Do tropical plants "signal for help" to natural enemies of herbivores?

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Introduction

The goal of this research was to determine whether plants in the genus *Piper* employ indirect defense mechanisms by releasing volatile compounds (chemical signals) in response to herbivory by caterpillars. Indirect defenses have been demonstrated in temperate systems to attract natural enemies of caterpillars, such as parasitoid wasps. To date there have been no studies which have evaluated whether tropical plants also utilize this form of defense. Most studies on the defense mechanisms of tropical plants have focused on direct defenses such as plant toughness, nitrogen and water content and concentrations of secondary metabolites, which can function as feeding deterrents. Thus, the proposed project aimed to address a novel aspect of plant defense research that is currently considered cutting edge in temperate biological control studies yet has been lacking in tropical regions.

This research was conducted at La Selva Biological Station in Costa Rica from July through the end of August. As I was unfamiliar with the forest, the first couple of weeks were spent locating *Piper* plants and collecting and rearing caterpillars to assess parasitism levels. Unfortunately, it wasn't long after collecting and rearing the caterpillars before I discovered that parasitism levels of these caterpillars appeared to be extremely low. This was a surprise as parasitism levels of these caterpillars are very high in Panama. I realized that the experiment I had planned probably would not be successful as a result of low parasitism. However, I decided to go ahead and carry out the research described in my proposal. This work involved locating *Piper* plants with and without caterpillars and placing a sticky trap to catch parasitoids on leaves where caterpillars were feeding.

The objective of this study was to determine whether parasitoids appeared more frequently on traps which were placed on leaves with caterpillars, compared to a) plants with no caterpillars and b) plants which had been artificially damaged and painted with caterpillar regurgitate. Caterpillar regurgitate was collected prior to fieldwork in the lab by gently squeezing the caterpillar until it regurgitated. The regurgitate was then collected with a pipette, placed in vials and kept in a cooler with ice during fieldwork. Regurgitate was also collected for chemical analyses at Tulane. Caterpillars from different species of *Piper* were also collected, starved for 24 hours and frozen. These caterpillars were taken to Tulane and analyzed for different plant compounds they may have sequestered during feeding. Leaves from each *Piper* species were also collected and dried and analyzed at Tulane for chemical compounds potentially used in defense against caterpillars.

To gather information regarding the general abundance of parasitoids I set out 4 large net traps to capture flying insects, these were set out for 2 days in patches of 2 species of *Piper*. The insects were collected in containers of alcohol and will be examined under a microscope where I will identify differences in the abundance of parasitoid wasps from the different patches of *Piper*. Sticky traps will also be examined under a microscope to determine whether parasitoid abundance is greater on plants where caterpillars were present.

As I was unsure about the success of the sticky trap study I decided to undertake several different projects to insure that I left Costa Rica with usable data. At the beginning of the summer, I flagged newly opened leaves on 40 plants of 3 *Piper* species and at the end of the summer I collected these leaves and calculated the amount of damage to assess differences in rates of herbivory between the *Piper* species using a leaf area measuring device. I also conducted a census on the 3 species of *Piper* and recorded percentage of herbivory, abundance of caterpillars and also predatory arthropods. To examine differences between caterpillar growth and digestive efficiency of the different *Piper* species I conducted a feeding efficiency experiment. This work involved collecting caterpillars in their early stages and rearing them on plants from which they were collected. Every 2 days, I recorded their weight, amount of leaf area consumed; mass of fecal matter and at the end of the study their pupal weight. These measures will allow me to calculate their rate of growth, efficiency with which they convert food into biomass and potential fecundity. In addition I conducted a feeding efficiency experiment to examine differences between old and young leaves. During my stay at La Selva, I was very fortunate to meet Dr. Van Zandt from Birmingham Southern University, Alabama who was very interested in my work and he and his undergraduate student helped me with the majority of these experiments. Dr Van Zandt and I have now decided to collaborate on these experiments, this work will provide research experience for an undergraduate student working with him in Costa Rica this summer (2008) while also providing further data for my own studies.

During the summer, I also spent time trying to breed the moths in order to establish my own colony to insure that I always have caterpillars to work with. This involved a lot of trial and error and a constant battle with foraging ants interested in eating the honey I provided for my moths! Eventually, I was successful and I have now figured out methods to breed these moths for future experiments.

Since leaving Costa Rica, I have conducted chemical analyses on the *Piper* leaves and also the caterpillars. I have identified compounds present in both the *Piper* leaves and the caterpillars that suggest the caterpillars sequester these compounds and may use them for their own defense against parasitoids. I am now planning on following up this research in Panama where I plan to examine differences in *Piper* chemical defenses and compounds sequestered by the caterpillars in forests along a rainfall gradient. These results will be compared with those collected by Dr Van Zandt and his student in Costa Rica. I am very grateful for this experience; it has broadened my perspective with regards to research questions, enabled me to establish a collaboration with a professor from a different university, it will provide summer research experience for an undergraduate and potentially others in the future and finally it has allowed me to develop methodology which I plan to use for experiments this summer.