


New Biorational Approaches for Pecan Pest Control: A Friendly Fungus Living in the Tree?

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Outline

- Introduction – key pecan insect pests
- Insect-killing fungi as inundative control agents for pecan insects
- Fungi as an endophyte, living in the plant?
- Progress thus far
- Future directions

Pecan Aphids

- <u>3 Species</u>:

black pecan aphid, *Melanocallis caryaefoliae*

blackmargined aphid, *Monellia caryella*

yellow pecan aphid, *Monelliopsis pecanis*

- Conserve natural enemies!

Pecan Weevil, *Curculio caryae*

- Key pest of pecan in SE US, TX, OK, KS, MO
- Life-cycle 2-3 yrs
- Adults emerge July-October

(but mostly mid-Aug to mid-Sept)

- Most crawl or fly to the trunk (Rainey & Eikenbary; Cottrell & Wood)
- Adults longevity (varies) average 20-30 d, up to 75 d

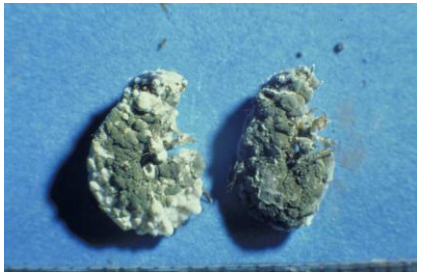
Traps used for monitoring

Pecan Weevil, *Curculio caryae*

- Adults feed & oviposit in nuts; pre-oviposition period ca. 7 d
- Average 3-4 eggs per nut, 35-55 eggs per female.
- 1 weevil damages approx 10-15 nuts
- Larvae drop to soil (late Sept to Dec), & form a soil cell at 3” to 10” depth
- About 90% of the larvae pupate after 1 yr in soil & emerge as adults the next yr
- The other 10% remain as larvae an extra yr (3 yr life-cycle)

One Potential Alternative Control Agent for Pecan Insect Pests: Entomopathogenic Fungi

- Focus on Hypocreales: includes *Beauveria bassiana, Metarhizium* spp., *Isaria fumosorosea*
- Penetrates the insect cuticle and proliferates in the host
- Grown on artificial media, commercially available
- Can control various white grubs, black vine weevil, Lepidoptera, grasshoppers, aphids, white flies, etc



Fungus Vs. Pecan Weevil

Endemic (native) Fungus in the Orchard: 30% to 50% pecan weevil mortality from endemic fungi, e.g., *B. bassiana* (Shapiro-Ilan et al., 2003)

Applied/Introduced Fungi:

- 80% mortality or more over a two week period of during peak weevil emergence
- Best treatments application of *B. bassiana* to trunk or to the ground with a cover crop - Sudan grass (Shapiro-Ilan et al., 2008; Hudson et al., 2010)
- Apply using standard spray equipment (>10^{13} conidia/ha)

Clover Can Enhance Beneficial Endemic Fungi

Persistence of *Beauveria bassiana*

- Endemic + clover

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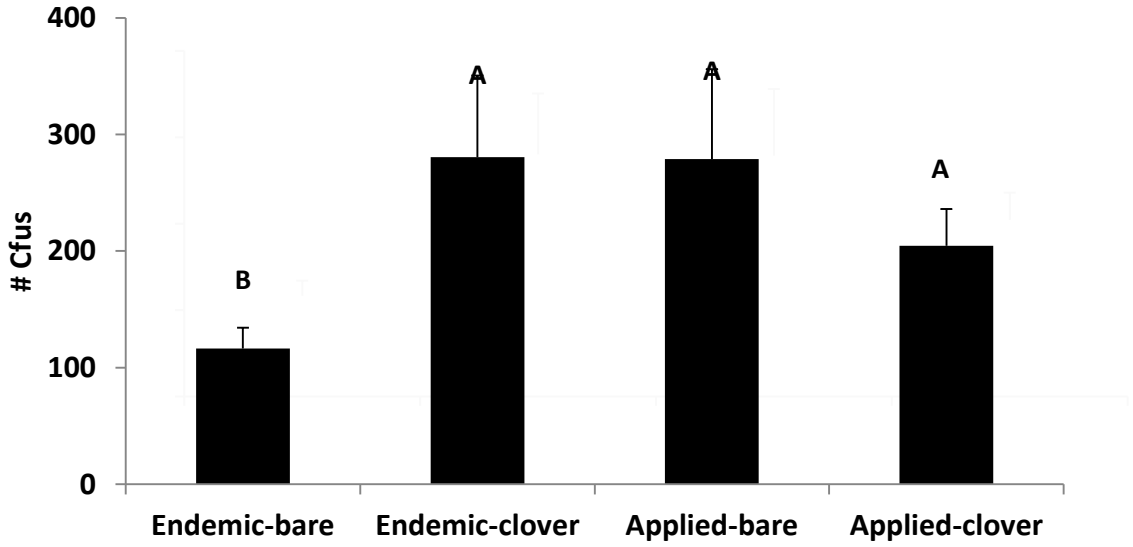
  Endemic w/o clover



Shapiro-Ilan et al., 2012. Environmental Entomology

Entomopathogenic Fungi as Inundative Agents for Control of Pecan Insect Pests

- Pecan weevil is a target (as shown in previous slides)
- Also, *B. bassiana*, *M. brunneum*, and *Isaria fumosorosea* can also kill all three species of pecan aphids (2008 J. Invertebrate Pathology)
- Yet the approach can be expensive
- What if the fungus could live in the tree and provide a “built-in” protection?

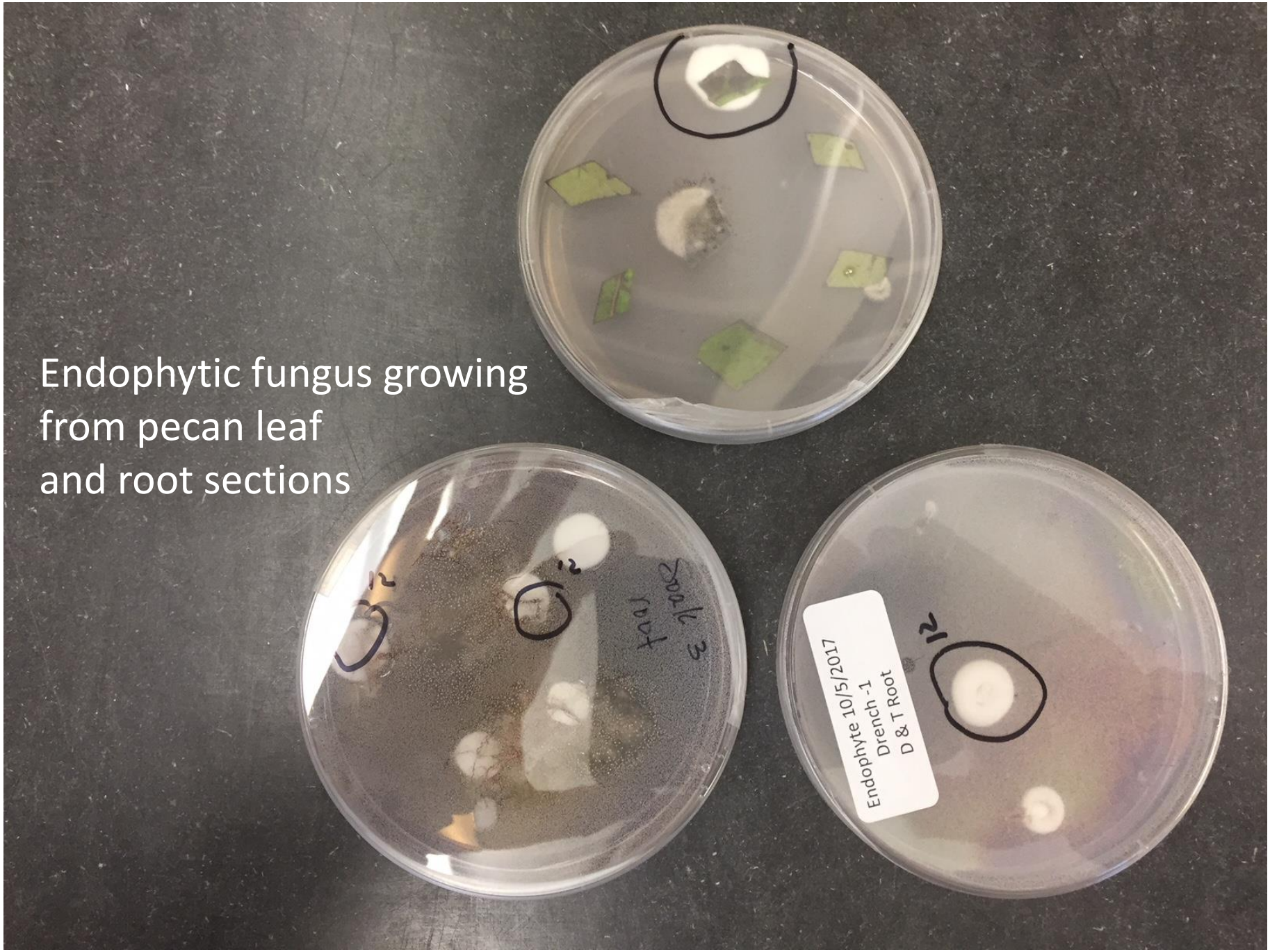
Beauveria bassiana as an Endophyte

- The fungus can also occur as an endophyte living inside the plant!
- First discovered suppressing corn borer (Bing and Lewis, 1991)

- Subsequently found to exist as an endophyte in other crops: bananas, beans, cacao, cotton, coffee, pine, tomato, wheat
- Documented to suppress insect populations (e.g., aphids in cotton, weevils in banana) and reduce diseases prevalence (e.g., *Fusarium, Pythium*)

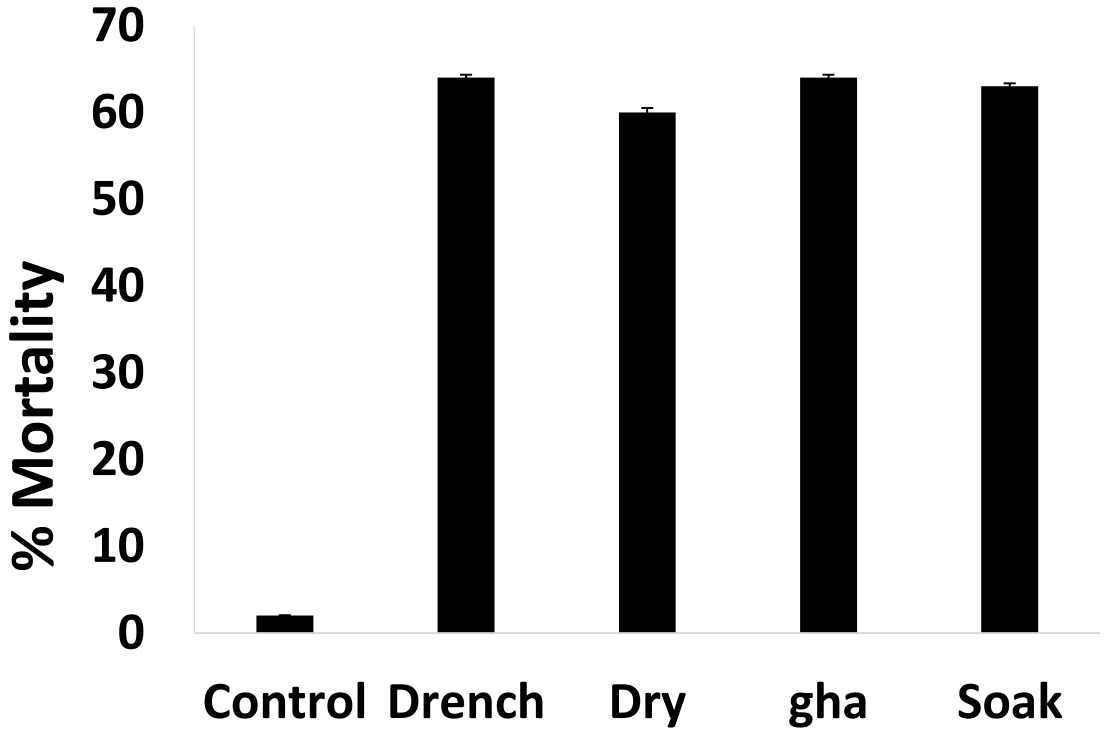
What about Pecan?
Yes: *B. bassiana* was successfully established in pecan!

- Methods: seed soak, seed roll, seedling drench
- Endophytic *B. bassiana* found in stem, leaves & root using all 3 methods
- Initial results confirmed by isolating the fungus and verifying infectivity in insects (*Tenebrio molitor* and *Galleria mellonella*)

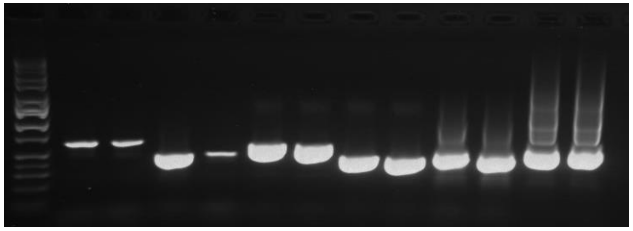

Endophytic fungus growing
from pecan leaf
and root sections
Endophyte 10/5/2017
Drench -1
D & T Root

Confirmation: Insect Mortality

- *Galleria mellonella* mortality



Also Confirmed via Molecular Techniques



- DNA extracted from endophyte plants (leaves, stem & roots) and control seedlings
- DNA also extracted from fungus grown out from plant tissue on nutritive agar plates (PDA)
- Two-stage nested PCR (Landa et al. (2013)
- Positive amplicons sequenced using Sanger sequencing
- PCR results directly from plant tissue showed positive results though sequencing results were variable (possibly due to secondary compound contaminants or low amounts of fungal DNA in the tissue)
- All samples isolated from the various plant parts and grown on PDA were confirmed as *B. bassiana* based on amplicon size

Endophytic *B. bassiana* in Pecan

Overall Goal: Determine the potential of beneficial endophytic fungi to contribute to insect pest and disease control

- Determine the longevity of fungal endophytes
- Is fungal virulence (killing-power) conserved when the fungus is in the tree?
- Determine the contribution toward control of insects and disease: initial focus - aphid control, then expand to other insects and disease (*Phytophthora*, scab)

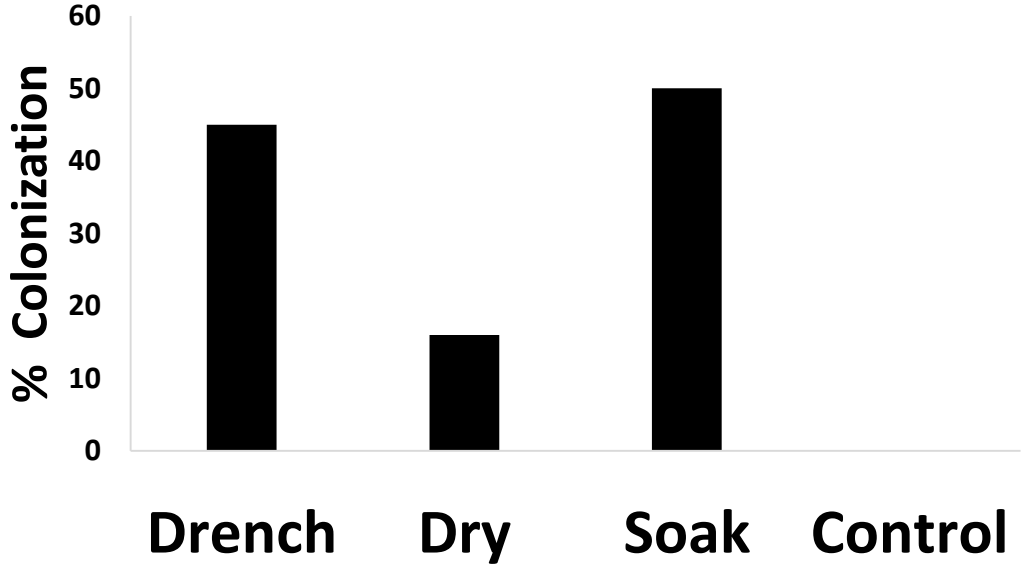
Virulence and Longevity

- A comparison of methods was made (drench, roll, soak)
- A comparative virulence assay was implemented (based on Shapiro-Ilan 2001). Pecan weevil larvae placed in 30 ml soil cups with $4x10^6$ conidia spores. Mortality assessed after 21 days. 30 insects per treatment: Commercial fungus from agar plates (GHA), fungus from an infected insects, fungus from pecan, control
- To assess longevity, every few months leaves were removed from inoculated trees to assess for endophyte activity (assay vs. insects).

- The longevity of the endophytic relationship of *B. bassiana* in pecan seedlings -so far 1.5 years (and continuing)

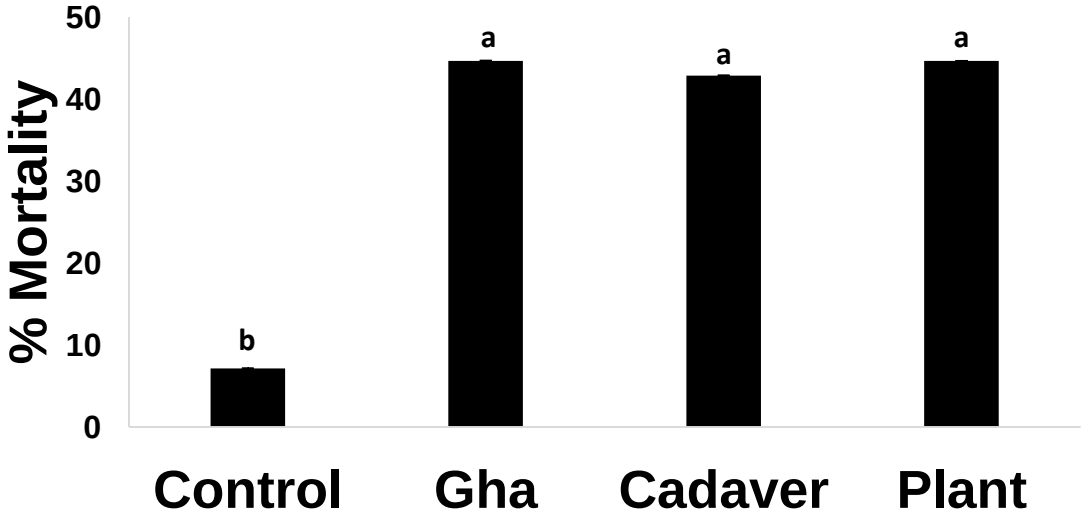
Results 2018

- Successful inoculation of endophytic fungus using various methods



- Virulence (killing power)

of the fungus is conserved in the plant

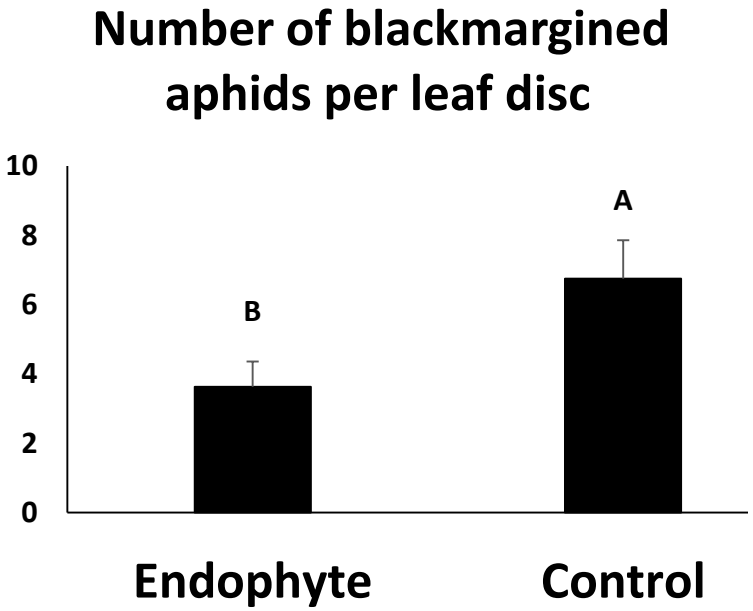


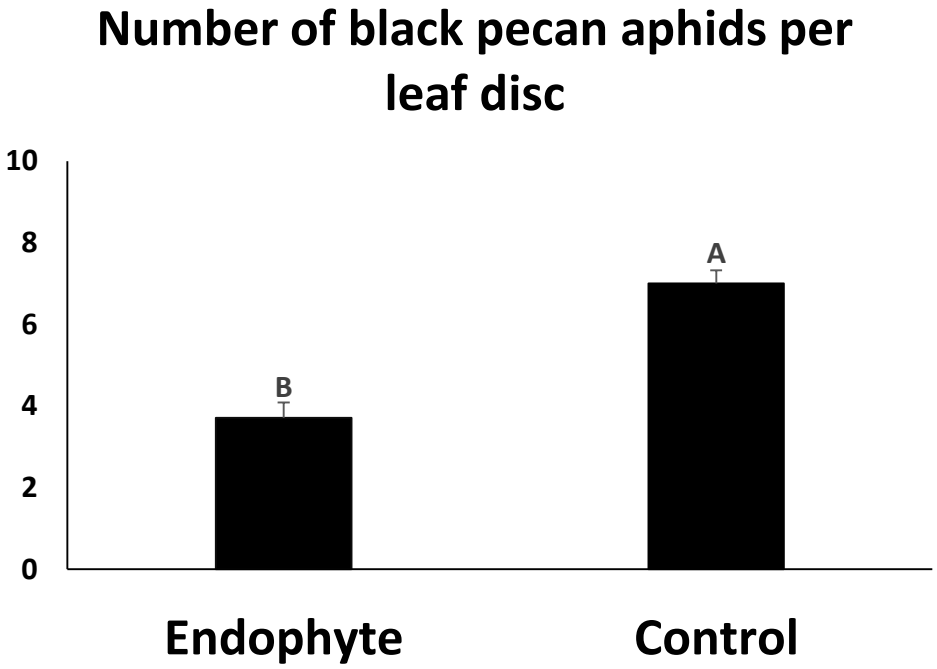
Impact on Aphids

- One trial was conducted for control of black pecan aphid and one for black margined aphid (based on Shapiro-Ilan et al., 2008).
- Four or five pecan leaf discs (2 cm diam) from each endophyte or control seedling were placed into Petri dishes (100 mm diam) half-filled with 1.5% water agar. Ten adult aphids were added to each dish
- The dishes were incubated under lights at room temperature (22 C ± 1 °C)
- After 5 days, the number of surviving aphids was recorded. There were 12 replicate seedlings of each treatment and control
- Analysis by t-tests

Results 2018

- Exposure to endophytic *B. bassiana* reduced black pecan aphid and the blackmargined aphid in laboratory studies (12 reps x 10 aphids per dish)





Conclusions thus far

- *Beauveria bassiana* can be inoculated into pecan trees via seeds or seedlings
- Initial research indicates the endophytic fungus can contribute to protection against pecan aphids
- Endophytic *B. bassiana* has been observed in nature

Objectives 2019

1. Expand efficacy tests on endophyte seedlings in the greenhouse to measure control against pecan insect pests and disease (initial focus on aphids and *Phytophthora*)
2. Measure the ability of endophytic pecan to suppress insects and disease in the field
3. Determine if drenching can establish fungal endophytes in mature pecan trees.
4. Determine the potential of *M. brunneum* to exist as an endophyte in pecan

Approach 2019

- Objective 1 (expand efficacy testing): Repeat aphid tests in the lab and also conduct leaf assays with *Phytophthora* (and possibly scab)
- Methods based on Shapiro-Ilan et al. (2008 J. Invertebr. Pathol.) and Shapiro-Ilan et al. (2014, Biological Control)

- Objective 2 (initiate field testing): Pecan seedlings containing endophytic *B. bassiana* and control seedlings (without endophytes) will be placed in an orchard in a replicated design. Insect pests and diseases (such as scab) will be monitored through the season

Approach 2019

- Objective 3 (inoculating mature trees): At least 20 mature pecan trees without endophyte will be drenched up to three times with *B. bassiana* and the success of inoculation will be assessed using molecular and re-isolation techniques on agar. The potential to inject trees will also be explored.
- Objective 4: *Metarhizium brunneum* will be inoculated to pecan using seed treatments and or drenching of seedlings in the same manner as was accomplished with *B. bassiana*. Initial tests on pest or disease suppression will be implemented as well. *M. brunneum* has been documented as an endophyte in other plants (e.g., potato) and has provided benefits

Future Directions for Endophytic Fungi in Pecan

- Cultivar effects
- Impact on plant growth, yield, other physiological effects (endophytic insect-fungi were shown to positively effect plant growth in other crops)
- Role of other endophytes in pecan

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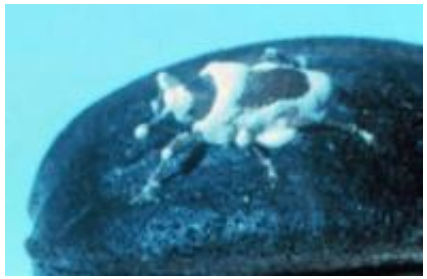

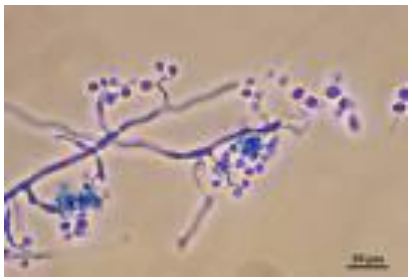

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