World's cocoa crop could get a big boost from a simple, non-toxic spray

July 2 2014, by Seth Palmer

Cocoa farmers this year will lose an estimated 30 to 40 percent of their crop to pests and disease, and with chocolate prices having risen globally by roughly two-thirds in the past decade, concern is growing about sustainability in cocoa production. Of particular concern are the environmental impact and human health risks of toxic agrichemicals – organochloride insecticides and heavy-metal-based fungicides – used in cocoa production to fight pests and disease.

But scientists at Penn State's Huck Institutes of the Life Sciences have found – in a safe, biodegradable compound – a potential alternative to the hazardous antifungal agents currently being used to combat one of the most damaging cacao diseases, Phytophthora pod rot (also known as Black Pod), responsible for an estimated 20 to 30 percent loss in yield annually.

Mark Guiltinan and Yufan Zhang, with Siela Maximova and in collaboration with Phil Smith of the Metabolomics Core Facility, have discovered that spraying the leaves of the Theobroma cacao tree with a low-concentration glycerol solution triggers the plant's defense response and enhances its natural disease resistance.

"Right now," says Guiltinan, "cocoa farmers are using fungicides and other chemicals that are very effective but are also highly toxic compounds, very persistent in the soil, and relatively expensive. Glycerol, on the other hand, is extremely non-toxic; it's super safe, super cheap, biodegradable, and it triggers the plants' defenses very efficiently – it only takes small amounts to trigger the whole plant defense system.

"The plant immune system," he continues, "is made up of many different components that – imagine, if you can – are like little micromachines. It has five or ten major components that all have little safeties on them, and safeties on the safeties, and things that turn them on and off and regulate them. Some of these components are always running at some level and the system is a complicated thing on a hair trigger, always ready to go. You just give it a little trip and off it goes – all the little micromachines will be activated in a certain sequence and the whole thing takes off, so glycerol is one of the ways we've found to come in and trigger this to happen."

Glycerol, a simple sugar-alcohol compound called a polyol, is a colorless, odorless, viscous liquid commonly used in soaps and other cosmetic

products and is produced in different ways, including as a byproduct of biofuel production where it is removed from plant or animal fats in a process known as transesterification.

"When you make biodiesel," Guiltinan says, "you end up with a massive amount of glycerol that nobody really has a good use for, and it's super cheap because of that."

Zhang adds that the production of glycerol from biofuels "is projected to increase ten-fold in the next ten years, as high as six times the projected demand, and people are already generating excessive amounts of glycerol that they don't know what to do with. There are journals focusing specifically on the use of glycerol and other biodiesel products, and research is being done on all different kinds of byproducts from the biodiesel industry to find out what how these compounds could be used."

Testing a hypothesis

"At the very beginning," says Zhang, "we weren't focusing on glycerol's effects on the cacao plant's defenses. We were more focused on its effects related to fatty acid biosynthesis – but digging deeper, we found that glycerol also has an effect triggering the plant's defense response."

Yufan Zhang, center, discusses data with Mark Guiltinan and Siela Maximova. Credit: Seth Palmer

The Guiltinan Lab studies two main aspects of the cacao tree: one has to do with the quality of the cocoa solids and fats being produced in the cacao seed, and the other is focused on disease resistance in the plant.

Zhang was working on a project to better understand what genes are implicated in the quality of fats in the cocoa bean, so it was "somewhat serendipitous," says Guiltinan, that he discovered a paper describing a potential intersection with disease resistance: in some model plant species, certain types of glycerol-related fatty acids triggered the plants' key defense mechanisms. Zhang decided to test this on the cacao tree.

"What we found," says Zhang, "is that a low (100 mM) concentration of glycerol sprayed on the cacao tree's leaves is sufficient to trigger its defense response. The concentration you use to spray the plants is really important because higher concentrations have a really bad effect, causing cell death and lesions on the leaf tips."

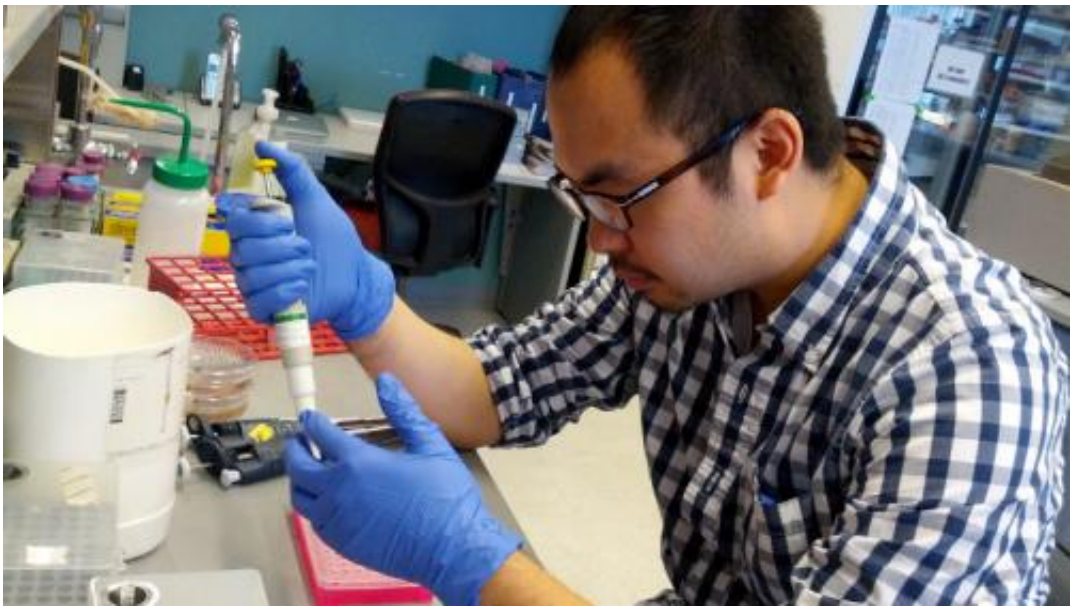
Normally plants wouldn't encounter glycerol being sprayed on them "It's an exogenous chemical that we're adding," Guiltinan explains, "but it's a pretty small molecule that can go through the cell wall and membranes and get into the cell body."

Zhang notes that all the leaf surfaces will absorb things – pesticides, micronutrients, other chemicals – and the plant's tissues will assimilate them. "We just dissolve the glycerol in water and add a little surfactant to break the cell extension on the leaf surface and make it easier to move the glycerol molecule into the cell," he says.

Collaboration

Because Zhang and the other researchers in the Guiltinan Lab work mainly with molecular biology techniques, they needed additional expertise in analytical chemistry in order to be able to assess glycerol's action in the cacao plant, and so they reached out to their long-time collaborator Phil Smith, co-director of the Metabolomics Core Facility.

"We've been doing things with Phil for many years on different projects," says Guiltinan. "For this study, we needed to be able to detect and quantify specific individual molecules of interest in a big mixture of all kinds of molecules, which is pretty hard to do."



Yufan Zhang prepares samples at a bench in the Guiltinan Lab. Credit: Seth Palmer

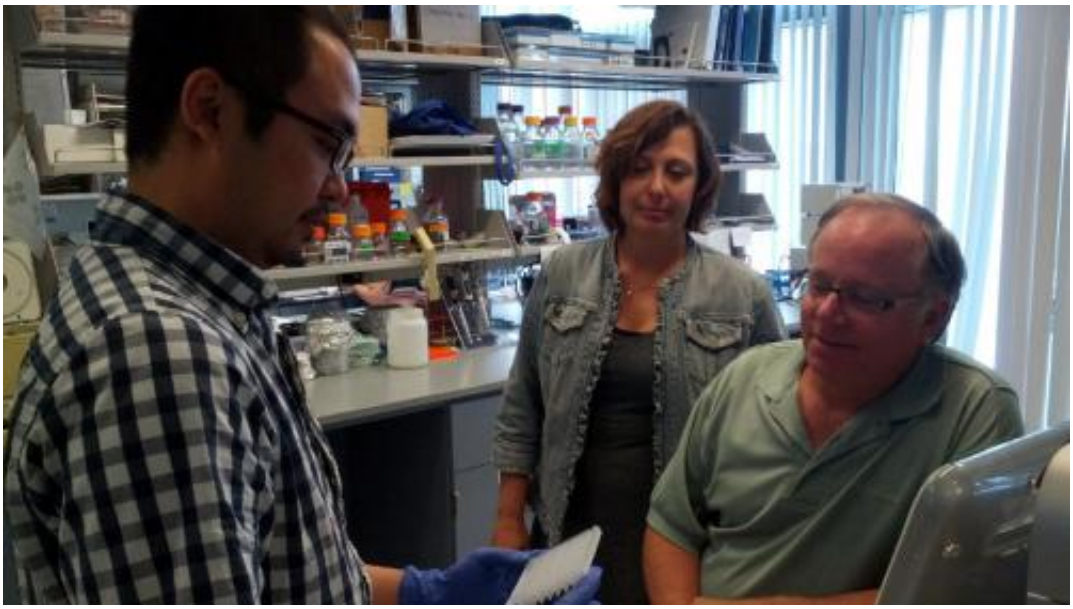
When leaves are sprayed or soaked with the glycerol solution, "you want to see whether the glycerol really gets into the cells and what kind of effect it has in the cellular environment," Zhang says. "We know that when the glycerol does penetrate into the leaf cells, it converts to a bioactive form known as glycerol 3-phosphate (G3P) which then reacts with a fatty acid species called oleic acid and changes the fatty acid profile of the cells. So we worked with Phil, using mass spectrometry to detect the concentration of G3P in the cells as well as the changes in the fatty acid profile."

Analyzing these data, Zhang was able to determine a concentration of glycerol solution sufficient to trigger the plant's defense response without causing adverse effects such as leaf burn.

"Basically," adds Guiltinan, "Phil and his machines allowed us to detect and separate and measure the amount of these molecules, which is the

most important thing – to be able to quantify them; it's not easy to do, and frankly, we couldn't do it without the facility and the equipment and – most importantly – Phil's know-how."

Potential impact



Yufan Zhang, Siela Maximova, and Mark Guiltinan discuss sample assays in the lab. Credit: Seth Palmer

As the Lab prepares to take its discovery to the field for trials, Guiltinan is hopeful that cocoa farmers will adopt the use of glycerol in their operations.

"Glycerol is really cheap," he says, "and it's going to get even cheaper the more biofuels are made. It's safe, biodegradable, and if it can yield good results in the field, then we'll have something really useful.

Phil Smith puts the final touches on a data analysis at the Metabolomics Core Facility. Credit: Mark Selders

Many cocoa farmers don't have access to agrichemicals or can't afford them. The only other alternative these farmers have is genetics – breeding for resistant strains – which is ongoing but slow. "There are tropical biofuel crops that can be grown with cocoa," Guiltinan says, "so theoretically a farmer could cultivate a small plot and make his own glycerol. With the price of cocoa going up, maybe some of these farmers will eventually be able to invest more in other chemicals, but I think it's a great idea to start promoting safer ones like glycerol as a better, more affordable alternative so maybe farmers will begin to move in that direction. That's our hope."

More information: "Applying Glycerol as a Foliar Spray Activates the Defense Response and Enhances Disease Resistance of Theobroma cacao." Zhang Y, Smith P, Maximova SN, Guiltinan MJ. *Mol Plant Pathol*. 2014 May 27. DOI: 10.1111/mpp.12158 . [Epub ahead of print] PMID: 24863347 [PubMed - as supplied by publisher]

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