

The role of chemical cues in *Crematogaster brevispinosa* and *Pseudomyrmex spinicola* defense response

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Abstract: Once a mutualistic relationship is established between species, selective pressures favor greater interdependence. Because of their greater dependence on *Acacia collinsii*, *Pseudomyrmex spinicola*, an obligate mutualist, may experience stronger selective pressures, leading to more complex mechanisms of coordinating defense responses to herbivores than *Crematogaster brevispinosa*, a facultative mutualist. Given this argument, we hypothesized that *C. brevispinosa* and *P. spinicola* would respond differently to plant-damage and conspecific chemical cues. We found that *Crematogaster brevispinosa* did not elevate their activity in response to either type of cue, suggesting *C. brevispinosa* does not respond to volatile chemical cues for defense. In contrast, *P. spinicola* showed a marked increase in activity when exposed to ant cues, but not to plant-damage cues. Thus, *P. spinicola* appears to utilize volatile chemical cues, especially those from conspecifics, for communicating during defense against herbivores. Further study is needed to examine how these different mechanisms used by *C. brevispinosa* and *P. spinicola* function in coordinating their defense against herbivores.

Key Words: *Acacia collinsii*, ants, chemical cues, communication, defense response mutualism

Introduction

Once a mutualistic relationship is established between species, selective pressures favor greater interdependence. A powerful incentive exists for maintaining strong mutualisms, since each member benefits from increasing the fitness of the other. The ant species *Crematogaster brevispinosa* and *Pseudomyrmex spinicola* are both involved in a mutualistic relationship with *Acacia collinsii*. In this system, *A. collinsii* trees provide food and shelter for a resident ant colony, and in return, the ants protect the plant from herbivory. Effective communication when mounting a defense response may play an important role in strengthening this mutualism. Although much is known about the various dispositions and behaviors exhibited by these two species of ants, little is known about the communication strategies they use in mounting a defense response.

Previous research suggests that obligate mutualists such as *P. spinicola*, defend their host plant against herbivory more aggressively than facultative mutualists such as *C. brevispinosa* (Agrawal and Rutter 1998). Due to their greater dependence on their host plant, selection should

favor more efficient defense mechanisms in obligate mutualists. One way that this may be accomplished is through the development of intraspecific communication techniques.

Previous studies have described behaviors in *P. spinicola* and *C. brevispinosa* that suggest that complex cues are involved in stimulating the defense response. *Pseudomyrmex spinicola* responds to both conspecific chemical cues (Janzen 1983) and chemical cues in crushed *A. collinsii* leaves (Alexander et al. 2002) by increasing activity and aggression. Response to ant cues represent intraspecific signals used in communication among ants for various reasons including reproduction, alarm, recruitment, and defense. Response to crushed plants is thought to be a way *P. spinicola* increases its defense of the acacia against herbivores, assuming the crushed plant gives off the same cue as when an herbivore feeds on the leaves. Thus, chemical cues produced by both ants and acacias may improve protection for the acacia against herbivory.

The role of chemical communication in *C. brevispinosa* does not appear to have been examined. A study by Wickre et al. (2003) found that ambient patrolling activity was higher in *C. brevispinosa* than in *P.*

spinicola; however, both species were found in equal numbers immediately following a disturbance. This suggests that these species may use different mechanisms for coordinating a defensive response. We hypothesized that *P. spinicola* and *C. brevispinosa* would respond differently to plant and conspecific chemical cues. Given results from previous studies, we predicted that *P. spinicola* would respond to both volatile plant and ant chemical cues with increased numbers and aggressiveness. However, based on pilot observations and data from Wickre et al. (2003), we predicted that *C. brevispinosa* would not respond to volatile plant-damage or to ant chemical cues.

METHODS

To determine whether *C. brevispinosa* and *P. spinicola* rely on chemical cues for communication, we sampled seven *A. collinsii* trees inhabited by *C. brevispinosa* and five with *P. spinicola*. We measured ant patrolling activity in response to three treatments: conspecific ant cue, plant cue and a control. The ant treatment consisted of exposing each colony to conspecific chemical cues, possibly secreted by the ants in response to damage arising from a herbivore. To test response to ant chemical cues, we first allowed a separate colony of *P. spinicola* or *C. brevispinosa* to sting a cotton swab for 1 min. Both species actively attacked by stinging and biting the swab. The plant cue treatment consisted of exposing each colony to a cotton swab coated in *A. collinsii* leaves that had been crushed with a mortar and pestle to simulate leaf damage that might occur as a result of herbivory. As a control for both experiments, we exposed the ants to an untreated swab.

For each treatment, we first measured ambient activity levels on a haphazardly chosen branch to control for the possible effect of the swab. To measure activity

levels, we counted the number of ants that crossed a specified point on the branch within a 30 s time period. We then waved a swab according to treatment type within 1 cm of the branch for 30 s. At no time did the swab physically contact the branch. Ant activity levels were measured both 30 s and 180 s after exposure to the swab.

We analyzed our data using separate two-way ANOVAs to examine the main effects and interactions between ant identity (*C. brevispinosa* vs. *P. spinicola*) and each of the two kinds of chemical cues.

RESULTS

Crematogaster brevispinosa patrolling levels did not change in response to conspecific ant chemical cues. However, *P. spinicola* showed a significant increase in activity when exposed to conspecific ant cues. There was a significant interaction between ant chemical cues and ant species at both 30 s (Fig. 1a; $F = 15.17$, $df = 2, 35$, $P < 0.001$) and 180 s (Fig. 1b; $F = 5.67$, $df = 2, 35$, $P = 0.008$) after treatment, showing that species identity is important in accounting for the response to chemical ant cues.

In contrast, neither species was affected by plant-damage stimuli compared to ambient and control activity levels. There was no significant interaction between plant cues and ant species identity (Fig. 2a; $F = 0.25$, $df = 2, 35$, $P = 0.78$). Independent of plant cues, *C. brevispinosa* had higher activity levels than *P. spinicola* (Fig. 2a; $F = 8.30$, $df = 1, 35$, $P = 0.007$; and Fig. 2b; $F = 10.18$, $df = 1, 35$, $P = 0.003$, respectively). Furthermore, there were no differences between ambient and control activity levels across each treatment, confirming the effectiveness of our methodology.

DISCUSSION

Our results demonstrated a clear difference in response to volatile ant cues

between *C. brevispinosa* and *P. spinicola*, suggesting that these species rely on different mechanisms to coordinate the defense against herbivores described by Wickre et al. (2003). *Crematogaster brevispinosa* activity level remained relatively constant across all treatments, implying that *C. brevispinosa*

does not recruit or respond to volatile chemical cues, either from conspecifics or from damaged plant tissues. In contrast, *P. spinicola* showed a marked increase in activity when exposed to volatile ant cues, indicating that conspecific chemical cues play a major role in recruiting ants to a disturbance.

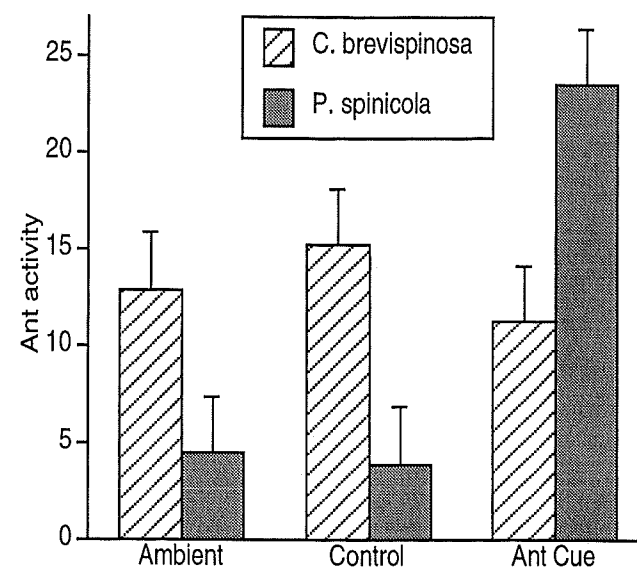


FIG. 1a. Ant activity (number of ants crossing a point on the branch in 30 s) of *C. brevispinosa* and *P. spinicola* 30 s after exposure to conspecific ant chemical cues.

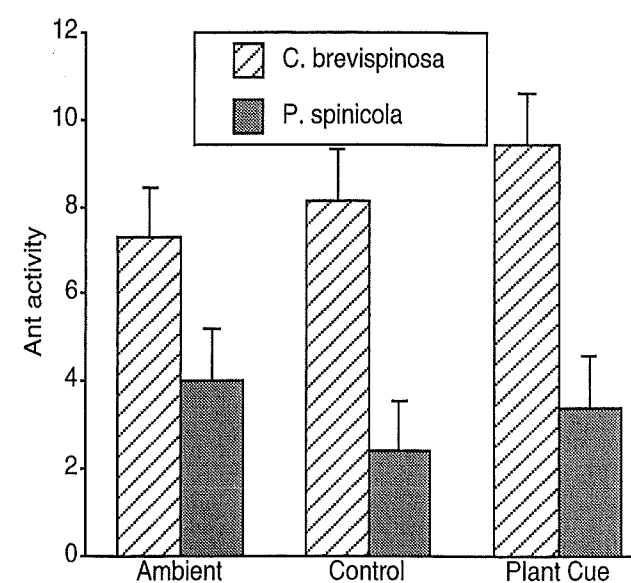


FIG. 2a. Ant activity (number of ants crossing a point on the branch in 30 s) of *C. brevispinosa* and *P. spinicola* 30 s after exposure to crushed *A. collinsii* plant chemical cues.

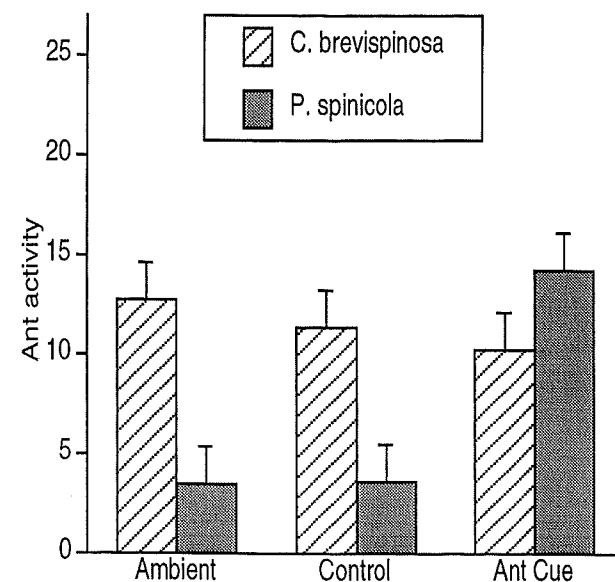


FIG. 1b. Ant activity (number of ants crossing a point on the branch in 30 s) of *C. brevispinosa* and *P. spinicola* 180 s after exposure to conspecific ant chemical cues.

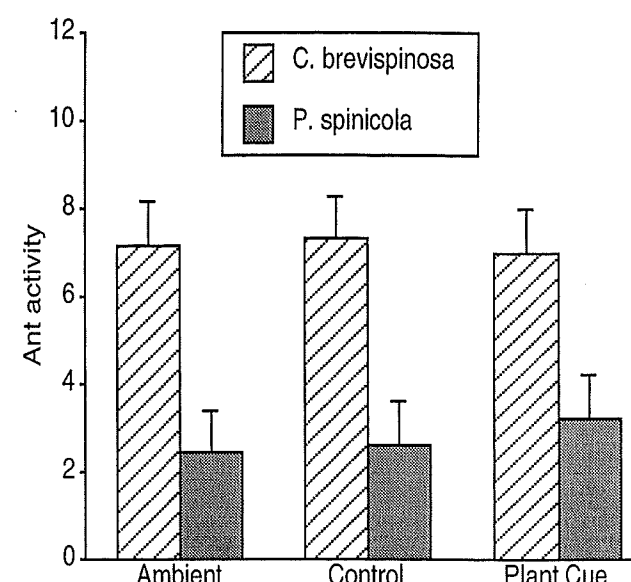


FIG. 2b. Ant activity (number of ants crossing a point on the branch in 30 s) of *C. brevispinosa* and *P. spinicola* 180 s after exposure to crushed *A. collinsii* plant chemical cues.

Our results are consistent with those of Agrawal and Rutter (1998) who reported that obligate mutualists tend to rely on conspecific chemical cues because there is strong selection for effective defense in obligate mutualisms. Since any damage to the plant is highly detrimental to the colony itself, selection should favor colonies that respond swiftly to damage. Facultative mutualists, however, may relocate to another host plant if the health of the current host deteriorates. Agrawal and Rutter (1998) showed that plants hosting facultative mutualists produce rewards to augment their protection. An example of such a reward was increased nectary production at the site of herbivory in order to attract the ants to the damage site (Agrawal and Rutter 1998).

In our test species, the absence of a response to volatile chemical cues in *C. brevispinosa* suggests a possible reliance on other sensory cues for mounting a defense response, such as tactile cues or tactile exchange of chemical cues. The latter possibilities are bolstered by our observations that *C. brevispinosa* seem to have a higher incidence of physical contact among conspecifics compared to *P. spinicola*. We observed that individual *C. brevispinosa* frequently made antennal contact while passing one another. Further research should examine the role of tactile interactions in *C. brevispinosa*, as well as the response of *C. brevispinosa* to possibly enhanced plant rewards, as proposed by Agrawal and Rutter (1998).

Contrary to findings by Alexander et al. (2002), we did not find a significant increase in patrol activity over ambient patrol levels in *P. spinicola* when exposed to volatile chemicals from crushed *A. collinsii* leaves. This discrepancy may be due to

methodological differences. Alexander et al. (2002) exposed crushed leaves to the colony for the duration (4 min) of observation. We, however, exposed the colony to the volatile cues for only 30 s, removed the stimulus, and then began to measure activity.

Our findings support the idea that obligate mutualists evolve different techniques for coordinating defensive responses, such as utilizing complex, volatile, chemical signals. Effective defensive coordination may result in a stronger mutualism. Further study is needed regarding the effectiveness of these mechanisms in defending against herbivores in obligate and especially facultative mutualist ant species.

LITERATURE CITED

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