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HOW CUCUMBER ROOTS CALL THEIR BODYGUARDS WITH ODOR SIGNALS

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Did you know that plants use smells to communicate? Some plants even use odors to send important information to other organisms in their environment. Scientists are now starting to understand how plants use odors underground, too. In this study, we discovered that the roots of cucumber plants release odors into the soil that change the way insects, and natural enemies of those insects, find their food. When below-ground caterpillars eat cucumber roots, the roots release specific smells into the soil that repel other insects. These same odors attract tiny insect-killing worms called nematodes. In a way, the plants recruit "bodyguards" to kill insects that are eating their roots and to prevent more caterpillars from eating them.

PLANTS, INSECT HERBIVORES, AND THEIR INTERACTIONS

Plants are important for many organisms, including insects. Insects that eat plants are called **herbivores**, and eating plants gives them energy to do everyday tasks, like reproducing and moving around to find other plants to feed upon. Insect herbivores use odors to locate their food, just as you might run to the kitchen to investigate when you smell your favorite desert baking in the oven. Since plants cannot run away from their enemies like some organisms can, they must defend themselves. The most obvious form of plant defense are thorns, which physically defend the plant. A second form of defense is the production of **plant odors**. After an insect herbivore damages a plant, plants often produce smelly odors that affect herbivore **foraging behavior** (To learn even more about how plants protect themselves, see this Frontiers for Young Minds article). Just as you might avoid someone who smells like they just came from gym class, insect herbivores also avoid plants that smell bad. These smelly odors can also have other effects. Scientists have found that aphids feeding on plants cause plants to attract "bodyguards" or predators that eat insect herbivores [1]. These predators, called **natural enemies** of insects, use plant odors to locate and hunt their prey [2]. Scientists have recently discovered that plant odors are also used below ground! The odors attract natural enemies to defend plant roots, where we cannot see insect herbivores [2, 3].

HERBIVORES

Organisms that feed on plants.

PLANT ODORS

Compounds produced by plants that have "smells".

FORAGING BEHAVIOR

Searching for food resources in the environment.

NATURAL ENEMIES

Organisms, like nematodes, that kill insects in their natural environment.

Scientists do not know if all plants use odors below ground or if just a few special plants can attract natural enemies in soil. In our work, we chose to use cucumber plants because their roots are very fragrant—if you remove cucumber roots from the soil and smell them, they have a strong odor. Cucumber roots are eaten by striped cucumber beetle larvae (Figure 1). However, cucumber plants still survive, because **insect-killing nematodes** in the soil kill striped cucumber beetles. In our study we had two hypotheses. First, we hypothesized that cucumber root odors produced after insect herbivores because root damage will change the overall odor of the cucumber roots. Second, we hypothesized that this change in root odors will change how cucumber beetle larvae and nematodes forage for resources. So, our overall starting hypothesis was that plant odors produced by roots damaged by insect herbivores would be stronger than odors produced by undamaged plants, and would thus attract the insect-killing nematodes. We also hypothesized that herbivores looking for a plant to eat would avoid these damaged plants, just as a person might avoid an area where they knew a lion was running loose.

INSECT-KILLING NEMATODES

A parasitic soil-dwelling roundworm that kills insects found in the soil.

DO CUCUMBER ODORS CHANGE AFTER INSECT HERBIVORY?

Before we could test our hypothesis, we had to see if cucumber odors change after herbivore insects eat them. To do this, we collected

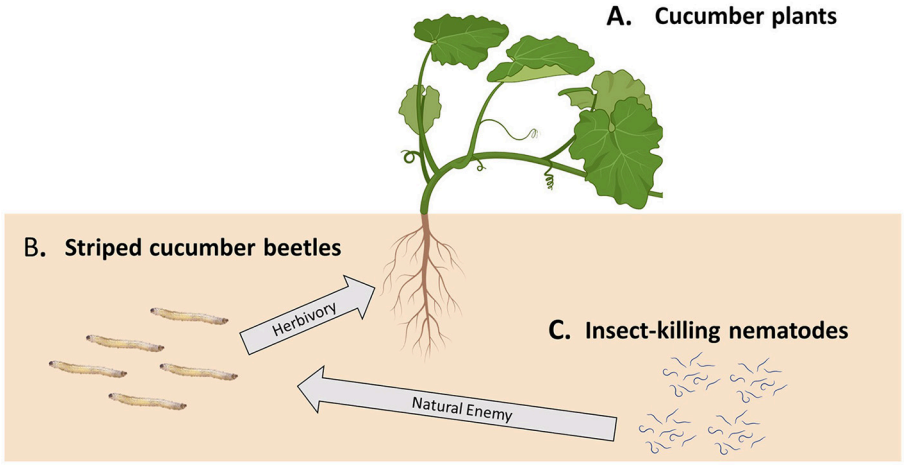

Figure 1

In our study, we used **(A)** cucumber plants, which are an important crop, **(B)** striped cucumber beetles, which are a major pest of cucumber plants, and **(C)** insect-killing nematodes, which are natural enemies of root-feeding herbivores like striped cucumber beetles.

root odors from plants that were eaten by insect herbivores and from plants that were not eaten by any insects. We used a special laboratory technique called **gas chromatography with mass spectrometry** to analyze the odor samples we collected. In short, this instrument tells us which chemicals make up the plant odors and how much of each chemical is present. Imagine a racetrack where different cars (molecules) travel at different speeds based on their size and weight. Gas chromatography separates the "cars" (odor molecules) by speed, while mass spectrometry weighs each "car" to identify what it is. Using this information, we can describe how smelly the roots are. We found that roots eaten by herbivore insects were much smellier than the roots of undamaged plants.

GAS CHROMATOGRAPHY WITH MASS SPECTROMETRY

A laboratory technique that uses the mass of molecules and the speed at which they can move to identify what kinds of molecules are present in compounds like odors.

DO CUCUMBER ROOT ODORS CHANGE STRIPED CUCUMBER BEETLE LARVAE BEHAVIOR?

Next, we hypothesized that striped cucumber beetle larvae looking for a plant to eat would avoid these damaged plants, just as someone might avoid an area where they knew a lion was set loose.

To test this hypothesis, we needed a way to test herbivore behavior. We decided to use a **two-choice arena,** where the insects could smell the plants and could choose to move toward one of the two plants—an undamaged plant or a damaged plant. The beetle larvae were placed in the middle, between two plants, and had to make their choice based only on smell because they could not touch or see the plant roots. We then counted the larvae in different parts of the arena, after they were given a short amount of time to make a choice and move (Figure 2A). We found that striped cucumber beetle larvae tended to move away from plants that were damaged by other larvae. This means that when larvae can smell plant roots but cannot see or touch them, they avoid insect-damaged roots.

TWO-CHOICE ARENA

An enclosed tube with a healthy plant at one end and a damaged plant at the other. Larvae are placed in the middle of the tube to see which way they move.

Figure 2

(A) We used a two-choice arena to test foraging behavior. One side had a healthy plant and the other side had a plant with roots damaged by herbivores. **(B)** Beetle larvae or nematodes were placed in the middle chamber, where they could smell the plants and move toward the plant they liked better, based on the smell. After some time, we counted the number of insects in each area of the device.

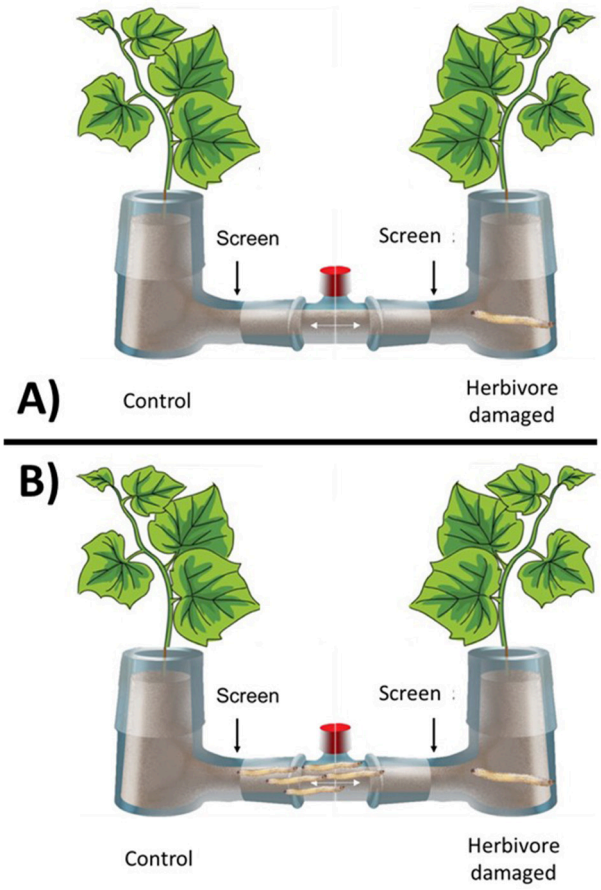

Figure 2

DO CUCUMBER ROOT ODORS CHANGE INSECT-KILLING NEMATODE BEHAVIOR?

Next, we wanted to know if insect-killing nematodes were attracted to the smell of cucumber roots with striped cucumber beetles feeding on them. For this experiment, we used a two-choice arena similar to the one we previously used, where the nematode could only smell the plant roots and had two choices within the arena. After 48 h, the nematodes were counted (Figure 2B). We found that more nematodes were located on the herbivore-damaged side of the arena than on the undamaged side. This tells us that the insect-killing nematodes were attracted to the herbivore-damaged plants.

WHAT DOES THIS ALL MEAN?

When we began this study, we knew about the importance of plant odors aboveground and in other types of plants. We knew that smelly plants tended to repel insects and attract natural enemies. In our study, we found the same thing below ground! We found that insect-damaged roots are smellier than undamaged roots. We also

saw that insect herbivores avoided these smells, while insect-killing nematodes (natural enemies) were attracted to these smells (Figure 3). Overall, while plant roots are not always easily seen, they have important roles—smelly roots protect plants!

Figure 3

When insect herbivores eat plant roots, the odors released attract natural enemies of the herbivores and repel additional root herbivores.

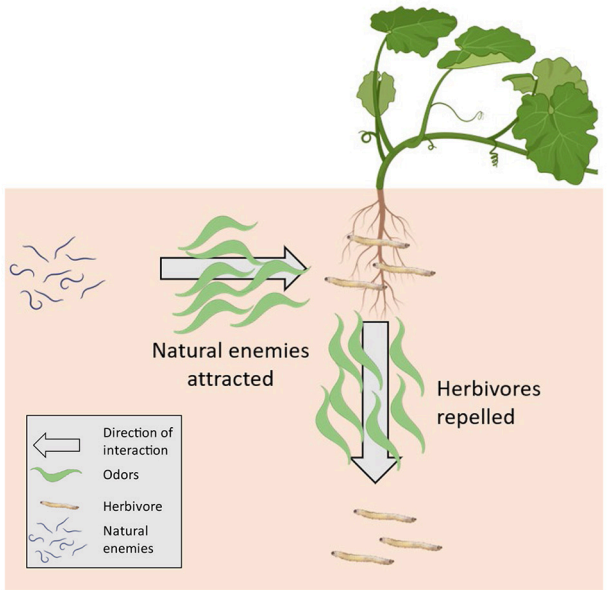

Figure 3

THE FUTURE OF STUDYING ROOT SMELLS

Understanding roots and their odors is a challenge, and many scientists are trying to learn more about their function in the environment. Roots odors might be recognized by the roots of other plants, similar to how insect herbivores and natural enemies recognize those smells. This could allow other plants to prepare for future attack and ready their defenses ahead of time. Also, the interaction of plant odors with microbes like bacteria has still not been studied very much, but it is becoming better known that roots can interact with microbial communities, too. Plant odors might help shape the microbial community as they also often have antimicrobial properties as well. Overall, there is still a lot that we do not know about root smells and their functions, but we are beginning to uncover some of their unique roles within their soil environment.

ORIGINAL SOURCE ARTICLE

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CONFLICT OF INTEREST: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Evan is passionate about discovering things in nature and science. Evan likes soccer and playing with his friends. He is fascinated by buildings and so wants to be an architect in the future. He likes reading. His favorite food is a local dish cooked with sugar beans.

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Liking for electricity and EV Grid Systems. Keen eye on utilization of renewable resources.

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Lina Bernaola is an assistant professor at Texas A&M University. Her research focus is on plant-microbe-insect interactions and she is currently working with rice and the insects attacking this crop. She has been fascinated by science her entire life, but her passion in the world of plants began when she joined the International Potato Center in Lima, Peru. She wants to use research to help develop more cost-effective management programs to fight insect pests.