Title: **Employing foliar endophytes as biocontrol agents**
Project Leader: **Dr. Taryn Bauerle**
Cooperator: **Dr. Cyd Hamilton**

Abstract:

Foliar fungal endophytes are microscopic fungi present in all plant species. These organisms can reduce pest feeding on plant hosts through the production of unpalatable chemicals. Endophyte-host effects have lead to the employment of fungal endophytes as biocontrols in grasses by successfully reducing pest loads while increasing host growth. However, utilization of endophytes in woody plants is in the nascent stages. Research is needed to identify which endophytes are present and how they reduce pest damage on important ornamental and horticultural plants. The proposed research is foundational for the effective use of fungal endophytes in integrated pest management.

Background and Justification:

Much of the research exploring the ecological consequences of foliar endophyte-plant relations have been limited to *Epichloë/ Neotyphodium* endophytes infecting agronomic and horticultural grasses. In these hosts *Epichloë* and *Neotyphodium* species increase host resistance to numerous plant pathogens and herbivores (Latch 1993, White et al. 1993, Gange 1996, Bultman et al. 2006, Sullivan et al. 2007, Kuldau and Bacon 2008, Tian et al. 2008), via production of alkaloids or mycotoxins (Ball et al. 1995, Frey et al. 1997, Arnold et al. 2003, Dingle and McGee 2003, Seto et al. 2007, Mejía et al. 2008). As such, particular endophytic compounds have been isolated for development of new pesticides (Ondeyka et al. 1997, Strobel 2002, 2006) and unique endophyte-host combinations have been and continue to be developed to increase forage and horticultural plant production (Siegel et al. 1987, Bouton et al. 2002, West and Piper 2008).

In woody plant species endophytes have been comparatively less well researched but initial results support their importance and potential application similar to those of *Epichloë/ Neotyphodium* endophytes (Kumar et al. 2008). For example, endophytes infecting woody species reduce survival of a suite invertebrate herbivores and retard fungal and bacterial pathogens (Sneh 1998, Arnold et al. 2003, Dingle and McGee 2003, Mejía et al. 2008). In total this research has lead to the suggested use of endophytes as an integrated pest management (IPM) approach. This is due to the expectation that fungal symbionts can reduce pesticide, fungicide, and fertilizer usage in woody crops similar to their use in grasses (Brimner and Boland 2003, Wicklow et al. 2005, Kumar et al. 2008). By integrating endophytes into IPM strategies the use of chemicals toxic to humans and the larger environment can be reduced. This type of research is timely because though little is known about the effects of endophytes on woody plants there is data to support the effective use of these symbionts in the development of IPM protocols. Our project proposes to capitalize on the recognized improvement of growth and herbivory tolerance in endophyte infected plants and target our results to the improvement of woody ornamental crops.

Objectives:

1. Remove systemic fungal endophytes from a model, woody, host plant to create endophyte infected (E+) and endophyte-free (E-) lines for experimental treatments.

2. Determine if endophytic infection alters host response to a generalist invertebrate herbivore and fungal pathogen.
3. Quantify changes in host biomass production as a general response by comparing E+ and E- controls.
4. Project Evaluation – presentation at the Green Industry Conference will provide opportunities to discuss application with growers and to get feedback from growers about how IPM application might impact them.

Procedures:

Initial research will target *Populus* sp. a fast growing, model species that is easily propagated (Lemus and Lal 2005**,** Das et al. 2009) and for which a rich data set exists, e.g. genetic maps, protein and metabolic expression profile. While we recognize *Populus* is only marginal in terms of importance in the ornamental industry, the enormous library of information on *Populus* will allow more rapid achievement of our proposed objectives. This will reduce the resources, including time, required to establish a primary outcome and will facilitate the adoption of our methods to other important ornamental woody species.

Populus clones will be hydroponically grown to remove fungal endophytes. Plants produced from the cloned, hydroponically grown tissues will be used to create two endophyte groups E+ and E-. Uninfected plants will be produced hydroponically as per Faeth and Sullivan (2003). All plants will be then grown in a soilless mix with a single application of a full-spectrum nutrient and watered as needed.

1. Identify fungal endophyte infection status and remove systemic fungal endophytes from an important woody ornamental.

To identify ubiquitous endophytes present in plant tissues, fungi will be identified directly from host tissues using common polymerase chain reaction method with a specific protocol developed by one of the authors (Hamilton and Faeth 2009).

2. Determine if endophytic infection alters host response to generalist invertebrate herbivores and fungal pathogens.

Generalist invertebrate herbivore treatments

A generalist invertebrate herbivore known to feed on *Populus* spp (e.g. gypsy moth caterpillars) will be collected from *Populus* in the field and reared on *Populus* tissue prior to experimentation to ensure their success on *Populus* (Frost et al. 2008). In a whole plant experiment 40 plants (20 per infection status) will be exposed to ten individual insects and net bagged as per Bultman et al. (2006).

Plants will be monitored for growth rate and biomass will be quantified for control versus insect infested plants. At two points during the experiment (before and after herbivory) plant tissue will be analyzed for total carbon (C), nitrogen (N) content. Herbivores will be evaluated prior to and after the feeding experiment for weight change and survival.

Fungal pathogen treatments

A ubiquitous fungal pathogen commonly infecting *Populus* spp. will be collected from *Populus* in the field. Leaf and stem tissue from E+ and E- hosts will be exposed to this fungus in *vitro*. Leaf tissue from 20 distinct plants (10 E+ and 10 E-) will be exposed to the pathogen and replicated five times. Growth of the fungal pathogen will be quantified by the size of the lesion formed.

3. Quantify changes in host biomass production as a general response by comparing E+ and E- controls.

The methods described in Objective #2 require the inclusion of controls, i.e. E+ and E- hosts not exposed to treatments. These hosts will provide the data to address this objective.

Results to date:

1. *Remove systemic fungal endophytes from a model, woody, host plant to create endophyte infected (E+) and endophyte-free (E-) lines for experimental treatments.*
    a. We have successfully identified, mapped, and collected plant materials (branch, green wood cuttings) from 15 mature *Populus deltoides* trees (represents 15 unique genotypes) from Tompkins County area.
    b. We have successfully rooted these cuttings in soil-less mix followed by successful maintenance of the cuttings in hydroponics. Success was defined by the emergence of leave along with continued growth of roots.
    c. Due to bacterial contamination resulting from blocked bubblers in 3 of the 4 hydroponics tubs we lost nearly all plants.
    d. We have purchased *Populus deltoides* trees from a supplier in Tennessee and will repeat the above procedures; anticipating plants will be ready for experimentation (Objective 2 and 3) by late February.
    e. We have corrected the bubbler situation to avoid a system breakdown in the future and made general improvements to the hydroponic set-up.
    f. We have isolated numerous fungal endophytes from leaf tissues and are maintaining pure isolate cultures in slants. Note: leaf tissues were collected in the autumn from the same trees as the green wood cuttings.
    g. We are in the process of optimizing DNA extraction and PCR protocols for identification of leaf endophytes.

2. *Determine if endophytic infection alters host response to a generalist invertebrate herbivore and fungal pathogen.*
    a. We have collected mature grasshopper herbivores from Tomkins County area and maintained these adults through mating.
    b. We have determined grasshopper adults and nymphs eat *Populus deltoides* leaves.
    c. We are currently maintaining eggs and developing nymphs produced from the original group of adult grasshoppers collected in the Fall of 2009
    d. We have successfully identified and collected leaf tissues harboring a fungal pathogen *Marssonina spp.*
    e. We have successfully isolated the fungus on plates and are maintaining colonies in slants until experimental treatments begin in the early spring.

3. *Quantify changes in host biomass production as a general response by comparing E+ and E- controls.*

4. *Project Evaluation – presentation at the Green Industry Conference will provide opportunities to discuss application with growers and to get feedback from growers about how IPM application might impact them.*
    a. Despite the delay we expect to have data available for presentation in the early summer of 2010.

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