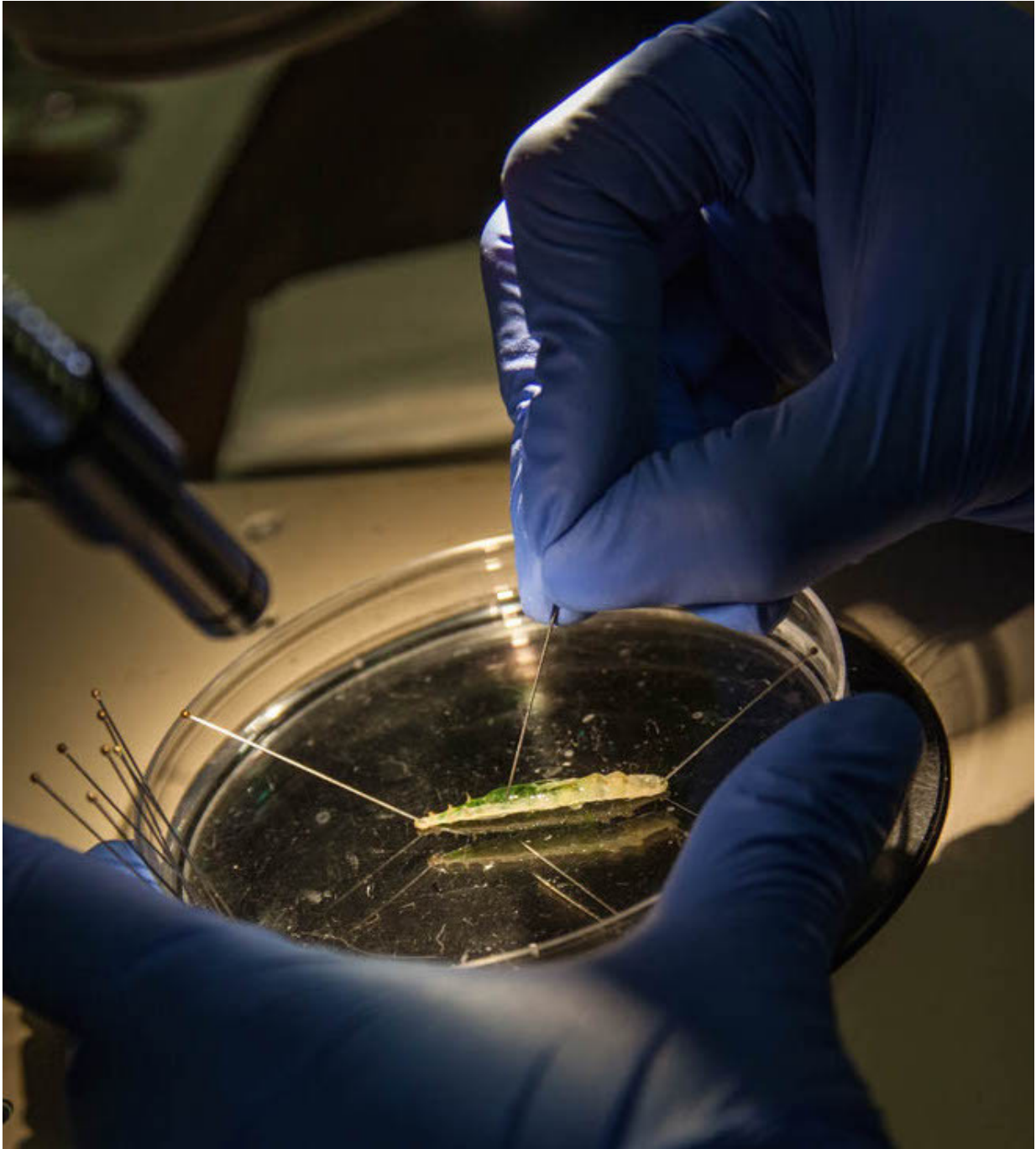


# **Crops vs. caterpillars: Insect spit a key weapon in ongoing agriculture war**

April 5 2016, by Brittany Dodson

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Loren Rivera-Vega pins a cabbage looper caterpillar in preparation for removing its salivary glands. Credit: Brittany Dodson

Next time you chew a stick of mint gum or pop a peppermint candy, think of insects.

That distinctive flavor comes from essential oils the mint plant makes to [defend itself](#) against hungry insects. Strong flavors and smells of other plants, such as basil and cabbage, are also plant defense compounds. These weapons halt insect feeding in many ways. Plant compounds can taste or smell bad, fortify cell walls so insects can't penetrate a leaf to feed, or affect digestion, eventually killing the attackers. But insects aren't helpless against these [plant defenses](#). They find ways to fight back—and one of their best weapons is their spit.

"It's all chemistry," says Loren Rivera-Vega, a doctoral candidate in entomology at Penn State. Plants may recognize physical damage caused by insect feeding and chemicals in insect feces or spit. "But some proteins in insect spit can suppress plant defenses instead of triggering them," she says. "In a way, the plant is fooled."

## Chemical warfare

Insects capable of this trickery are most often specialists that eat one type of plant. Generalists – insects that have broader tastes and eat many different plants – are usually more sensitive to plant defenses than specialists. "It's difficult for an insect to eat multiple plant species because different plants have different defenses," Rivera-Vega says. Yet some of our worst agricultural pests are generalists. Rivera-Vega studies one of them, the caterpillar of the [cabbage looper moth](#), *Trichoplusia ni*. Despite their name, these caterpillars can eat and damage more than forty vegetable crops and ornamental plants. Rivera-Vega wants to find a new way to control them. "If you understand how the plant responds to the insect and how the insect avoids defenses, you can try to manipulate that to your advantage," she says. "My question is, how can the cabbage looper eat so many different plants and still survive?"



Caterpillar of the cabbage looper (*Trichoplusia ni*), one of the most destructive pests on agricultural crops in the U.S. Credit: Brittany Dodson

She thinks the secret may be in their spit. She's studying how the spit changes when cabbage loopers feed on different plants. To set up an experiment, Rivera-Vega fills a growth chamber with trays of tiny plastic cups containing two or three newly hatched cabbage looper caterpillars each. She makes baby caterpillar food for them by blending pinto beans, brewer's yeast, and other nutrients. Her caterpillars eat this artificial diet for 10 to 12 days, until they're big enough for the experiment. So far, Rivera-Vega has tested them on cabbage and tomato plants. These crops are easy to grow in the lab, and they contain different defense chemicals that affect cabbage loopers in different ways. The caterpillars are

healthiest eating cabbage, their preferred food source. They survive eating tomato, but grow more slowly. She explains, "It's a nice comparison between a host where they do really well versus a host where they don't do as well."

Once the caterpillars are big enough, Rivera-Vega takes them to the lab greenhouse where her cabbage and tomato plants are growing. She can't haphazardly release the caterpillars -- if she did, they'd all head for the cabbages. To prevent runaways and keep each caterpillar eating its prescribed diet, she clips small homemade cages onto the leaves and places a single caterpillar in each cage. She lets the insects feed for two to three days and then harvests both the leaves and the caterpillars.

## **Caterpillar innards**



Cabbage loopers usually stay on the undersides of leaves. Credit: Brittany Dodson

She grinds up the leaves and does chemical assays on them to look for defense compounds. Her results with cabbage are unclear, so she is repeating the experiment. But she found that her loopers did suppress the production of defense compounds by tomato plants. To find out whether something special in the looper spit interfered with the plant response, she has to analyze the spit—and to do that, she first has to collect it.

Accumulating enough spit from caterpillars that are less than an inch long is no easy task, so instead, Rivera-Vega collects whole salivary glands. She pins both ends of a caterpillar to a padded dissecting dish, and then, with a tiny set of scissors, she cuts the caterpillar open from end to end.

"Pffft," she says, puffing out her cheeks and her stomach. "And the gut just pops out because it's big and so full of food." Then she uses forceps to pluck out the two long translucent salivary glands, places them on another dish, and applies a salt solution to draw out the compounds inside for identification with molecular techniques.



Cabbage loopers eat about three times their own weight each day. This photo shows the damage done to a cabbage leaf by one looper in just 6 hours. Credit: Brittany Dodson

Though dissecting hundreds of these tiny caterpillars might seem tedious, Rivera-Vega enjoys the process. She has dissected many insects for class and previous research, including larval flies smaller than a grain of rice. "I hope I don't sound psycho," she laughs. "It's really cool when you open them up and you see all this stuff that's going on inside."

## **Nixing nicotine**

Rivera-Vega identified 400 proteins in cabbage looper salivary glands that have known functions in other insects, including some that make the insects invisible to plant defenses. For instance, the loopers make enzymes similar to those used by another generalist, the [corn earworm](#). The earworm spits glucose oxidase on tobacco plants to prevent deployment of the fatal toxin, nicotine. This similarity between spit enzymes that work against tomato and tobacco plant defenses is not surprising. Both plants belong to the same family and have similar defense chemicals—including nicotine.





Caterpillars are raised in small plastic cups until they're big enough for experiments on crops. Credit: Brittany Dodson

She also found that loopers make another type of protein that battles plant defenses in a different way. It breaks down defense chemicals when the insect ingests leaves, rendering them harmless. In Rivera-Vega's favorite example of this type of counter-attack, the tobacco hornworm regularly eats tobacco plants by using detoxifying gut proteins to break apart and excrete nicotine. The detoxifying proteins are so effective that the hornworm can safely eat loads of nicotine—if humans chowed down on as much nicotine for our size, [it would kill us](#). Much is known about these proteins in the guts and spit of some insects, but not in caterpillar spit.

Rivera-Vega discovered that cabbage looper spit does change depending on the type of plant the insects are eating. Caterpillars on both crops had the same spit proteins, but levels of the proteins were different.

"Defenses of cabbage and tomato are very different, so dealing with those defenses is not necessarily the same," she says. "The insect would need different amounts of proteins to deal with them." Other caterpillars, like the tobacco hornworm, are able to increase levels of particular proteins in response to changes in plant chemical doses. Rivera-Vega thinks the cabbage looper may increase production of certain proteins depending on which plant chemical it's facing.

## **Next steps**



Loren Rivera-Vega attaches a caterpillar cage to a cabbage leaf. Credit: Brittany Dodson

There are more experiments to be done. "So far, I've been focusing on the insect," says Rivera-Vega. "Now I need to look at the plant and see if the interaction is really the way I think it is." She will test whether spit proteins she identified truly combat tomato and cabbage plant defenses. She plans to do that in two ways. To see if a specific protein fools the plant, she will first damage a leaf with an embosser tool to simulate the

physical damage done by a chewing insect. Second, she will treat both injured and uninjured leaves with the protein and then measure levels of plant defense compounds. To see if a specific protein detoxifies plant compounds after ingestion, Rivera-Vega can block production of the spit protein before putting the caterpillar on the plant. If the missing protein is a detoxifier, the caterpillar would be susceptible to toxic defense compounds and would grow more slowly than usual or even die.

Rivera-Vega will study her caterpillar spit with other crops as well. "The idea was to do the big analysis on two crops. Now I can specifically study key proteins and test those in a broader range of crops," she says. By understanding how insects feed on many crop types, scientists may find new ways to stop them. Through natural breeding and genetic engineering, many crops have already been developed to better defend themselves against [insects](#). Rivera-Vega comments, "The future of research is to understand the basics so we can find tools we haven't even thought about. But research is still behind with respect to caterpillar spit and how it helps the insect feed on [plants](#)."

Turns out, there's a lot left to learn about caterpillar spit.



Closeup of a caterpillar cage attached to a cabbage leaf. Credit: Brittany Dodson

Provided by Pennsylvania State University

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