CITRUS RESEARCH BOARD

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Quantitative image analysis of photographed citrus roots.
(Image courtesy of Hana You - Volder Laboratory, University of California, Davis.)

Response of Citrus Roots and Their Microbiota to Soil Amendments

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Project Summary

This article summarizes the outcomes of a project to explore the inter-relationships between the citrus "rhizobiome" (root-associated microbial communities) and the availability and uptake of soil compounds by citrus root systems. Research confirmed that the composition of citrus tree root communities varies with location and soil amendments, which is consistent with the idea that it matters where trees are planted, grown and managed in terms of the microbiota with which their roots interact. We showed that soil drenches with blackstrap molasses reduced the uptake of imidacloprid and that liquid organic fertilizer did not significantly modify root system architecture. We also demonstrated that at least some California soils harbor microbial species known to benefit citrus tree health (for example, arbuscular mycorrhizal fungi) and believe that these species represent interesting candidates as root inoculants and an exciting opportunity for future research.

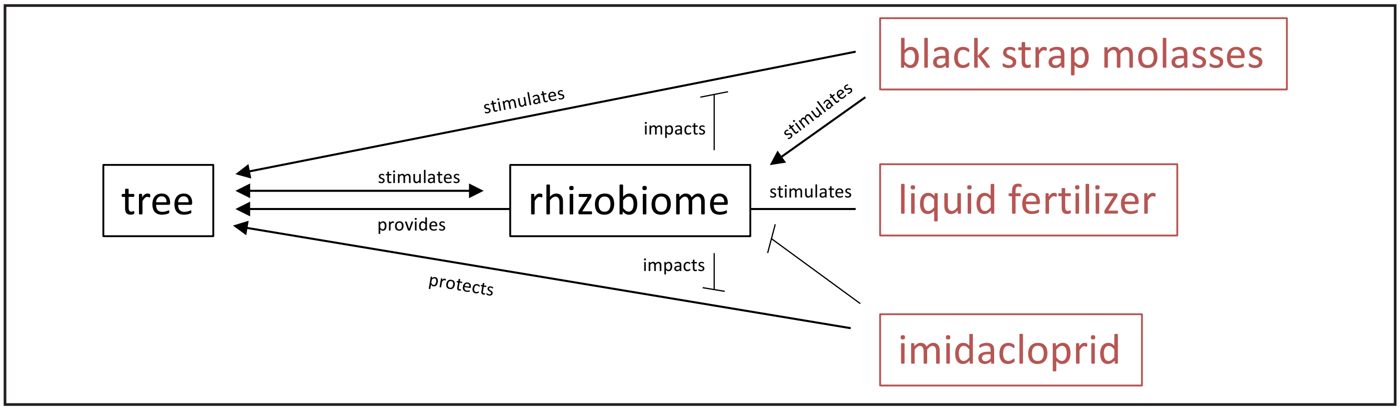

Figure 1. Presumed relationships between three types of soil amendments (blackstrap molasses, imidacloprid, liquid organic fertilizer), the citrus tree and its root microbiome (or rhizobiome). The graph highlights various direct and indirect effects of the rhizobiome and soil amendments on citrus tree health.

The plant-root surface represents a critical interface where water, minerals, nutrients and other compounds such as insecticides are taken up from the soil for distribution to other parts of the plant. The plant root surface also is home to a large and diverse microbial community (also known as the rhizobiome) that influences soil compound uptake or availability. There is a limited but growing understanding of what the rhizobiomes of citrus trees look like (Zhang et al. 2017; Blaustein et al. 2017). However, very little is known about the ways in which these root-associated communities of bacteria and fungi affect root function and the access to soil compounds or how those communities might be managed to grow healthier trees.

Our main objective was to describe and compare the structure of citrus tree root systems and/or their associated microbial communities in response to three different soil amendments:

- blackstrap molasses – a rich source of carbon, calcium, magnesium, iron, potassium and many micronutrients, it stimulates root growth and soil microbial activity (Schenck zu Schweinsberg Mickan and Müller 2009),
- a liquid organic fertilizer – used as an alternative to traditional mineral fertilization (Martínez-Alcántara et al. 2016) and
- imidacloprid – a systemic insecticide that is taken up by the roots and transported into the foliage to become effective against foliar insects; however, microorganisms in the soil may degrade imidacloprid (Hu et al. 2013) before it can be taken up by the tree.

We were interested in identifying which microorganisms are enriched on the root surface by these soil amendments. In addition, we wanted to know if and how these amendment-triggered changes in the root microbial community correlated with root structure and function (**Figure 1**).

What's on a Root?

To establish a baseline understanding of the citrus rhizobiomes for this project, we analyzed microbial communities of root samples from grapefruit, navel orange, blood orange and lemon trees growing in various settings across California, Texas and Florida. **Figure 2** shows a subset of these samples and highlights important principles about citrus rhizobiomes.

First, there is substantial variation in root-associated fungal communities between trees from different locations. This is most likely due to local differences in scion/rootstock cultivar, soil type and microbiota, tree health status, environmental conditions, soil management practices and other factors.

Second, many of the most abundant root-associated fungal "operational taxonomic units" (OTUs) have representatives that are known pathogens of citrus, either soil-borne (for example, certain *Fusarium* species have been linked to citrus rot and wilt diseases) or affecting fruit or foliage (for example, *Alternaria, Penicillium* and *Mucor*). Because the majority of sampled trees looked healthy at the time of soil collection, we suspect that these fungal OTUs represent either non-pathogenic strains or pathogenic strains whose abundance or activity falls below the threshold required to cause disease.

Third, our profiling of the fungal communities of citrus tree roots in the field revealed low relative abundances of representatives of the species *Paraglomus occultum, Diversispora spurca* and *Claroideoglomus claroideum*. As arbuscular mycorrhizal fungi (AMFs), these species benefit citrus trees by enhancing tolerance to salt stress (Wu et al. 2010) and soil aggregate stability (Wu et al. 2014). AMFs are specialized fungi that symbiotically colonize feeder roots, modulate their density and length to stimulate uptake of nutrients and water, and provide tolerance to several stresses, including drought (Wu et al. 2013) and soilborne

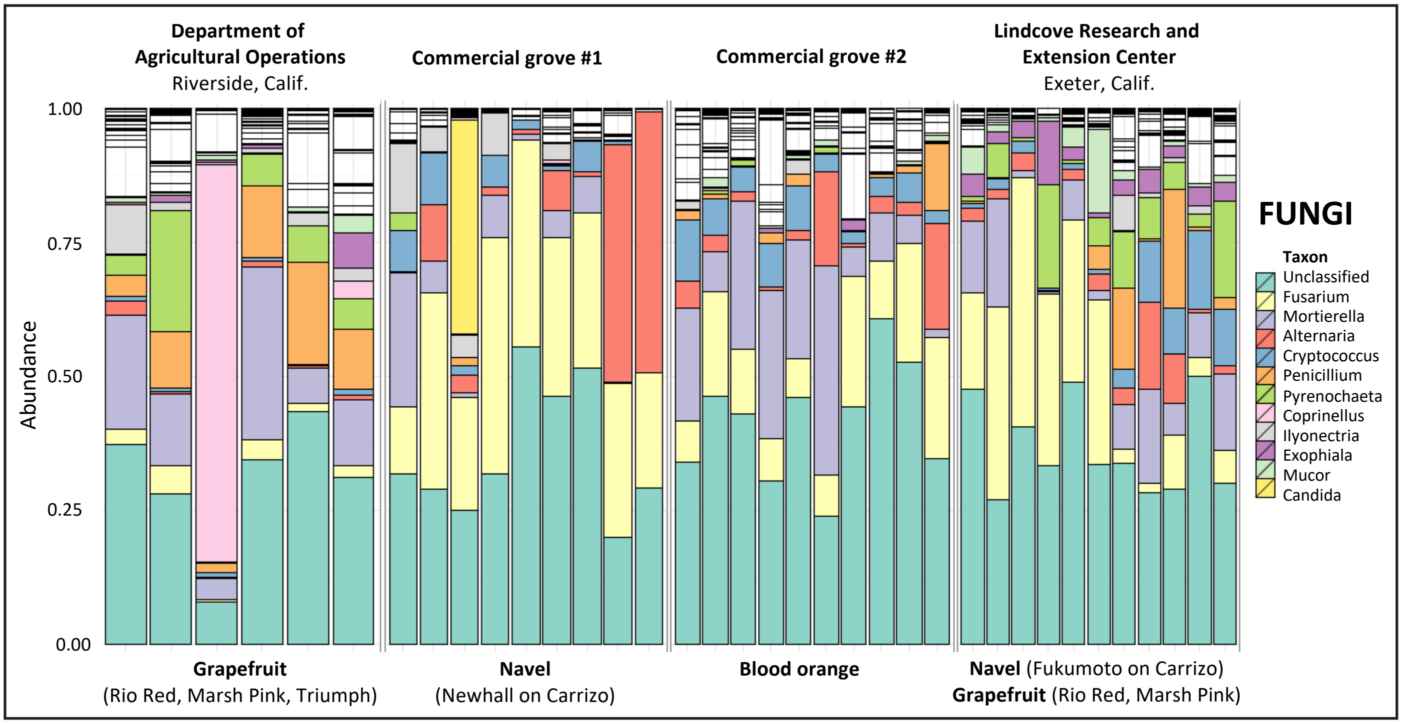

Figure 2. Stacked bar graph showing the relative abundances of fungal taxa (see color legend) in 36 root soil samples from grapefruit, navel and blood orange trees growing in four different locations in California.

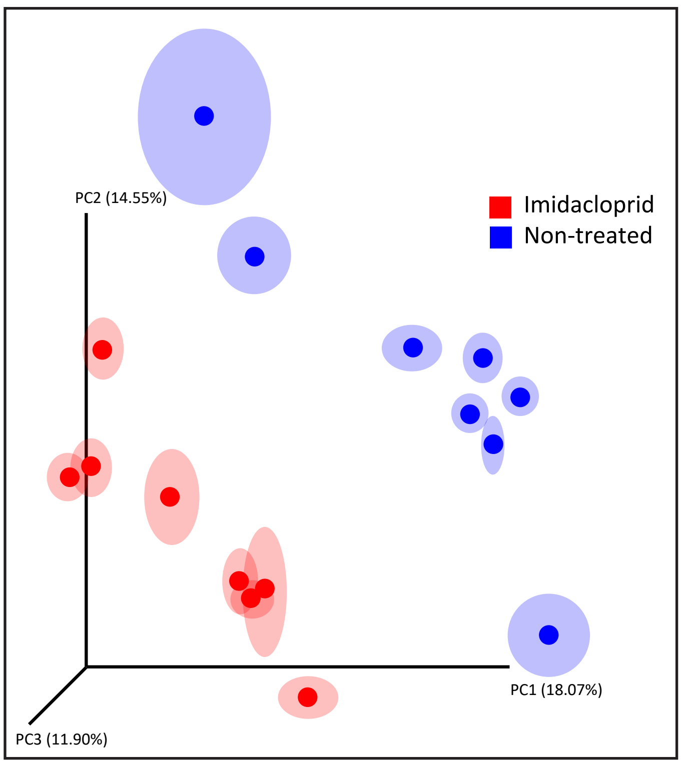

Figure 3. Principal Coordinates Analysis (PCoA) plot in which each one of 16 tree root soil samples is represented by a data point in red or blue – red if the sample came from a field treated with imidacloprid (two fields in Redlands) and blue if it came from a field that was not treated (one in Yorba Linda, one in Riverside). PCoA is a method to explore and visualize similarities or dissimilarities of data. The closer two points are to each other, the more similar they are in terms of microbial community composition. Blue data points cluster together, as do red data points, suggesting an effect of imidacloprid application on microbial community composition.

pathogens (Watanarojanaporn et al. 2011). The identification of AMF species native to California citrus trees represents an exciting entry for future studies into their practical use as tree inoculants.

Effect of Imidacloprid on Rhizobiomes

To assess the impact of imidacloprid on microbial community structure, soils were analyzed under 16 citrus trees in the greater Riverside metro area. The results (**Figure 3**) suggest that imidacloprid application and microbial community composition are correlated and that imidacloprid application alters the microbial composition of citrus rhizobiome. The underlying mechanisms for this change remain unclear. We tried extensively but were unable to isolate from the imidacloprid-treated soils bacteria or fungi with the ability to grow on artificial nutrient media containing imidacloprid as has been reported previously (Hu et al. 2013). It is unlikely, therefore, that the imidacloprid-induced differences in the microbial community are due to an enrichment of such microorganisms. Possibly, the observed differences are the result of variation among soil microbes in their susceptibility to imidacloprid (Devashree et al. 2014). Alternatively, imidacloprid may have an indirect effect, for example, by altering the quantity and/or quality of root exudates that shape the root microbiota.

Effects of Soil Amendments on Root Function

Anecdotal reports indicate that under-tree soil drenching with stimulants such as blackstrap molasses can increase

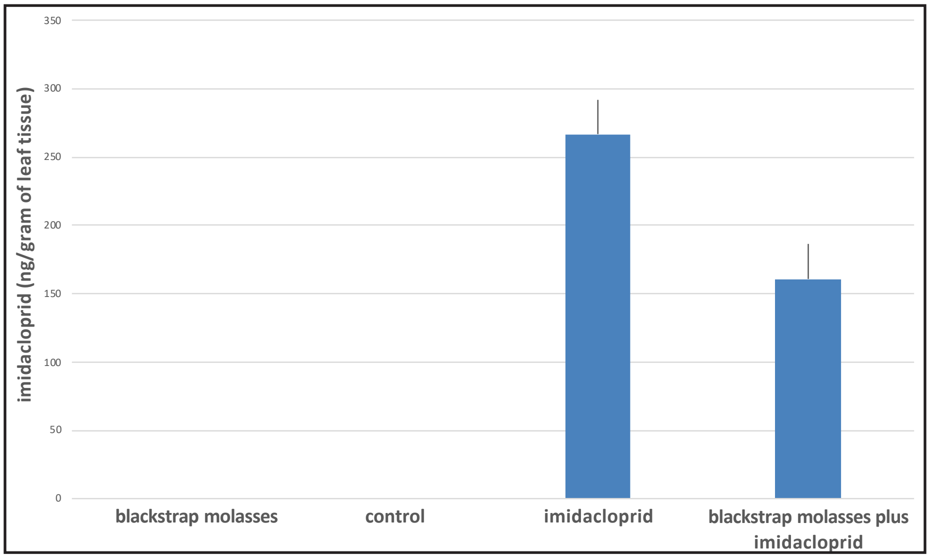

Figure 4. Concentrations of imidacloprid in leaves of citrus trees that, 30 days prior, had been soil-drenched with blackstrap molasses (500 mL at a concentration of 10 g/L), water, imidacloprid (500 mL Merit® at a imidacloprid dosage of 0.037g/L) or a mixture of blackstrap molasses and imidacloprid (same total volume and same concentration as in the single drenches).

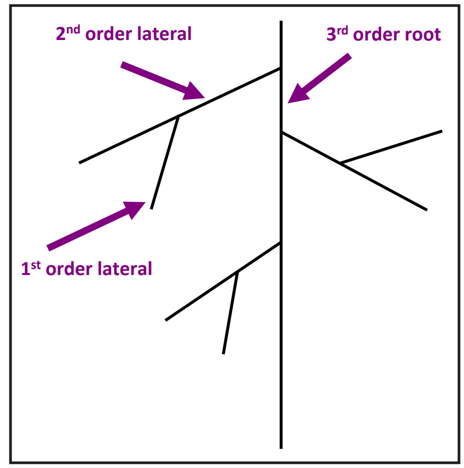

Figure 5. Schematic showing the concept of root order. Individual root sections are classified as first, second, third or higher root order based on their relative position in the root architecture.

citrus tree productivity, presumably through increased root activity. There have been grower reports about possible failure of citrus trees in some California field settings to take up imidacloprid. To test whether blackstrap molasses may be used to stimulate root uptake of imidacloprid, we conducted a greenhouse experiment in which navel orange trees were drenched with one of four treatments (imidacloprid, blackstrap molasses, a mixture of the two or water-only control). Thirty days after drenching, the imidacloprid concentration in leaves was measured by Frank Byrne, Ph.D. The results are shown in **Figure 4**. As expected, imidacloprid was undetectable in the foliage of trees treated with blackstrap molasses only or with water, while imidacloprid-treated trees showed an average foliar concentration of 267 nanograms (ng, or one-billionth of a gram) per gram of fresh leaf material. An unexpected finding was that the imidacloprid concentration in trees treated with a mixture of blackstrap molasses and imidacloprid was significantly lower at 161 ng per gram of fresh leaf material. We do not currently have an explanation for this molasses-induced 40-percent reduction in uptake, and this finding needs further investigation.

Effect of liquid fertilizer on root structure

Tree root functions include uptake of nutrients, water transport and structural support. These functions are not distributed equally within the structure of the root system, but instead vary with the position of an individual root on the branching architecture. This concept is known as "root order" (Pregitzer et al. 2002) (**Figure 5**), where lower-order, typically short-lived feeder roots are crucial for nutrient and water absorption, while higher-order, longer-lived roots are responsible for water and nutrient transport to other parts of the tree.

The root order concept permits a quantitative description of root system architecture, as it relates to tree productivity (e.g. fruit yield) or effects of a soil amendment. To assess how this concept might apply to citrus roots, we collected and analyzed root samples from a citrus grove that featured an on-going trial (from a Tulare County Clementine mandarin grove) comparing the effects of soil amendment with an organically certified, commercially available liquid organic fertilizer. Roots were collected from "treated" trees and compared to those from "untreated" trees that did not receive the liquid fertilizer. In the laboratory of Astrid Volder, Ph.D., root branches were photographed and analyzed for root length, diameter and length within each branching order. The roots belonging to orders 1 and 2 were the finest roots and accounted for about 85-90 percent of the total root system length (**Figure 6**). Treatment with liquid fertilizer resulted in roots with smaller diameters within the same order, particularly higher order roots. There were no significant differences seen between populations of roots treated with liquid fertilizer compared to untreated roots. The expectation was that liquid fertilizer would yield a higher relative proportion of first and second order roots (responsible for nutrient and water uptake), but perhaps that proportion was already maximal under these field conditions.

Conclusion and outlook

This project provided new insights into the complexity of citrus rhizobiomes, i.e., the communities of bacteria and fungi that are associated with citrus tree roots. The composition of these communities can vary with the location where the citrus trees are grown. This is consistent with the idea that root microbiota are selected from among the members of the microbiota in the soil through which these roots grow. Also, a soil amendment such as imidacloprid can change the root microbial community structure. Experimental data confirming this observation in a controlled greenhouse

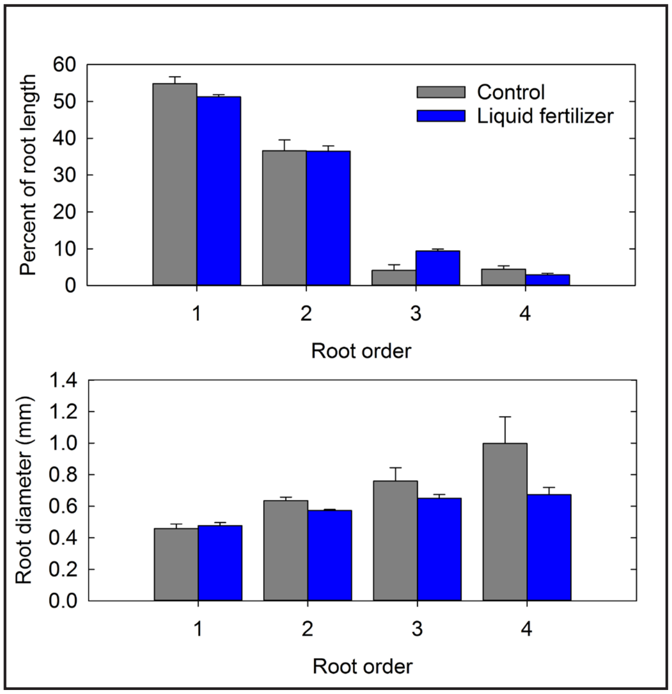

Figure 6. Proportion of total root length (in percent, top panel) and root diameter (in mm, bottom panel) as a function of root order (1 through 4) for field-grown citrus trees treated with liquid organic fertilizer (blue) or not treated (i.e. control, in grey). Error bars represent standard deviation around the mean.

experiment are pending. Profiling of citrus rhizobiomes revealed an abundance of taxa that might represent species pathogenic to citrus; however, also identified, albeit at much lower numbers, were potentially beneficial microorganisms, including AMFs. These candidates may represent valuable leads for future research efforts that seek to test and compare these strains as root inoculants to improve tree health in the face of biotic and abiotic stresses. We were intrigued by the use of root order as a metric to quantify citrus root health. Future projects could exploit this or other root architecture metrics to assess how they correlate with canopy size or fruit yield, together with data on the microbiota that associate with different root orders, into prediction or management models for tree performance.

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