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Evaluation of Volatile Metabolites in a Plant Symbiosis: Does the "smell" of Plant Stress Affect Symbiotic Fungal Growth

William N. Setzer
University of Alabama in Huntsville

Evaluation of Volatile Metabolites in a Plant-Fungus Symbiosis: Does the "smell" of plant stress affect symbiotic fungal growth?

A Proposal for the Research or Creative Experience for Undergraduates (RCEU) Program
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Faculty Research Mentor: William N. Setzer, Department of Chemistry, MSB 315. Phone: 6519
E-mail: wsetzer@chemistry.uah.edu.

Project Summary: Plant-fungal symbioses are essential to how plants colonize land, how plant communities develop via ecological succession (*e.g.*, abandoned farm field → grassland → forest), and ~90% of all plants have fungal sybionts.[1] Our preliminary work with a liverwort (a type of primitive non-vascular land plant, *Conocephalum salebrosum* is the species), indicated geographic variation in the volatile metabolites produced with in the liverwort, and the volatile metabolite methyl (*E*)-cinnamate increased in concentration after stress.[2] Another study found that *Conocephalum conicum*, also elicited this stress response following similar treatment by another lab group. Liverworts in the *Conocephalum* genus are known to harbor symbiotic fungus (arbuscular mycorrhizae, Glomeromycota), but it is unclear how volatile metabolites like methyl (*E*)-cinnamate affect these fungal symbionts. We hypothesize that: 1) volatile metabolites produced during stress could inhibit or stimulate the growth of symbiotic fungi and 2) methyl (*E*)-cinnamate could also be synthesized by the fungi, and 3) volatile metabolites produced during stress could alter the fungal density in cultures of the fungi and the liverwort (*i.e.*, liverwort-fungus whole organism). The overall goal of this research is characterize biological activity of methyl (*E*)-cinnamate and extracts from stressed and wild *C. salebrosum* against strains of fungus symbiotic with the liverwort, and other strains not found in liverworts. There have apparently been few previous studies on this plant in regard to biological activity screening or natural products characterization. The research will involve collecting and extracting *C. salebrosum* using hydrodistillation, screening the crude volatile extracts for biological activity (antibacterial, antifungal, and arbuscular mycorrhizae), carrying out gas chromatographic – mass spectral (GC-MS) analyses, and identifying bioactive compounds relevant to the plant-fungus interaction. ***We are very experienced in bioactivity screening,***[3] ***and identification of volatile components by GC-MS***.[4]

The student will be supervised by Dr. Setzer and Ph.D. student Jonathan Craft every day during the conduct of this research. Dr. Setzer's office (MSB 315) is directly across the hall from the laboratory (MSB 312) and next door to the departmental computer facility (MSB 317), so he will be available at all times during the day and evenings for consultation. The student's work will not only fit directly into our overall research in natural products, essential oils, and chemical ecology, but also is a stand-alone project with the scope suitable for undergraduate summer research; the plant, extraction, activity screening, and GC-MS analysis will belong to the student. Dr. Setzer has a good track record in working with undergraduate researchers (more than 170 individuals at UAH) and publishing their results (56 peer-reviewed publications based on undergraduate research have appeared since 2010).

Student Prerequisites: There are no coursework or academic standing prerequisites. We will instruct and oversee all the student needs to carry out the project. This project does involve field work as well as handing organic solvents and chemicals; some understanding of chemical handling and safety would be beneficial.

Student Duties:

Collection and Extraction of Conocephalum salebrosum. *C. salebrosum* is a ubiquitous liverwort in our area; collection will not be problematic. The student will carry out extraction of dried bark using hydrodistillation / extraction (Likens-Nickerson apparatus with continuous extraction using dichloromethane).[4] The collection and extraction of plant materials will be carried out under the direct supervision of Dr. Setzer and Ph.D. student Jonathan Craft.

Bioactivity Screening. Bioactivity screening (antibacterial, antifungal, and arbuscular mycorrhizae) on the volatile extracts and purified compounds[3] will be carried out in our laboratories in the Shelby Center (SST 324) under the supervision of Jonathan Craft.

Gas Chromatographic – Mass Spectral Analysis. The RCEU participant will be instructed on the proper use and care of the GC-MS instrument. Volatile extracts will be analyzed using the protocols and structural databases in the Natural Products Group.[2,4] The student will work very closely with Dr. Setzer collecting GC-MS data as well as interpretation to determine structures of components. This RCEU project provides hands-on experience with GC- MS instrumentation.

Manuscript Preparation. Dr. Setzer encourages all undergraduate student researchers to write up their results in the form of a manuscript for publication. The RCEU participant, under the supervision of Dr. Setzer, will help prepare the manuscript(s), which may include data from other undergraduate or graduate students.

Expected Results and Deliverables. The *Conocephalum salebrosum* will be wild collected at the beginning of the summer, flash frozen, and stored below −20 °C. The first week will be spent culturing arbuscular fungal isolates from different liverwort populations and species. Large batch cultures of fungi will be grown for GC-MS analysis of volatile metabolites. Replicates of *C. salebrosum* exposed to methy (*E*)-cinnamate, arbuscular fungi, fungal extracts, and control specimens with be extract using hydrodistillation. GC-MS data for hydrodistillation extracts will be collected after the hydrodistillation extractions have been completed.

Mentor Supervision and Interaction: Collection and extraction of plant materials, bioactivity screening, GC-MS measurements and interpretation, and manuscript preparation will be supervised by Dr. Setzer and Jonathan Craft. We will hold regular group meetings each week. Note: In addition to the bioactivity screening we do here at UAH, we also collaborate with several other groups for additional screening (antileishmanial, antitrypanosomal). This project is particularly suitable for an undergraduate summer researcher and will provide hands-on experience with several laboratory techniques not typically encountered as well as experience with modern analytical instrumentation.

References

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2. Craft, JC, Harrelson D, Setzer WN. Chemotypic variation of *Conocephalum salebrosum* in the Southeastern Appalachian Range: A Search for Cryptic Plant Biodiversity Around the Tennessee River Valley. *Natural Products Communications,* **2016**, *11*, 1009-1014.
3. Setzer MC, Newby JS, Moriarity DM, Setzer WN. A phytopharmaceutical survey of Abaco Island, Bahamas. *American Journal of Essential Oils and Natural Products*, **2015**, *2(5)*, 10-17.
4. da Silva JKR, Maia JGS, Dosoky NS, Setzer WN. Antioxidant, antimicrobial, and cytotoxic properties of *Aniba parviflora* essential oils from the Amazon. *Natural Product Communications*, **2016**, *11*, 1025-1028.