

Anthidium manicatum, an invasive bee, excludes a native bumble bee, *Bombus impatiens*, from floral resources

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Abstract *Anthidium manicatum* is an invasive pollinator reaching widespread distribution in North America. Male *A. manicatum* aggressively defend floral territories, attacking heterospecific pollinators. Female *A. manicatum* are generalists, visiting many of the same plants as native pollinators. Because of *A. manicatum*'s rapid range expansion, the territorial behavior of males, and the potential for female *A. manicatum* to be significant resource competitors, invasive *A. manicatum* have been prioritized as a species of interest for impact assessment. But despite concerns, there have been no empirical studies investigating the impact of *A. manicatum* on North American pollinators. Therefore, across a two-year study, we monitored foraging behavior and fitness of the common eastern bumble bee (*Bombus impatiens*) in

response to *A. manicatum* presence. We found that *B. impatiens* avoided foraging near *A. manicatum* in both years; but despite this resource exclusion, we found no evidence of fitness consequences for *B. impatiens*. These results suggest *A. manicatum* pose as significant resource competitors, but that *B. impatiens* are likely able to compensate for this resource loss by finding available resources elsewhere.

Keywords Exotic species · Resource competition · Interspecific competition · Foraging behavior · Pollination

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 the limited resources available to combat species invasions, it is important to estimate impact of exotic species; unfortunately, 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

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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), while the majority of introduced bees are unmanaged and solitary (Russo 2016). This leaves 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 a native pollinator (*Bombus impatiens*).

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; Graham and MacLean 2018). *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 (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 (Wirtz et al. 1988). 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 *A. manicatum*'s

interactions with 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, some bumble bee populations are showing decline worldwide, with several species in North America showing a drastic population decline 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 and significant range contraction, the first North American bumble bee, *B. affinis*, was recently designated endangered by the United States government (U.S. Fish and 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 the 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 *A. manicatum*'s native range (Wirtz et al. 1988), and it is expected that there is a similar effect in *A. manicatum*'s introduced North American range (Severinghaus et al. 1981; Russo 2016), though this has not been explicitly tested. Avoidance of floral resources due to presence of *A. manicatum* could have a significant effect on a bumble bee colony's ability to produce reproductives due to resource scarcity. A reduction in reproductive output will have a significant effect on colony fitness, as reproductives are the colony's only opportunity to pass on genetic material.

Concerns about the introduction of *A. manicatum* in North America garnered it specific mention in the 2015 Pollinator Research Action Plan (PRAP), put forth following a 2014 Presidential Memorandum "Creating a Federal Strategy to Promote the Health of Honey Bees and Other Pollinators" (Pollinator Health Task Force 2015). Within the PRAP was a call to better understand the impacts of introduced bees on native communities, with specific interest in species that are rapidly increasing in abundance and distribution, such as *A. manicatum*. Here, we investigate the impact of *A. manicatum* presence on the foraging

behavior and fitness of a native pollinator, *B. impatiens*. This is the first study to test if significant competition for resources exists between *A. manicatum* and a native pollinator. We predict *B. impatiens* will avoid foraging at resources when *A. manicatum* are present, and that this will lead to a significant decrease in colony reproductive fitness due to resource shortage.

Methods

Study design

In order to manipulate the encounter rate of *B. impatiens* and *A. manicatum*, we used screened research enclosures (3 m × 3 m across, and 2.1 m tall) set up in two rows in an open, mowed field (tents were spaced 3–5 m apart within each row, and rows were 10 m apart; Fig. S1). 12 enclosures were used in 2015, and 14 enclosures in 2016. We planted flowering plants (*Nepeta x faassenii*, *Salvia farinacea*, and *Agastache foeniculum*) visited by both *B. impatiens* and *A. manicatum* (pers. obs., Payette 2001) in each enclosure. All plants were purchased in June 2015 from a local nursery and planted with the same orientation and spacing within each enclosure. All plants are perennials and flowered the following year; therefore, the same plants were used in 2015 and in 2016, with additional plants purchased from the same nursery for the two added enclosures in 2016. We also added in 20 *Vicia villosa* plants in pots (four plants per pot) to each enclosure in 2016, which were grown from seeds.

We placed one commercially reared bumble bee hive (*B. impatiens*) just outside the enclosure, and *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. S2). *B. impatiens* colonies had the following differences between the years. In 2015, we ordered 12 *B. impatiens* colonies from Koppert Biological Systems Inc. (Howell, MI). These colonies arrived around peak production with approximately 75 workers, a queen, and brood, and were setup as “observation colonies” with clear observation lids and no cotton insulation over brood. In 2016, we ordered 14 *B. impatiens*

colonies from Biobest U.S.A. Inc. (Leamington, Ontario). These colonies were significantly younger, with newly emerged workers (~ 10), a queen, and brood. Colonies used a “field deployment” setup, with grated lids (not observation lids) and cotton insulation over brood. We switched companies in 2016 due to pricing differences, and used younger colonies in 2016 to allow us to evaluate changes in colony growth across the season due to treatment (presence/absence of *A. manicatum*). We switched to a field deployment setup because workers were observed in 2015 gathering materials from outside the colony (mulch, grass, etc.) to insulate the brood. We worried this behavior may have been due to the impact of environmental stressors (namely high temperatures) on the development of brood. Since the health of colonies was being directly tested in 2016, we wanted to limit the potential influence of environmental stressors. To ensure that we accounted for new gynes produced by the colony, queen excluders were fitted to each hive.

After *B. impatiens* colonies were installed at the field site (13 July 2015/14 June 2016), we gave them 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 (50 ml conical tubes filled with a 50% sucrose solution inverted on petri dishes). At the end of the acclimation week, *B. impatiens* workers from all colonies were foraging at enclosure plants, and were also observed returning with pollen loads after foraging outside the enclosure. At that time, we removed nectar access from the hive and removed feeding stations from the enclosures.

Changes in *Bombus impatiens* foraging behavior in response to *Anthidium manicatum* presence

We began treatments following the week of acclimation, and treatments alternated by enclosure placement (Fig. S1). Half the enclosures had *A. manicatum* present (1–2 male *A. manicatum* and 2–3 female *A. manicatum*) throughout the study, and we collected *A. manicatum* using aerial nets in the surrounding urban residential area. If any *A. manicatum* died or went missing during the study, we quickly replaced them through additional collections. *A. manicatum* densities in the enclosures matched similar densities observed at flower patches in the surrounding environment. The

other half of the enclosures had no *A. manicatum* present.

We used two data collection methods to assess differences in *B. impatiens* foraging behavior between the two treatments (Table S1): (1) number of *B. impatiens* foraging on enclosure plants, and (2) proportion of foragers entering the enclosure compared to the surrounding environment.

1. We counted the number of *B. impatiens* on enclosure plants 1–3 times per week for five (2016) or six (2015) weeks. Counts were instantaneous as we scanned the enclosure. All counts were made between 09:00 and 14:00 h, on days when the temperature was between 21 and 32 °C with no rain and when both *B. impatiens* and *A. manicatum* were observed to be actively foraging. Enclosures were observed in random order.
2. To monitor forager choice between the enclosure and the field, we monitored each hive entrance for 30 min 1–2 times per week. Observations were made between 09:00 and 12:30 h, and only during conditions when both *B. impatiens* and *A. manicatum* were actively foraging (as described above). We counted the number of *B. impatiens* exiting the hive, as well as where they were traveling (to either the research enclosure or the surrounding field). While making observations, we stood approximately 1 m from the hive with a good view of the hive entrance and exit tubes. Again, order of observations at each enclosure were done randomly.

Changes in *Bombus impatiens* colony growth and reproduction due to interactions with *Anthidium manicatum*

In 2016, we also collected data on correlates of colony fitness. We measured weight of colonies 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). We weighed colonies (Ohaus Ranger 3000, accurate to 0.002 kg) at night (21:00–23:00 h) when the majority of individuals were likely to be in the colony. Treatments ended when the first reproductives (males or gynes) were observed. When reproductives were

first seen (16 August 2016) we collected all colonies at night and freeze-killed them at -20 °C. The colonies were then stored in a -20 °C freezer until dissections.

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

Data analysis

We completed all analyses using R version 3.3.1 (R Core Team 2016), and graphs were created using GraphPad Prism 7 (GraphPad Software 2017). Generalized linear mixed models (GLMM), linear mixed models (LMM) (lme4 package) or linear models (LM) (stats package) were used to test treatment effects, and model assumptions were tested prior to model building to improve fit (DHARMA package). We conducted model comparison for each analyses such that full models were compared to models with factors excluded (Table S2). The best model for each analyses was then selected through comparison of AICc scores using the Ictab function (bbmle package) (Burnham and Anderson 2002) (Table S2). If competing models were within 2.0 Δ AICc, we chose the simpler model. We then calculated both the marginal R^2 (R^2_m) and the conditional R^2 (R^2_c) using the sem.model.fits function (piecewiseSEM package). R^2_m describes the proportion of variance explained by the fixed factor(s). R^2_c describes the proportion of variance explained by both the fixed and random factors (Nakagawa and Schielzeth 2013). We calculated p values using the Anova function (car package).

We first compared the average number of *B. impatiens* foraging at enclosure plants. Given the different initial life stages of the colonies at the start of each year, we looked at the effect of treatment separately for each year, instead of combining data across years, except when explicitly testing differences between the years (such as overall foraging effort, see below). The full model included treatment and week, as well as their

interaction, as fixed effects and enclosure was included as a random effect (Table S2). Data (number of *B. impatiens* counted on enclosure plants) were log transformed to attain equal variances and were normally distributed (family = Gaussian).

Second, we compared the proportion of *B. impatiens* foragers exiting the hive and entering the enclosure (out of total foragers exiting the hive) between treatments. We removed any data points with no foraging activity from the data set, as the reason for inactivity could not be determined. Inactivity was rare and not correlated with hive or treatment. The full model included treatment and week, as well as their interaction, as fixed effects and enclosure was included as a random effect (Table S2). Models were created with a Negative Binomial distribution to adjust for dispersion.

Third, in order to better interpret differences between 2015 and 2016, we compared the average foraging effort (total foragers exiting the colony during the 30 min observations) between years. This allowed us to better understand differences in average colony foraging activity between the years, and to test if treatment had any overall impact on foraging effort. The full model included year (2015/2016) and the interaction of treatment and week as fixed effects (Table S2). Data were log transformed to attain equal variances and were normally distributed (family = Gaussian).

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 gynes, and gyne pupae). The full model included treatment and demographic variable type as interacting factors, with colony as a random effect (Table S2). Data were log transformed to attain equal variances and were normally distributed (family = Gaussian).

We then compared “reproductive success” (RS), as defined by Pelletier and McNeil (2003): $RS = \text{Males (adult males and pupae)} + 3 * \text{Queens (adult new gynes and gyne pupae)}$, between the treatments. Calculation of reproductive success accounts for the disproportionate foraging effort needed to produce queens compared to males. The full model included treatment as the factor of interest (Table S2). Data were log transformed to attain equal variances and were normally distributed (family = Gaussian).

We compared average weight of workers, queens, and males between treatments. The full model

included treatment as factor and colony as a random effect (Table S2). Data followed the assumptions of a linear model.

Finally, we calculated change in colony weight 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.

Results

Changes in *B. impatiens* foraging behavior

In both years, significantly fewer *B. impatiens* foraged on enclosure plants when *A. manicatum* were present (LMM; 2015: $R^2_m = 0.24$, $R^2_c = 0.74$; $X^2 = 5.50$, $df = 1$, $p = 0.019$; 2016: $R^2_m = 0.29$, $R^2_c = 0.56$; $X^2 = 4.01$, $df = 1$, $p = 0.045$; Table S2). This effect was constant across weeks in 2015 ($X^2 = 18.66$, $df = 14$, $p = 0.18$; Fig. 1), however, the effect increased over time in 2016 ($X^2 = 15.33$, $df = 1$, $p < 0.001$; Fig. 1).

To further understand how foraging behavior may have changed due to presence of *A. manicatum*, we also compared the ratio of all foragers entering the enclosures compared to the surrounding environment. In 2015, a significantly lower proportion of total *B. impatiens* foragers leaving the colony went into enclosures with *A. manicatum* present compared to those without *A. manicatum* (GLMM; $R^2_m = 0.13$, $R^2_c = 0.36$; $X^2 = 5.14$, $df = 1$, $p = 0.023$; Fig. 2; Table S3A), and this effect did not change over time ($X^2 = 0.04$, $df = 1$, $p = 0.840$). However, in 2016, an equal proportion of foragers went into the enclosures versus outside the enclosures, with no effect of treatment ($R^2_m = 0.13$, $R^2_c = 0.36$; $X^2 = 1.69$, $df = 1$, $p = 0.194$).

There were significantly fewer foragers (all foragers leaving the colony during 30 min observations) in 2016 compared to 2015 (LM; $R^2 = 0.38$, $X^2 = 125.23$, $df = 1$, $p < 0.001$; Fig. 3). This is not surprising, as we started with much smaller colonies in 2016. Treatment (*A. manicatum* presence) did not affect average foraging effort, with equivalent numbers of total foragers leaving colonies associated with *A. manicatum* present enclosures, as those without *A. manicatum* ($R^2 = 0.38$, $X^2 = 1.51$, $p = 0.22$).

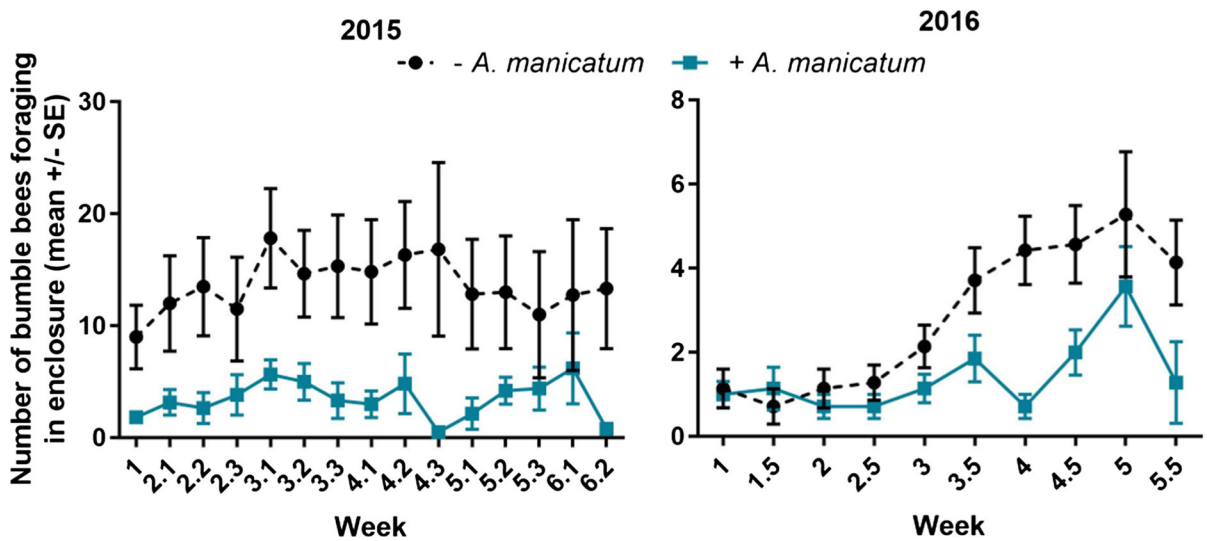


Fig. 1 Average number of *Bombus impatiens* foraging on enclosure plants (*Nepeta x faassenii*, *Salvia farinacea*, and *Agastache foeniculum*) in 2015 and 2016. Error bars indicate standard error from the mean. Half the enclosures (six in 2015, seven in 2016) had *A. manicatum* present throughout the experiment, while the other half had no *A. manicatum* present.

Number of *B. impatiens* on enclosure plants were counted 1–3 times per week for five (2016) or six (2015) weeks. In both years, significantly fewer *B. impatiens* foraged on enclosure plants when *A. manicatum* were present (LMM; 2015: $R^2m = 0.24$, $R^2c = 0.74$; $X^2 = 5.50$, $df = 1$, $p = 0.019$; 2016: $R^2m = 0.29$, $R^2c = 0.56$; $X^2 = 4.01$, $df = 1$, $p = 0.045$)

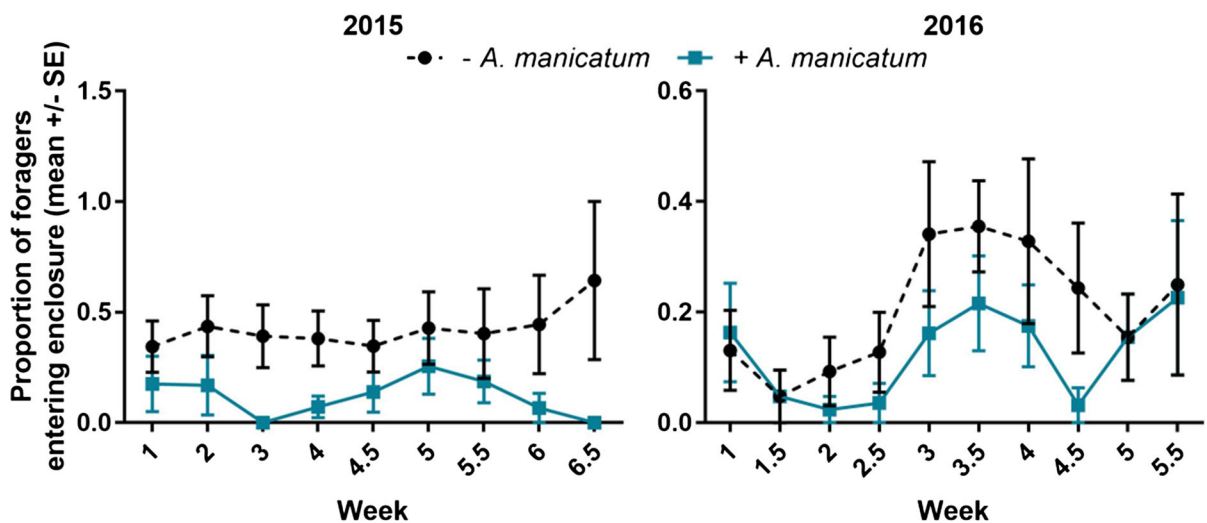


Fig. 2 Average proportion of total *Bombus impatiens* foragers entering the enclosure compared to the surrounding field in 2015 and 2016. Error bars indicate standard error from the mean. Half the enclosures (six in 2015, seven in 2016) had *A. manicatum* present throughout the experiment, while the other half had no *A. manicatum* present. Foragers exiting each colony were observed for 30 min 1–2 times per week for five (2016) or six (2015) weeks. In 2015, a significantly lower proportion of total

B. impatiens foragers leaving the colony went into enclosures with *A. manicatum* present compared to those without *A. manicatum* (GLMM; $R^2m = 0.13$, $R^2c = 0.36$; $X^2 = 5.14$, $df = 1$, $p = 0.023$). In 2016, an equal proportion of foragers went into the enclosures versus outside the enclosures, with no effect of treatment ($R^2m = 0.13$, $R^2c = 0.36$; $X^2 = 1.69$, $df = 1$, $p = 0.194$)

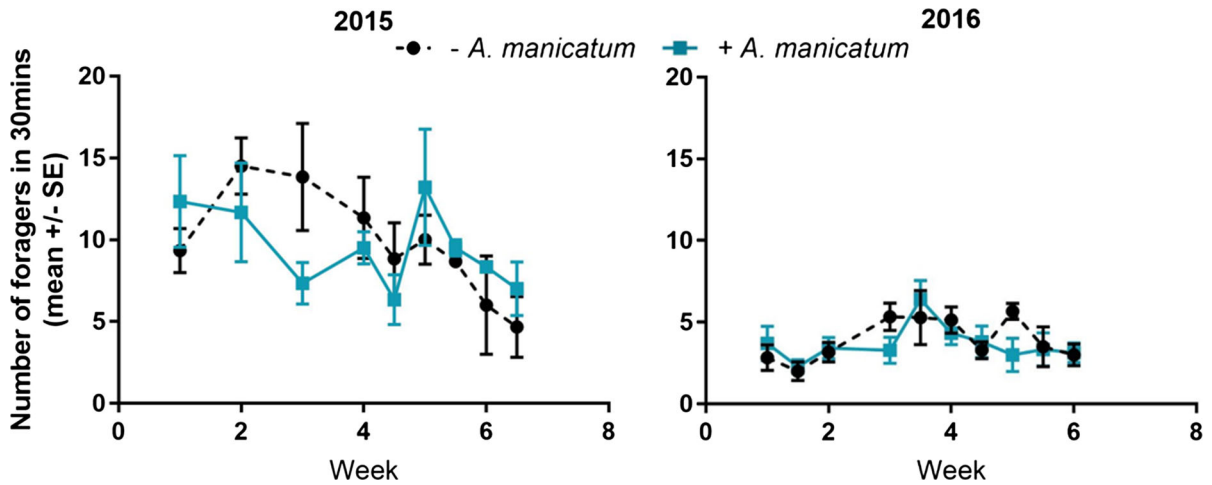


Fig. 3 Average total *Bombus impatiens* foragers exiting the colony (to the research enclosure and the surrounding field) in 2015 and 2016. Error bars indicate standard error from the mean. Half the enclosures (six in 2015, seven in 2016) had *A. manicatum* present throughout the experiment, while the other half had no *A. manicatum* present. Foragers exiting each colony

were observed for 30 min 1–2 times per week for five (2016) or six (2015) weeks. There were significantly fewer foragers (all foragers leaving the colony during 30 min observations) in 2016 compared to 2015 (LM; $R^2 = 0.38$, $X^2 = 125.23$, $df = 1$, $p < 0.001$), but with no significant effect of treatment across the years ($R^2 = 0.38$, $X^2 = 1.51$, $p = 0.22$)

Table 1 Comparing *Bombus impatiens* colony demographics between treatments

Fitness metric	+ <i>A. manicatum</i> (average ± SE)	– <i>A. manicatum</i> (average ± SE)	Statistical significance
# of workers	50.4 ± 8.6	61.3 ± 9.9	No, $p = 0.27$
# of queens ^a	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 ^a)	4.9 ± 2.8	4.6 ± 3.4	No, $p = 0.42$
Reproductive success ($RS = M^b + 3Q^a$)	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 ^c weight, dry (g)	0.273 ± 0.090	0.398 ± 0.118	–
Male weight, dry (g)	0.071 ± 0.008	0.048 ± 0.001	–

Weights were measured after they were lyophilized for 24 h. Statistics were not performed for individual reproductive groups (males or queens) due to low sample sizes

^aNew adult queens and queen pupae

^bAdult males and non-queen pupae (Pelletier and McNeil 2003)

^cOriginal queen with new adult queens

Bombus impatiens colony fitness effects (2016)

Number of queens or males were not compared individually, as very few were produced (Table 1). Instead, we combined them as “reproductives.” There was no significant effect of treatment on number of

workers, pupae, larval clumps, or reproductives (LMM; $R^2 = 0.79$; $F_{1,60} = 0.19$, $p = 0.663$; Table 1). There was also no significant effect of treatment on Reproductive Success (RS) (LM; $R^2 = 0.00$, $F_{1,12} = 0.04$, $p = 0.853$). Average weights of queens and males were not compared due to low sample sizes.

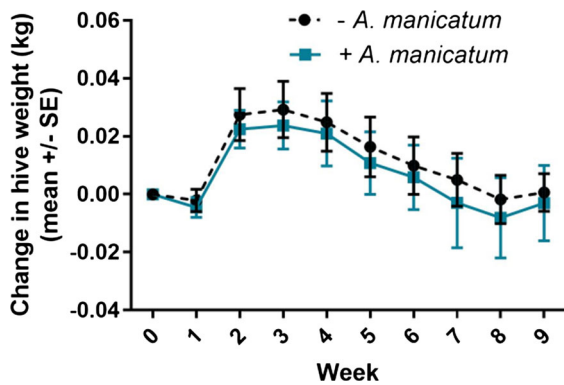


Fig. 4 Average change in hive weight compared by treatment. Hives were weighed once a week for 9 weeks. Change in weight was compared to their initial weight (Week 0), and compared between treatments: *A. manicatum* present throughout experimental period or *A. manicatum* absent in research enclosures. Error bars indicate standard error from the mean. There was no significant effect of *A. manicatum* presence on change in hive weight (repeated measures 2-way ANOVA; $F_{1,135} = 0.40$, $p = 0.527$)

Average weight of workers was compared between treatments, with no significant effect of treatment (LMM; $R^2_m = 0.00$, $R^2_c = 0.73$; $X^2 = 0.08$, $df = 1$, $p = 0.782$; Table 1).

There was also no significant effect of treatment on average change in colony weight (repeated measures 2-way ANOVA; $F_{1,135} = 0.40$, $p = 0.527$). Colonies from both treatments followed a similar growth trajectory throughout the experiment (Fig. 4).

Discussion

While the effects of invasive populations of *A. manicatum* on native pollinators have been speculated (Colla 2016; Russo 2016), this is the first study to explicitly test these effects in North America. In manipulating the presence of *A. manicatum*, we demonstrated that *A. manicatum* excludes *B. impatiens* from floral resources. *B. impatiens* avoided foraging on enclosure plants when *A. manicatum* were present across two years of testing. In the second year (2016), we also found that *B. impatiens* avoided the enclosures with *A. manicatum* more strongly over time. However, it is likely that this influence of time is due to the growth of the colonies in 2016, as colonies started out very small (with ~ 10 foragers) and grew over the course of the season. Measuring an effect at the start of

the season may therefore have been challenging, given the low number of foragers. As such, we favor the interpretation that avoidance of *A. manicatum* is relatively consistent over time (as suggested by the 2015 data); however, the possibility that *B. impatiens* will increase avoidance behavior with increased exposure cannot be ruled out.

In the first year, we also found that a lower proportion of foragers entered enclosures when *A. manicatum* were present. This suggests that foragers may be increasing foraging efforts in the surrounding environment to compensate for exclusion from resources in *A. manicatum* enclosures. However, the same pattern was not found in the second year. One possible explanation for this is that use of smaller colonies, with a smaller number of total foragers, limited our ability to detect a difference. An alternate explanation is that bumble bees did not compensate for reduced access to enclosure resources by increasing foraging trips to the surrounding environment. However, we do not favor this interpretation, as average foraging effort between treatments did not differ. If no compensation was occurring, you would expect reduced overall foraging effort at colonies associated with enclosures with *A. manicatum*, since we did find fewer foragers in *A. manicatum* enclosures.

Avoidance of *A. manicatum* did not appear to affect *B. impatiens* colony growth or production of reproductives. Colonies grew at similar rates, and we found no difference in colony demographics at the end of the season. It appears that, at least within the context of this experiment, resource exclusion due to presence of *A. manicatum* did not carry over into effects on colony fitness. These results are encouraging, though unexpected, as presence of heterospecific resource competitors (such as honey bees) have been shown to have significant negative effects on *Bombus* colony growth and production of reproductives (Thomson 2004; Elbgami et al. 2014). However, limitations with study design may have restricted our ability to fully capture the impact on reproductive output.

B. impatiens produce reproductives over several weeks. Due to concerns about males leaving the nest before we were able to record their presence, we froze all colonies at the first appearance of reproductives. This only allowed us to sample the first round of reproductives produced, though pupae were also included in analyses with no effect of treatment. To fully rule out any impact of *A. manicatum* presence on

colony fitness, additional studies on colonies where you can observe colony development directly would need to be completed. This can be accomplished using “observational colonies” with observation lids and uncovered brood, though care would need to be taken that colonies with this type of setup are not subjected to increased environmental stressors, which could potentially mask any effect of treatment. Nonetheless, within the context of this study, it seems that *B. impatiens* colonies were able to gain enough resources from unguarded flowers in the surrounding environment to maintain healthy colony growth, and that there was no impact on reproductive output.

However, if *A. manicatum* become more abundant in the environment, as has been predicted by some habitat suitability models (Strange et al. 2011, though see Graham and MacLean 2018), competition for resources is likely to increase. Given the patchy distribution of territorial male *A. manicatum*, it is possible that *A. manicatum*'s ability to exclude native pollinators from resources will cause cryptic habitat fragmentation. We define cryptic habitat fragmentation as a non-traditional fragmentation of the habitat, where otherwise “good” bee habitat becomes unusable or less attractive to native pollinators due to presence of *A. manicatum*. This could be particularly problematic in urban areas where *A. manicatum* presence is predicted to be high (Graham and MacLean 2018), and resources are already relatively limited and patchy in distribution (urban gardens, landscaping, etc.).

Our results show season long disruption of foraging activity in a native pollinator due to presence of *A. manicatum*. Given the rapid spread of *A. manicatum* in North America and the results of this study, further research should be done to understand *A. manicatum*'s impact on other native species, particularly vulnerable species with niche overlap. While social Apidae (namely *Apis* and *Bombus*) have been the most commonly recorded heterospecifics having negative interactions with *A. manicatum* (Severinghaus et al. 1981; Wirtz et al. 1988; Soper and Beggs 2013), there is much left unknown about their interactions with other groups, especially other Megachilidae. Native Megachilidae could share a more similar ecological niche, and competition for floral or nesting resources may be more important for this group. Additionally, exclusion from resources or negative interactions with territorial male *A. manicatum* may be more significant

for solitary species where females are solely responsible for provisioning nests. *Bombus* may be less vulnerable because they are generalist foragers able to take advantage of a wide variety of resources in the landscape and eusociality buffers the effects of individual forager loss. As management of Megachilidae (namely *Osmia lignaria* and *Megachile rotundata*) for pollination services increases in popularity, it may become easier to test these types of questions (though it should be noted that *M. rotundata* is native to Europe).

Studies testing the impacts of exotic bees are lacking in general. This is largely due to challenges associated with manipulative experiments in wild bees (Goulson 2003). Those that have been carried out have largely focused on the impact of intentionally introduced social species, honey bees (*Apis* sp.) and bumble bees (*Bombus* sp.). However, even with the focus on these genera, the literature is often mixed about overall impact directionality, with many studies only hypothesizing potential impacts, and many others with inconclusive results (reviewed in Russo 2016). Of the 67 documented non-*Apis* or *Bombus* introduced bees, only eight species have associated studies which empirically tested impacts (Russo 2016). Increased attention needs to be paid to understudied exotic bees, especially Megachilidae, which are the most widespread unintentionally introduced family of bees (Russo 2016). This study is the first to empirically test the impact of *A. manicatum* in North America, and we hope that it serves as a catalyst for future assessment of this and other exotic bees.

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Compliance with ethical standards

Conflict of interest The authors have no conflicts of interest to report.

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