Probing the phytobiome to advance agriculture

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The Colorado potato beetle had Gary Felton stumped. Felton, an entomologist at Pennsylvania State University, has built his career on revealing how plants defend themselves against voracious insects. Plants often detect chemicals in an insect's oral secretions and respond by producing proteins that wreak havoc on insect digestion and nutrient absorption.

But the Colorado potato beetle was different. Felton found that oral secretions from its larvae actually prevented potato and tomato plants from launching a proper defense. He tested chemical factors in the secretions that might help the beetle foil the plant, but came up short. "Maybe there is something else here that we've totally overlooked," he recalls thinking.

That something else turned out to be bacteria. If he applied antibiotics, the plants could launch a defense and inhibit potato beetle larvae growth (1). Bacteria in the insect's oral secretions were tricking the plants into defending against microbial invaders instead of insect ones. Kill the bacteria and the cover is blown.

Myriad factors affect crop health, such as genetics, insects, microbes, weather, soil nutrients, weeds, fertilizer, tilling. Until recently, scientists typically studied one variable at a time, says plant pathologist Jan Leach of Colorado State University. "When a plant is sitting in the field, it's not just exposed to one pathogen, one temperature, one insect. It's exposed to everything at once," says Leach. "If we want to understand how plants

Bacteria in the oral secretions of Colorado potato beetle larvae can trick potato and tomato plants into defending against microbes instead of the insect pest. Image courtesy of Nick Sloff (Pennsylvania State University, State College, PA).

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respond to a single pathogen, we really need to take the whole system into account."

Leach calls this whole system the phytobiome, a term that encompasses plants, the environment they inhabit, and the surrounding community of organisms. Leach attached a name to this concept in 2013 during a meeting hosted by the American Phytopathological Society. The group had brought together scientists to address a looming global food crisis. Among the major challenges afoot: the human population will increase by 2.4 billion by 2050, and with increases in food crop-yields slowing, many researchers fear food demand will soon outpace supply (2). The scientists concluded that for them to optimize crop productivity to meet increasing needs in a sustainable way, researchers could no longer focus on any factor affecting plant health in isolation. They would have to contend with many interrelated factors. They would have to explore the phytobiome.

An Alliance Forms

Following the meeting, researchers developed a Phytobiomes Roadmap, a strategic plan for "acquiring knowledge of what constitutes a healthy, productive, and sustainable agroecosystem and translating that knowledge into powerful new tools in our crop management toolbox" (3). Phytobiome researchers realized a key component of that plan in October 2016 with the launch of the Phytobiomes Alliance.

The Alliance's vision is to empower farmers to choose the best combination of crops and management practices for a specific field in a specific year based on detailed knowledge of the environmental and biological components of phytobiomes, says Kellye Eversole, the Alliance's executive director. "It's like precision agriculture in a precision medicine sense." Some farmers already use precision agricultural technologies to understand the physical components of the phytobiome (4). They might place sensors in soil to monitor moisture levels or conduct soil surveys to track nutrient levels.

Eversole would like to see more biological data as part of the equation. Scientists might, for example, design sensors that quantify microbial communities in real-time, or produce cheaper soil and plant sampling kits that can tell farmers which microbes are present. Combined with detailed records of crop yield over space and time, all of this information could help farmers predict which crops do best under which abiotic and biotic conditions, and how many plants a given plot of land can support.

The Phytobiomes Alliance includes academics, small agricultural start-ups, and large companies like Monsanto and Bayer that have all agreed to collaborate in what is known as a precompetitive space, an agreement in which industry players recognize the value of putting aside intellectual property interests to advance a fledgling idea. "It means that industry agrees that we have a specific need that individually we cannot achieve," says Magalie Guilhabert, head of Biologics Crop Efficiency and Seed Growth Indication in the Crops Science division of Bayer. In principle, industry and academic partners share data, results, and ideas.

Agricultural companies are already developing products that hinge on understanding the inner workings of the phytobiome. Bayer, for example, offers BioAct, a fungal product that infects the eggs and larvae of a broad range of parasitic nematodes that attack crop roots. Indigo Agriculture, a Boston-based agricultural start-up, is developing methods to coat seeds with beneficial microbes that improve crop growth.

Piecing Together the Parts

Scientists and farmers have long known that many interacting factors affect crop health. But until recently, they didn't have the technological capacity to quantify them or the analytic and computational power to tease them all apart. "We always assumed it would be complex," says plant pathologist Linda Kinkel of the University of Minnesota, who is also the associate editor-in-chief of *Phytobiomes*, a journal launched this year. "With genomics tools, we can begin to put these pieces together in ways we couldn't do 10 or 20 years ago."

Metagenomics, for example, now enables researchers to collect soil samples from the field and identify the suite of bacterial and fungal residents based on gene sequences. "When we could only identify microbes by culturing them in the [laboratory], we didn't know many of them were there," says Kinkel. Using metaproteomics, researchers can also get a sense of how these microbes are responding to their environments. They could, for example, compare how different crops affect the quantities of proteins that microbes produce in response to stress (5).

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>
> **—Linda Kinkel**

Drawing on these technologies, Kinkel and others are diagraming the working parts of the phytobiome. Kinkel studies what she calls the "Russian doll story" of microbes and plants. She wants to understand how the diversity of a plant community affects how an individual plant influences the microbes colonizing that plant's roots. "You can't understand the potential to resist plant pathogens unless you measure all of these layers," she says.

In one study, her laboratory explored the bacterial communities associated with four different prairie plants: when the plants were grown in monoculture versus when they were grown with 3, 7, or 15 other species (6). The researchers found that as plant community richness increased, bacterial diversity actually decreased, as did the proportion of antibiotic-producing bacteria that fend off plant pathogens. So the monoculture encouraged more beneficial bacteria. Kinkel is now looking for ways to encourage these beneficial bacteria without actually relying on monocultures, which can drain

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soil nutrients and leave crops vulnerable to rapid disease spread.

Building on his previous work, Felton recently discovered that the bacterial community inside the guts of Colorado potato beetles is shaped by the plant species on which the larvae feed (7). It turns out that for many plants, including eggplant, the bacterial community can't help the insect evade detection. "There is more specificity involved in the interaction than we initially thought," Felton says.

Leach is teasing apart the complex interactions between plant genetics, pathogen susceptibility, and temperature. "Traditionally, in order to protect plants from disease, plant breeders will introduce single resistance genes that recognize pathogens and mount a defense response in the plant," Leach says. "The problem is that at high temperatures, many of those genes aren't effective." But Leach discovered that one gene in rice makes the crop more resistant to pathogens as temperatures rise (8). For 11 years, she and her team monitored a rice field infected with a bacterial blight. Rice that carried a resistance gene, known as *Xa7*, were protected from disease more effectively in warmer months. It's important to identify how resistance genes respond to temperature so that we can develop crops that maintain resistance in a warming climate, says Leach.

Power in a Name

Many challenges remain. Researchers need better molecular techniques for identifying viruses and fungal species. They need standardized sampling techniques so they can compare results across studies. And researchers need a better sense of how often they should sample organisms and environmental parameters to capture a changing phytobiome.

Even selecting which types of data to collect is challenging. "Do we really need to know the soil particle size to understand how a microbe signal impacts plant health and productivity at high versus low temperatures?" asks Leach. "Maybe that dataset does not significantly influence or is not useful to train a model. But we don't yet know."

Kinkel, Felton, and others say that having the name "phytobiome" makes interdisciplinary work toward tackling these challenges possible. Previously, Kinkel could always call an atmospheric or computational biologist for input. "But now we have an integrative framework where we can articulate our goal in a way that is more understandable." Felton says he's invited to meetings in disciplines that he wouldn't have been before. "It has opened my eyes to possibilities."

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