Smells like herbivory: role of chemical stimuli in acacia tree ant defense

Kathryn M. Alexander, Michael L. Brosnahan, Matthew T. Burke, Jennifer L. Butcher and Erica B. Close

Abstract: *Acacia collinsii* may be actively defended from herbivory through mutualistic interactions with ant species such as *Pseudomyrmex spinicola*. Prior studies have suggested that defense activity by *P. spinicola* is induced by simulated herbivory. We hypothesized that such induced defensive activity by acacia ants could be caused by volatiles emitted from either damaged leaf tissue or from the herbivore itself. We measured ant activity before and after exposure of branches to putative volatile stimuli from damaged leaf tissue and from a common herbivore (a katydid, genus *Orophus*). Ant activity increased 3-fold within 4 min of exposure to crushed leaf volatiles, but was unaffected by exposure to katydid volatiles. Further studies are required to explore the importance of chemical stimuli relative to the previously well-established effect of mechanical stimuli.

Key Words: Acacia collinsii, *herbivory defense, induction, mutualism,* Pseudomyrmex spinicola

Introduction

Plants often utilize a variety of chemical, physical and/or biotic defenses against herbivory. These defenses may increase plant fitness by making the plants less susceptible to herbivory. Janzen (1967) found that the tree species *Acacia collinsii* utilizes the presence of mutualistic ant species such as *Pseudomyrmex spinicola* for protection from herbivores. In recent studies, Macintosh et al. (2000) demonstrated that the aggressive defense response of *P. spinicola* could be induced by simulated herbivory on attached leaves. One possible explanation is that ants respond to the presence of volatiles released from damaged leaf tissue. We tested this hypothesis with experiments that isolated the potential effects of damaged leaf volatiles on ant patrolling behavior. We also tested the hypothesis that chemical volatiles emanating from insect herbivores themselves might induce a response from the ants.

Methods

The study was conducted on 7 January 2002 in Palo Verde National Park, Costa Rica. We observed activity levels of *P. spinicola* on randomly selected *A. collinsii* trees at an acacia stand ~0.5 km east of the OTS Biological Station on a sunny, windy morning. Trees ranged from 2 - 5 m in height.

We quantified ant activity levels on randomly selected branches 1 - 2 m from the ground on *A. collinsii* trees. We recorded the number of times that ants crossed a point midway along each branch. Ants were observed during 10 sec intervals for 4.5 min, starting 30 sec prior to introduction of the stimuli and for 4 min afterwards. Stimuli (freshly damaged leaves or insect herbivores) were placed within 5 cm of the branch at the imaginary line of observation, but not touching the branch or any part of the plant.

In our first experiment, we tested chemical cues from damaged leaf tissue. Leaves from neighboring *A. collinsii* trees were ground with a mortar and pestle. The pestle (with leaf mull present) was presented as a stimulus at the observation point (within 1 cm but not touching the branch). For the control, we simultaneously presented a clean pestle next to the observation point on nearby branches. These paired tests were performed on 10 trees.

In our second experiment, we tested the response of ants to chemical volatiles associated with a potential herbivore. A live katydid (*Orophus* sp.) was placed within a mesh bag and held adjacent to the observation point. We simultaneously observed a branch on the opposite side of the tree using an empty bag as a control. These paired observations were repeated on 8 trees.

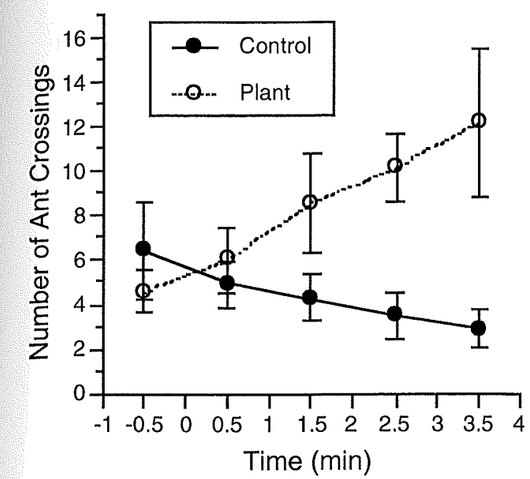

FIG. 1. Mean number (± SE) of *P. spinicola* crossing an observation point per minute in the presence of a plant chemical stimulus (damaged leaf volatiles) and control. Values at -0.5 min represent basal ant activity.

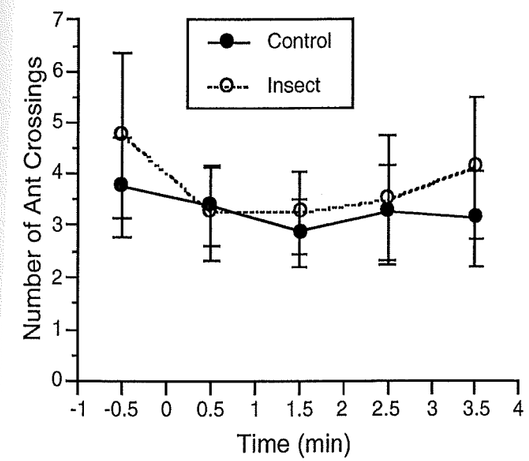

FIG. 2. Mean number (± SE) of *P. spinicola* crossing an observation point per minute in the presence of a putative herbivore (katydid) and a control (empty bag). Values at -0.5 min represent basal ant activity.

For a statistical analysis, we first standardized our data by subtracting the baseline ant activity (recorded before introduction of the stimulus) from the experimental ant activity. Data were then summed in 1 min increments, and analyzed by MANOVA.

Results

Plant Chemical Stimulus

Ant numbers increased in response to plant chemical stimulus relative to the control (Fig. 1). The mean number of *P. spinicola* crossing the observational line after the introduction of the plant chemical stimulus was greater in the experimental treatment than in the control ($F_{1,18} = 8.68$, $P = 0.009$). The ant response to the stimulus gradually increased throughout the 4 min sampling period, while there was no increase in ant activity in the controls (Fig. 1).

Insect Chemical Stimulus

Ants did not respond to insect chemical stimulus (Fig. 2). There was no significant difference in *P. spinicola* activity between the control and experimental treatments for the insect chemical stimulus ($F_{1,14} = 0.81$, $P = 0.38$).

Discussion

Activity of *P. spinicola* increased in response to presentation of chemical volatiles of damaged leaves, but not to those associated with a common herbivore (katydid). This stronger response to leaf volatiles may be an efficient means of defending the host *A. collinsii* trees. *A. collinsii* are probably threatened by many different herbivore species, and it seems improbable that its mutualistic ants could develop sensitivity to specific chemical volatiles associated with each potential herbivore. Sensitivity to volatiles associated with damaged leaves, on the other hand, ensures that ant activity will increase in response to all threats to host *A. collinsii* that involve consumption of leaf tissue.

Ants respond to both mechanical and chemical stimuli, but the corresponding patterns of response are qualitatively different. Mechanical disturbance produces high activity very quickly (e.g., 5-fold increase in 15 sec) but the activity declines immediately after this increase (Macintosh et al. 2000). In contrast, ant activity increased relatively slowly upon presentation of damaged leaf volatiles but the response continued to increase for at least 4 min, at which point ant activity was 3-fold higher than controls (see Fig. 2). These differences make sense given the quick transmission and dissipation of mechanical stimuli relative to volatile stimuli.

The response curve of ants to volatile cues for damaged leaf tissue in our study was similar to that from the leaf-cutting results reported by Macintosh et al. (2000). This similarity suggests that chemical volatiles associated with leaf damage may be important stimuli, especially in disturbances by small herbivores where little mechanical disturbance is generated. Future study of ant response to herbivory of *A. collinsii* should consider the interaction of these two stimuli and the conditions that induce maximal ant response.

Literature Cited

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