Microbial Control Approaches for Pests of Fruit and Nut trees

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the in-house research arm of the U.S. Department of Agriculture

Barriers to Expanded Use of Microbial Control Agents

- Cost 
- Sensitivity to environmental extremes (heat, desiccation, UV)

- Quality, consistency in field performance
- Basic knowledge

Approaches to Advancing Microbial Control: Overcoming Hurdles

- Strain improvement & stability
- Improving mass production
- Improving formulation & application technology
- Environmental manipulation
- Enhanced understanding the microbial biology/ecology

- <u>Focus</u>: due to time constraints, I will provide just a few of <u>many</u> examples involving entomopathogenic nematodes (EPNs), entomopathogenic fungi (EPF), & bacteria
- <u>Focus crops</u>: Pecan & peach

PECANS

Pecan Weevil, *Curculio caryae*

- Key pest of pecan,
- Life-cycle 2-3 yrs
- Adults emerge July-October

(but mostly mid-Aug to mid-Sept)

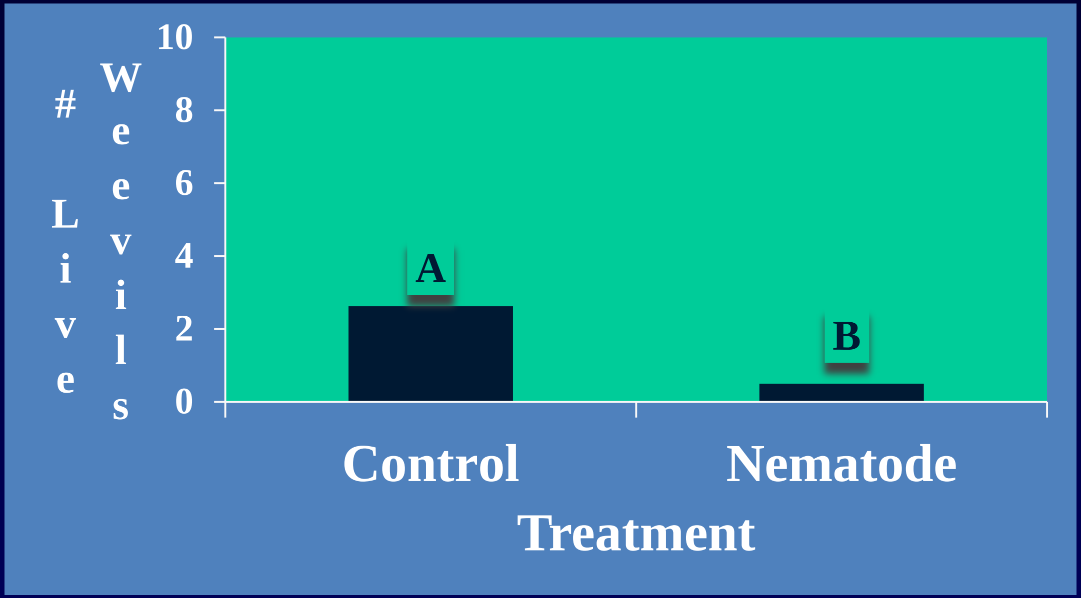
- Most crawl or fly to the trunk
- Larvae drop to soil (late Sept to Dec), & form a soil cell at 3” to 10” depth
- About 90% of the larvae pupate after 1 yr in soil & emerge as adults the next yr
- The other 10% remain as larvae an extra yr (3 yr life-cycle)





Traps used for monitoring

Suppression of Pecan Weevil Prior to Emergence: [Improved Application Techniques]

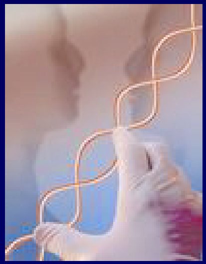


- *Steinernema carpocapsae* is highly virulent (especially to adult weevils)
  - Applied *S. carpocapsae* 3X per yr (25 per cm^2 minimum)
    - Less than 1% weevil survival in treated pots after 2 yrs
  - Lots of natural mortality (as expected); nematode provide 81% additional control (Shapiro-Ilan & Gardner, 2012)

Enhancing Biological Control Efficacy by Improving the Organism

- Discovery: New strains and species greatly expand biocontrol utility!
- Artificial Selection: directed genetic selection e.g., for entomopathogenic nematodes (EPNs) selection successful for host finding, nematicide resistance, environmental tolerance (Gaugler et al. 1989; Glazer et al. 1997; Anbesse et al 2013;
- Hybridization: controlled crosses, e.g., for environmental tolerance (Shapiro-Ilan et al. 1997, 2005; Mukuka et al 2010)
- Genetic modification: using molecular methods (Gaugler et al. 1997; also mining the organism for genes– BT crops!)

[**Strain Improvement**]

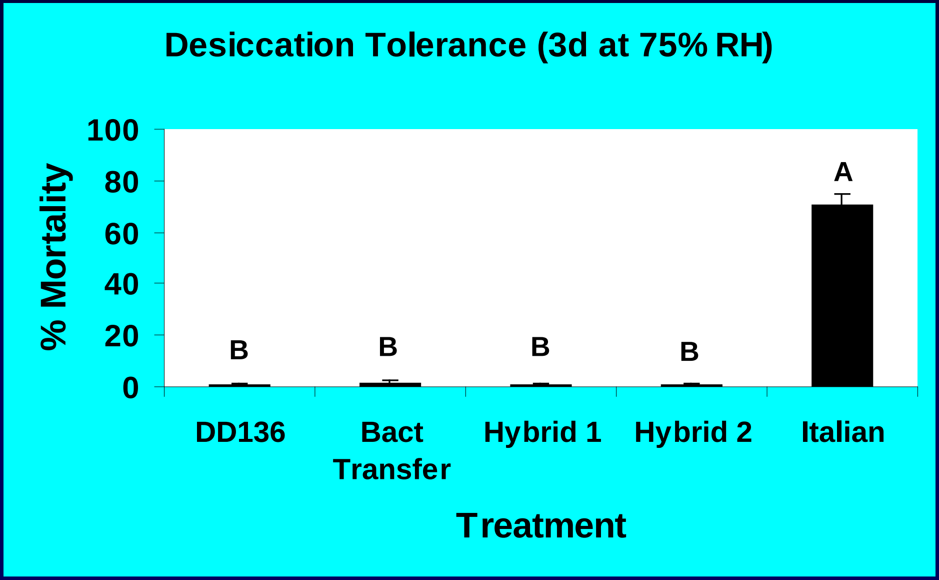


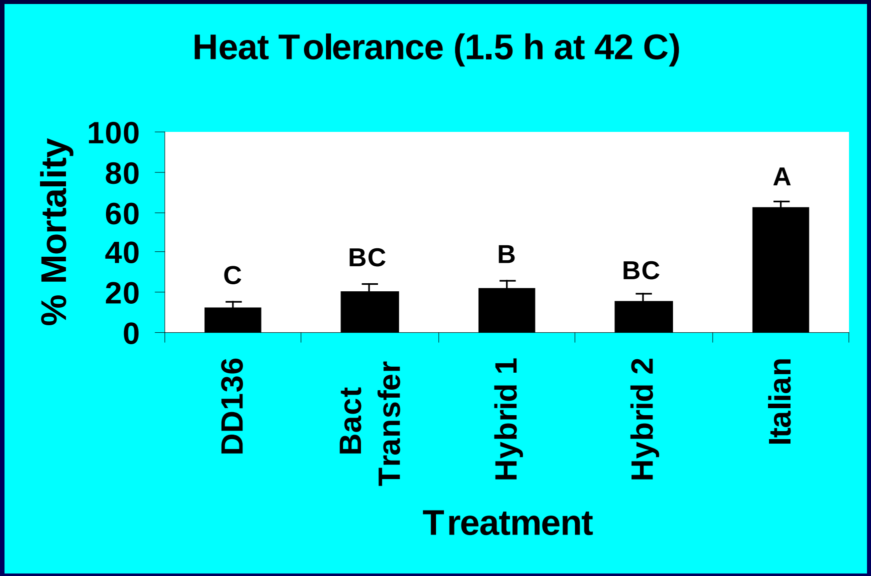
<u>Hybridization</u>: Making A Better Nematode Strain for Pecan Weevil Control

- *S. carpocapsae*, Italian strain, exhibits high virulence to adult weevils but poor environmental tolerance, whereas *S. carpocapsae*, DD136 strain, exhibits high environmental tolerance but low virulence (Shapiro-Ilan et al. 2003)
- Transfer bacteria for better environmental tolerance (Italian strain bacteria into DD136)
- Hybridize Italian strain for better environmental tolerance (Italian x DD136)

Improved Strains: Environmental Tolerance

(Shapiro-Ilan, Stuart & McCoy, 2005, Biol Control)





Environmental tolerance improved & virulence not compromised

- Also improved Hb for heat tolerance using marker mutations (Shapiro et al. 1997, Biol. Control)
- Ehler's group using hybridization & selection (Mukuka et al 2010)

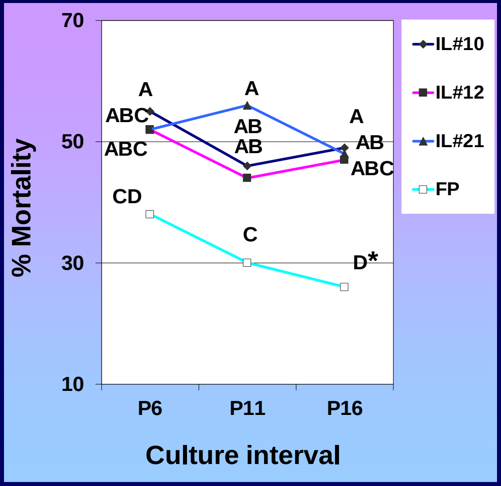
<u>Improving Strain Stability</u>: Once you have a good strain, how do you keep it stable?

- **When biocontrol agents are cultured repeatedly, deterioration can occur in beneficial traits** e.g., virulence, fecundity, environmental tolerance, etc. (Hopper et al., 1993; Shapiro et al., 1996; Wang & Grewal 2002)
- For EPNs the cause is largely genetically based; evidence indicates inbreeding depression (Bai et al., 1995; Chaston et al., 2011 Int. J. Parasitology; Adhikari et al. 2009 BMC Genomics)
- **<u>Overcoming the Problem</u>: <u>Use homozygous lines</u>!**

1. Generate an array of inbred lines
2. Select the best line(s) for continued research & commercialization (Bai et al. 2005)

- Thus implementing stable strains with superior biocontrol potential

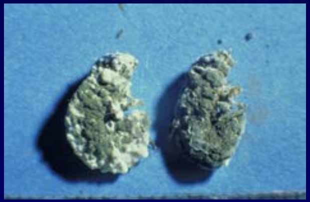
- Commercial companies adopting this approach



Improving Application of Entomopathogenic Fungi for Control of Pecan Weevil

- To enhance persistence and efficacy, compared application of *B. bassiana* to ground, trunk, cultivation, cover crop; <u>best results observed</u> with trunk or cover crop treatment; 80% mortality (Shapiro-Ilan, Behle et al. 2008)

- Trunk bands also effective (*Metarhizium anisopliae*)



- Clover cover crop enhanced persistence of endemic fungi!

<u>[Improved Application Techniques]</u>

<u>[Environmental Manipulation]</u>

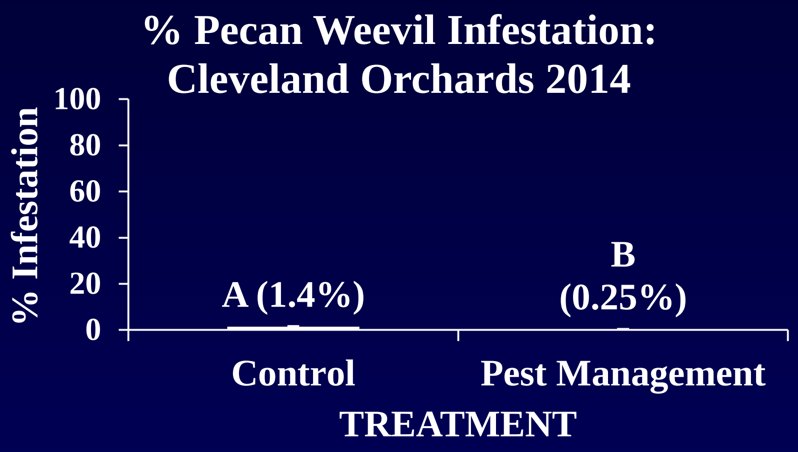
I: Integrated Organic Approach

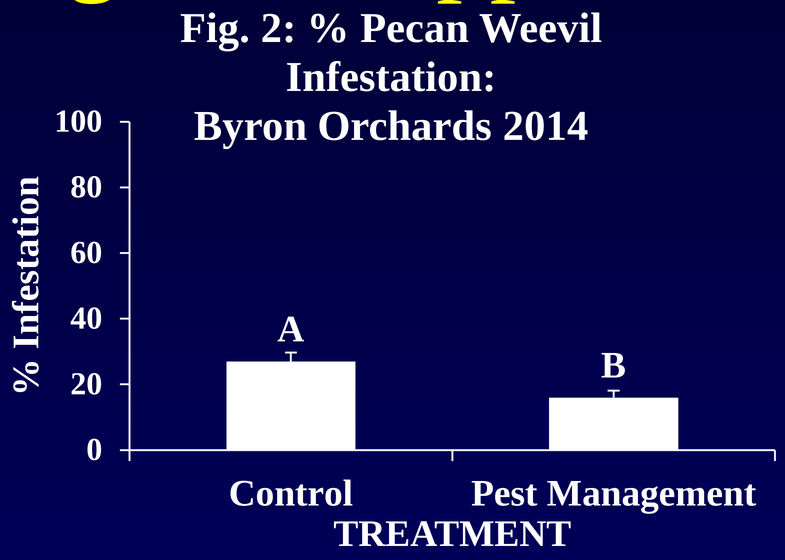
- Large Plot Studies at Two locations: 1) Byron (USDA, 3 reps), 2) Fort Valley (Cleveland, 8 reps)
- 1.5 acres per replicate; Treatments = Pest mgt vs control
- Pest management regime:
  - o Nematodes (*S. carpocapsae* from E-Nema, at 2 billion per A) applied to soil under the canopy, May-June,
  - o Fungus application (*B. bassiana* Mycotrol®O applied a 2×10^{12} spores per A), applied to soil September-October

  - o Grandevo® applied at 3 lbs per acre to the canopy 3 times from mid-August to mid-September (when the pecan weevil is emerging)

- The combined pest management regime (3 tactics) was compared to a non-treated control

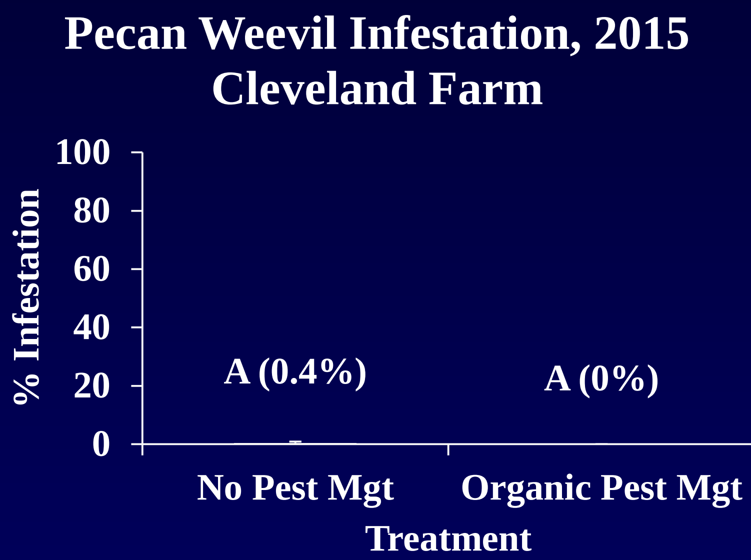
Results Year 1: Integrated Approach

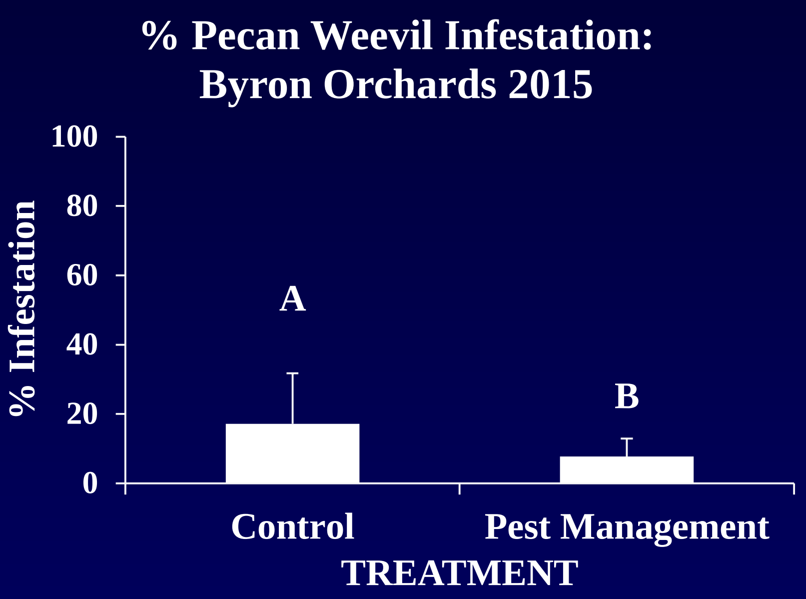




- Pest management treatments significantly reduced weevil infestation in both orchards (sample 100 nuts per rep)
- Microbial populations persisted about 2 months at both locations

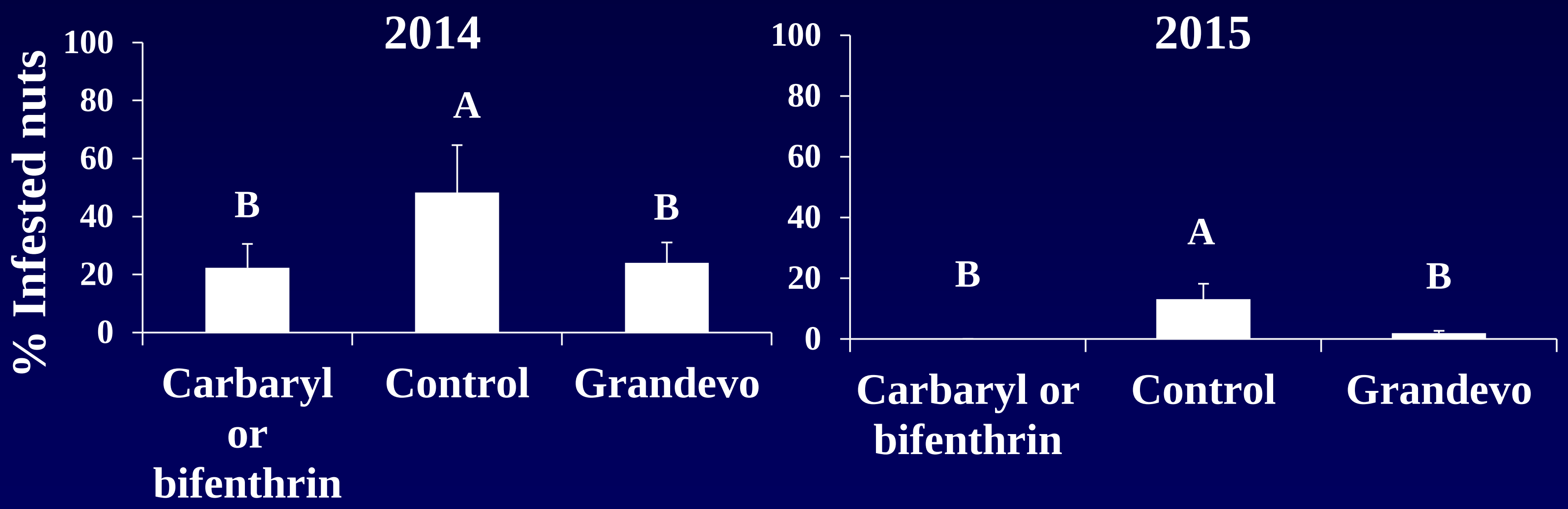
Results Year 2: Large Plot Test





Thus, Grandevo can be used as canopy spray (curative) and nematodes & fungi applied to soil prior to weevil emergence (Apply to weevil hot spots)

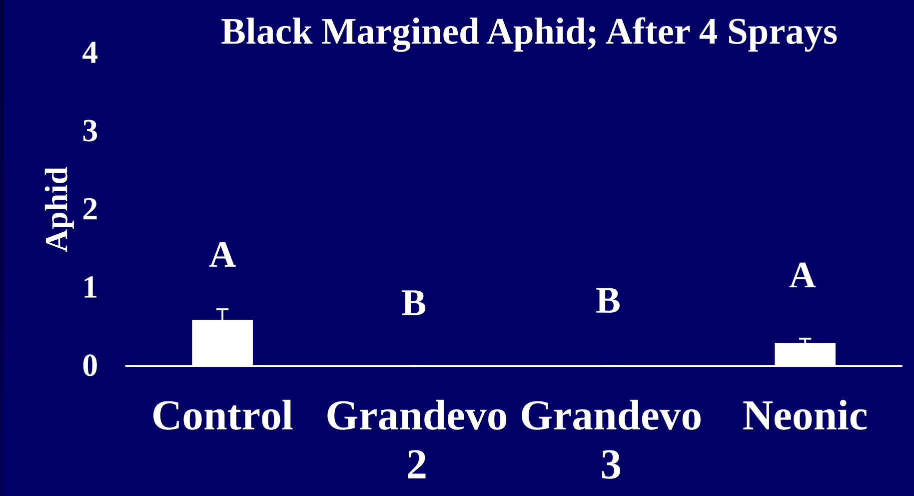
Grandevo® Performs as Well as the Chemical Insecticide Standards

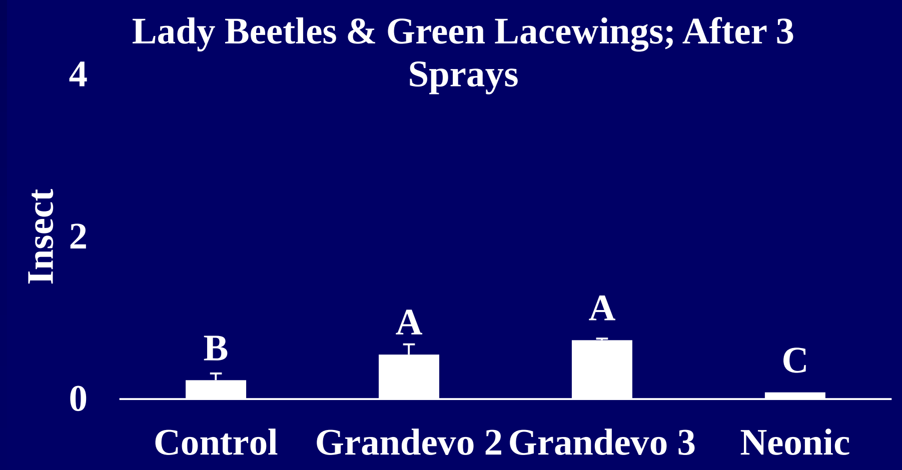


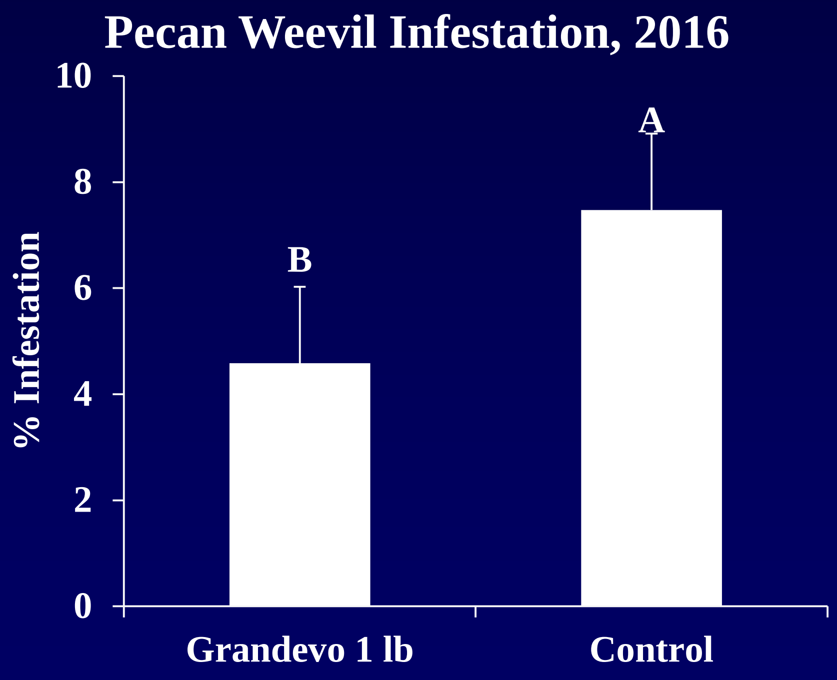
- 3 lbs per acre of Grandevo compared with standard (carbaryl/pyrethroid) applications

(D. Shapiro-Ilan, T. Cottrell & R. Mizell)

Grandevo: Pecan Aphid Suppression, Conservation of Natural Enemies & Lower Rates



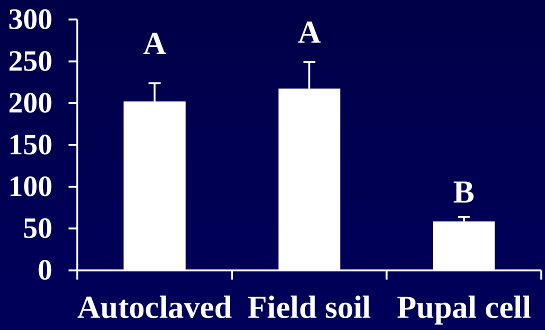




Self-Defense: An Insect Pupal Cell with Antimicrobial Properties that Suppress an Entomopathogenic Fungus

Shapiro-Ilan & Mizell, J. Invertebr. Pathol. 124 (2015) 114–116

- The pupal cell of pecan weevil was found to possess antimicrobial properties
- The pupal cell suppressed growth and or germination of *Beauveria bassiana*
- This is the first report of antibiosis associated with an insect soil cell
- Other weevil pupal cells show the same property
- Causal agents may be biological or chemical (and used to our benefit?!)
- The findings expand our knowledge of invertebrate host defense systems
- Potential for disruption?



[Enhanced understanding the microbial biology/ecology]

Suppression of Key Peach Pests with Microbial Control Approaches

D. Shapiro-Ilan[1], T. Cottrell[1],
R. Mizell[2], Tracy Leskey[3], & D. Horton[4]

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[2]University of FL
[3]USDA-ARS: Kearneysville, WV
[4]University of GA



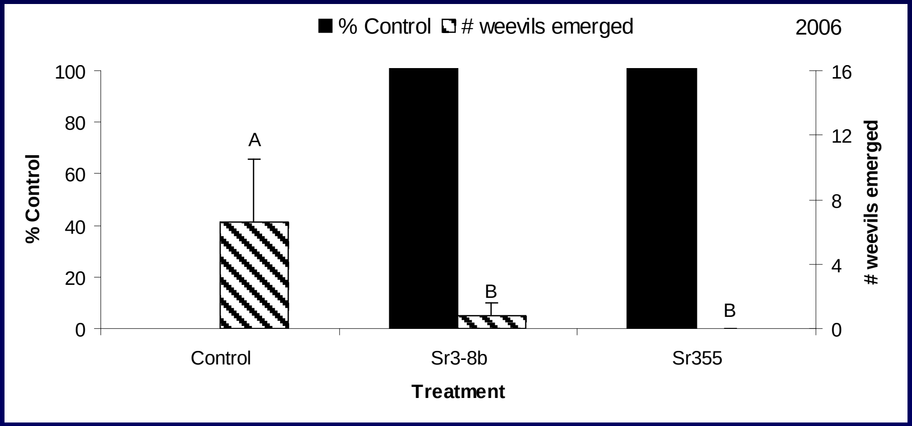

Plum Curculio, *Conotrachelus nenuphar*

- Larval control with Sr is highly efficacious!
- Problem? = adult is the damaging stage and large numbers can potentially enter from external overwintering sites (Jenkins et al. 2006 Env. Ent)
- Solution =

Set up trap crops/sentinel trees

And develop integrated program targeting each stage

(Leskey et al)

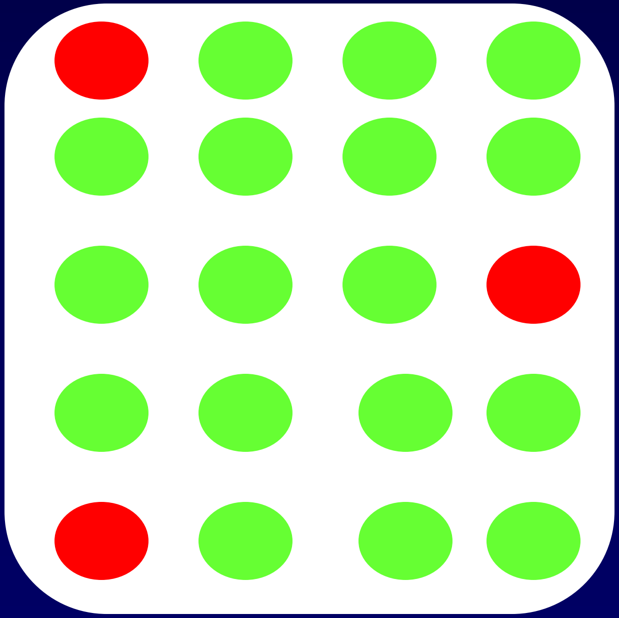


>95% larval suppression with Sr (Shapiro-Ilan et al. 2004, 2008) (81-88%, Pereault et al. 2009)

Integrated Program for Control of Plum Curculio

Leskey, Shapiro-Ilan, Zhang, et al

- Attract insects to trap trees on the border of orchards (trap trees in red) (Reduce spray by 90%!)
- Apply curatives if possible…
- Some fruit drop is anticipated…so then apply EPNs to the soil to kill larvae and protect the crop (Shapiro-Ilan et al., 2012 J. Nematol., 2013 Biol Control)
- Excellent results in apples=> working on peaches



[Improved Application Techniques]

Improving Formulation/Application

Case of the Lesser Peachtree Borer (LPTB)

Synanthedon pictipes (Lepidoptera: Sesiidae)

- Like all microbials, EPNs are sensitive to UV; & also are sensitive to desiccation (and LPTB attacks above ground)
- Thus, as expected when EPNs were applied in aqueous to LPTB wounds, the treatment failed
- However, application of a protecting formulation, Barricade® firegel + EPNs caused high levels of control (= chemical standard)! (Shapiro-Ilan, Cottrell, Mizell & Horton, 2010)
- The gel can be applied as a cover or tank-mix on the tree (Shapiro-Ilan et al. 2016)

Peachtree borer – A great target for Nematodes!

Peachtree Borer (PTB)

Synanthedon exitiosa
(Lepidoptera: Sesiidae)

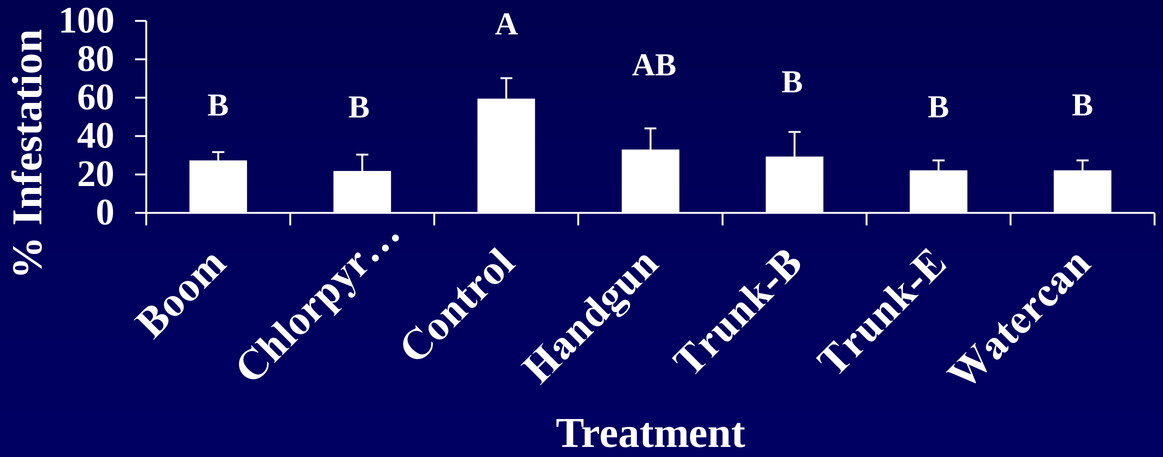
- Larval feeding occurs at or below soil level on the trunk and roots

- Highly damaging pest, especially to young trees

- Late season application of chlorpyrifos to trunks is effective against the single generation of this pest

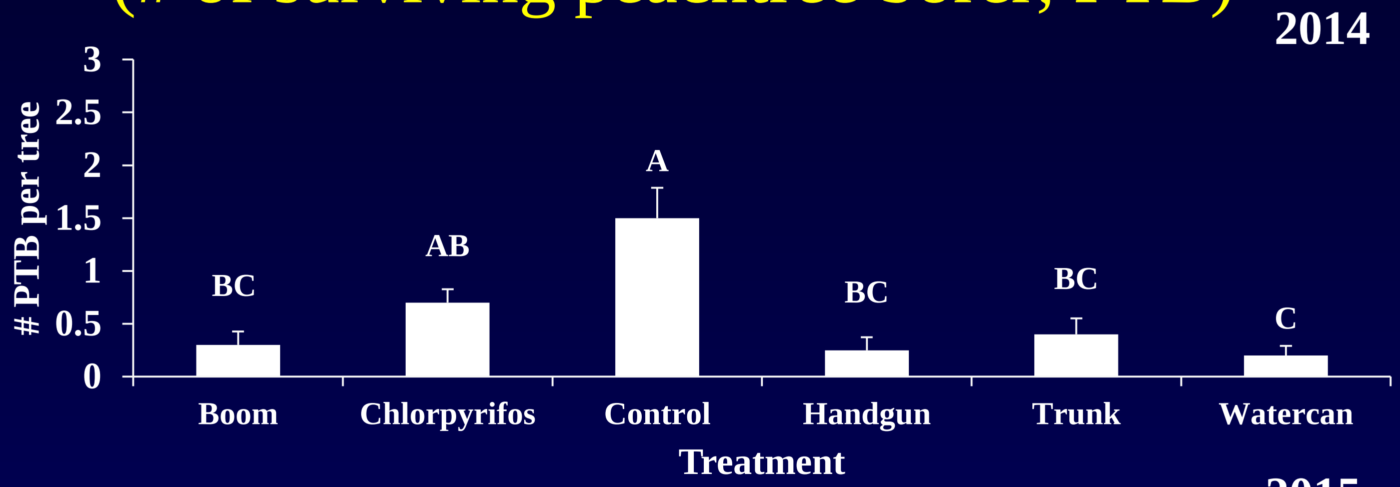
- **High levels of control** with *S. carpocapsae* Vs. young and mature larvae (88-100%), can be curative or preventative
- **<u>Very economical </u>**– Apply only to base of tree (Cottrell & Shapiro-Ilan, 2006; Shapiro-Ilan et al., 2009)
- <u>Recent research</u>: <u>Comparison of standard equipment handgun, boom sprayer, trunk sprayer - all work well!</u>

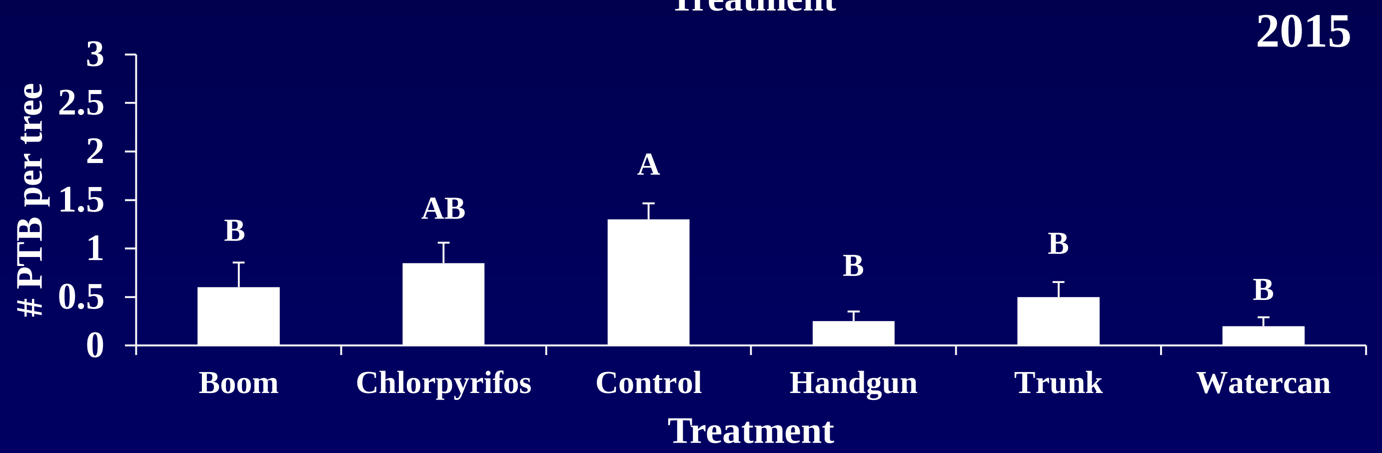
(Shapiro-Ilan et al. 2015 Biol Control)



- Sprayable gel can also enhance soil surface applications

Curative applications for control of peachtree borer (# of surviving peachtree borer, PTB)





- Beneficial nematodes suppressed the pest but chlorpyrifos did not (Shapiro-Ilan et al., 2016 J. Nematology)

Conclusions:

- Pecan Weevil: Grandevo (*C. subtsugae*) alone or combined with *S. carpocapsae* and *B. bassiana* provide high levels of control
- Plum curculio: The trap tree (sentinel tree) incorporating nematodes (*S. riobrave*) approach reduces orchard sprays and controls the pest
- Lesser peachtree borer: High levels of control can be obtained if nematodes (*S. carpocapsae*) is used with a protective formulation
- Peachtree borer: *S. carpocapsae* applied curatively or preventatively can provide equal or better control than chlorpyrifos

Conclusions

- Microbial control can be enhanced by improving strains (& stability), formulation/application, environmental manipulation & basic research

- Future Directions:

- Optimize microbial applications in pecan & peach via novel/improved strains, formulations, etc.
- Leverage basic knowledge on microbial agents, e.g., soil cell; nematode signaling (manipulating the wolf pack)

- For more on understanding Ecology of Microbials see:

Ecology of Invertebrate Pathogens, Hajek & Shapiro-Ilan Eds. , 2017 (Wiley)

Acknowledgments

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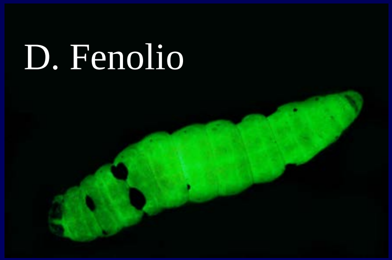
- **Surendra Dara for Inviting Me!**



Mention of a proprietary product name does not imply USDA's approval of the product to the exclusion of others that may be suitable

Questions?