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PLANT-ANIMAL INTERACTIONS - ORIGINAL PAPER

Consumption of a nectar alkaloid reduces pathogen load in bumble bees

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Abstract Diet has a significant effect on pathogen infections in animals and the consumption of secondary metabolites can either enhance or mitigate infection intensity. Secondary metabolites, which are commonly associated with herbivore defense, are also frequently found in floral nectar. One hypothesized function of this so-called toxic nectar is that it has antimicrobial properties, which may benefit insect pollinators by reducing the intensity of pathogen infections. We tested whether gelsemine, a nectar alkaloid of the bee-pollinated plant *Gelsemium sempervirens*, could reduce pathogen loads in bumble bees infected with the gut protozoan *Crithidia bombi*. In our first laboratory experiment, artificially infected bees consumed a daily diet of gelsemine post-infection to simulate continuous ingestion of alkaloid-rich nectar. In the second experiment, bees were inoculated with *C. bombi* cells that were pre-exposed to gelsemine, simulating the direct effects of nectar alkaloids on pathogen cells that are transmitted at flowers. Gelsemine significantly reduced the fecal intensity of *C. bombi* 7 days after infection when it was consumed continuously by infected bees, whereas direct exposure of the pathogen to gelsemine showed a non-significant trend toward reduced infection. Lighter pathogen loads may relieve bees from the behavioral impairments associated with the infection, thereby improving their foraging efficiency. If the collection of nectar secondary metabolites by pollinators is done as a means of self-medication, pollinators may selectively maintain secondary metabolites in the nectar of plants in natural populations.

Keywords Antimicrobial · *Bombus impatiens* · *Crithidia bombi* · Gelsemine · Toxic nectar

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Introduction

The severity of pathogen infections is often dictated by the quality of an animal's diet and, for species that forage on plants, secondary metabolites are a common dietary component that can greatly alter infection intensity (Cory and Hoover 2006). The post-ingestive effects of secondary metabolites for many animals are often deleterious and include reduced growth (Blau et al. 1978; Isman and Duffey 1982), inhibited uptake of nutrients (Slansky 1992) and altered organ structure and function (Berenbaum 1988), all of which can leave individuals more vulnerable to disease. However, pathogens may be just as susceptible as their hosts to the negative consequences of secondary metabolites, the consumption of which can often improve an infected host's survival or life expectancy (Berenbaum 1988; Price et al. 1980). For animals fighting infection, the curative benefits of secondary metabolites may thus outweigh the costs associated with the consumption of these noxious compounds.

Secondary metabolites are not limited in distribution to leaves, but are also found in the floral nectar of plants. This so-called toxic nectar is paradoxical given that floral nectar is usually interpreted as attractive, not deterrent, to pollinators. Secondary metabolites, including tannins, phenols, alkaloids and terpenes, have been found in floral nectar

across 21 angiosperm families (Adler 2000). The prevalence and diversity of secondary compounds across the angiosperms suggest that nectar secondary metabolites have some adaptive function for plants. Hypothesized functions of secondary metabolites in nectar include deterrence of nectar robbers, increased constancy of effective pollinators, or protection against deleterious microbes (see Adler 2000 for full review; Rhoades and Bergdahl 1981). Of these, the antimicrobial hypothesis is perhaps the most general because microbes are ubiquitous and nectar is an ideal medium to support a variety of microorganisms. Although many secondary metabolites have microbicidal properties (Cowan 1999), and diverse microorganisms often occur in floral nectar (Brysch-Herzberg 2004; Ehlers and Olesen 1997; Golonka 2002), few studies have tested whether nectar secondary metabolites actually suppress microbes (but see Manson et al. 2007).

Pollinators, principal consumers of floral nectar, are exposed to a variety of pathogenic microorganisms that can reduce their survival and foraging efficiency. For example, bumble bees (*Bombus* spp.) in Europe and North America frequently carry the intestinal protozoan *Crithidia bombi* (Colla et al. 2006; Lipa and Triggiani 1988; Schmid-Hempel 2001), which elevates their mortality rate under food stress (Brown et al. 2000) and impairs their associative learning, flower handling, and foraging efficiency (Gegear et al. 2005, 2006; Otterstatter et al. 2005). Horizontal transmission of *C. bombi* occurs at flowers, when infected bees deposit "free-living" pathogen cells that are subsequently ingested by susceptible foragers (Durrer and Schmid-Hempel 1994). Since *C. bombi* is known to occur in the nectar of wild flowers (Durrer and Schmid-Hempel 1994), nectar secondary metabolites may influence the survival and infectivity, and consequently the transmission, of this pathogen. Furthermore, for gut pathogens such as *C. bombi*, host diet can significantly affect the severity of infection by altering immunocompetence, metabolic processes, or by limiting nutrient availability for the parasite (Cory and Hoover 2006; Logan et al. 2005; Wink and Theile 2002). Hence, in flowers or in the guts of flower visitors, nectar secondary metabolites may benefit pollinators via antimicrobial action.

The nectar of the Carolina jessamine (*Gelsemium sempervirens* L.) contains the indole alkaloid gelsemine, a secondary metabolite that is highly toxic to vertebrates (Blaw et al. 1979). Gelsemine appears to have little effect on the fitness or physiology of bees (Elliott et al. 2008; Manson and Thomson 2009) and no effect on non-pathogenic floral yeasts (Manson et al. 2007). Although gelsemine-rich nectar can be distasteful and deterrent to pollinators (Adler and Irwin 2005; Gegear et al. 2007), *G. sempervirens* consistently attracts a number of floral visitors, including the bumble bees *Bombus impatiens* and *Bombus bimaculatus*.

In the present study, we examined the putative antimicrobial properties of the nectar alkaloid gelsemine on the bumble bee pathogen *C. bombi*. First, we asked, does consumption of alkaloid-rich nectar by bumble bees reduce the severity of intestinal infections by *C. bombi*? Second, given that this pathogen is naturally transmitted at flowers, we asked, does alkaloid-rich nectar directly reduce the infectivity of *C. bombi* cells? We then discuss the ecological impact of nectar alkaloids on pollinator–pathogen dynamics.

Materials and methods

Our experimental protocol is illustrated in Fig. 1. In both experiments, we exposed the pathogen *C. bombi* to either a gelsemine solution (Alkaloid) or plain sucrose solution (Control) and then compared the intensity of developing infections in inoculated bumble bees (*Bombus impatiens* Cresson). In experiment 1, bees were first inoculated with *C. bombi* and then fed on a daily diet of Alkaloid or Control solution. In experiment 2, we exposed *C. bombi* cells to Alkaloid or Control solutions for varying durations before inoculating bees, feeding them on a sucrose-only solution for the balance of the experiment.

We designed this study to test the interaction between pollinator pathogens and nectar alkaloids using natural conditions to define the parameters of each experiment. Concentrations of gelsemine in the nectar of *G. sempervirens* range from 5.8 to 246.1 ng/µl in natural populations (Adler and Irwin 2005) and we used the highest naturally occurring concentration of the nectar alkaloid throughout the study. In experiment 1, some bees were fed exclusively on an Alkaloid solution to simulate foraging conditions in early spring, when *G. sempervirens* is one of the few plants in bloom and bumble bees frequently collect its nectar (Pascarella 2007). With both alkaloid concentration and amount of alkaloid consumed, we created treatments that represent the upper limits of natural conditions while still providing ecologically meaningful results.

We made artificial alkaloid-rich "nectar" by mixing gelsemine hydrochloride [purchased from Chromadex (Irvine, Calif.), hereafter referred to as "gelsemine"] into a 30% w/w aqueous sucrose solution at a concentration of 250 ng/µl. Alkaloid solutions were refrigerated at 4°C when not in use and stored for up to 2 days, although they were usually prepared immediately before use.

We prepared pathogen inocula from the gut tracts of four "donor" *B. impatiens* workers from each of five hives infected by *C. bombi* (provided by a commercial rearing company). Following the general protocol of Otterstatter and Thomson (2006), gut tracts were excised and crushed in a microcentrifuge tube containing 300 µl of distilled

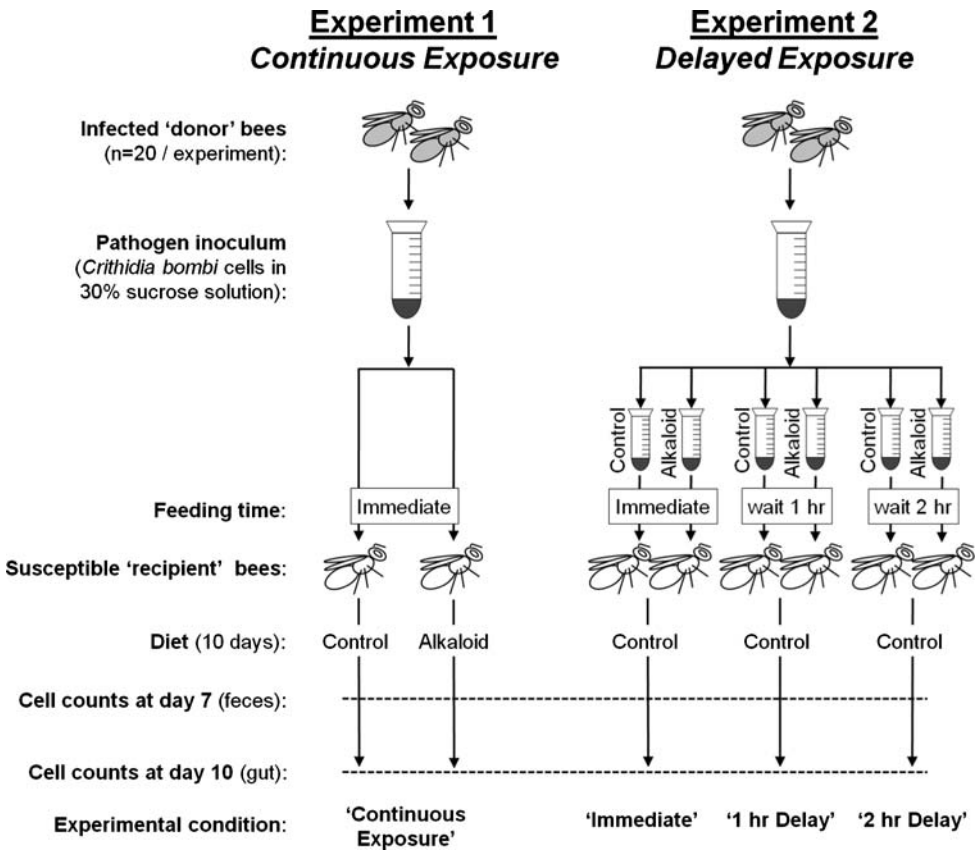

Fig. 1 Diagram of the experimental design. Throughout, *Alkaloid* refers to a 30% sucrose solution containing 250 ng/µl of the alkaloid gelsemine and *Control* refers to a 30% sucrose-only solution. In experiment 1, bees harboring the gut pathogen *Crithidia bombi* were fed a daily diet of Alkaloid or Control solution, whereas in experiment 2, bees were fed *C. bombi* cells that had been pre-exposed to Alkaloid or Control solutions for varying durations

water. The mixture was allowed to settle at room temperature for 3 h, after which the supernatant was removed and mixed thoroughly. Supernatants were diluted to the appropriate density of *C. bombi* cells (Neubauer hemocytometer counts) and sucrose was added to a concentration of 30%. In each of the two experiments (described below), we used 20 new donor bees from five new hives; thus, within experiments, all bees received the same cocktail of *C. bombi* strains (genotypes), but between experiments, inocula may have contained different pathogen genotypes.

We obtained susceptible "recipient" *B. impatiens* workers from pupal clumps originating from commercially reared hives (same supplier as above). Previous studies have found that *C. bombi* infections are not acquired until workers emerge (Otterstatter and Thomson 2007), making new workers naïve to *C. bombi* regardless of the infection status of the source colony. Newly emerging (<24 h old) worker bees were placed in containers according to their hive of origin and given 30% sucrose solution and pollen ad libitum. After 2 days, workers were starved overnight, weighed (±0.1 mg), and then arbitrarily assigned to an experimental group. We ensured that each of the Alkaloid and Control groups in both experiments contained recipient bees from at least three hives, in roughly equal numbers.

In experiment 1, "continuous exposure" bees inoculated with *C. bombi* were allowed to feed daily on gelsemine, simulating the continual ingestion of nectar constituents by an infected foraging bee. Each bee was initially fed a 2 µl drop containing 10^4 *C. bombi* in 30% sucrose solution and we monitored individuals until the entire drop was consumed. This dose falls within the range of *C. bombi* cells shed in the feces of infected bees in previous studies (Logan et al. 2005; Schmid-Hempel and Schmid-Hempel 1993), and therefore simulates cells available for transmission to naïve individuals. Bees were reared in individual 15-ml vials and received either a 0.5-ml solution of 250 ng/µl gelsemine in 30% sucrose (Alkaloid bees, $n = 35$) or 0.5 ml of 30% sucrose only (Control bees, $n = 35$) along with a pollen lump daily for 10 days.

In experiment 2, "delayed exposure" *C. bombi* was exposed to gelsemine for various durations prior to host ingestion, simulating direct exposure of the pathogen to nectar in a flower. We placed 10^4 *C. bombi* (in 2 µl of 30% sucrose solution) into each of 60 microcentrifuge tubes: 30 of these contained 8 µl of a 250 ng/µl solution of gelsemine in 30% sucrose (Alkaloid), and 30 contained 8 µl of 30% sucrose only (Control), which were divided equally amongst three treatments. In the "immediate" group, we fed the Alkaloid and Control pathogen mixtures to recipient bees immediately; each bee was housed individually and received only one dose, yielding ten Alkaloid bees and ten Control bees. In the "1-h delay" and "2-h delay" groups, we

left the Alkaloid and Control pathogen mixtures at room temperature (~21–24°C) under fluorescent lighting for 1 and 2 h, respectively, before feeding them to recipient bees (as before, ten Alkaloid bees and ten Control bees per group). The two treatments are based on realistic delays between pollinator flower visits in natural *G. sempervirens* populations (Adler and Irwin 2005; Pascarella 2007) and simulate the period between the deposition of *Crithidia* cells by infected bees and the next flower visit by a naïve bee. In this experiment, we compensated for evaporative water loss by starting with more dilute sucrose solutions that evaporated to a concentration of 30% sucrose after 1 or 2 h (dilutions calculated from a preliminary study). Following the inoculation with *C. bombi*, bees were kept in individual vials and given 0.5 ml of 30% sucrose solution and a fresh pollen lump daily.

In both experiments, we quantified infection intensities of all bees at day 7 and day 10 post-inoculation, as these periods bookend the period in which pathogen load is saturated (Otterstatter and Thomson 2006; Schmid-Hempel and Schmid-Hempel 1993) On day 7, all bees were transferred to clean vials without food and left until they defecated. The density of *C. bombi* in each bee's feces was determined with a hemocytometer. On day 10, all bees were sacrificed and the total density of *C. bombi* in their gut tracts was determined with a hemocytometer following Otterstatter and Thomson (2006).

Statistical analyses

Our final sample sizes were lower than the original design due to mortality from unknown causes (9% of bees died before day 10; subsequent examinations did not reveal unusually intense *C. bombi* infections), missing fecal samples (17% of bees did not produce enough feces for analysis on day 7 post-inoculation), and the failure of certain bees to develop an infection (10% of bees remained uninfected throughout the experiment). We excluded all of these bees from further analyses. Likelihood ratio (G) tests showed that, in each case, the proportion of "excluded bees" did not differ between Control and Alkaloid groups ($P > 0.20$ in all cases), suggesting that these were not serious sources of bias. In total, we analyzed the infection intensities of 43 (day 7) and 55 (day 10) bees in experiment 1, and 47 (day 7) and 57 (day 10) bees in experiment 2.

Given their differing designs, experiments 1 and 2 were analyzed separately. In both cases, we used an analysis of covariance, including both categorical and continuous explanatory factors, with repeated measures on bees, to determine whether or not gelsemine reduced the intensity of gut infections. The repeated measures component accounted for the non-independence of observations on the same individuals at day 7 and day 10. Although this analysis does not produce test statistics for the repeated factor (because Bee is not a true explanatory variable in the statistical model), it reduces the *df* below the actual number of observations. In order to directly compare a bee's intensity of infection at day 7 (measured as *C. bombi* cells/µl host feces) and day 10 (*C. bombi* cells/µl of gut fluid) post-inoculation, fecal counts were converted to estimated gut counts using the linear regression: gut count = $-6.3455 + 0.6955 \times$ feces count ($F_{1,41} = 268.93$, $P < 0.001$, $R^2 = 0.93$) based on data in Otterstatter and Thomson (2007). We treated pathogen counts (square-root transformed to satisfy the standard assumption of normally distributed errors) as our dependent variable, and whether or not *C. bombi* was exposed to gelsemine (Alkaloid or Control group), time (day 7 or day 10 post-inoculation), and bee body mass, as explanatory factors in our analyses. In experiment 2, we also included Delay as an explanatory factor, i.e., the duration that *C. bombi* was exposed to gelsemine prior to host inoculation (no delay, 1-h delay, 2-h delay). We originally included hive of origin as an explanatory factor, but it had no significant effect and was removed from the analysis. Preliminary analyses showed that infection intensity in Control and Alkaloid groups did not meet the standard assumption of homoscedasticity (F-test for equal variance, day 7, $F = 3.96$, $P = 0.004$; day 10, $F = 2.07$, $P = 0.067$); we therefore used a heterogeneous variance model (Proc MIXED, SAS Institute 2006) to account for this deviation. For both experiments, we began with a saturated model and removed non-significant effects via backward stepwise elimination. Akaike information criterion (AIC) values were used to compare candidate models; ultimately, the model with an AIC value at least 2 units lower than any simpler competing models was chosen as the best fit to the data. We used linear contrasts (t-tests) in our regression models to compare the average infection intensities of Control and Alkaloid bees at day 7 and day 10. Finally, we used Kolmogorov-Smirnov (K–S) two-sample tests to compare the distributions of infection intensities between Control and Alkaloid bees; P-values for K–S tests were computed using Monte Carlo estimation (Proc NPAR1WAY; SAS Institute 2006).

Results

In experiment 1, an alkaloid-rich diet reduced the intensity of *C. bombi* infections in bumble bees. Our regression analysis revealed significant main effects of gelsemine (Alkaloid or Control diet), time since inoculation, and bee body size on infection intensity (Table 1). At 7 days post-inoculation, bees receiving dietary gelsemine had infections that were, on average, 2.2 times less intense than bees receiving the control diet ($t = 2.45$, df = 36, $P = 0.019$; Fig. 2).

Table 1 Experiment 1: mixed model statistics describing the effect of an alkaloid-rich diet on the intensity of *Crithidia bombi* infections in bumble bees. Bee was included in each model as a repeated factor to account for the non-independence of sequential observations on individuals. Bees were inoculated with pathogen cells and then fed a daily diet of either alkaloid or control solution (*Gelsemine*). Pathogen counts were done at 7 and 10 days post-inoculation (*Time*). Numerator and denominator *df* are shown for each explanatory factor

Explanatory factor	*F*	*df*	*P*
Gelsemine	4.65	1,57	0.035
Time	32.97	1,36	<0.001
Bee body size	7.61	1,57	0.008
Gelsemine × Time	0.88	1,36	0.36

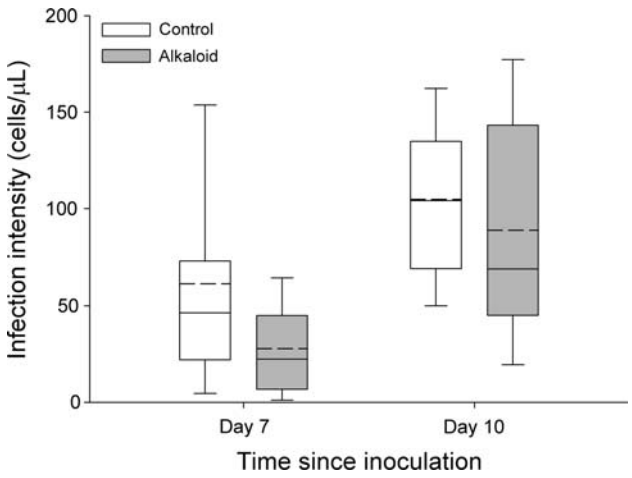

Fig. 2 Experiment 1: effect of an alkaloid-rich diet on the intensity of *C. bombi* infections in bumble bees. Bees were inoculated with a standard dose of pathogen cells and then fed a daily diet of either a gelsemine or control solution. The *lower* and *upper edges* of each *box* indicate the 25th and 75th percentiles, respectively, the *solid* and *dashed lines* within a *box* indicate the median and mean values, respectively. *Error bars*, where visible above and below a *box*, indicate the 90th and 10th percentiles, respectively. Infection intensities have been square-root transformed

Indeed, gelsemine completely prevented heavy infections in bees by day 7: whereas infections in the Control group ranged from 0 to 51,500 cells/µl, the most intense infection in the Alkaloid group was only 5,300 cells/µl (significantly different distributions of infection intensity, K–S test: $D = 0.42$, $P = 0.02$). Infection intensities increased significantly from day 7 to day 10, and this effect did not differ between Control and Alkaloid groups (non-significant Gelsemine × Time effect; Table 1); this increase is expected, as *C. bombi* infections generally increase for the first 8–10 days before leveling out (Schmid-Hempel and Schmid-Hempel 1993). At 10 days post-inoculation, although average infection intensities were similar in Alkaloid and Control groups ($t = 0.96$, $df = 36$, $P = 0.342$; Fig. 2), the distribution of infection intensities was skewed to significantly lighter infections among bees receiving gelsemine compared to bees receiving the control diet (K–S test: $D = 0.35$, $P = 0.02$). For example, while Alkaloid and Control bees exhibited similar ranges of infection intensity at day 10 (150–39,188 vs. 150–35,250 cells/µl, respectively), the median infection intensity of Alkaloid bees was less than half that of Control bees (4,775 vs. 10,850 cells/µl, respectively). Overall, in experiment 1, larger bodied bees developed lighter infections than small bees, independently of alkaloid treatment (Table 1).

Given that a continuous diet of gelsemine reduced infection intensity in bumble bees, we asked in experiment 2 if exposing *C. bombi* cells to gelsemine *prior* to host inoculation would also reduce infections. Exposing *C. bombi* inocula to gelsemine did not have a clear effect, however. Gelsemine did not significantly reduce average infection intensity (non-significant Gelsemine effect; Table 2), nor did the distribution of infection intensities differ between Control and Alkaloid groups for any of the treatments (K–S tests: $P > 0.60$ in all cases). Average infection intensity increased significantly over time (from day 7 to day 10) when *C. bombi* was fed to bees immediately ($t = 3.40$, $df = 41$, $P = 0.002$; Fig. 3a), but this effect decreased when pathogen cells sat for 1 h before inoculation ($t = 1.91$, $df = 41$, $P = 0.064$; Fig. 3b), and disappeared when pathogen cells sat for 2 h before inoculation ($t = 1.60$, $df = 41$, $P = 0.118$; Fig. 3c) (significant Delay × Time interaction; Table 2). There was no indication that gelsemine affected this variation in infection intensity over time in any of the three experimental treatments (non-significant Gelsemine × Delay × Time interaction; Table 2).

Table 2 Experiment 2: mixed model statistics describing the effect of exposing *C. bombi* cells to gelsemine for varying durations prior to bumble bee inoculation on infection intensity. Bee was included in each model as a repeated factor to account for the non-independence of sequential observations on individuals. Pathogen inocula were mixed with either an alkaloid or control solution (*Gelsemine*) and fed to bees immediately, or after either a 1- or 2-h delay (*Delay*). Pathogen counts were done at 7 and 10 days post-inoculation (*Time*). Numerator and denominator *df* are shown for each explanatory factor

Explanatory factor	*F*	*df*	*P*
Gelsemine	0.11	1,51	0.74
Delay	10.48	2,51	<0.001
Time	3.74	1,41	0.06
Gelsemine × Delay	0.1	2,51	0.91
Gelsemine × Time	1.1	1,41	0.3
Delay × Time	6.15	2,41	0.005
Gelsemine × Delay × Time	1.06	2,41	0.36

Bee body size was non-significant and excluded from the final model ($F = 1.53$, $P = 0.22$)

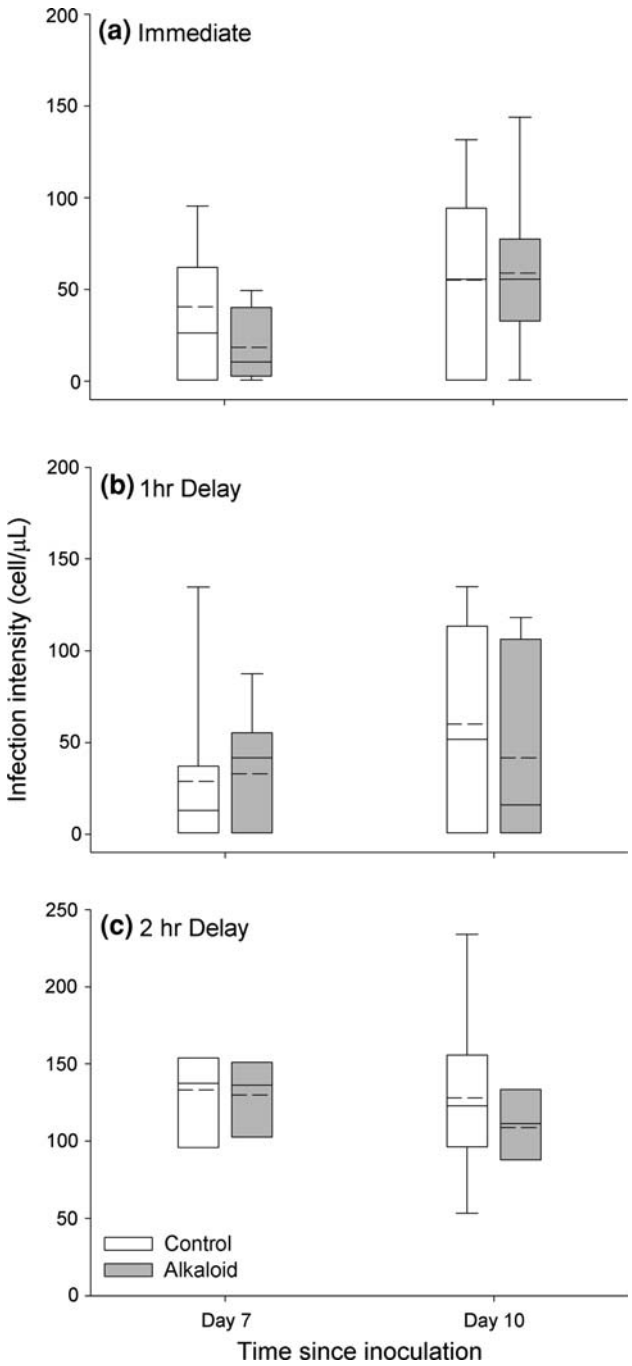

Fig. 3a–c Experiment 2: effect of exposing *C. bombi* cells to the alkaloid gelsemine for varying durations prior to bumble bee inoculation. *Boxes* are as described in Fig. 2. Pathogen inocula were mixed with either a gelsemine or control solution and fed to bees **a** immediately, **b** after a 1-h delay, or **c** after a 2-h delay

Discussion

Insect pollinators regularly feed from flowers that contain alkaloid-rich nectar but the consequences of such nectar for pollinators and plants remain unclear (Adler 2000). Our results demonstrate for the first time that artificial nectar containing a naturally occurring nectar alkaloid reduces the severity of gut infections in pollinators. Bumble bees (*B. impatiens*) inoculated with the intestinal parasite *C. bombi* developed less intense infections when feeding on the alkaloid gelsemine for several days (Fig. 2). In particular, the distribution of infections differed substantially between treatments, with most gelsemine-consuming bees experiencing far lighter infections than the control bees. However, the infectivity of *C. bombi* inocula was unaffected when pathogen cells were exposed to gelsemine outside of the host. These results suggest that alkaloid-rich nectar can act as a microbicide against a protozoan pathogen of pollinators when ingested, but does not directly interfere with pathogen viability. Given that *C. bombi* is deposited at flowers by infected foragers, and can spread between bees via contaminated floral nectar, alkaloid-rich nectar could have substantial effects on the transmission of this pathogen both within the hive and through bumble bee populations.

Our experiments were conducted under laboratory conditions, which facilitated the careful manipulation of both alkaloid and pathogen. However, the artificiality of the lab may also have limited certain aspects of our study. Natural floral nectar is rarely as simple as the artificial nectar that we used; thus, our experiment may have eliminated some of the subtle interactions between other chemical components of nectar and *C. bombi*. Similarly, bees may not forage on a single nectar source continuously for 10 days, as we simulated in experiment 1. Nevertheless, *G. sempervirens* flowers very early in the spring (Pascarella 2007) and therefore represents one of the few nectar sources for early emerging bumble bees. Our study also simulates how bumble bee pathogen loads are affected by nectar alkaloids under the most extreme gelsemine concentrations found in nature, and the effects of more moderate nectar alkaloid concentrations on *C. bombi* may be less substantial. Finally, by isolating bees in individual vials, we may have disrupted important aspects of infection dynamics that naturally occur within hives, such as the exchange of pathogen cells and strains among nestmates.

The effects of plant secondary metabolites on host–pathogen interaction are understudied and poorly understood (Cory and Hoover 2006; Price et al. 1980). Plant-derived alkaloids appear to have anti-protozoal properties that are effective against human parasites, such as *Trypanosoma brucei rhodesiense*, the causative agent in African sleeping sickness (Freiburghaus et al. 1996). In bumble bees, the plant-derived alkaloid gelsemine appears to have similar anti-protozoal effects on *C. bombi*, another trypanosome parasite. Although the underlying mechanism is not yet clear, it may be that when a host's gut contains substantial concentrations of alkaloids, C. *bombi* cells suffer reduced growth and replication because of costs associated with

alkaloid tolerance. Similar reductions in pathogen proliferation have been reported for secondary metabolite-tolerant plant pathogens (Vanetten et al. 2001). Alternatively, the consumption of alkaloids might alter the host's gut environment, making it less hospitable for pathogen cells. Logan et al. (2005) proposed this mechanism after pollen consumption altered the rate at which *C. bombi* populations increased within hosts, perhaps by affecting their adherence to the gut wall. Consumption of alkaloids may also increase gut pH, which could be deleterious to pathogen cells (Stiles and Paschke 1980). Finally, an alkaloid-rich diet may increase a bee's excretion rate, effectively "flushing" *C. bombi* cells from the gut wall. Indeed, animals that consume secondary metabolites often deal with the inherent toxicity through rapid excretion (Despres et al. 2007; Wink and Theile 2002), and alkaloid-rich nectar in particular has been shown to increase excretion rates in a nectarivorous bird (Tadmor-Melamed et al. 2004). Gelsemine does not, however, seem to hinder a bee's immunocompetence toward *C. bombi*, since this would result in a pattern opposite to what we observed, i.e., *higher* levels of infection in the gelsemine-consuming bees.

The impact of nectar secondary metabolites on pathogens could be ecologically significant both to bumble bees and the plants they pollinate. Although *C. bombi* is often considered a benign pathogen (Schmid-Hempel 1998), it renders foragers less able to provide food for their colonies. For example, infected workers have reduced foraging rates, a decreased capacity to learn floral cues, and difficulty manipulating complex flowers (Gegear et al. 2005, 2006; Otterstatter et al. 2005). The severity of these impairments increases with infection intensity; bees with *C. bombi* infections higher than 1,000 cells/μl were significantly less efficient, more prone to error and slower to learn how to manipulate flowers compared to bees with no or low (<1,000 cells/μl) parasite loads (Gegear et al. 2005). The prevalence of colonies infected with *C. bombi* rises dramatically as the flowering season progresses (Schmid-Hempel 2001) and *C. bombi* often infects the majority of foragers within a hive (Imhoof and Schmid-Hempel 1999), which can result in reduced foraging proficiency for much of the colony's workforce and has the potential to severely reduce colony success (Otterstatter et al. 2005). Although dietary gelsemine does not appear to cure *C. bombi* infections, it could curtail the adverse affects of the pathogen on host behavior by reducing infection intensity. Bumble bee queens might derive the greatest benefit from nectar secondary metabolites. In the spring, queens that emerge from hibernation harboring *C. bombi* are less likely to found a colony than healthy queens (Brown et al. 2003). It is possible that a gelsemine-rich diet would suppress a queen's pathogen load to the extent that she could establish a viable colony. In the south-eastern United States, *Bombus impatiens* and *Bombus bimaculatus* queens often collect alkaloid-rich nectar from *G. sempervirens* in the spring (J. S. Manson, personal observation); whether or not these queens receive a "medicinal" benefit from this nectar is an important topic for further study. The medicinal properties of nectar secondary metabolites might also have consequences for plant communities, as parasitic infections are known to alter pollen collection (Schmid-Hempel and Schmid-Hempel 1991) and plant species choice (Schmid-Hempel and Stauffer 1998) in bumble bees.

Our demonstration that gelsemine can mitigate infections raises the possibility that infected bees might actively self-medicate. There is mounting evidence that infected insects alter their foraging strategies in order to fight pathogens. Some insects adjust basic nutrient intake to improve their overall immune response (Lee et al. 2006), whereas others actively seek compounds that have antimicrobial properties. The active collection of non-nutritive secondary metabolites, or "pharmacophagy" (Boppre et al. 2005), is often associated with a significant shift in diet. For example, parasitoid-infested *Platyprepia virginalis* caterpillars preferentially consume hemlock instead of lupine, their primary host plant, in field choice experiments; this diet switch was correlated with higher survival rates amongst infected individuals, while the consumption of hemlock reduced the survival of healthy caterpillars (Karban and English-Loeb 1997). Parasitized *Grammia geneura* caterpillars also choose a mixed diet of plants rich in secondary metabolites rather than a nutrient-rich, but toxin-poor, single-plant diet (Singer et al. 2004). Singer et al. (2009) elegantly demonstrated that *G. geneura* self-medicate with pyrrolizidine alkaloids to reduce parasite infections and increase caterpillar survival, despite the fact that the alkaloid reduces fitness in unparasitized individuals. The preferential consumption of secondary metabolites in parasitized *G. geneura* is caused by an increase in the firing rates of the animal's taste receptors, which results in increased consumption of pyrrolizidine alkaloids (Bernays and Singer 2005), although the generality of this mechanism is unknown. Several social insect species, including wood ants and honey bees, are known to collect antimicrobial resins to prevent microbe growth within their hives (Chapuisat et al. 2007; Christe et al. 2003; Konig 1988; Marcucci 1995). In the current study, we did not allow bees to choose their diet, so we were unable to test for a gelsemine preference amongst infected individuals. We know of no reports of self-medication by pollinators; the possibility warrants study.

Plants experience multidirectional selection on secondary metabolite concentrations. Strong chemical defenses that reduce herbivory may also reduce pollinator attraction, unless secondary compounds confer a fitness benefit to pollinators via reduced pathogen loads. Indeed, Price et al. (1980)

proposed that the very multifunctional nature of plant defenses may shape the concentration of plant secondary metabolites. The evolutionary origin of nectar secondary metabolites may well be linked to the role of secondary metabolites as herbivore defenses (Adler 2000; Strauss and Whittall 2006); however, the function and, consequently, the concentration of these compounds has undoubtedly been shaped by nectarivorous animals. If pollinators benefit from, and even seek out, nectar rich in secondary metabolites, selection on plants to decrease alkaloid compounds in nectar may be minimal, and potentially even countered by stabilizing selection from pollinators (Clayton and Wolfe 1993). Few studies have attempted to tease apart the various forces that select for or against nectar secondary metabolites in plants (but see Irwin et al. 2004); however, the potential impact of this unusual trait on both plant and pollinator fitness suggests that it merits further investigation.

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