I. Cover Sheet

Faculty Name: Evan Lampert  
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Department: Biology

Student Name: Audrey Barrett  
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Major: Biology

Title of Proposal: Catalpol Sequestration and Trophic Ecology of the Catalpa Sphinx

II. Narrative

- Description of proposed research (by Audrey):

  Background: Catalpa sphinx larvae (caterpillars) are a common pest species across the Southeastern United States, especially in North Georgia. Larvae feed exclusively on the leaves of the catalpa tree. Feeding can be extremely messy in landscape trees, and the larvae can reach plague proportions during the season. They are known to completely defoliate trees, sometimes more than once in a season. Additionally, the larvae themselves are locally known as ‘tawba worms and are most frequently used as panfish bait, specifically catfish. Their bright colors and pungent smell are thought to attract fish while deterring invertebrate and avian predators.

  Catalpa sphinx are showing to be an emerging model for studying chemical ecology, in particular herbivore sequestration of plant compounds as a method of defense. Larvae sequester a toxin called catalpol, and catalpol and its derivatives are known to stimulate feeding. Catalpol and its derivatives are present in amounts varying among catalpa trees. It is believed this may explain finding larvae only present on some trees but not on others. This phenomenon is notable due to the fact that the larva will defoliate one tree and not consume any of the other tree. Sequestration is also notable, in that other caterpillars that sequester catalpol are rejected by predators.

  Catalpa sphinx larvae have an interesting relationship with the parasitic wasp Cotesia congregata. Cotesia congregata attacks the larvae by injecting a virus into its body cavity, then laying several dozen eggs just under the surface of the epidermal tissue of the larva. Wasp larvae then feed on the caterpillars body fluids (hemolymph) before emerging to pupate inside cocoons on its epidermis (seen in the photo above). Like the larva is not present on all trees, C. congregata are not present in all catalpa sphinx populations. Top-down pressure from C. congregata should select for a stronger immune response in populations with the parasitoid present. The stronger immune response has been proven to be negatively correlated with toxin sequestration; the more catalpol larvae sequester the weaker their immune response (Lampert 2012). It is interesting that the larvae would sequester high amounts of catalpol (>20% dry mass), which reduces their immune system and increase susceptibility to the enemy that provides the greatest top-down pressure.

  Other research has shown that leaf catalpol and larval catalpol sequestration are related (Lampert et al. 2010, 2011), thus we can assume that if catalpol sequestration deters catalpa sphinx predators we would assume that the amount of toxin in the leaves would affect predation. If predation is reduced, we hypothesize that high chemistry levels in the plant are actually bad for the plant because the larvae are both stimulated to consume more leaves and more protected from predators.

  We plan to conduct three research projects, exploring separate aspects of catalpa sphinx chemical ecology. I have already researched this species summer and early fall 2012.

  Project A: This project explores the relationship between metabolic processes and catalpol; specifically we use CO2 production of the larva paired with chemical analyses of larval hemolymph. This project would be performed with two experimental sites to explore the chemical and metabolic differences between larvae with top-down pressure and larva without top-down pressure from C. congregata. I have already identified two locations from
which to collect larvae and leaves. One is the University of Georgia Horticulture Farm in Watkinsville, Georgia and the other is the home of Dr. Timothy Howell (Coordinator of Chemistry, Gainesville campus) in Murrayville. I have already explored the effects of catalpol sequestration on metabolic processes, following an assumption that the more they consume the more toxin will be present. This experiment did not show an affect of toxin sequestration on respiration; however, the data are incomplete without chemical analysis.

**Methods:**
- The CO₂ production of 150 total caterpillars from each population would be measured at 5 instars (growth stages). We have 3 CO₂ sensors, and Vernier LoggerPro software is already installed on lab computers on the Gainesville campus science building.
- We will harvest 30 larvae from each instar for chemical analysis after measuring CO₂.
- Chemical analysis would be performed through gas chromatography at the Dahlonega campus. We plan to determine if amount of catalpol sequestered is correlated with metabolic rate.
- A negative correlation would provide a possible causal mechanism for the negative relationship between sequestration and immune response. The strength of the relationship will be compared in populations with and without *C. congregata*

**Project B:** Next, we are interesting in testing predation pressure, and how well catalpol sequestration defends larvae. Previously I performed ant (*Solenopsis invicta*, “fire ant”) assays with hemolymph, where the catalpol is stored, gut contents, catalpol extract from leaves, and ten percent sucrose solution as a control. The ants showed an obvious deterrence to any extract containing catalpol even if they initially fed on one of the experimental solutions.

**Methods**
- Live larvae and pooled extracts (ant only) would be used ant, spider, and predatory stinkbug assays to determine the effect of the level of catalpol sequestered on predation. Deterrence is shown when the proportion of ant visits to larval samples is reduced significantly, or when predators reject intact larvae.
- Chemical analyses of all larvae and samples will be necessary to determine the relationship between predation and the amount of catalpol sequestered. We plan to use logistic regression to determine what level of sequestration is “too much” for each predator.
- In order to measure predation (mainly social wasps and birds) in the larva’s natural habitat, we would tie one end of a 1m piece of monofilament fishing line to a branch of a tree acceptable for larvae to feed on and glue the other to the dorsal thorax of the larvae. This would be done with thirty 5th instar larvae from each site and I will return to the site after 24, 48, and 72 hours to determine if predators have removed larvae. We will extract 5μL of hemolymph from larvae before tethering to determine catalpol content of all larvae in the experiment; previous research has shown that larvae can survive several days after a small hemolymph volume has been removed. We will compare hemolymph between larvae that were removed by predators to recovered larvae, in order to test the hypothesis that larvae that sequester more are rejected.

**Project C:** Dr. Karen Kester, Associate Professor of Biology, Virginia Commonwealth University, is researching the chemistry of trees to determine what mechanisms explain the paradoxical lack of larvae on some trees and population outbreaks on others. We are collaborating on both leaf collections and chemical analyses.

- **Significance of the proposed work (by Lampert):**
  - Catalpa sphinx are an exceptional local model system in chemical ecology and community ecology; in particular, the role of chemical defense in food webs and selection from predators and parasite. I have previously published several articles in this field in highly-regarded international publications.
  - The data from all three projects would contribute to future research and proposals in broader projects concerning chemical ecology and predator-prey relationships, and encourage external collaboration.
  - Locally the “tawba worm” is notable for both its ability to completely defoliate catalpa trees (a popular landscaping tree) and exceptional use as panfish bait. This research will contribute to understanding why some trees are defoliated, and future research plans to examine catalpol sequestration and fishing.
  - This research is multidisciplinary, and will initiate and increases collaboration among departments. Mike McGinnis and Dan Thompson (Chemistry Department) have been contacted for GC use.
  - Other students will be recruited to assist Audrey for Biology 2901 or 4800 course credit. This will increase high-impact education practices on our campus.
- **Goals and expected products (by Lampert and Audrey):**


  - *Project B:* Publication in *Journal of Insect Science* (current impact factor: 0.95) or *Insects* (2012). Both are rigorously peer-reviewed, open-access online international publications that attract and publish only high-quality submissions from entomologists.

  - One major goal is to promote future research and proposals in broader projects concerning chemical ecology of predator-prey relationships. Audrey has already presented 2012 research at the Entomological Society of America 2012 conference (an international conference that is the largest annual conference in this field) and plans to present her complete data at conferences in 2013 and 2014. Both regional and international conferences are a great ways for student-faculty teams to network and learn more about our fields of study.

  - The proposed research may also potentially contribute to an in-progress manuscript reviewing how catalpol and similar compounds affect food-web interactions. This article is coauthored by a group led by Deane Bowers, Professor and Curator of Entomology at the University of Colorado Museum of Natural History.

  - Lampert would also use the data obtained in a broader project concerning non-technical description of catalpa sphinx larvae, including local lore about fishing.

  - All projects will provide data essential for future proposals examining this system and similar systems.

- **Plan for faculty-student collaboration and mentorship:**

  Audrey: Dr. Lampert and I will schedule weekly meetings to discuss progress on each of the projects and to discuss data collection and manuscript writing. Dr. Lampert will be helping me to learn how to write in a suitable fashion for the scientific community. The knowledge obtained from performing these experiments will help me to become familiar with biological topics that range outside of entomology, such as ecology, evolution, and biochemistry. The knowledge from the experiments and the guidance for writing will be extraordinarily helpful in being accepted to a graduate school and then publishing research later on that pertains to my preferred field of study.

  Lampert: Audrey has been involved in researching this system since May 2012. In the last 9 months, Audrey has generated data that have been presented at an international conference and the GSC STEM poster symposium. Audrey has excelled in data collection and analysis, and shown superlative work ethic and dedication to research. We are recruiting other students to participate in catalpa sphinx and entomology research (for work study or course credits), and given Audrey’s experience she will be allowed to mentor the day to day activities of the other student researchers. I will participate in collections in June, and mentor Audrey in chemical analysis in Dahlonega in July and beyond. In addition to discussions, Audrey will produce a draft of her methods after 4 weeks, and a draft of one manuscript including a bibliography at the end of the support period. We are also excited for the networking and presentation opportunities with FUSE peers, which will greatly enhance Audrey’s research experiences.

  Following FUSE support, Audrey and I will produce several publications. Audrey will be expected to produce drafts of each, which I will revise afterward. She will also submit and act as corresponding author on the manuscripts for project 2, which will provide invaluable experience for her planned scientific career.

  **III. Budget and timeline**

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• **Rationale:**
  - Methyl alcohol is used to extract catalpol from carcasses. Each sample requires 6mL total.
  - Ethyl ether is used to separate catalpol from hydrophobic compounds (fats, chlorophyll). Each sample requires 6 mL total.
  - Larvae can be reared in plastic Ziploc containers with snap-on lids. Holes must be drilled into lids so larvae don’t asphyxiate.
  - Although the majority of driving to field sites would be in June-August, we propose a “fuel stipend” with estimated total mileage up front to cover the cost. Dr. Howell’s house is 30 mile round-trip from the Gainesville campus, while UGA horticulture farm is approximate 100 miles round-trip.

Although the total budget exceeds $500, we intend to seek other sources of support by early summer, including larger proposals to cover equipment/consumable costs.

• **Timeline (by Lampert):**
  - Spring 2013 Semester: Obtain support and collaboration for GC use. Submit other proposals for supplies.
  - May: Locate other populations, and collect if possible.
  - June: Survey known populations, collect larvae and conduct field experiments and lab assays depending on larva availability.
  - July: Survey known populations, collect larvae and conduct field experiments and lab assays depending on larva availability, prepare samples for chemical analysis. Complete chemical analysis. A set of 30 samples (1 GC run) requires ~8-10 “man hours” to complete.
  - August and beyond: Continue chemical analysis. Present results to FUSE peers. Rigorous publication-quality should be totally completed by end of December 2013, while manuscript preparation will ideally be completed Spring 2014 semester. Our goal is to submit before Audrey’s anticipated graduation, May 2014.

**IV. Certifications**

• **Faculty certification.** “I hereby certify that Evan Lampert will teach no more than 8 course hours during each summer session spanned by the FUSE program. Evan Lampert is committed to mentoring Audrey Barrett on a continual basis during the period of the FUSE program.”

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Evan Lampert, Faculty Mentor

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Nancy Dalman, Department Head

• **Student certification.** “I hereby certify that I, Audrey Barrett, will commit at least 40 hours per week to the scholarly project described in this application. I also certify that I am not enrolled in more than 4 course hours during each summer session spanned by FUSE. I am aware that failure to comply with these two requirements may result in the forfeiture of my summer stipend.”

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Audrey Barrett