**The Dilemma of a Plant, a Pollinator, and a Parasite: The Evolution of Cooperation**

*Key Words: mutualism, pollination syndrome, prisoner's dilemma, Silene, Hadena, anther smut*

**Introduction**: Mutualisic interactions and their evolutionary stability have been a conundrum in science theory since Darwin[1,2,3]. Darwin (1859)[4] hypothesized that "Natural selection cannot possibly produce any modification in any one species exclusively for the good of another species." Nevertheless, ecological examples of mutualisms are prevalent[1,2,3,5]. Previous models seeking to test the evolutionary stability of mutualisms presented an iterated adaptation of the prisoner's dilemma game[1,2,6]. This game defined cooperation between distantly related individuals (or separate species) as being stable if individuals could make assessed decisions, interacted with a high probability, and could not define the number of interactions that would occur[1,2]. Examples given by Hamilton and researchers that followed illustrate plant/pollinator relationships as model systems to study mutualisms[1,2,3,5]. I plan to incorporate a modified version of this game, in conjunction with empirical data on floral scent, to examine the relationship between a plant (*Silene latifolia*), a pollinator (*Hadena bicruris*) and a parasite (*Microbotryum violaceum*- anther-smut fungus).

The role of volatile emission (scent) and the effect of a third party parasite (anther-smut fungus) on the mutualism between *S. latifolia* and *H. bicruris* can be utilized to test an advanced application of Hamilton's game. Floral scent is an integral component of the relationship between plants and their insect pollinators. This association has been demonstrated as essential for effective pollen transfer for some species, and it may provide reproductive isolation between closely related species[7,8,9,10,11]. In addition, it has been shown that minute differences in floral scent can have a dramatic effect on plant reproductive fitness[12,13]. I will incorporate an adapted version of the prisoner's dilemma game with the results of an empirical study to test the following hypothesis: The nursery pollination mutualism between *Hadena bicruris* and *Silene latifolia* is only stable if transmission of the anther-smut fungus *Microbotryum violaceum* aborts fruits containing developing larva (in a random or frequency-dependant fashion) and *H. bicruris* moths can alter behavior based on perceived smut infection in *S. latifolia* females.

**Objectives:** This research will employ an interdisciplinary approach to science. Furthermore, it will incorporate both empirical methods and theory. First, I will analyze the scent of infected and healthy *S. latifolia* flowers. Second, this data will be used in conjunction with an adapted model to test stability in *S. latifolia*'s mutualism with *H. bicruris*.

**Study Species:** The dioecious plant species *S. latifolia* and the nocturnal moth *H. bicruris* interact in a nursery-pollinator mutualism. As a dioecious plant, *S. latifolia* has male (staminate) and female (pistilate) flowers on different plants. *H. bicruris* will pollinate *S. latifolia* and oviposit on the ovaries of female flowers. *H. bicruris* uses scent in guiding ovipositing[7,14]. After hatching, *H. bicruris* larvae consume the developing seeds [15,16]. Nevertheless, *H. bicruris* is a primary pollinator of *S. latifolia* [15,17]. However, the parasitic fungus *M. violaceum*, anther-smut, complicates the evolutionary consequences of this relationship[15,16].

*M. violaceum,* anther-smut, infections cause *S. latifolia* flowers to become sterile and either kill or arrest the growth of developing *H. bicruris* larva[16,18,19]. Furthermore, the success of the anther smut is due in large part to the existing mutualism between *S. latifolia* and *H. bicruris*. *H. bicruris* is the primary vector for *M. violaceum* infection in *S. latifolia*. Infected *S. latifolia* produce the same amount of nectar as healthy individuals and thus attract *H. bicruris*. However, *H. bicruris* oviposits six times less when visiting *M. violaceum* infected flowers[14,19,20].

**Experiment 1:** **Do qualitative and/or quantitative differences exist between smut-infected and uninfected *S. latifolia* flowers?**

This experiment will provide the empirical data necessary to evaluate models on *S. latifolia*'s relationship with *H. bicruris* and anther-smut. This method will elucidate any chemical differences in the volatiles released after flowers become infected with *M. violaceum*.

Plants will be grown in a common-greenhouse; individuals will be randomly selected and infected after producing their first set of true leaves. Transferring the plants to a water-filled Petri dish, scaring the leaf, and exposing the damaged tissue to fungal spores will transmit infection. To assay scent, I will utilize methods learned through my previously outlined research in the Delph Lab at Indiana University. Specifically, I will be looking for alterations in volatile emission shown by Dotterl et al. 2006[14] to be the primary scent cues for *H. bicruris* ovipositing, especially lilac aldehydes.

**Experiment 2:** **Can the prisoner's dilemma game be applied to understand the mutualism between *S. latifolia* and *H. bicruris*?**

The prisoner's dilemma game is not directly applicable to this relationship. In order to adapt it to this scenario, it will be necessary to account for the frequency of anther-smut infection based on ovipositing vs. pollination. Additionally, the model must be robust to the increased pressure for mutualism breakdown imposed by the anther-smut fungus on the already tenuous relationship between *S. latifolia* and *H. bicruris*. I plan to utilize analytical modeling and computer simulation to test different adaptations for ecological significance.

**Significance and Intellectual Merit:** Understanding theoretical conundrum is at the heart of current evolution research. This research will directly examine the stability of complex mutualisms. This project will embrace both theoretical and empirical approaches to research, including advanced chemical techniques.

**Broader Impacts:** The methods and results of this research can be applied to medical and agricultural research. Understanding the evolution of mutualisms and the role of parasites in ecological stability can have a profound effect on current problems in medicine. These likely include: drug resistant bacteria, emerging infectious diseases, and HIV. In addition, it may aid in understanding how biological controls can be employed in lieu of chemical methods and in the face of currently ineffective treatments. In addition, I will foster the development of future generations of researches by actively engaging undergraduates in this scientific process. Due to its importance, teaching and mentoring will be an integral part of my graduate research.

*This work is a product of my original thoughts and ideas*

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