. The Kappa technique was used to calculate KHAT and confidence intervals for each model. Comparison of the Land Cover Model matrix to the Bioclimatic Model matrix was performed through a Z test (Congalton 1991, Congalton and Green 2009).

Results

Land Cover Model

During the creation of the model, land cover type had the highest gain of the environmental inputs. However, percent impervious surface showed the highest contribution to the model (70.4%). The training AUC score was 0.842, and the test AUC score was 0.783, showing moderate predictability of the model given the presence data provided (Fig. 5.2). Results of the density distributions for impervious surface level indicate that levels 2-10 have a high probability of suitability (Fig. 5.3). For land cover type, density distributions suggest that developed land (open space, low, medium and high intensity) has a high probability of suitability (Fig. 5.3). Predicted habitat suitability across the NE was visualized using ArcGIS v10.2, and followed closely with our prediction that suitable habitat would correlate with human disturbance (Fig. 5.2).

Bioclimatic Model

During creation of the model, the bioclimatic variable Precipitation Seasonality (Coefficient of Variation) had the highest gain and contributed the most to the model (38.8%). The training AUC score was 0.957, and the test AUC score was 0.957, showing good predictability of the model given the presence data provided (Fig. 5.4). For a more exhaustive analysis of bioclimatic variable correlations with *A. manicatum* habitat suitability please see Strange et al. (2011).

Presence-absence data

Out of the 100 sampling locations from the 2013 sampling effort, we were able to gather data at 82 sites, with 18 sites deemed inaccessible upon arrival. Of those 82, *A. manicatum* were only found at three locations, and only able to be successfully netted at one site to confirmed identity (Table S5.1).

In 2014, we used a stratified random sampling scheme with a smaller geographic focus (I-495 corridor in Massachusetts) to test the probability of detecting *A. manicatum* using our 2013 sampling strategy. Of the 60 sampling locations chosen, we were able to gather data at 58. Of the 58, *A. manicatum* was found, and confirmed through aerial netting, at four (Table S5.1). The placement of bee bowls at half of these sites in 2014 resulted in no captures of *Anthidium* spp.

The total number of *A. manicatum* presence locations for both the 2013 and 2014 sampling efforts was seven out of 140 total visited sites.

Testing Accuracy of Models

Both models showed overall poor predictive ability (Bioclimatic Model KHAT = -0.094 and Land Cover Model KHAT = 0.023) (Table 5.1). Models were also not significantly different from each other (Z = 0.548).

Discussion

We used maximum entropy methods to create two SDMs of *A. manicatum* in the NE using different sets of environmental variables. Our Land Cover Model estimates of habitat suitability fit with our predictions of high correlation between *A. manicatum* and disturbed habitat. However, when we compared the ability of each model to accurately predict presence/absence of *A. manicatum*, both models show low predictive ability, and were not significantly different from each other. When looking at why the models performed poorly, they appear unpredictive for opposite reasons. The Bioclimatic Model predicted "low" suitability at each presence data point collected during the sampling effort, showing an overall under-prediction of habitat suitability. Conversely, the Land Cover Model predicted 6/7 of the presences correctly, but an inability to accurately predict absences (38/127 absences correctly predicted), or an over-prediction of habitat suitability.

Our findings suggest neither model is sufficient for accurately predicting *A. manicatum*'s invasive range in the northeastern United States. However, given currently available presence/absence data, the Land Cover Model likely holds better utility given its finer scale predictions and tendency to over-predict habitat suitability. When estimating exotic species spread, it is generally considered better to over-predict habitat suitability than to under-predict (Jiménez-Valverde et al. 2011). Though each study must weigh the risk of Type I error versus Type II error. Given that the Bioclimatic Model severely under-predicted suitable habitat for *A. manicatum*, we do not recommend use of this model alone for estimating current presence of *A. manicatum* or future spread. Furthermore, concerns about the underlying assumptions of bioclimatic models should be considered when choosing environmental factors.

Bioclimatic models do not account for habitat requirements or restrictions in animal movement (Jeschke and Strayer 2008). Within this system, *A. manicatum*'s association with exotic ornamental plants, and the high likelihood of aided distribution by humans make landscape features particularly important when considering habitat suitability for this species. Another common limitation of bioclimatic SDMs are their coarse predictions. Previous models have estimated highly suitable habitat for *A. manicatum* to include the entire northeast (Strange et al. 2011). While our regional Bioclimatic Model further focuses the area of highest probability for presence to southern New England and New York, our sampling results still suggest *A. manicatum* is not currently occupying predicted "suitable" habitats. Additionally, *A. manicatum* was found in areas the Bioclimatic Model predicted to have low suitability. These results suggest that bioclimatic variables are not the limiting factor for *A. manicatum* habitat occupancy.

The low accuracy of both models may be indicative of a more universal problem with presence-only SDM creation. Use of presence-only modeling programs such as Maxent for predicting invasive species spread is relatively common. However, model assumptions of unbiased sampling are often violated when using occurrence data acquired through unstructured sampling (Yackulic et al. 2013). While this can be a problem for any occupancy model, it appears to be commonly violated when performing presence-only modeling as lack of absence data is generally due to a lack of structured sampling. This is almost certainly true for the presence-only data currently available for *A. manicatum*. Almost all occurrence records were gained through unstructured sampling, and therefore have high likelihood of sampling bias. Furthermore, geographically restricted occurrence records likely biased model predictions. This geographic bias likely accounts somewhat for the highly localized habitat suitability predictions in the Bioclimatic Model. Suitable habitat is centered around the highest aggregation of occurrence records (CT, MA, and RI). Whether this truly indicates restricted habitat suitability can only be confirmed with additional sampling, though the lack of presence locations within these areas suggests that *A. manicatum* is not restricted by these bioclimatic variables.

Our structured sampling effort is the first recorded for this species in North America (to the best of our knowledge). Unfortunately, we did not attain enough presence records to build a presence-absence model. Such a model would allow for more accurate representation of suitable habitat by decreasing the effect of sampling bias. We do provide the first records of sampled absences, however, which may provide additional inputs for future presence-absence modeling efforts.

Given the large number of absence records and few presences records attained through our sampling efforts, our data suggest that the current range of *A. manicatum* is less than previously estimated (Strange et al. 2011). This is encouraging given the predicted impact of *A. manicatum* on native species (Graham et al. 2017b). However, we must caution optimism. Given the world-wide invasion of *A. manicatum*, we predict that *A. manicatum* may have been present but undetected at some sites. Due to inherent limitations in our sampling effort, in both time and space, and the sometimes cryptic behavior of *A. manicatum*, we may not have been at the "right time and place", so to speak. While male *A. manicatum* tend to be relatively predictable in location due to territorial behavior at floral resources, females and non-territorial males are more transient within the habitat (Starks and Reeve 1999). Therefore, presence of females and non-territorial males within the habitat may have been missed. During the 2014 sampling effort, bee bowls were used in an attempt to decrease the likelihood of false absences, and estimate detection probability using our 2013 sampling methods. However, no *A. manicatum* were caught in the bee bowls, even at sites where *A. manicatum* were netted, indicating this sampling technique may not be particularly useful for estimating presence or abundance of this species. Alternative longer term sampling methods should be explored.

Given the low accuracy of our presence-only models, we join others in a call to increase overall sampling efforts (Schmeller et al. 2015), particularly use of probability sampling for species of concern (invasive species and threatened species). While the increasing availability of open-sourced occurrence records has provided abundantly more resources for tracking species distributions, we must caution the wide-spread use of SDMs built from non-probability sampling data. Habitat suitability models for *A. manicatum* based on land use data show promise, but can only be developed with a more widespread structured sampling regime, particularly if it results in both presence and absence data.

Acknowledgements

We thank Sydney Cameron and Jeffrey Lozier for providing us with their *Bombus impatiens* suitability map. We would also like to thank Philip Starks, Michael Reed, and Elizabeth Crone for their advice on project design and interpretation of data. This project was funded through a Babson College Faculty Research Fund (awarded to MGM) and a Tufts Institute of the Environment Travel Grant (awarded to KKG).

Figures

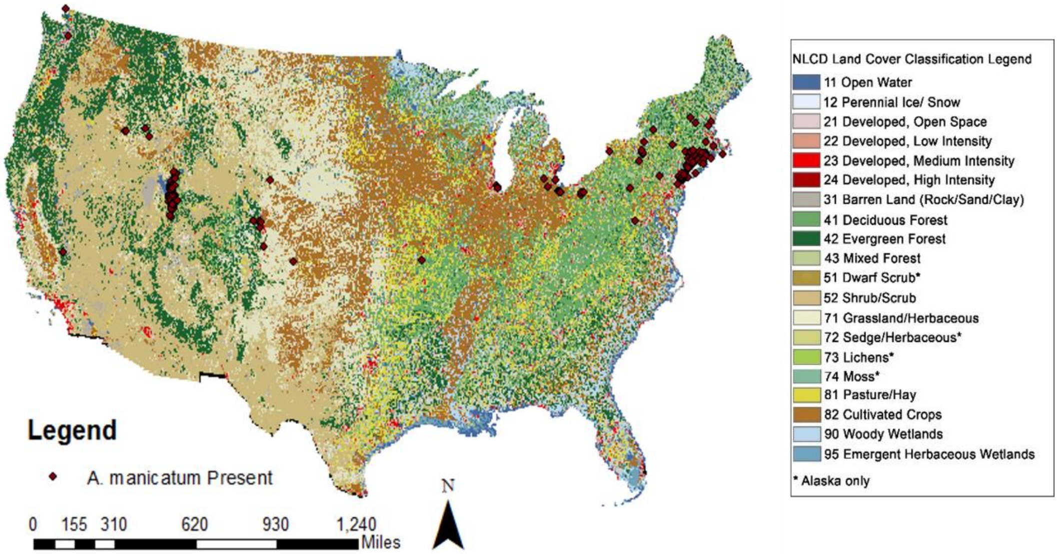

Figure 5.1 Presence of *Anthidium manicatum* in the continental US. Occurrence records were aggregated from open access data sources such as Discover Life (Ascher and Pickering 2011) and published records (Maier 2009, Griswold et al. 2014). NLCD 2011 Land Cover Type included as the background layer.

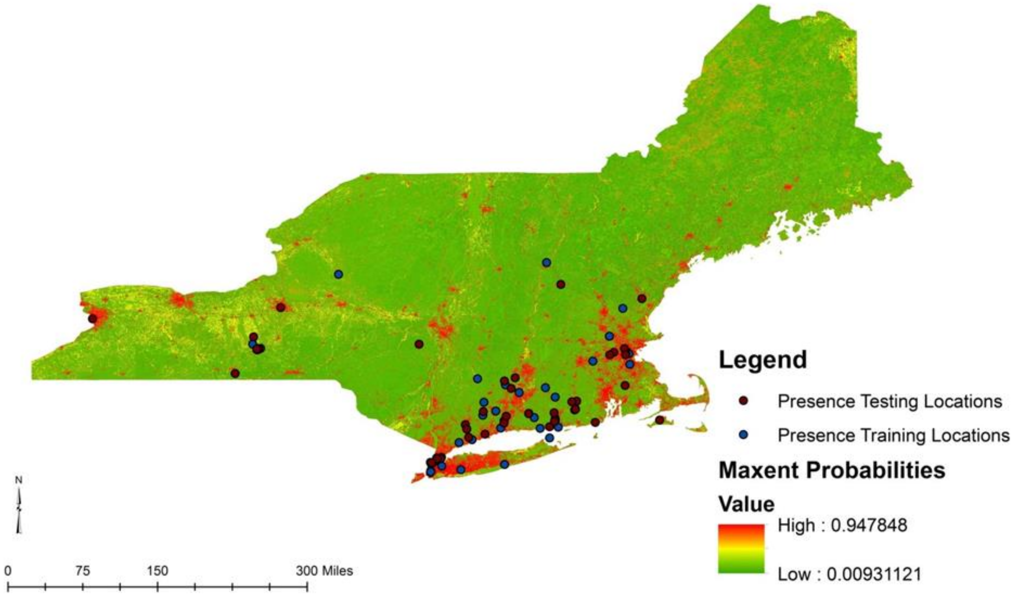

Figure 5.2 Land Cover Model - suitability distribution model for *Anthidium manicatum* in the northeast United States. Model was created using two environmental inputs – NLCD 2011 land cover type and percent impervious surface. A total of 87 occurrence records were included in the model, with 20% of data withheld to test the model. Red indicates high estimated habitat suitability, and green indicates low estimated habitat suitability. The model was created using the Maxent software (v3.3) (Phillips et al. 2006, Elith et al. 2011) and visualized using ArcGIS v10.1 (ESRI 2011).

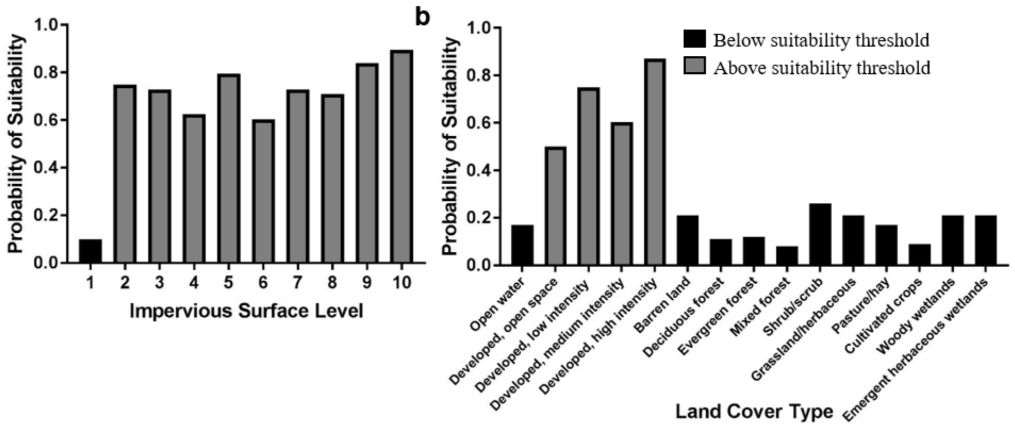

Figure 5.3 Probability of habitat suitability of *Anthidium manicatum* for (a) each impervious surface level (reclassified from NLCD 2011), and (b) each land cover type. A probability score of 0.5 or higher was classified as “suitable” for *A. manicatum*. Outputs were created using probability densities in Maxent (Phillips et al. 2006).

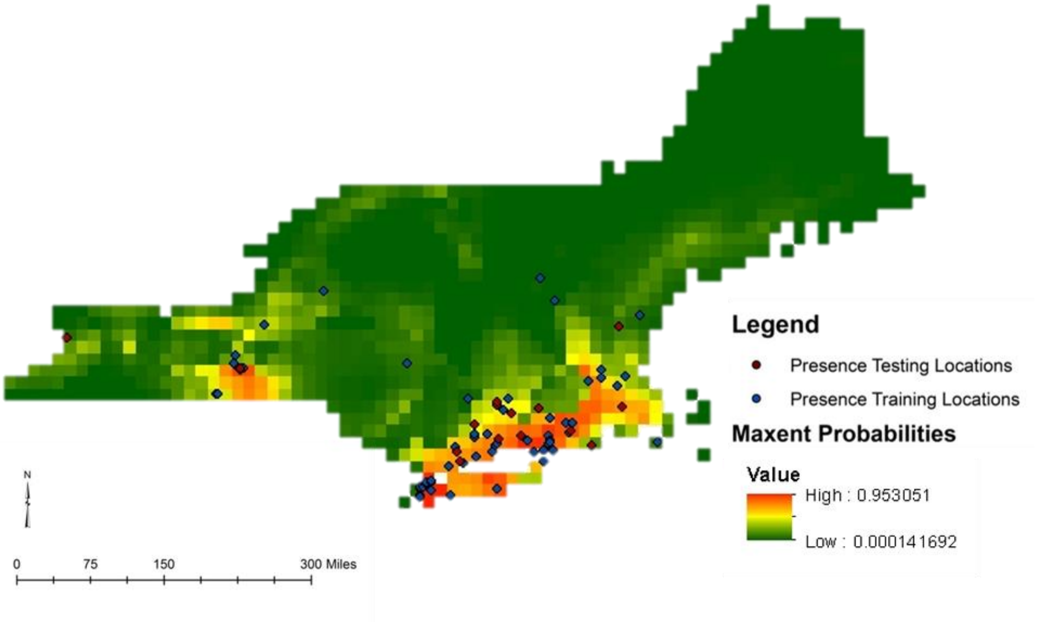

Figure 5.4 Bioclimatic Model - suitability distribution model for *Anthidium manicatum* in the northeast United States. Model was created using ten

environmental inputs – mean temperature diurnal range, temperature annual range, mean temperature of wettest quarter, mean temperature of driest quarter, mean temperature of warmest quarter, precipitation seasonality, precipitation of wettest quarter, precipitation of driest quarter, precipitation of warmest quarter, and precipitation of coldest quarter (WorldClim database - http://worldclim.org/). A total of 87 occurrence records were included in the model, with 20% of data withheld to test the model. Red indicates high estimated habitat suitability, and green indicates low estimated habitat suitability. Model was created using the Maxent software (v3.3) (Phillips et al. 2006, Elith et al. 2011) and visualized using ArcGIS v10.1 (ESRI 2011).

Table 5.1 Error matrices for two models created in Maxent. *(a)* Land Cover Model, which uses two landscape environmental variables - NLCD 2011 land cover type and percent impervious surface. *(b)* Bioclimatic Model, which uses 10 bioclimatic variables as described in Strange et al., (2011).

a. Land Cover Model		Maxent prediction	
		Absent	Present
Sampling results	Absent	38	89
	Present	1	6
KHAT = 0.023 (+/- 0.319)			
b. Bioclimatic Model		Maxent prediction	
		Absent	Present
Sampling results	Absent	95	32
	Present	7	0
KHAT = -0.094 (+/- 0.267)			

Supplementary figures

Table S5.1 Locations and results of sampling efforts in 2013 and 2014.

ID	Year	Long.	Lat.	*A. manicatum* (Present/ Absent)	Confirmed through netting?	Bee bowl sampling (Yes/No)
1	2013	-71.25283	42.34353	Absent		No
2	2013	-70.93005	42.53612	Absent		No
3	2013	-70.91502	42.53689	Absent		No
4	2013	-70.95778	42.5157	Absent		No
5	2013	-73.34125	42.27343	Absent		No
6	2013	-73.28348	42.35701	Absent		No
7	2013	-73.68676	42.5781	Absent		No
8	2013	-76.75893	43.10387	Absent		No
9	2013	-77.02855	42.6301	Absent		No
10	2013	-76.95999	42.88008	Absent		No
11	2013	-76.96269	42.87584	Absent		No
12	2013	-76.39609	42.90935	Absent		No
13	**2013**	**-76.79206**	**42.09355**	**Present**	**Yes**	**No**
14	2013	-76.26937	42.29307	Absent		No
15	**2013**	**-77.51041**	**43.194**	**Present**	**No**	**No**
16	2013	-71.36145	43.23252	Absent		No
17	2013	-70.86534	43.1628	Absent		No
18	2013	-70.71049	43.50869	Absent		No
19	2013	-70.45127	43.39969	Absent		No
20	2013	-74.19635	41.56663	Absent		No
21	**2013**	**-73.83428**	**41.54198**	**Present**	**No**	**No**
22	2013	-73.90939	41.74997	Absent		No
23	2013	-73.93096	41.76037	Absent		No
24	2013	-73.97641	41.87438	Absent	*A. oblongatum* present	No
25	2013	-73.98296	42.20602	Absent		No
26	2013	-72.48732	42.14439	Absent		No
27	2013	-72.53932	42.14362	Absent		No
28	2013	-72.63638	42.27805	Absent		No
29	2013	-72.63523	42.31156	Absent		No
30	2013	-72.6098	44.19199	Absent		No
31	2013	-72.8101	44.18891	Absent		No
32	2013	-71.07064	42.45292	Absent		No
33	2013	-71.0949	42.49298	Absent		No
34	2013	-71.1573	42.5939	Absent		No

35	2013	-71.40343	42.62779	Absent		No
36	2013	-75.72512	42.9887	Absent		No
37	2013	-70.86071	42.55037	Absent		No
38	2013	-70.97319	42.59428	Absent		No
39	2013	-70.98359	42.63549	Absent		No
40	2013	-71.40613	42.33698	Absent		No
41	2013	-71.21238	42.30771	Absent		No
42	2013	-71.10569	42.28691	Absent		No
43	2013	-71.10915	42.33891	Absent		No
44	2013	-71.67536	42.27497	Absent		No
45	2013	-71.84638	42.22798	Absent	*A. oblongatum* present	No
46	2013	-71.8718	42.05157	Absent	*A. oblongatum present*	No
47	2013	-71.87912	41.92484	Absent		No
48	2013	-71.47122	41.99918	Absent		No
49	2013	-71.48316	42.13168	Absent		No
50	2013	-71.15961	42.03	Absent		No
51	2013	-71.04444	42.05388	Absent		No
52	2013	-70.96664	42.1105	Absent		No
53	2013	-70.75518	42.13592	Absent		No
54	2013	-70.73784	42.04348	Absent		No
55	2013	-71.60103	42.39014	Absent		No
56	2013	-71.76241	42.54575	Absent		No
57	2013	-72.43955	41.85936	Absent		No
58	2013	-72.5813	41.75845	Absent	*A. oblongatum* present	No
59	2013	-72.59979	41.79311	Absent		No
60	2013	-72.72266	41.75806	Absent		No
61	2013	-72.75656	41.6972	Absent		No
62	2013	-72.87095	41.59012	Absent		No
63	2013	-72.96956	41.60515	Absent		No
64	2013	-72.89869	41.3205	Absent		No
65	2013	-72.84553	41.27081	Absent		No
66	2013	-73.13595	41.22421	Absent		No
67	2013	-73.12902	41.59359	Absent		No
68	2013	-72.50157	41.58512	Absent		No
69	2013	-73.87627	40.64413	Absent		No
70	2013	-73.92826	40.58943	Absent		No
71	2013	-73.9352	40.65183	Absent		No

72	2013	-73.93019	40.73773	Absent		No
73	2013	-73.51112	40.64297	Absent		No
74	2013	-73.55233	40.70075	Absent		No
75	2013	-73.67597	40.68611	Absent		No
76	2013	-73.67636	41.03624	Absent		No
77	2013	-70.04337	41.98185	Absent		No
78	2013	-71.41999	41.85821	Absent		No
79	2013	-71.66342	41.45839	Absent		No
80	2013	-71.42538	41.59128	Absent		No
81	2013	-71.42153	41.59244	Absent		No
82	2013	-71.18272	41.74265	Absent		No
83	2014	-71.11031	42.36125	Absent		Yes
84	2014	-71.13342	42.35586	Absent		Yes
85	2014	-71.08643	42.34623	Absent		Yes
86	2014	-71.08797	42.34546	Absent		No
87	**2014**	**-71.10184**	**42.33043**	**Present**	**Yes**	**No**
88	2014	-71.18773	42.53034	Absent		Yes
89	2014	-71.14767	42.5107	Absent		Yes
90	2014	-71.04483	42.53381	Absent		No
91	2014	-70.96587	42.46216	Absent		Yes
92	2014	-71.0013	42.39707	Absent		No
93	2014	-70.98898	42.41941	Absent		No
94	2014	-71.01979	42.41825	Absent		Yes
95	2014	-71.11329	42.5157	Absent		No
96	2014	-71.05061	42.52418	Absent		No
97	2014	-71.06948	42.47526	Absent		No
98	2014	-71.17936	42.48528	Absent		No
99	2014	-71.04675	42.2118	Absent		No
100	2014	-71.01633	42.24608	Absent		Yes
101	2014	-71.0067	42.24993	Absent		Yes
102	2014	-71.03558	42.27497	Absent		Yes
103	2014	-71.3287	42.34045	Absent		Yes
104	2014	-71.38802	42.32119	Absent		Yes
105	**2014**	**-71.42924**	**42.30848**	**Present**	**Yes**	**Yes**
106	2014	-71.50011	42.42711	Absent		No
107	2014	-71.41498	42.40554	Absent		No
108	2014	-71.31946	42.3963	Absent		No
109	**2014**	**-71.44464**	**42.42095**	**Present**	**Yes**	**Yes**
110	2014	-71.42038	42.47488	Absent		Yes
111	2014	-71.54171	42.33544	Absent		Yes
112	2014	-71.5602	42.34353	Absent		Yes
113	2014	-71.59525	42.35354	Absent		Yes
114	2014	-71.58485	42.24839	Absent		No

115	2014	-71.60873	42.27035	Absent		No
116	2014	-71.529	42.28999	Absent		No
117	2014	-71.46814	42.27266	Absent		No
118	2014	-71.15615	42.27959	Absent		Yes
119	2014	-71.19843	42.24955	Absent		Yes
120	**2014**	**-71.21431**	**42.19601**	**Present**	**Yes**	**No**
121	2014	-71.22702	42.24223	Absent		No
122	2014	-71.25013	42.23375	Absent		No
123	2014	-71.28788	42.26187	Absent		No
124	2014	-71.25668	42.27458	Absent		No
125	2014	-71.25745	42.28036	Absent		No
126	2014	-71.27324	42.56924	Absent		Yes
127	2014	-71.34719	42.47873	Absent		No
128	2014	-71.3445	42.48335	Absent		No
129	2014	-71.34257	42.4899	Absent		No
130	2014	-71.3341	42.47218	Absent		No
131	2014	-71.26823	42.58773	Absent		No
132	2014	-71.54903	42.17713	Absent		Yes
133	2014	-71.48817	42.15248	Absent		Yes
134	2014	-71.44195	42.15826	Absent		Yes
135	2014	-71.45235	42.18753	Absent		Yes
136	2014	-71.39534	42.17636	Absent		No
137	2014	-71.11878	42.15248	Absent		No
138	2014	-71.3237	42.2195	Absent		Yes
139	2014	-71.37608	42.22451	Absent		Yes
140	2014	-71.08027	42.44291	Absent		Yes

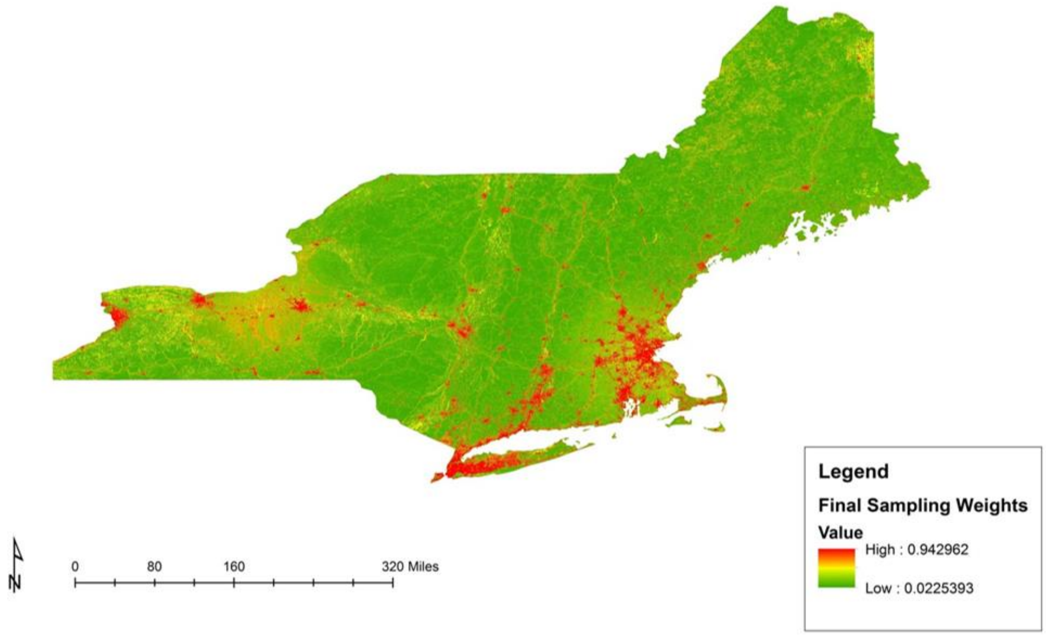

Figure S5.1 Weighted sampling scheme for Sampling in 2013. Weights were based on the likelihood of presence of *Anthidium manicatum* (40%), likelihood of *Bombus impatiens* (40%), and accessibility (20%).

Chapter 6

Conclusion

This dissertation is the most complete assessment of *Anthidium manicatum* within its invaded territory to date. Results suggest *A. manicatum* is impacting both pollinators and plants in its invaded range. I have provided evidence that female *A. manicatum* cause significant chemical changes in lamb's ear plants (*Stachys byzantina*) when they remove trichomes for nesting material. This removal of trichomes and subsequent change in plant chemistry is also likely to attract other *A. manicatum*, leading to additional carding damage. I have also shown that male *A. manicatum* impact native bumble bees (*Bombus impatiens*) through interference competition, and that female *A. manicatum* impact *B. impatiens* through exploitative competition. Additionally, my data suggests presence of *A. manicatum* causes decreased seed production in a forage crop, hairy vetch (*Vicia villosa*) and may impact seed germination rates. Finally, I have modeled habitat suitability for *A. manicatum* in the northeastern United States and found an association between *A. manicatum* and developed habitat.

Taken together, these results provide evidence that invasive *A. manicatum* are having a negative impact on the native community. However, the magnitude of impact is somewhat more encouraging. Across two studies measuring fitness impact of *A. manicatum* on *B. impatiens*, I found somewhat conflicting results. In Chapter 3, results suggest a fitness impact on *B. impatiens* colonies; however, this was only in comparison to average colony demographics reported in another study (Cnaani et al. 2002). In Chapter 4, I found no evidence of fitness consequences in *B. impatiens*. Given the larger scale of Chapter 4, and the inclusion of true controls, these results are likely more ecologically relevant. Additionally, my sampling effort found far fewer *A. manicatum* than predicted, suggesting *A. manicatum* are not as widespread as previously thought (Strange et al. 2011). In combination, these results suggest *A. manicatum* is having a relatively low magnitude impact. Though *A. manicatum*'s impact on *B. impatiens* foraging behavior remains concerning. Previous studies have found significant fitness costs for *Bombus* spp. due to interspecific resource competition (Thomson 2004, 2006, Elbgami et al. 2014). If *A. manicatum* continue to become more abundant, I would predict fitness consequences in *B. impatiens*. Though continued monitoring of this species would be needed to predict future range expansion or increases in abundance. Furthermore, other native pollinators who are more vulnerable to habitat disturbance may also be more vulnerable to resource exclusion, which is an area of research that should be explored.

Fitness of plants in association with *A. manicatum* also remains a concern. I found decreased seed production in *V. villosa* associated with *A. manicatum*. However, while this is a strong trend, I did not find a significant difference between treatment groups. Given that other studies have suggested high rates of heterospecific pollen transfer by female *A. manicatum* (Soper and Beggs 2013), additional studies looking specifically at how *A. manicatum* visits impact seed production should be explored.

Success of *A. manicatum* as an invasive species is likely due to several factors. *A. manicatum*'s family, Megachilidae, is the most represented among exotic bees (Russo 2016). It has been suggested that invasion of Megachilidae is aided due to their nesting habits, particularly those of cavity nesters (Gibbs and Sheffield 2009, Russo 2016). Cavity nesting bees nest in pithy stems, branches, and rotting wood. Many will also readily use provided nesting habitat (e.g. bamboo, or holes bored into wood blocks), making them useful for management purposes (Pitts-Singer and Cane 2011). *A. manicatum* also readily accept provided nesting habitat (Payne et al. 2011), and have even been observed nesting in the lock of a metal garden gate (Kirby and Spence 1843). This nesting behavior and flexibility almost certainly aids in their widespread invasion, as they are more likely to find pathways of introduction. Furthermore, *A. manicatum*'s near world-wide distribution and rapid rates of range expansion suggest strong propagule pressure. Distribution within their invaded habitat is also likely to be aided through accidental human transport, though this hypothesis remains to be tested.

Once established in invaded habitat, *A. manicatum* may benefit from novelty (Saul and Jeschke 2015, Graham et al. 2017c). Though another invasive bee *A. oblongatum* shares much of *A. manicatum*'s invaded range in the northeastern United States (Miller et al. 2002, Maier 2009), there are no native *Anthidium* spp. and certainly no other bees that display the same level of territorial aggression. This may allow *A. manicatum* to more easily obtain access to floral resources, and outcompete native pollinators. Comparison studies between *A. manicatum* in both their native and their invaded ranges should be undertaken to further explore advantages *A. manicatum* may exploit within its invaded habitat.

It is my hope that this dissertation serves to inspire further exploration of this charismatic invader, as much is still left to be discovered. Nonetheless, I believe this dissertation provides evidence that this species is one of concern, particularly due to resource competition with native bees. Further monitoring of this species is suggested to better predict its present and future range. Additionally, little is known about how these, and other exotic bees, are being introduced to non-native ranges. Uncovering common pathways of introduction would help limit any current and future impact of *A. manicatum* and other exotic bees.

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