A call to arms:

protecting and summoning native nematodes to kill Diaprepes root weevil

By Larry Duncan and Lukasz Stelinski

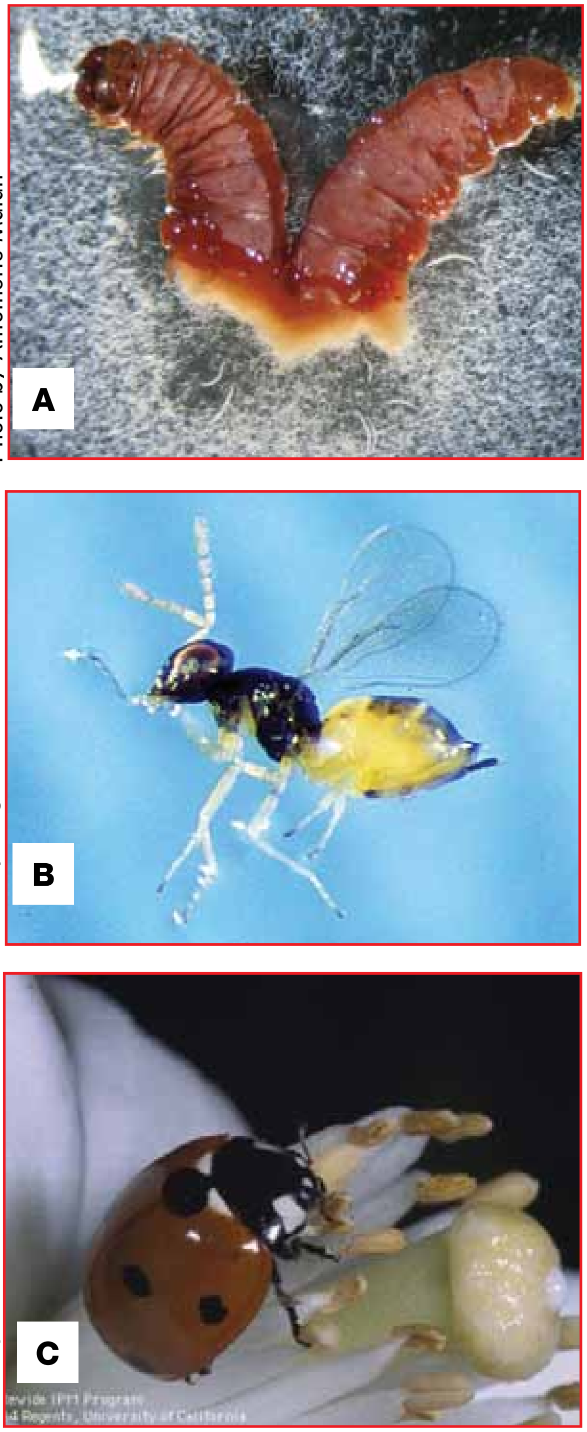

Photo by Antoinette Malan

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***Figure 1.** Can conservation of native, subterranean natural enemies be as effective as protecting natural enemies in the tree canopy for control of insect pests? Photos show subterranean, infective entomopathogenic nematodes emerging from the cadaver of an insect larva (A); above-ground, parasitic wasps (B); and predators (C).*

Research to control *Diaprepes* root weevil decreased dramatically following the arrival of huanglongbing in Florida. Nonetheless, several recent projects studied the question of whether growers can encourage native natural enemies to provide greater biological control of *Diaprepes* larvae in soil by simply changing some agricultural practices. This approach, called conservation biological control, is well understood by citrus growers who have long recognized the importance of protecting insect parasitoids and predators in the canopies of their trees in order to reduce the expense and environmental risks of over-reliance on pesticides. Less evident is the potential to exploit organisms in soil to help control below-ground pests or diseases. Even scientists know amazingly little about how insects interact with their natural enemies in soil. However, it is becoming apparent that soils in Florida's citrus groves harbor natural enemies of insects in an abundance and diversity unknown in most other parts of the world.

How important are these organisms in controlling *Diaprepes* on their own? Can we do things that enhance their activity against root weevils? Could these subterranean biocontrol agents be as effective as their counterparts in the tree canopy? These are some of the questions we're studying.

We recently surveyed more than 150 sites that included citrus groves and adjacent natural areas to identify and quantify Florida's naturally occurring entomopathogenic nematodes (EPNs) and some of the natural enemies of nematodes. EPNs are part of the functionally diverse community of nematodes that inhabit all soils. Unlike other nematodes, EPNs can only develop inside arthropods. They require the assistance of pathogenic bacteria that the nematodes carry inside themselves while they migrate through soil in search of prey. Once EPNs penetrate through an insect cuticle or body opening, they release the bacterium which kills the insect. Both nematode and bacteria produce abundant progeny that eventually enter the soil to continue the cycle (Figure 1).

Our previous research demonstrated that EPNs are important natural enemies of *Diaprepes* root weevil. We wanted to know whether these insect-killing nematodes are more prevalent in some regions or soil types than others. If EPNs are associated with specific physical properties of soil, it might be possible to create favorable habitats for them.

We detected 11 species of EPNs in the survey, with at least one species present in every grove we sampled. Such a high degree of EPN occurrence and species diversity has not been reported anywhere else in the world. We also found regional differences in both the complexity of EPN communities and where particular species of EPNs are likely to occur. Groves in flatwoods soils had less diverse communities of EPNs than did groves on the deeper sandy soils of the central Ridge. Provided they don't prey on one another (which EPNs do not), greater species richness and diversity of natural enemy communities can increase the rate at which they kill insect pests.

We also learned that some of the EPN species that are most effective and persistent at killing *Diaprepes* in controlled experiments dominate EPN communities on the central Ridge, whereas the EPNs that dominate flatwoods communities are somewhat less effective in killing root weevils. These observations support the idea that *Diaprepes* tend to be much more abundant in some flatwoods groves than in groves on the central Ridge, in part due to greater EPN activity on the central Ridge.

We characterized the grove soils in terms of more than 30 physical (soil texture, water-holding capacity etc.), chemical (nutrients, heavy metals, pesticides) and biological (fungal and bacterial natural enemies of nematodes) properties. The properties which best explained EPN occurrence and abundance were all related to soil moisture (soil clay and organic matter content, water-holding capacity and depth to groundwater). The most effective EPN species tended to be associated with drier soil conditions.

MOVING RIDGE SAND TO THE FLATWOODS

On the basis of results such as these, we conducted an experiment to determine if using Ridge sand as a planting medium in a flatwoods site with heavy weevil pressure might provide better conditions for biocontrol of weevils by EPNs (Figure 2). The sand soil was used to fill large planting holes for 50 trees. Fifty additional trees were planted in conventional planting holes in the native soil. Some plots of trees in both soil types were inoculated with EPN species that were not endemic at the site. Trees were grown for two years before measurements were begun to give weevils and other soil organisms the opportunity to establish and for communities to equilibrate in the imported soil.

During the third and fourth years of the trial, the numbers of EPN species and the species diversity were always higher in sand than in the native soil. Although the total numbers of EPNs did not differ between the soils, the rate at which caged and buried *Diaprepes* larvae (sentinels) became infected by EPNs was significantly higher in sand than in native soil. More adult weevils emerged from native soil than from sand, especially from those plots with low sentinel infection rates. During four years, just three trees in sandy soil died due to *Diaprepes* damage compared to 21 trees in native soil. Trunks of trees in sand were 60 percent larger and the trees produced 85 percent more fruit than did those in native soil.

***Figure 2.** Citrus trees growing in sand (foreground) and native soil (background) in a grove that is heavily infested with Diaprepes root weevil. Trees in sand grew significantly larger and produced more fruit than trees in native soil. EPNs killed fewer Diaprepes larvae in the native soil and more adult weevils emerged from the native soil than from the sand. Traps to capture emerging adults are shown adjacent to each tree.*

We cannot know all of the ways in which the two soils affected weevils and trees at this site, but substitution of sand for native soil was an effective means of conserving EPNs. Some growers already fill planting holes with sand to improve drainage, suggesting the practicability of adapting the practice for *Diaprepes* management in flatwoods groves with a history of weevil damage to trees.

EPNs IN ACPS

Just as important as finding ways to increase EPN services, is to not reduce them inadvertently. The Advanced Citrus Production System (ACPS) has tremendous potential as a tactic to manage greening disease by growing trees much more efficiently and quickly. The improved tree growth requires daily fertigation which creates soils with moisture and chemical properties very different than those managed conventionally. But how do these changes affect EPN communities?

During 12 months in collaboration with Arnold Schumann of the University of Florida-IFAS, we detected five-fold more EPNs in plots under conventional management than under ACPS in an ongoing experiment near Auburndale. We also measured higher densities of some natural enemies of EPNs in ACPS plots (Figure 3, page 9). In the subsequent year, nearly three times as many *Diaprepes* adults emerged from soil in ACPS compared to conventional plots. Not surprisingly, with greater weevil pressure, *Phytophthora nicotianae* levels are also significantly higher under ACPS.

Following these discoveries, we have found evidence from controlled studies of the soils that the chemical changes from ACPS affected EPNs directly and possibly indirectly by increasing the activity of some natural enemies of EPNs. As we narrow the search to focus on individual chemical changes, we intend to identify specific aspects of ACPS that can be modified to avoid this serious non-target effect.

Besides protecting and facilitating these nematodes, are there other ways to exploit their potential? An additional recent area of *Diaprepes* research that has been conducted at the Citrus Research and Education Center in Lake Alfred has dealt with the effect of root damage by the larval stage of this beetle underground on induced plant defense. It turns out that when insects or pathogens damage plants, often plants will respond to this damage in diverse ways to defend themselves.

One manner in which plants commonly respond to insect damage is called "induced response." Specifically, the damage caused by the insect induces that plant to release very specific chemicals. These induced chemicals serve to attract natural enemies of the pest and increase populations of those natural enemies in the immediate area where the pest is doing the damage. This increases the mortality of the damaging pests and thus reduces their density, which results in less damage to the plant.

PLANTS "CALLING OUT FOR HELP"

Another way of putting it is that plants actually "call out for help" to beneficial insect predators or parasites when they are being damaged by pests. This phenomenon has been extensively documented for pests that feed on above-ground portions of plants or trees, such as the leaves. For example, when larval insects feed on leaves, those damaged leaves begin to release volatile chemicals that attract parasitic wasps or predators, such as ladybird beetles, to the damaged area which then parasitize or eat the pest, reducing pest density and indirectly protecting the plant.

We wondered whether something similar could be happening in the soil where larval *Diaprepes* cause their damage to citrus roots. We knew that *Diaprepes* severely damages citrus roots by feeding. We also knew that EPNs are a potent natural enemy that attack *Diaprepes* larvae and can keep their populations in check. So, our question was whether the roots damaged by *Diaprepes* larvae released a signal to "call in the troops" by attracting EPNs that occur naturally in the soil to the specific place where *Diaprepes* larvae were doing their damage.

Following this line of reasoning, we determined that citrus roots that were being damaged by *Diaprepes* larvae did, in fact, attract significantly more EPNs than undamaged, control roots. To our surprise, this effect occurred broadly, meaning that all of the species of EPNs that we tested were attracted to these damaged roots. Furthermore, we were able to show that this root signal that attracts EPNs following *Diaprepes* damage also increases the mortality of the feeding beetle larvae and the call for help indeed aids the citrus plant because the EPNs move to that area and kill the afflicting beetles.

Next, we identified the actual chemical that attracts the EPNs. We identified a chemical called pregeijerene (1, 5-dimethylcyclodeca-1, 5, 7-triene) from citrus roots that causes this effect. Although this chemical is not yet commercially available, we were able to obtain sufficient quantities of it by extraction from plants for field research. By doing so, we confirmed, in the field, that application of pregiejerene does increase kill of *Diaprepes* larvae as

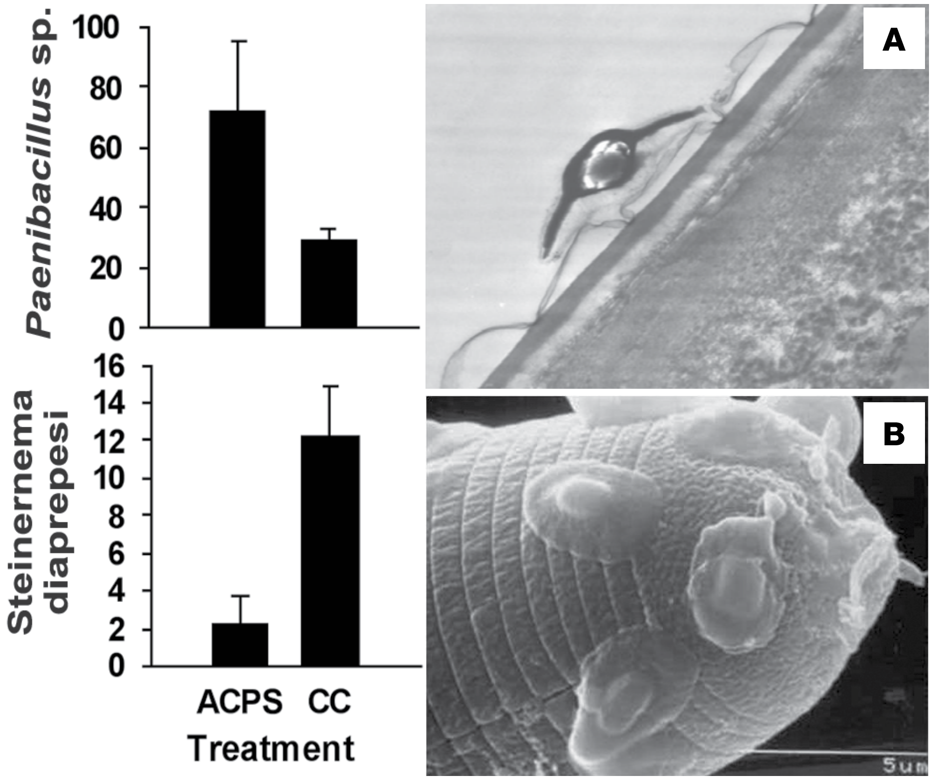

Figure 3. *Effects of advanced citrus production system (ACPS) vs. conventional citriculture (CC) on the native entomopathogenic nematode. Steinernema diaprepesi and Paenibacillus sp., a bacteria natural enemy of S. diaprepesi. Spores of the bacterium adhere to the nematode cuticle, impeding its ability to move through soil and locate insect hosts. Photos show transmission (A) and scanning (B) electron microscope images of the bacterial spores on the nematode cuticle.*

compared with untreated controls. We were also able to detect this chemical in the soil in the immediate area where mature citrus trees were being damaged by *Diaprepes*. Furthermore, we tested the generality of this root-zone "call-for-help" signal by also testing pregeijerene in blueberry fields in New Jersey. Just like in citrus, mortality of blueberry pests was again significantly increased by attracting naturally occurring populations of EPNs with this chemical. Thus, this specific belowground signal attracts natural enemies of widespread root pests in distinct agricultural systems and may have broad potential in biological control of root pests.

Our current efforts are focusing not only on application of synthetic attractants in the field so as to enhance root-zone biological control, but also on potentially engineering plants so that they are better capable of "calling in" or attracting beneficial nematodes in response to insect attack, with particular focus on *Diaprepes*. However, practical application of this technique is still a long-term goal and a useful tool for the field is still in very early stages of development.

Larry Duncan is a professor and Lukasz Stelinski is an assistant professor — both with the University of Florida-IFAS at the Citrus Research and Education Center in Lake Alfred.