Pursuit - The Journal of Undergraduate Research at The University of Tennessee

Volume 2
Issue 1 *Spring 2011*

Article 10

March 2011

Quality vs Quantity: The Effect of Aristolochic Acids on Preference and Performance of a Non-Specialist Herbivore

Samantha Eileen Jeude
sjeude@utk.edu

Pursuit: The Journal of Undergraduate Research at the University of Tennessee

Pursuit

Quality vs Quantity: The Effect of Aristolochic Acids on Preference and Performance of a Non-Specialist Herbivore

SAMANTHA JEUDE
Advisor: Jim Fordyce

Department of Ecology and Evolutionary Biology,
University of Tennessee, Knoxville

As immobile organisms, plants have evolved many strategies for defense against herbivores. These defenses can be physical, such as thorns, or chemical, such as antifeedant compounds. Most plants possess chemical compounds that serve a deterrent function against at least some herbivores. Aristolochic acids are alkaloids characteristic of plants of the genus Aristolochia. *Although their toxicity and efficacy as herbivore deterrents have been documented, it is unknown whether different kinds of these compounds elicit different responses in herbivores. In this study, we use the generalist caterpillar* Spodoptera exigua *as a bioassay to evaluate both qualitative and quantitative effects of four aristolochic acids. Preference and performance were measured using artificial diets containing different aristolochic acids at different concentrations, all within the lower end of the natural range found in* Aristolochia. *We observed that some aristolochic acids (AAI and AAIII) were avoided at higher concentrations, while others (AAII and AA7-OH) had little or no effect on herbivore feeding patterns at any concentration. Performance tests were consistent with choice tests; those aristolochic acids that were stronger deterrents also had a stronger effect on larval growth. These results suggest consistent variation in the effectiveness of various aristolochic acids on both preference and performance.*

Introduction

Nearly all plants possess at least some chemical compounds that are believed to function as defenses against herbivores, at least under specific conditions (e.g., the presence of natural enemies) (Harborne, 1993; Feeny, 1977; Rhoades and Cates, 1977; Pasteels and Rowell-Rahier, 1992). Such allelochemicals typically function as toxins or feeding deterrents for non-specialist herbivores (Blau, 1978; Harborne, 1993; Whittaker, 1971). However, to

http://trace.tennessee.edu/pursuit

coevolved herbivores that specialize on a plant, its chemicals may pose no threat or may even provide some kind of biological benefit (Bowers, 1988b; Ehrlich and Raven, 1964; Fordyce, 2001; Jeffords, 1979; Pasteels and Rowell-Rahier, 1992; Sime, 2002; Whittaker, 1971). In fact, chemical similarity among related plants, alongside herbivore specialization, has been proposed as a facilitator of diversification of phytophagous insects (Ehrlich and Raven, 1964; Futuyma, 1976). For example, furanocoumarins, a class of phototoxic allelochemicals found in several plant families, are metabolized several times more efficiently and effectively by specialist lepidopteran herbivores than by non-generalist lepidopterans (Berenbaum, 1991; Hung, 1995; Zangerl, 2003).

Although host plant chemistry has historically been divided into broad chemical categories (e.g., total cardenolides, total iridoids, etc.), plants may contain one or more compounds of a particular chemical category (Harborne, 1993). These chemically related compounds might vary in their biological activity, thus varying in their effectiveness against herbivores (Messiano, 2008; Pacheco, 2009). For example, Hung and colleagues (1995) observed that angular and linear furanocoumarins induce a unique suite of cytochrome P-450 oxygenases in the guts of a specialist herbivore. Variation in the ability to induce P-450's specific to particular forms of furanocoumarins create a selection mosaic, where populations differ in their ability to disrupt the DNA crosslinking ability of these compounds. Thus, quantitative variation (i.e., linear vs. angular forms) plays a substantial role in determining host plant use patterns, rather than solely quantitative variation (Berenbaum and Zangerl 1998). Qualitative biological differences, particularly pertaining to antifeedant effectiveness, have also been found in groups of chemically related diterpenes, clerodanes, and lignans found in *Aristolochia* (Messiano, 2008; Pacheco, 2009), as well as iridoids in *Plantago* (Bowers, 1988a; Bowers, 1988b) and cardenolides in *Asclepias* (Fordyce and Malcom, 2000; de Roode, 2008). The ratios of these differentially effective compounds may change among different plants of the same species, or even among different structures on the same plant (Bowers, 1996; Bowers and Stamp, 1992). Thus, both qualitative and quantitative variation should be considered in the study of plant allelochemicals.

Aristolochic acids are nitrophenanthrene carboxylic acids found exclusively in plants of the genus *Aristolochia* (Aristolochiaceae). These toxic alkaloids are tolerated by *Aristolochia* specialists such as swallowtail butterflies in the tribe Troidini, and are even sequestered by the larvae, rendering larvae and adults chemically defended against many natural enemies (Fordyce, 2001; Jeffords, 1979; Sime, 2002). However, aristolochic acids as a group are known to have antifeedant properties on non-specialist herbivores (Ikemoto, 1995; Jbilou, 2006; Jbilou, 2008; Lajide, 1993) and on the natural enemies of sequestration specialists (Brower 1960; Jeffords, 1979; Fordyce and Nice, 2008).

At least nine forms of aristolochic acid have been described (Chen, 1987). In nature, aristolochic acids typically occur as mixtures rather than isolated compounds, and most experiments examining aristolochic acid have used mixtures isolated from plants. Qualitative variation among compounds within the category has not been extensively explored; consequently, it is unknown whether aristolochic acids differ in their biological activity.

In this study, we examined the qualitative and quantitative effects of four aristolochic acids (AAI, AAII, AA7-OH, and AAIII) on a generalist herbivore, *Spodoptera exigua*. *S. exigua* has been used extensively as a bioassay for exploring the effectiveness of plant defenses (e.g., Thaler 1999; Agrawal 2003; Barrett & Agrawal 2003) and does not feed on *Aristolochia* in nature (Greenberg *et al.* 2001 and references therein). Specifically, we asked the following questions: 1) Does feeding preference vary across four aristolochic acids at different concentrations? 2) Are there synergistic effects on preference when two aristolochic acids occur in a mixture? 3) Does development performance vary across four different aristolochic acids at different concentrations?

Materials and Methods

Rearing and Diet

Spodoptera exigua eggs were obtained from Benzon Research, Inc (Carlisle, PA). Neonate *S.exigua* were placed into 60mL plastic cups with sealable lids. Artificial *Spodoptera* diet was purchased from Southland Products, Inc. (Lake Village, AR) and prepared according to the manufacturer's instructions. Aristolochic acid is not present in this artificial diet. Aristolochic acid content of the diet was manipulated by adding 2mL of an ethanol solution containing aristolochic acids to the diet while it was still in solution (approximately 95°C). The ethanol boiled off, leaving the aristolochic acid in the diet. Control diets received 2mL of 100% ethanol without aristolochic acid.

Preference Test

To examine the qualitative and quantitative effects of aristolochic acids on preference, we conducted choice tests for each of four types of aristolochic acid (AAI, AAII, AA7-OH and AAIII) at four different concentrations. For each choice test, 50 groups of approximately 10 neonate *S.exigua* larvae were placed into 60mL plastic cups with four equal-sized disks of artificial diet cut with a 9mm cork borer. Each disk had a different concentration (zero, 0.0001, 0.002, and 0.004 μg/mg dry weight) of the same aristolochic acid. At four 24-hour intervals, the observed feeding position of each larva was recorded as the response variable indicating larval preference. These observed feeding positions were then summed across observations and analyzed using a Friedman test to determine whether their preference for any one diet differed from a random expectation. During the experiment, the replicates were randomly sorted into several boxes, which we blocked as a random variable in the Friedman test. Implementation of the Friedman test and post-hoc test for multiple comparisons followed Conover using a script written in R (Conover, 1999).

Test for Synergistic Effects

To determine whether synergistic effects occur with a mixture of aristolochic acids, we conducted a choice test in which larvae were presented with four diets:control, AAI (0.005 μg/mg), AAII (0.005 μg/mg), and a 1:1 mixture of AAI and AAII (0.005 μg/mg, 0.0025μg/mg of each AA). As before, groups of 10 neonate *S.exigua* were placed in each of 50 cups. Each cup contained four equal-sized disks of artificial diet, cut with a 9mm cork borer. As before, the individuals' observed feeding positions were recorded at 24-hour intervals, and analyzed using a Friedman test as above.

Performance Test

To examine the qualitative and quantitative effects of aristolochic acids on performance, we set up four no-choice rearing tests in which each type of aristolochic acid (AAI, AAII, AA7-OH and AAIII) was used to raise individual *S.exigua* larvae at four concentrations. Here, we define performance as the ability of a caterpillar to develop successfully to an adult moth. Adult and pupal weight were used as an additional measure of performance, as it is generally regarded as an effective correlate for reproductive potential in Lepidoptera (Price, 1997). For each aristolochic acid, 80 larvae were placed into individual 60mL plastic cups and fed a continuous diet of only one concentration (20 replicates each of zero, 0.0001, 0.002, or 0.004 μgAA/mg dry weight). Rearing cups were placed in 48.6cm x 81.6cm plastic boxes with each box containing approximately 60 cups. Performance was measured as pupal weight and adult dry weight. Sex was determined after pupation by examining the genital scar, anterior to the anal scar on the ventral side of the pupa. Those with significant genital scarring are male (Scoble, 1992). The effect of each type and concentration of aristolochic acid on pupal and adult weight was analyzed using a mixed model factorial ANOVA. The model included sex, aristolochic acid concentration of the diet, the interaction between diet concentration and sex, and rearing box as a random effect. ANOVAs were implemented using the statistical software JMP 8 (SAS Institute Inc., Cary, NC). Post hoc comparisons of performance among diet treatments were examined using Tukey's HSD at ($\alpha = 0.05$). Each aristolochic acid was analyzed with a separate factorial ANOVA and each had its own control group.

Results

Preference Test

Overall, preference varied qualitatively among acids, and quantitatively across concentrations for each aristolochic acid. Typically, the higher concentrations were avoided in favor of the control (0 μg/mg). AAI was the strongest deterrent, avoided at all concentrations ($T_2 = 6.84$, df = 3, 147, $P \leq 0.001$; Figure 2a). Interestingly, those larvae reared on AAII consistently avoided the medium concentration (0.002 μg/mg) the most ($T_2 = 6.38$, df = 3, $P \leq 0.001$; Figure 2b), rather than the highest concentration, even in repeated experiments ($T_2 = 20.20$, df = 3, 147, $P \leq 0.001$; Figure 2c). AA7-OH had no detectable effect on preference at any concentration ($T_2 = 2.00$, df = 3, 147, $P = 0.088$; Figure 2d). AAIII had little effect at the lowest concentration (0.0001 μg/mg), but was avoided at the higher concentrations ($T_2 = 10.89$, df = 3, 147, $P \leq 0.001$; Figure 2e).

Test for Synergistic Effects

There was no detectable preference among the AAI, AAII, and 1:1 mixture diets, and larvae consistently preferred the control diet ($T_2 = 6.76$; df = 3, 147; $P \leq 0.001$; Figure 2f). There was no evidence that the mixture of AAI and AAII had a stronger deterrent effect compared to either of the aristolochic acids in isolation. This suggests that there are no synergistic effects when AAI and AAII are combined at this concentration. However, we cannot extrapolate this result out to combinations at different concentrations, because 0.001μg/mg is within the "strange behavior range" for AAII. In choice tests of just AAII, the diet at 0.002μg/mg was a more effective deterrent than diets at both higher and lower concentrations. *see suggestion 4*

Performance Test

Fifty individual *S.exigua* were raised on each of the diets described in the preference test. Pupal weight was highly correlated with adult dry weight for both sexes (Pearson Product Moment: $r = 0.82$, $P < 0.001$). There was variation in the time between eclosion and adult weighing (some adults had more time to lose weight, lay eggs, or batter themselves against the lid). Thus, all results presented here are based on analysis of pupal weight. The highest concentration (A) of AAI produced a much higher pupal weight, although the other concentrations did not differ significantly from each other or the control (Figure 3a). There was no detectable effect of AAII on pupal weight at any concentration (Figure 3b). As with

AAI, only the highest concentration (A) of AAIII produced a significantly higher pupal weight (Figure 3d). The three concentrations of AA7-OH did not significantly differ in their effects on pupal weight (Figure 3c).

Discussion

We used a model, generalist herbivore to examine the effects of four types of aristolochic acids on caterpillar performance and preference. Our intent was not to explore the consequences of aristolochic acid on *S.exigua,* but rather to explore whether different forms of aristolochic acid vary in their effectiveness against herbivores. Our results showed that significant variation exists among these four aristolochic acids in terms of deterrence against *S. exigua* and their effects on larval growth.

We found that there was variation among aristolochic acids in efficacy as an herbivore deterrent. The most effective deterrent was AAI, one of the most common aristolochic acids found in *Aristolochia*. AAIII was almost as effective as AAI; both AAIII and AAI were most effective at higher concentrations, suggesting that quantitative variation is important to deterrence. We detected no deterrence effect for AAII or AA7-OH. This is interesting, because AAII is commonly found in *Aristolochia*, both in isolation and mixed with other aristolochic acids (Fordyce, 2000; unpublished data). Further studies should be conducted on plants that contain primarily AAII to determine their susceptibility to generalist herbivores.

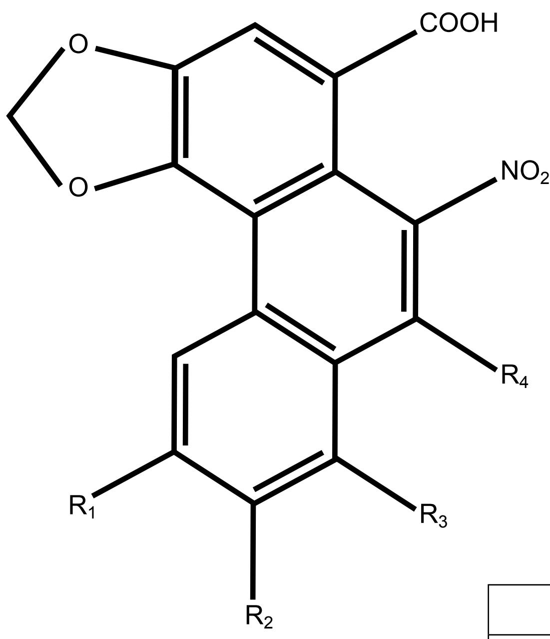
We tested for synergistic effects in aristolochic acid mixtures, and were unable to detect any at the concentrations analyzed. We found no evidence that an AAI-AAII mixture was a more or less effective deterrent than either AAI or AAII alone. Again, this is interesting because we find AAII commonly in nature, and yet we are unable to detect any deterrent effects either alone or combined with another common aristolochic acid. Additionally, aristolochic acids isolated from plants are usually in mixtures; such a tendency to combine aristolochic acids suggests that a mixture might be more effective at deterring herbivory than a single compound, yet this study does not support this hypothesis. However, the concentrations of AAII used in the synergistic effects experiment fall into a "strange behavior range" for AAII. That is, in the above choice tests, we detected a deterrence effect of isolated AAII at concentrations similar to those used in this synergistic effects experiment, yet not at higher or lower concentrations. Therefore, we cannot extrapolate this lack of synergistic effects to other concentrations of AAII, because AAII may behave differently at those concentrations. Additional studies should be conducted comparing different aristolochic acid mixtures to their isolated components to find out if combinations other than 1:1 AAI-AAII produce synergistic effects.

Finally, we determined that aristolochic acids do vary in their effects on herbivore growth. Both AAI and AAIII caused significantly increased pupal weight at the highest concentrations, while we failed to detect an effect of both AAII and AA7-OH on weight at pupation. Note that higher concentrations of the effective aristolochic acids produce the highest pupal weights; this implies that pupal weight is not necessarily positively correlated with good health or growth performance. Rather, the toxic diets could be causing water retention, or causing changes in time spent in the larval stage and thus increased diet consumption. Regardless of the biological significance of the higher pupal weight, it is clear that there are qualitative differences among the aristolochic acids with respect to their effects on herbivore performance. The performance tests are consistent with choice tests, in that those aristolochic acids with the strongest efficacy as deterrents also have the strongest effect on pupal weight. Thus, the effects on growth may be caused directly by the behavioral deterrence itself, rather than any separate factors such as toxicity. Indeed, it is not always known whether deterrence implies toxicity, or vice versa (Bowers, 1988b).

It is known that plants often invest in a variety of compounds within each chemical class. Other experiments have shown that variation often exists among these chemically related compounds (Bowers, 1988a; Bowers, 1988b; Fordyce and Malcom, 2000; Messiano, 2008; Pacheco, 2009; de Roode, 2008); in this study, we found consistent differences in antifeedant efficacy among four aristolochic acids against the model herbivore, *S. exigua*. Such significant differences imply that qualitative variation is an important aspect of plant defense chemistry. The pattern of variation detected in this study might not be universal; that is, variation in the efficacy of these various compounds might vary among herbivore species. Thus, treating quantitative variation of a broad chemical category as a measure of efficacy might be insufficient to fully understand the functional role of plant secondary compounds. Future studies of plant defense chemistry should focus on qualitative as well as quantitative variation.

Figures

Figure 1. Chemical structure of aristolochic acid.



	R_1	R_2	R_3	R_4
AAI	H	H	OCH3	H
AAII	OH	H	H	H
AAIII	H	OH	OCH3	H
AA7-OH	H	H	H	H

Figure 2. Proportion of larvae found on each diet type. Different letters indicate significantly different preference at α = 0.05 following post-hoc comparison test recommended in Conover (1999).

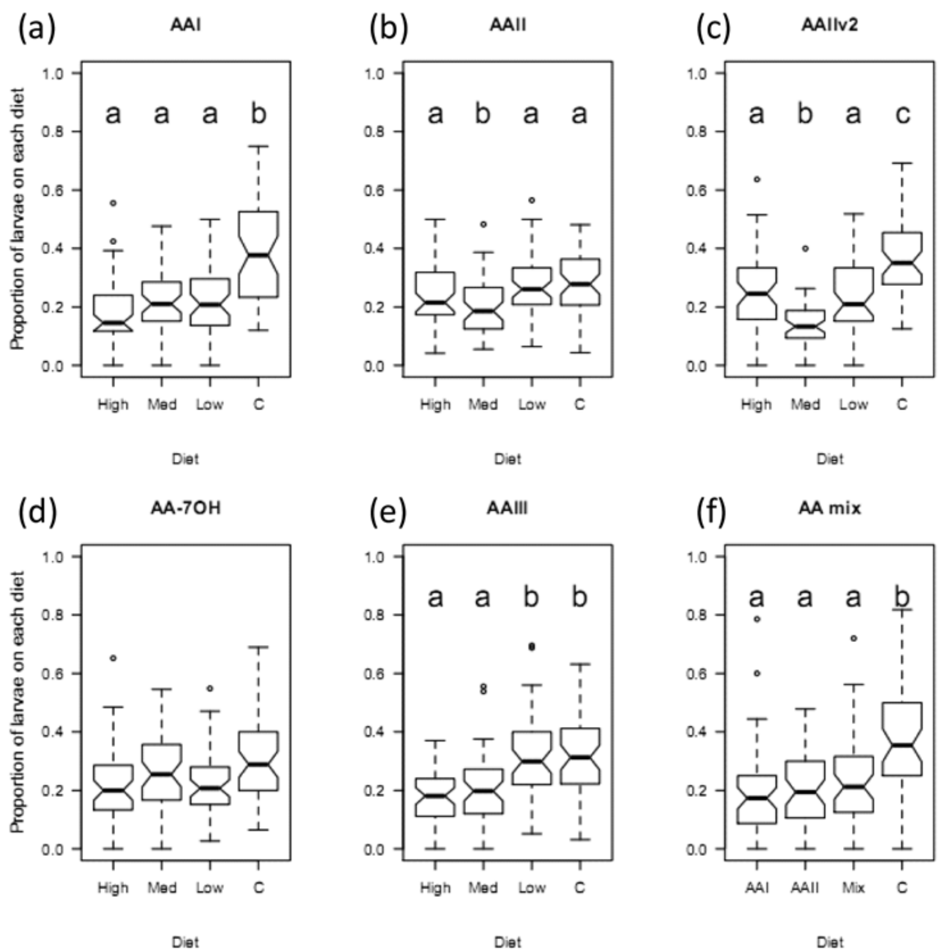
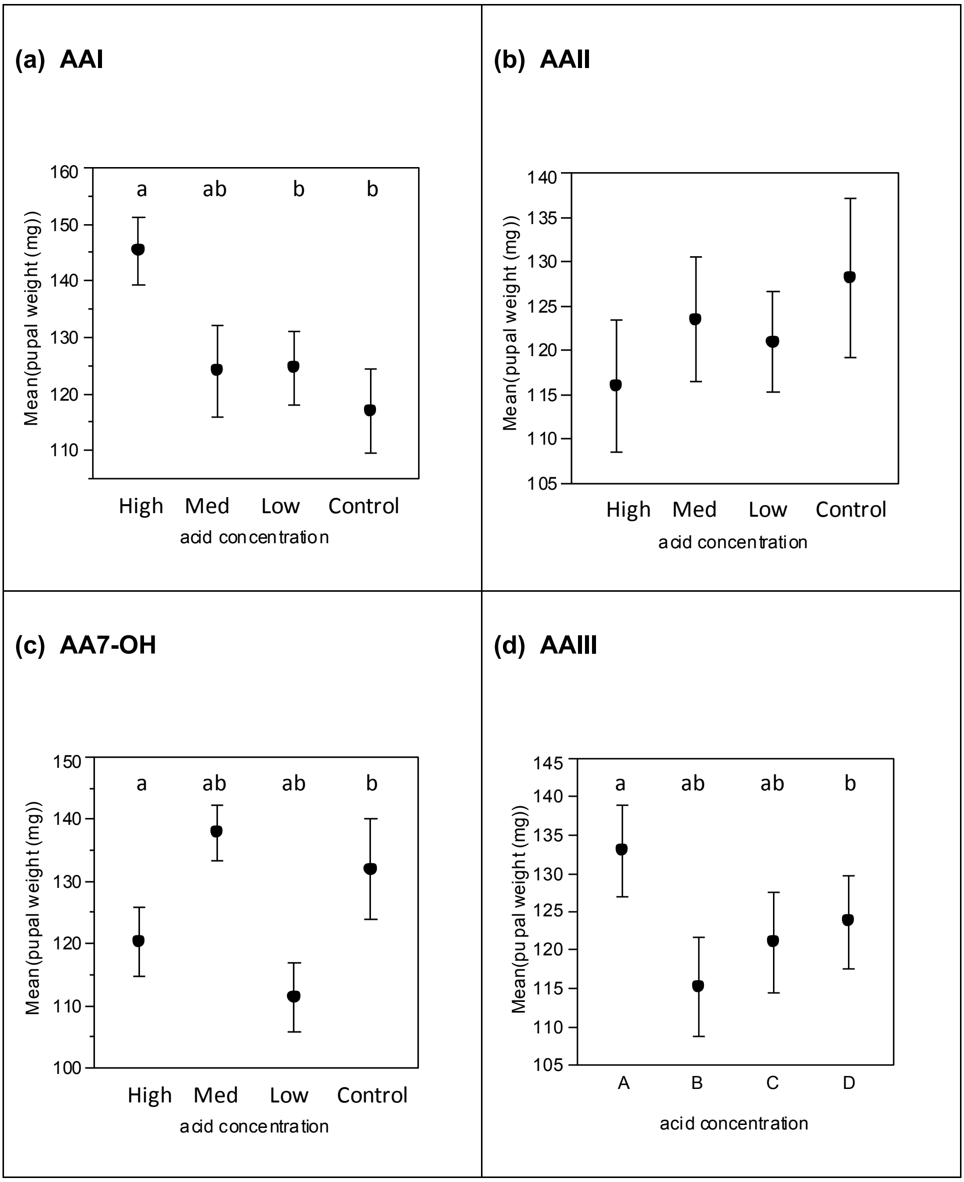


Figure 3. Pupal weight across diets and concentrations. Different letters indicate significant differences at α = 0.05 (Tukey's HSD).



References

Agrawal , A.A. 2003. Specificity of induced resistance in wild radish: causes and consequences for two specialist and two generalist caterpillars. Oikos 89(3):493-500

Barrett, R.D.H. and Agrawal, A.A. 2004. Interactive Effects of Genotype, Environment, and Ontogeny on Resistance of Cucumber (*Cucumis sativus*) to the Generalist Herbivore, *Spodoptera exigua*. Journal of Chemical Ecology 30:37-51

Berenbaum, M.R. 1991. Comparative Processing of Allelochemicals in the Papilionidae (Lepidoptera). Archives of Insect Biochemistry and Physiology 17:213-221

Berenbaum, M. R. and Zangerl, A. R. 1998. Chemical phenotype matching between a plant and its insect herbivore. Proceedings of the National Academy of Sciences of the United States of America 95:13743-13748.

Blau, P.A. et al. 1978. Allylglucosinolate and Herbivorous Caterpillars: A Contrast in Toxicity and Tolerance. Science 200:1296-1298

Bowers, M.D. and Puttick, G.M. 1988a. Response of generalist and specialist insects to qualitative allelochemical variation. Journal of Chemical Ecology 14:319-334

Bowers, M.D. and Puttick, G.M. 1988b. Effect of qualitative and quantitative variation in allelochemicals on a generalist insect: iridoid glycosides and the southern armyworm. Journal of Chemical Ecology 14:335-351

Bowers, M.D. and Stamp, N.E. 1992. Chemical variation within and between individuals of *Plantago lanceolata* (Plantaginaceae). Journal of Chemical Ecology 18(7):985-995

Bowers, M.D. 1996. Variation in iridoid glycosides in a population of *Plantago patagonica* Jacq. (Plantaginaceae) in Colorado. Biochemical Systematics and Ecology 24(3):207-210.

Brower, J.Z. 1960. Experimental studies of mimicry IV: the reactions of starlings to different proportions of models and mimics. The American Naturalist. 94(877):271-282

Cates, R.G. and Rhoades, D.F. 1977. Patterns in the production of antiherbivore chemical defenses in plant communities. Biochemical Systematics and Ecology 5:185-193.

Chen, Z. and Zhu, D. 1987. "Aristolochia Alkaloids" in *The Alkaloids: Chemistry and Physiology*, ed. Arnold Brossi. 31:29-66. Academic Press, Inc., San Diego, CA.

Conover, W.J. 1999. Practical Nonparametric Statistics, 3rd ed.

De Roode, J.C. et al. 2008. Host plant species affects virulence in monarch butterfly parasites. Journal of Animal Ecology 77:120-126.

Ehrlich, P.R. and Raven, P.H. 1964. Butterflies and plants: a study in coevolution. Evolution 18:586-608.

Feeny, Paul. 1977. Defensive Ecology of the Cruciferae. Annals of the Missouri Botanical Garden 64(2):221-234.

Fordyce, J.A. 2000. A model without a mimic: Aristolochic acids from the California pipevine swallowtail, *Battus philenor hirsuta*, and its host plant, *Aristolochia californica*. Journal of Chemical Ecology 26:2567-2578

Fordyce, J.A. and Malcom, S.B. 2000. Specialist weevil, *Rhyssomatus lineaticollis*, does not spatially avoid cardenolide defenses of common milkweed by ovipositing into pith tissue. Journal of Chemical Ecology 26(12):2857-2874.

Fordyce, J.A. 2001. The lethal plant defense paradox remains: inducible host-plant aristolochic acids and the growth and defense of the pipevine swallowtail. Entomologia Experimentalis et Applicata 100:339-346.

Fordyce, J.A. and Nice, C.C. 2008. Antagonistic, stage-specific selection on defensive chemical sequestration in a toxic butterfly. Evolution 62:1610-1617.

Futuyma, D.J. 1976. Food Plant Specialization and Environmental Predictability in Lepidoptera. The American Naturalist 110(972):285-292.

Greenberg, T.W. *et al*. 2001. Feeding and Life History of *Spodoptera exigua* (Lepidoptera: Noctuidae) on Different Host Plants. Annals of the Entomological Society of America 94(4):566-575.

Harborne, J.B. 1993. *Introduction to Ecological Biochemistry*. 4th ed. Academic Press Inc., San Diego, CA.

Hung, Chien-Fu and Berenbaum, M.R. et al. 1995. Differential Induction of Cytochrome P450 Transcripts in *Papilio polyxenes* by Linear and Angular Furanocoumarins. Insect Biochemical and Molecular Biology 25(1):89-99

Ikemoto, Y. et al. 1995. The Effect of Antifeedants against the Level of Biogenic Amines in the Central Nervous System of the Lepidopteran Insect (*Spodoptera litura*). Pesticide Biochemistry and Physiology 52:60-70.

Jbilou, R. et al. 2006. Insecticidal activity of four medicinal plant extracts against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). African Journal of Biotechnology 5:936-940.

Jbilou, R. et al. 2008. Insecticidal effects of extracts of seven plant species on larval development, α-amylase activity and offspring production of *Tribolium castaneum* (Herbst) (Insecta: Coleoptera: Tenebrionidae). Bioresource Technology 99:959-964.

Jeffords, M.R. et al. 1979. Batesian mimicry: field demonstration of the survival value of pipevine swallowtail and monarch color patterns. Evolution 33(1):275-286.

Lajide, L. et al. 1993. Antifeedant Activity of Metabolites of *Aristolochia albida* against the Tobacco Cutworm, *Spodoptera litura*. Journal of Agricultural and Food Chemistry 41:669-673

Messiano, G.B. et al. 2008. Evaluation of Insecticidal Activity of Diterpenes and Lignans from *Aristolochia malmeana* against *Anticarsia gemmatalis*. Journal of Agricultural and Food Chemistry 56:2655-2659

Pacheco, A.G. et al. 2009. C-NMR Data of Diterpenes Isolated from *Aristolochia* Species. *Molecules* 14:1245-1262

Pasteels, J.M. and Rowell-Rahier, M. et al. 1992. Chemical defense in Chrysomelid leaf beetles: storage of host-derived pyrrolizidine alkaloids versus de novo synthesized cardenolides. Naturwissenschaften 79:521-523.

Price, P.W. 1997. Insect Ecology, 2nd edn. Wiley-Interscience. New York.

Scoble, M.J. (1992) *The Lepidoptera: Form, Function and Diversity*. Oxford University Press, New York, New York.

Sime, K. 2002. Chemical defence of *Battus philenor* larvae against attack by the parasitoid *Trogus pennator*. Ecological Entomology 27:337-345.

Thaler, J.S. 1999. Jasmonate-inducible plant defences cause increased parasitism of herbivores. Nature 399:686-688

Whittaker, R.H. and Feeny, P.P. 1971. Allelochemics: Chemical Interactions between Species. Science. 171:757-770

Zangerl, A.R. and Berenbaum, M.R. 2003. Phenotype matching in wild parsnip and parsnip webworms: causes and consequences. Evolution 57(4):806-815.

About the Author

Samantha Jeude graduated *magna cum laude* from the University of Tennessee in 2010 with a Bachelor of Science degree in Ecology and Evolutionary Biology. This paper stemmed from research she conducted under Dr. Jim Fordyce over several semesters of her undergraduate career.

About the Advisor

Dr. J.A. Fordyce is an associate professor in the Department of Ecology and Evolutionary Biology. His research focuses broadly on ecological factors that affect gene flow among populations and how these factors might ultimately affect rates of diversification. He has worked extensively on how plant chemistry affects herbivore behavior and life history evolution, with particular emphasis on aristolochic acid alkaloids. Dr. Fordyce's work at UT is supported by the National Science Foundation, and he received the Chancellor's Citation for Professional Promise in Research and Creative Achievement in 2007. He is currently the faculty mentor for the Naturalist Club, the undergraduate organization in the Department of Ecology and Evolution.