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Bumble Bee Avoidance of Argentine Ants and Associated Chemical Cues

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Abstract Bees provide pollination services in both agricultural and natural ecosystems. However, invasive ants that exploit floral nectar in these landscapes can compete with bees for resources, with implications for pollinator resource acquisition, plant fitness, and, ultimately, ecosystem function. While interference competition has been described between bees and nectivorous, non-pollinator antagonists, the behaviors and sensory mechanisms involved remain largely unresolved. Here, we studied the mechanisms by which invasive Argentine ants influence bee nectar foraging behavior. In a series of laboratory assays, we assessed the foraging behavior of bumble bees (*Bombus impatiens*) in response to live Argentine ants (*Linepithema humile*) or to a subset of ant chemical cues. Bees were clearly deterred by live ants at a nectar source: they consumed less, fed less frequently, and fed for a shorter duration when live ants were present. Bees were also deterred by a combination of olfactory and gustatory ant chemical cues, consistent with both innate and learned avoidance behavior. Naïve and ant-experienced bees were deterred through chemosensation of ants, feeding less from nectar infused with ant chemicals as compared to nectar lacking ants or their associated cues. Some ant-experienced bees showed a unique behavior, displaying aggression toward ants as well as in response to ant chemicals. The marked effects of this invasive ant on bee foraging behavior—through physical interaction and chemical cues—highlights Argentine ants as a serious pest whose control should be considered when developing pollinator conservation and management strategies.

Keywords Bumble bees · pollinators · Argentine ants · invasive species · competition · species interactions · chemical ecology · behavioral ecology

Introduction

Pollinators are both ecologically and economically important organisms, responsible for aiding the reproduction of the majority of wild plant species and providing vital pollination services in agriculture (Ashman et al. 2004; Potts et al. 2010). Unfortunately, key pollinators, like bees, face many threats, including invasive species (Vanbergen et al. 2013). The Argentine ant, *Linepithema humile*, is a globally

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distributed pest which is known to outcompete organisms encountered in its introduced range (Human and Gordon 1996; Wetterer et al. 2009). Given the Argentine ant's capacity to harass pollinators (Hanna et al. 2015; Sidhu and Wilson Rankin 2016), it is critical that we understand the magnitude and mechanisms of invasive Argentine ant impacts on bees.

Niche overlap between invading ants and resident bees may manifest as exploitative or interference competition (Miller 1967). Interspecific exploitative competition entails the consumption of a limiting common resource by one species, making it less available to competitor species; while interspecific interference competition occurs when a species hinders or prevents successful resource collection by another species, often through aggression (Fellers 1987). While exploitative competition with nectar-thieving ants would primarily affect nectar-collecting pollinators, interference competition may inhibit both nectar and pollen foraging by bees.

To avoid aggressive floral visitors, pollinators may use various sensory cues, such as direct visual or chemical cues or more persistent, indirect chemical traces that indicate the identity and previous presence of the aggressor (Kats and Dill 1998). Bumble bees are known to use vision during both foraging (e.g., Nityananda and Pattrick 2013; Telles and Rodriguez-Girones 2015), and the evasion of predators (Gonçalves-Souza et al. 2008; Nityananda and Chittka 2015). However, the importance of visual cues in bee evasion of non-predatory aggression remains largely unexplored. Here we wanted to examine how a combination of visual and olfactory cues as well as chemical cues alone affected bee foraging behavior.

Chemosensation plays a role in the foraging of both insect and avian pollinators. In most insect species, cuticular hydrocarbons (CHCs) cover the insect cuticle and serve as an important means of chemical communication (Howard and Blomquist 2005). Such chemically-mediated communication has been particularly well documented in social Hymenoptera (Blomquist and Bagneres 2010), where CHC profiles can indicate nestmate status and dominance rank in ants, bees and wasps (Liebig 2010). For example, eusocial honey bees (*Apis mellifera*) (Châline et al. 2005) can discriminate among CHC compounds, while bumble bees deposit CHC footprints on flowers and may utilize them when selecting a resource (Witjes and Eltz 2009). In addition, pheromones are extensively used by social insects in a variety of contexts (Leonhardt et al. 2016), including defense (Leonhardt 2017), social harmony (Le Conte and Hefetz 2008) and foraging (Czaczkes et al. 2015). The detection of ant-associated CHCs or pheromones may allow bees to avoid aggressive, nectar-thieving ant competitors. Although the olfaction and gustatory literature on pollinators is largely dominated by insects, there is evidence that nectar-feeding birds also respond to ants on flowers and feeders. Rankin et al. (2018) found that several species of hummingbirds reduced visitation to nectar resources with live Argentine ants and were strongly repelled by combined tactile and gustatory ant cues.

Here, we quantify how bumble bee nectar foraging changes in the presence of invasive Argentine ants, as well as identify the sensory mechanisms involved in bumble bee recognition and learning in this context. We hypothesized that competitive interference occurs between bumble bees and Argentine ants, and we predicted that bees would show avoidance of (1) nectar sources with live ants and (2) ant chemical cues in the absence of live ants. We performed a series of choice assays in the laboratory to ascertain the foraging behaviors and preferences of common eastern bumble bees (*Bombus impatiens*) in response to live Argentine ants and several ant-associated chemical cues.

Materials and Methods

Colony Maintenance

Bumble Bee Colonies We conducted a series of behavioral trials with foragers from 20 *Bombus impatiens* colonies obtained from Biobest USA (Romulus, MI). In the lab, each colony was maintained in a plywood nest box (30 × 30 × 30 cm) and provided 40.6% (w/w) sucrose solution and pollen (Brushy Mountain Bee Farm, Inc., Moravian Falls, NC) *ad libitum.* To allow for individual identification, each bee was marked by adhering a small, unique number tag to the thorax. Bees were deprived of sucrose solution for at least 15 min prior to each trial in order to encourage participation and feeding.

Argentine Ant Microcolonies We collected queens, workers and brood *Linepithema humile* from the University of California, Riverside (UCR) Biological Control Grove (33.973387, -117.318344), housed in a plastic container (42.5 L x 30.2 W x 17.8 H cm, Sterilite Corporation, Townsend, MA), and regularly supplemented, as needed, with workers collected from additional nests of the same source supercolony. From this queenright lab colony, we created queenless experimental microcolonies by relocating approximately 200 ants and at least five larvae into a smaller plastic container (18.5 L x 13.5 W x 10 H cm, Target Corporation, Minneapolis, MN) lined with Insect-a-Slip Insect Barrier — Fluon (PTFE-30, DISP30, BioQuip Products, Inc., Compton, CA). Ants were provided *ad libitum* with sucrose solution, water and diced German cockroaches (*Blattella germanica*) (Online Resource 1: Fig. S1). Microcolonies were randomly selected at the beginning of each trial, however no microcolony was used in more than one experiment a day.

General Experimental Set Up

In all experiments, we provided bees with a 1.2 M sucrose solution as experimental nectar in each of two feeders, which were presented as a choice test between a control and ant-treatment. Each feeder consisted of a 5.9 cm diameter jar lid, through which a 5 cm nectar-soaked dental cotton wick protruded from a 3.5 mL vial of sucrose solution (Online Resource 1: Figs. S1 and S2). Each prepared feeder was placed in a 100 × 15 mm plastic petri dish (Fisher Scientific International, Inc., Hampton, NH), and feeder pairs were placed in the center of a plastic arena (42.5 L x 30.2 W x 17.8 H cm, Sterilite Corporation). Each bee colony was trained to forage in this arena for a minimum of eight hours before their first experimental trial. Bees could access feeders by flying or by crawling on a Y-shaped, metal mesh bridge. The placement of treatment and control feeders for each trial was randomized by using an online digital coin flip simulator (Google.com).

During each 30-minute trial, we recorded: bee identity, number of visits, time spent on each feeder per visit, feeding duration, and, if applicable, the number and nature of ant interactions. A visit to the feeder was defined as landing or walking on a feeder for > 1 s, while a feeding event occurred when a bee extended its proboscis and fed for > 1 s. To determine nectar consumption, feeders were weighed using a Mettler PC 180 balance (Mettler-Toledo, LLC, Columbus, OH) before and after each trial. After each trial, all feeders underwent a chemical rinse with hexane, ethanol, and dH_2O (see Online Resource 2 for additional details). At all times, feeders were handled with sterile, disposable gloves to ensure that the control feeders were free of contamination from any ant treatment materials. No feeder was ever used in consecutive trials, and all arenas were cleaned with 70% ethanol and dH_20 between trials.

Live Ant Presence Experiments

To test the effect of live ant presence on bee foraging behavior, a queenless *L. humile* microcolony foraged freely on the treatment feeder only, while a queenless *B. impatiens* colony had access to the entire foraging arena, including control and treatment feeders. Prior to each trial, an ant microcolony was allowed at least 15 min to establish on the treatment feeder before a bee colony was given access to the arena. In order to prevent ant escape, the petri dishes in which feeders sat were coated with Fluon. Each of five bee colonies underwent three 30-minute trials on three consecutive days, for a total of 1.5 h foraging time with live ants. A sixth colony required a fourth trial with ants due to low participation (i.e., less than five bees interacting with ants) in an earlier trial.

Ant Cues Experiments

To measure the effects of ant chemical cues and to assess bee learning, we tested both naïve and ant-experienced bee colonies with one of several ant treatments: CHC footprints (N = 12 colonies), ant bodies in nectar (N = 12 colonies), or (Z)-9 hexadecenal pheromone (N = 6 colonies). For all of the following experiments, bee colonies were classified as “naïve” if they had never made contact with Argentine ants and classified as “experienced” once they had at least 1.5 h of foraging time in the live ant arena.

CHC Footprints as an Indicator of Prior Visitation by Ants Ants, similar to other insects, passively deposit hydrocarbon footprints while walking (Lenoir et al. 2009; Wust and Menzel 2017). A fully prepared treatment feeder was placed into an ant microcolony for one hour to ensure that a minimum of 20 ants walked upon the filter paper and fed, depositing any chemical cues associated with visitation—e.g., footprint hydrocarbons as well as any pheromones associated with feeding. During this hour, the control feeder sat outside of the ant colony. After one hour, the treatment feeder was retrieved, the ants gently and quickly removed with a paint brush, and both treatment and control feeders were placed in the arena to begin the trial. Data were collected as described above for the general experimental setup.

Ants in Nectar Drowning is a foraging risk ants face (Zhou et al. 2020, 2022), potentially polluting the nectar quality or taste. To simulate this situation, 50 frozen ants (0.018 g ± 0.000 g) were placed into a tissue homogenizer and crushed into 5mL of 40.6% sucrose solution. This is the equivalent of 1 ant/100 µl, a ratio shown to deter hummingbirds (Rankin et al. 2018). One cotton wick was placed in the ant-infused nectar while a control wick was placed in 5mL of unaltered sucrose solution. The remaining 3.5 mL of ant nectar and 3.5 mL of control (ant-free) nectar were poured into the treatment and control feeders, respectively. These wicks allowed for the sugar solutions and ant chemicals to be consumed by the bees but prevented the bees from ingesting any part of the ant carcass. Feeders were then placed in the arena and data were collected as described above.

Ant Pheromone (Z)-9-Hexadecenal This aggregation pheromone elicits increased foraging behavior by Argentine ants (Welzel and Choe 2016) and has been examined for its potential role in forager recruitment (Choe et al. 2012). We applied 100µL of 100% ethanol (solvent control) in four equally spaced 25 µL droplets to the filter paper of the control feeder. On the treatment feeder, we applied 100µL (Z)-9-hexadecenal in 100% ethanol solvent at a concentration of 1ng/µL (Sidhu and Wilson Rankin 2016) in four equally spaced 25 µL droplets to the filter paper of the treatment feeder. Based on Choe et al. (2012), we estimate that this represents 4.3 ant-equivalents. The feeders remained in the fume hood for five to seven minutes to allow evaporation of ethanol, while retaining the less volatile pheromone. Using gas chromatography – mass spectrometry (GC-MS), we verified detectability of the 1 ng of pheromone on filter paper and found that 46% of the pheromone applied was still present after 30 min (Online Resource 2). A series of experiments found that bees did not discriminate between an unmanipulated control and a feeder treated with 100µL of 100% ethanol ($X^2_2 = 1.01$, $p = 0.60$; Online Resource 1: Fig. S3).

Statistical Analyses

All statistical analyses were conducted in R v. 4.1.0 (R Core Team 2022). Outliers were identified and, when appropriate, removed using the romr.fnc function in the LMERConvenienceFunctions package (Tremblay and Ransijn 2015) prior to data analysis. All generalized linear mixed-effects models (GLMMs) were done using the lme4 package (Bates et al. 2015). To assess bee colony-level visit frequency in response to live ants or ant chemical cues, we used GLMMs with a negative binomial error structure (function *glmer.nb*) as data were overdispersed. A visit to a feeder was defined as a bee landing or walking on the feeder for more than one second irrespective of whether the bee fed during the visit. For live ant presence, visit frequency was the response variable, treatment and trial were the fixed effects, and bee colony ID and date were random effects. For each of the ant cue experiments, visit frequency was the response variable, treatment, trial and bee colony's ant experience status (naïve or experienced) were the fixed effects, and bee colony ID, and date were random effects.

To assess bee colony-level consumption by bees in the presence of live ants or ant chemical cues, we used GLMMs with a gamma error structure. For live ant presence, the weight of nectar consumed in grams was the response variable, feeder treatment and trial were the fixed effects, and bee colony ID, and date were random effects. For each of the ant cue experiments, the weight of nectar consumed in grams was the response variable, feeder treatment, trial, bee colony's ant experience status (naïve or experienced) and the interaction between treatment and experience status were fixed effects, and bee colony ID, and date were random effects. We conducted post-hoc tests on any significant interactions using package "lsmeans" (Lenth 2016).

To assess per capita consumption by bee colonies in the presence of live ants or ant chemical cues, we used GLMMs with a gamma error structure and an offset of pre-trial feeder weight. For live ant presence, grams of nectar consumed per capita was the response variable, feeder treatment and trial were the fixed effects, and bee colony ID, and date were random effects. For each of the ant cue experiments, grams of nectar consumed per capita was the response variable, feeder treatment, trial, bee colony's ant experience status (naïve or experienced), and the interaction between treatment and experience status were fixed effects, and bee colony ID, and date were random effects. We conducted post-hoc tests on any significant interactions as described above.

To assess frequency and duration of foragers' feeding in response to live ants or ant chemical cues, we used GLMMs with a negative binomial error structure. For live ant presence, the response variable was either feeding frequency or feeding duration, fixed effects were treatment, trial, and foragers' ant experience level (naïve or experienced), and random effects were bee ID, bee colony ID, and date. For each of the ant cue experiments, the response variable was either feeding frequency or duration, fixed effects were treatment, trial, bee colony's experience status (naïve or experienced), the interaction between treatment and experience status and individual foragers' ant experience level (bitten or not), and random effects were bee ID, bee colony ID, and date. We conducted post-hoc tests on any significant interactions as described above. All model outputs are reported in Online Resource 1: Tables S1 – S21.

Results

Live Ant Presence

Bumble bee foragers showed a strong avoidance of live ants. While colony-level *visit frequency* did not differ between the two feeders (Table S1, $F_{1,299}$ = 1.25, $p = 0.27$), foraging bees avoided *feeding* on the ant-occupied feeder. Nectar consumption at both the colony and individual forager levels were greater for the control feeder as compared to the live ant feeder (Fig. 1a: total consumption: $F_{1,35} = 21.31$, $p < 0.0001$, Table S2; Fig. 1b: per capita consumption: $F_{1,33}$ = 7.91, $p = 0.0082$, Table S3). Similarly, individual foragers fed 70% more frequently and for twice as long on the control feeder relative to the live ant treatment

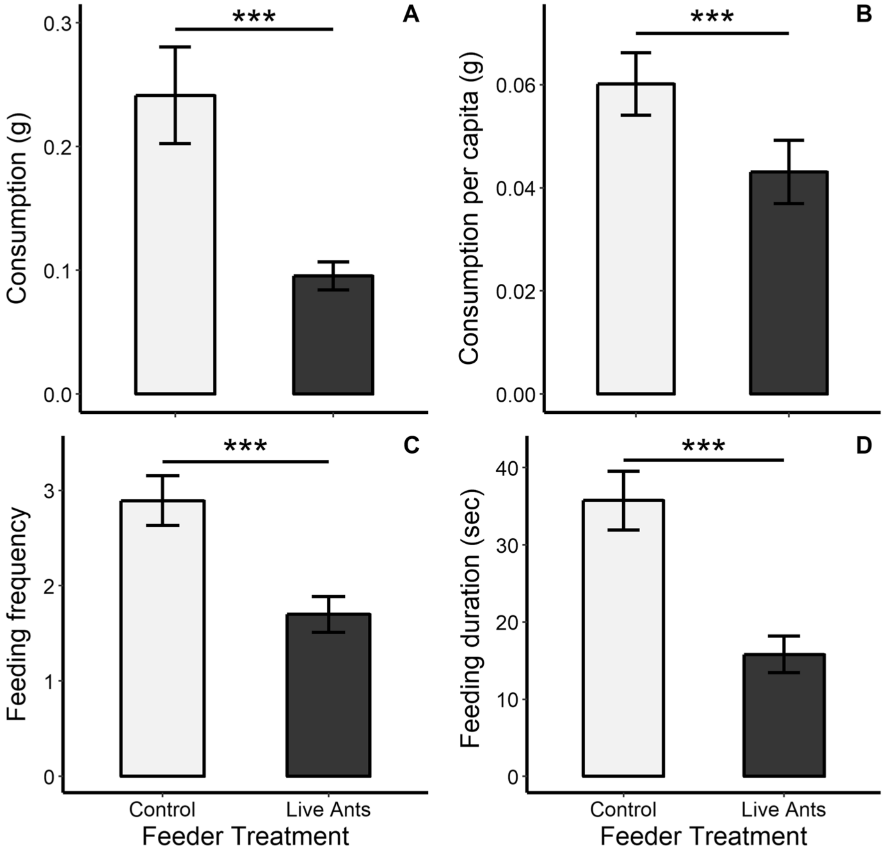

Fig. 1 Live ant presence treatment feeders exhibited **a** decreased total nectar consumption in grams (g) by bee colonies, **b** decreased per capita nectar consumption in grams compared to control feeders, **c** decreased forager feeding frequency, and (**d**) decreased forager feeding duration in seconds (sec) compared to control feeders. Error bars represent standard error. NS indicates $p > 0.05$, * indicates $p < 0.05$, ** indicates $p < 0.01$, *** indicates $p < 0.001$

feeder (Fig. 1c: feeding frequency: $F_{1,150} = 15.69$, $p = 0.00012$, Table S4; Fig. 1d: feeding visit duration: $F_{1,380} = 23.01$, $p < 0.0001$, Table S5).

CHC Footprints as an Indicator of Prior Visitation by Ants

Here, foraging bees did not discriminate between unmanipulated feeders and those that had been previously visited by ants with regard to visitation frequency at the colony-level (Table S6: $F_{1,63} = 0.92$, $p = 0.76$), consumption at the colony level (Fig. 2a: $F_{1,60} = 0.36$, $p = 0.55$, Table S7), per capita consumption (Fig. 2b: $F_{1,61} = 3.31$, $p = 0.074$, Table S8), frequency of feeding (Fig. 2c: $F_{1,493} = 0.002$, $p = 0.96$, Table S9), or feeding duration (Fig. 2d: $F_{1,974} = 0.62$, $p = 0.43$, Table S10). However, an individual forager's previous history with ants affected the frequency at which they fed (Table S9: $F_{1,494} = 10.35$, $p < 0.0001$), such that foragers who had been bitten in the past fed more frequently (mean ± SE: 3.86 ± 0.3 feeding bouts) than foragers with no prior ant experience (3.43 ± 0.1 feeding bouts).

Ants in Nectar

Bee foragers did not discriminate between the control nectar and the ant-infused nectar at the colony level with regards to their colony-level visit frequency (Table S11: $F_{1,75} = 0.02$, $p = 0.89$), nectar consumption at the colony level (Fig. 3a: $F_{1,63} = 2.21$, $p = 0.14$, Table S12), or per capita nectar consumption (Fig. 3b: $F_{1,60} = 1.51$, $p = 0.22$, Table S13). However, there was a significant effect of treatment at the individual level. Individual foraging bees fed more frequently from the control feeder than treatment feeder (Fig. 3c: $F_{1,675} = 4.25$, $p = 0.040$, Table S14). There was an interaction with colony experience level (Table S14: $F_{1,675} = 3.86$, $p = 0.05$), such that foragers from naïve colonies fed more frequently on the control feeder than the treatment feeder (Table S15, $Z = 2.67$, $p = 0.038$). Feeding frequency was also associated

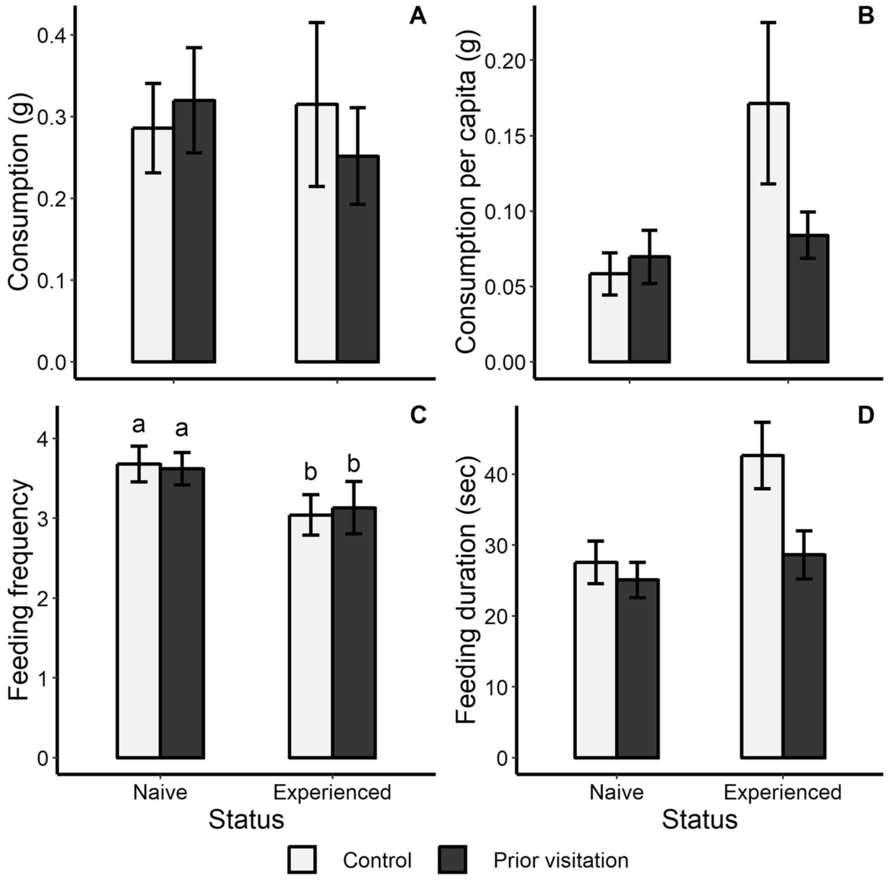

Fig. 2 CHC footprints as an indicator of prior visitation by ants to treatment feeder. Control feeders and treatment feeders exhibited no difference in **a** total nectar consumption in grams (g) by bee colonies, **b** consumption per capita in grams, **c** forager feeding frequency, and **d** forager feeding duration in seconds (sec). Error bars represent standard error. Different letters indicate significance, $p < 0.05$

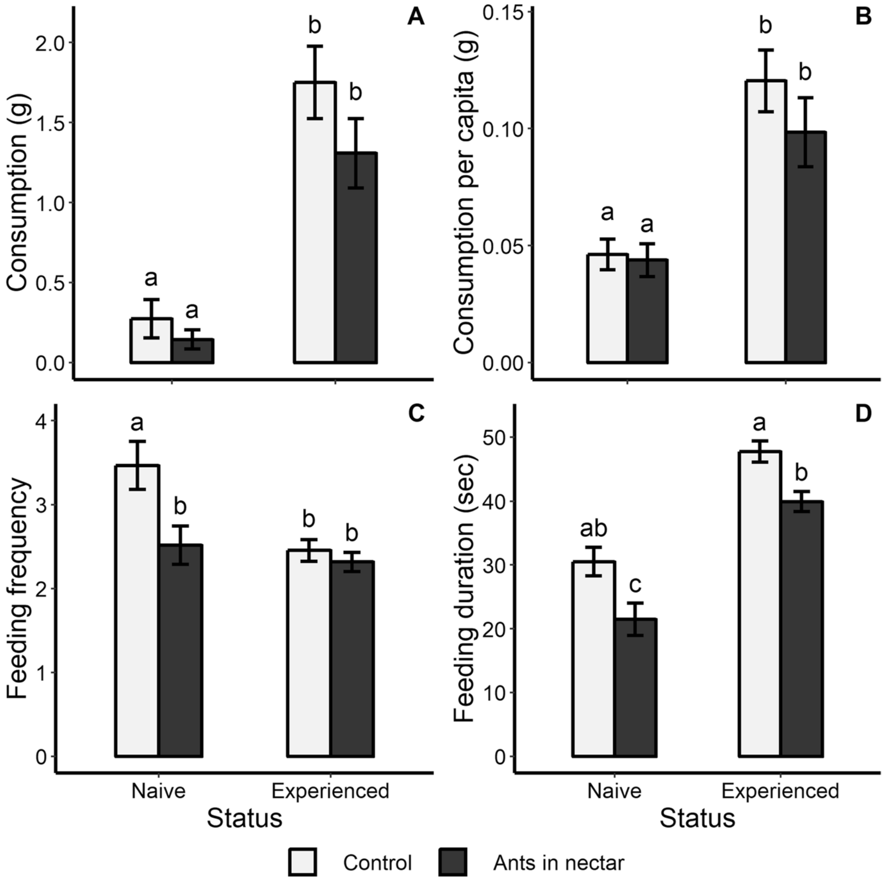

Fig. 3 Ants in nectar as treatment. Control feeders and treatment feeders exhibited no statistical difference in **a** total nectar consumption in grams (g) by bee colonies, or **b** consumption per capita in grams. However, treatment feeders had **c** decreased forager feeding frequency in naïve colonies, and **d** decreased forager feeding duration in naïve and experienced colonies in seconds (sec) compared to the control feeder. Error bars represent standard error. Different letters indicate significance, $p < 0.05$

with the individual foragers' experience with ant bites (Table S14: $F_{1,675}$ = 12.61, $p < 0.0001$), such that foragers fed more frequently if they had prior experience of being bitten (mean ± SE: 3.38 ± 0.24 feeding bouts) than foragers with no ant experience (2.40 ± 0.09 feeding bouts). In addition, foragers from naïve and experienced colonies fed for 20% and 42% longer respectively on the control feeder than the treatment feeder (Fig. 3d: $F_{1,1908}$ = 24.15, $p < 0.0001$, Table S16).

Ant Pheromone, (Z)-9-Hexadecenal

In this experiment, we observed no avoidance of the Argentine ant aggregation pheromone. Bees did not discriminate between the two feeders with regard to their visit frequency (Table S17: $F_{1,67}$ = 0.77, $p = 0.38$), consumption at the colony level (Fig. 4a: $F_{1,66}$ = 0.054, $p = 0.82$, Table S18), consumption per capita (Fig. 4b: $F_{1,66}$ = 0.35, $p = 0.56$, Table S19), forager feeding frequency (Fig. 4c: $F_{1,740}$ = 0.08, $p = 0.78$, Table S20), or feeding duration (Fig. 4d: $F_{1,2084}$ = 0.22, $p = 0.64$, Table S21). However, feeding frequency was associated with foragers' experience with ant bites (Table S20: $F_{1,740}$ = 8.41, $p = 0.00025$), such that foragers with prior experience of being bitten fed more frequently (mean ± SE: 3.43 ± 0.27 feeding bouts) than foragers with no ant experience (2.60 ± 0.09 feeding bouts).

Discussion

Here, we demonstrate that live Argentine ants deter bumble bees foraging on sucrose solution, and that a subset of ant-associated chemical cues elicit this avoidance in the physical absence of ants. Both exploitation and interference mechanisms of competition enable invasive Argentine ants to displace native ant species (Human and Gordon 1996). Similarly, both forms of competition may occur in bumble bee interactions with Argentine ants (Buys 1987),

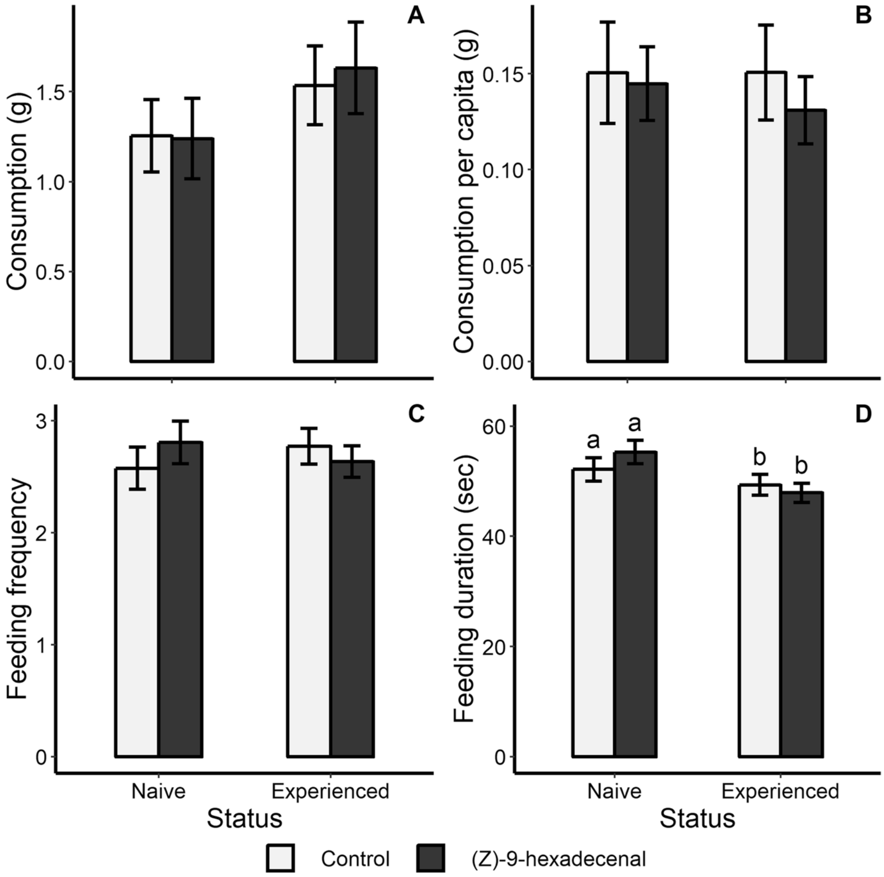

Fig. 4 Ant pheromone (Z)-9-hexadecenal as treatment. Control feeders and treatment feeders exhibited no difference across treatment in **a** total nectar consumption in grams (g) by bee colonies, **b** consumption per capita in grams, **c** forager feeding frequency, or **d** forager feeding duration in seconds (sec). Error bars represent standard error. Different letters indicate significance, $p < 0.05$

Myrmica rubra (Cembrowski et al. 2014), *Crematogaster dentinodus and Forelius pruinosus* ants (Schaffer et al. 1983). In this study, we isolated interference competition; the nectar wells of our experimental feeders provided nectar *ad libitum* to exclude the possibility of exploitative competition with ants. We subsequently observed that bumble bees modified their foraging behavior in response to past aggressive encounters with ants and detectable chemical cues associated with their ant antagonists, supporting the hypothesis of interference competition.

In all four experiments, colony-level visit frequency demonstrated that bee foragers approached both feeders at similar rates regardless of treatment. However, for the live ant presence and ant-infused nectar experiments, we observed forager preference for the control feeders upon arrival. This preference indicated (a) the importance of physical contact with ants and short-range chemical cues at a nectar resource and (b) that select chemical cues in the absence of visual cues are sufficient to elicit a response from bumble bees in the context of interference competition with ants. Such avoidance of ants (or some of their cues) is exhibited by pollinator species, including bumble bees (Cembrowski et al. 2014; this study), honey bees (Lach 2008a; Li et al. 2014; Sidhu and Wilson Rankin 2016), megachilid bees (Wilson Rankin et al. 2020) and hummingbirds (Rankin et al. 2018).

The average feeding frequency and feeding duration of *B. impatiens* decreased significantly both with the presence of live ants and with ant-infused nectar. These findings are consistent with reports of decreased floral visitation by honey bees (Sidhu and Wilson Rankin 2016) and wild bees (Hanna et al. 2015) in the presence of live Argentine ants. Moreover, ant-driven decrease in feeding corresponds with the general trend of experienced colonies' lower consumption of nectar containing crushed ants, but decreased nectar consumption was only statistically significant when live ants were present. These findings demonstrate that chemical cues specifically associated with ant bodies clearly shape forager decision-making, while the physical presence of ants has the greatest overall effect on bumble bee foraging behavior.

Experience with enemies is known to shape subsequent forager decisions in bumblebees (Jones and Dornhaus 2011). We observed that foragers from naïve bee colonies had a stronger avoidance of ant-infused nectar and a stronger preference for ant-free nectar compared to experienced colonies. This effect of experience may be due in part to bumble bees learning when they can ignore the chemical cues of ants (Ballantyne and Willmer 2012). By feeding less frequently and for shorter durations of time, foragers may be limiting their exposure to potential predators, however, this may also decrease the amount of resources being brought back to the nest. Further investigation is required to determine whether the level of decreased resource collection observed here would be sufficient to decrease colony longevity or productivity.

Interestingly, some foragers on the feeder with ant-infused nectar displayed similar aggressive behaviors (i.e. rapidly gaping mandibles and attacking) that they showed toward live ants. Gaping of the mandibles is an aggressive behavior observed in a diversity of ant (e.g., Saar et al. 2018; Sasaki et al. 2014) and bee species (e.g., Harrison et al. 2019; James et al. 2022; Nieh et al. 2005). This occurred before the bee made any physical contact with the ant-infused nectar, which suggests scent may be an important aspect of ant-experienced bees' recognition of Argentine ants. At least one bumble bee in each of the six colonies gaped their mandibles while on the ant-infused nectar treatment feeder, and in three of those colonies at least one bumble bee aggressively bit the cotton wick as though it were attacking an ant. This behavior of attacking the wick was highly distinctive since it was not observed during any other ant cue trials, including naïve bees with ant-infused nectar.

By crushing ants in nectar, we exposed bees to all the external and internal chemicals that they might encounter from ants drowned in nectar. Drowning while foraging for liquids is a risk ants (Zhou et al. 2020, 2022) and other insects face (e.g., Robertson 1946; Smith et al. 2017; Thien et al. 2009). Even in the absence of the physical threat of ants, these chemical stimuli elicited similar responses by bees to when live ants were present. These behaviors may be due to an innate aversion of naïve bees to the contamination of nectar with the flavor of dead insects. Any such aversion may be reinforced by bees learning to associate ant flavors and smells with their aggressive interactions with ants (as may be the case for experienced colonies tested). Foraging bumble bees may be deterred from flowers as a response to both tactile chemosensation (e.g., inadvertently licking or antennating an ant) and close-range scent (e.g., ant pheromone, CHC's).

Ant-infused nectar was the only ant chemical cue experiment in which bees exhibited a feeder preference. For the other two cues—prior visitation and the ant pheromone (Z)-9-hexadecenal—feeding duration and frequency were consistent irrespective of feeder and colony-level ant experience. The marked differences between bee responses to these two chemical cues and to the live ant treatment demonstrate that bumble bees may be unable to detect if Argentine ants have fed from a nectar source beforehand. Bees did not avoid feeders that had previously been visited by ants, suggesting that bees were not avoiding ant CHC footprints specifically. However, CHC's from other parts of the ant body could have been detected in the ant-infused nectar.

The lack of response by bumble bees to the ant pheromone, (Z)-9-hexadecenal, is particularly interesting, given that it elicits avoidance in free-foraging honey bees (Sidhu and Wilson Rankin 2016). (Z)-9-hexadecenal evokes trail following behavior in both the Argentine ant and the Southeast Asian ant, *Dolichoderus thoracicus* (Attygalle et al. 1998; Key and Baker 1982). This substance is also found in other insect taxa where it can function as a sex pheromone (Kainoh et al. 1991) or as a kairomone (DeLury et al. 1999). While Choe et al. (2012) found that (Z)-9-hexadecenal was present in the bodies of Argentine ants, it was not detected in naturally-laid ant trails. Rather Argentine ant trails were characterized by two iridoids (Choe et al. 2012), which Welzel et al. (2018) found to be used in combat with native ant species. Recent research found that solitary *Megachile rotundata* and *Osmia lignaria* both avoided sugar solutions containing iridomyrmicin, an iridoid Argentine ant pheromone, while the social *Apis mellifera* showed no attraction or avoidance of this pheromone (Wilson Rankin et al. 2020). Future studies could test the response of these bee species to these compounds in the context of foraging in the field or defense at nests.

Bees' aggression toward the ant-infused nectar prior to making physical contact suggests that they do use olfactory cues in their recognition of Argentine ants, although the cue eliciting this behavior is not (Z)-9-hexadecanal. It may be that *B. impatiens* does not detect (Z)-9-hexadecenal, does not use it as a foraging cue, has a higher response threshold compared to honey bees, or responds differently based on experience level with ants. As such, further studies of bee response thresholds regarding ants and ant chemical cues are warranted. In comparison with Sidhu and Wilson Rankin (2016), we have shown that there is a clear difference between two eusocial bee species. Both the honey bee and the common eastern bumble bee inhabit areas where the Argentine ant has invaded (Ruggiero et al. 2018; Tsutsui and Suarez 2003; Williams et al. 2014), while only honey bees currently overlap in distribution with Argentine ants in the ant's native range (Maggi et al. 2016). While we may posit that shared evolutionary history with Argentine ants may influence bee responses to these ant pheromones, we still lack any data about the responses of many other non-*Apis* and non-*Bombus* bees to (Z)-9-hexadecanal and other ant pheromones (but see Wilson Rankin et al. 2020). These results provide a reminder that we should be cautious and not make definitive predictions about all bees based on the behaviors of social bees.

Most of our insights into the chemical ecology of competition between ants and bees comes from studies of social bees. Social insects tend to have highly evolved chemical communication through odor (Leonhardt et al. 2016), including the recognition of colony and nest mates and the organization of their societies (Blomquist and Bagneres 2010; Nunes et al. 2009, 2011). There are just a handful of bee species, including solitary ones, whose behavioral responses to the scent signals left on flowers by previous intra- and inter-specific floral visitors have been studied (Ballantyne and Willmer 2012; Barônio and Del-Claro 2018; Gawleta et al. 2005; Yokoi and Fujisaki 2009). Furthermore, bees of different body sizes may behave differently in the physical presence of aggressive, ant floral visitors (e.g., Gonzálvez et al. 2013). Thus, different bee species may respond in a non-uniform fashion to invasive ants, such as the Argentine ant. Future research should investigate the foraging impacts and differences in sensory perception across a variety of bee species regarding antagonistic, non-pollinator competitors. With this information we could better predict community-level responses or resilience to ant pests.

While the constant laboratory environment obviously differs from variable field conditions, bumble bees often exhibit similar foraging decisions in the lab and field. For example, bumble bees will expand their foraging area when conspecifics are experimentally removed in lab (Makino and Sakai 2005) and field studies (Thomson et al. 1987). Moreover, *Bombus* foragers use scent marks to identify and avoid previously visited resources in arena assays (Saleh et al. 2007; Witjes and Eltz 2007) as well as in field trials (Goulson et al. 2000). Such studies suggest that bumble bees will reliably avoid the same cues while foraging in controlled or wild conditions. While there is research showing bees avoid ants (or their cues) while free-foraging in the wild (e.g., Barônio and Del-Claro 2018; Ibarra-Isassi and Oliveira 2018; Lach 2008a; Lach 2008b), these particular bee species are rarely studied under laboratory conditions. Future experiments could assess how *Bombus* species respond to Argentine ants in the field to confirm the avoidance behavior observed here is conserved.

Here, we documented that bumble bees avoid feeders with live ants and avoid collecting nectar that is infused with ants. We examined several chemical cues associated with the presence of ants and determined that the physical interaction with ants and the chemosensation of ants in the nectar were sufficient to elicit avoidance. Predator simulation has shown that after bumble bees experience physical attack, they will decrease foraging or switch to a less rewarding nectar resource (Jones and Dornhaus 2011). Experience with Argentine ant harassment may elicit similar responses that result in resource collection of a lower quality or quantity. Decreased nectar collection has serious implications for the maintenance of healthy pollinator colonies and populations. Furthermore, pollinator-ant interactions could shape plant-pollinator community dynamics, perhaps in a manner similar to predator-prey interactions (Binz et al. 2014; Dukas 2001). Thus, efforts to conserve and manage pollinators and pollination services may benefit from integrating ant control into their strategies.

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Declarations

Conflict of Interest The authors have no competing interests to declare that are relevant to the content of this article.

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