To: Research and Grants Committee
From: Deborah Marr
Date: June 8, 2003
Re: Final report for Faculty Research Grant

Dear Research and Grants Committee,

This is the final report for my 2002-03 Faculty Research Grant "The role of insects and fungal pathogens in the genetics and ecology of seed production in *Hydrophyllum appendiculatum*."

Description of grant-supported activity

In my Faculty grant proposal I requested funds for a pilot project exploring the effect of a fungal pathogen and insect herbivore on the quantity and quality of seeds produced by the understory forest herb *Hydrophyllum appendiculatum*. This plant is unusual in that individual plants can produce seeds that vary 10-fold or more in size, despite the advantages that larger seeds have in germination and seedling vigor. Damage from pathogens and herbivores can potentially affect the quality and quantity of both male and female contributions to seed production. I proposed to address three questions

1. Does the natural variation in seed size differ among populations that vary in levels of pathogen and herbivore attack?
2. Is the paternal contribution to seed fitness affect by fungal pathogens or insect herbivory?
3. Is the maternal contribution to seed fitness affected by fungal pathogens or insect herbivory?

In summer of 2002 over 95% of the *H. appendiculatum* plants at the study sites (Bendix Woods and St. Patrick's County Park) did not flower. In addition, populations in Urbana-Champaign, IL and Indianapolis, IN did not flower well either. The unusually cold weather and hard frosts that occurred in early May apparently killed the buds of *H. appendiculatum* in many areas throughout northern IN and IL. The lack of flowering made it impossible to do the proposed experiments. However, two other species of *Hydrophyllum* occur in this region and no studies have been done on seed size variation in these species. Therefore, I changed the focus of my work to determining whether other *Hydrophyllum* species also show high variability in seed size and whether fungal pathogens that cause wilt in *H. appendiculatum* are also present in other *Hydrophyllum* species. Specifically the questions that I addressed were:

1. Do other species of *Hydrophyllum (H. virginianum and H. canadense)* also produce seeds of variable size?
2. What is the frequency of wilting disease in *H. virginianum, H. appendiculatum, H. canadense*?
3. Which fungal species cause wilt in species of *Hydrophyllum*?
4. Do healthy and diseased individuals in *H. canadense* differ in their reproductive success?
5. Do seed germination rates differ between small and large seeds in *H. virginianum, H. appendiculatum, H. canadense*?

Results of Project

Below I briefly describe the main results of this work and the role of undergraduates who contributed to these studies.

1. Do other species of *Hydrophyllum (H. virginianum and H. canadense)* also produce seeds of variable size?

Hydrophyllum appendiculatum and *H. canadense* have significantly more variation in seed mass within individuals compared to *H. virginianum* Seeds produced by *H. appendiculatum* individuals varied the most in mass; 35% of the individuals produced seeds that varied 9-20 fold in seed mass.

Approximately 40% of the *H. canadense* individuals produced seeds that varied 8-16 fold in seed mass, and only 7% of *H. virginianum* individuals produced seeds that varied 7-8 fold in seed mass.

John Martz worked as a research assistant on this project. John had planned on presenting his results at the IU Undergraduate Research conference in the fall; however, the conference conflicted with the date of the GRE subject test. I presented this work at the Indiana Academy of Sciences conference in October 2002 and John was a co-author on this paper.

2 & 3. What is the frequency of wilting disease in *H. virginianum, H. appendiculatum, H. canadense,* and which fungal species cause the wilting disease?

Frequency of wilting disease was greater than 20% in populations of *H. appendiculatum* and *H. canadense* in St. Patrick's park and Bendix Woods. No wilting was observed in populations of *H. virginianum* in these two locations. The absence of wilt and relatively small variation in seed size observed in *H. virginianum* is intriguing because this species is more distantly related to the other two species.

One of the challenges of this work has been identifying the fungal species that cause the wilting disease. Studies by other researchers noted that *H. appendiculatum* was susceptible to a wilting disease, but their efforts at isolating the pathogen were unsuccessful. My initial isolation of fungi showed that there were 5+ fungal species present in or on stem and root tissue. To help narrow down the species of fungi that were non-pathogenic (had no role in disease) from those that were pathogenic, I sent plant tissue samples to Gloria Abad, a soil pathogen expert at North Carolina State University. Isolation of fungi associated with the stems and roots of *H. canadense* and *H. appendiculatum* showed that *Fusarium* (possibly *Fusarium oxysporum*) and an unknown species of *Rhizoctonia* are most likely involved in causing the wilting disease.

In Fall 2002 and Spring 2003, Aubrey Truex, an IUSB undergraduate, did an L490 project that involved sampling plant tissue from more *H. canadense* individuals, isolating and culturing fungi from this plant tissue, and then identifying fungi that were most common in stem and root tissue . We found that *Fusarium* sp. were common in both root and stem tissue, but *Rhizoctonia* appeared to be more common in root tissue samples. This summer I will be expanding this work to include *H. appendiculatum* and isolating fungi from more populations of both *H. appendiculatum* and *H. canadense*.

4. Do healthy and diseased individuals in *H. canadense* differ in their reproductive success?

Hydrophyllum canadense did flower in 2002 so I compared reproductive success in healthy and diseased individuals. Diseased individuals produced significantly fewer fruits. However, seed mass and number of pollen grains germinating on styles did not differ between flowers from healthy and diseased plants. Tim Greenlee worked as a research assistant on this project. Due to changes in Tim's personal circumstances he was unable to complete the final stages of this project. Tim was also a co-author on the paper presented at the Indiana Academy of Sciences.

5. Comparison of seed germination in small and large seeds in *H. virginianum*, *H. appendiculatum*, and *H. canadense*.

In spring 2003 I set up a seed germination experiment to compare germination rates between small and large seeds for all three *Hydrophyllum* species. This experiment is not complete yet. *Hydrophyllum* seeds require a regimen of both warm and cold temperatures before they will germinate. Seeds should begin germinating in late summer and into this fall. Stuart Orr worked as a research assistant on this project this spring.

Dissemination of Results from Project

I presented results from part of this work in October 2002 at the Indiana Academy of Sciences (title listed below). I will present more of this work at the Ecological Society of American meetings (a national meeting) in August 2003. The abstract of the Ecology meeting paper is attached.

Four undergraduates were involved in this research this past year. The Faculty Research Grant allowed me to hire three undergraduate research assistants, and one student did an L490 project. I plan to submit a grant to the National Science Foundation in Spring 2004. The data collected from this pilot project combined with results from this summer's research will serve as the foundation for this NSF proposal. In addition, this pilot project will contribute to at least two manuscripts; one manuscript describing the fungi that cause wilt and their effects on reproduction in *H. canadense* and a second manuscript comparing seed size variation and effects of wilt on the three species of Hydrophyllum.

Paper presented at Indiana Academy of Sciences meeting, October 2002.

Marr, Deborah L., Timothy Greenlee, and John Martz. October 2002. Ecological causes of phenotypic variation in seed production: a comparison of seed size variation in 3 species of *Hydrophyllum*. Indiana Academy of Sciences, Indianapolis, IN.

Abstract of paper that will be presented at the Ecological Society of America meetings in Savannah, GA, August 2003.

Marr, Deborah L August 2003. The role of fungal pathogens on seed mass variation and seed production in 3 species of *Hydrophyllum*.

ABSTRACT - Many plant species exhibit little variation in seed size, however there are some species in which seed size varies 10 fold or more. The persistence of this variation is intriguing because both empirical and theoretical studies predict that seed size should be less variable compared to other reproductive traits because larger seeds generally have higher fitness within species. I studied seed mass variation in three species of *Hydrophyllum*, *H. appendiculatum*, *H. canadense*, and *H. virginianum* to better understand the range of natural variation found in these closely related species. These species coexist as understory herbs in deciduous forests throughout the midwestern United States. The range in seed mass produced by individual maternal plants varied widely among the three species. The greatest amount of seed mass variation was observed in *H. appendiculatum* and *H. canadense*; over 30% of maternal plants in both species produced seeds that varied at least 8 fold in size. However, fewer than 7% of the *H. virginianum* maternal plants produced seeds that varied 7 fold in size. Both *H. appendiculatum* and *H. canadense*, were frequently infected by fungal pathogens (*Fusarium* sp. and *Rhizoctonia* sp.) that caused stems and inflorescences to wilt. In contrast, wilting was not observed in *H. virginianum* in the study sites. To test whether seed production and seed mass were affected by the disease status of the individual in *H. canadense*, seeds were collected from healthy and diseased individuals that were hand-pollinated in the field with supplemental pollen. Diseased individuals produced significantly fewer fruits, but no difference in seed mass variation between healthy and diseased maternal plants was found. Further studies of all three species in which maternal environment is controlled will help determine whether pathogens that cause wilt contribute significantly to the unusual seed size variation in *Hydrophyllum*.