New mechanism of action found for agricultural pesticide fludioxonil

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A fungicide commonly used by the agricultural industry to protect grains, fruit and vegetables from mold damage seems to kill fungi by a previously uncharacterized mechanism that delivers a metabolic shock to

cells, new research finds.

The fungicide, fludioxonil, was originally devised to protect seeds during storage but was so effective at limiting mold damage that it is now widely used to treat produce after harvest to extend its shelf life. While scientists have long known that a protein unique to fungi is required for fludioxonil to kill fungal cells, the exact mechanism by which fludioxonil works has remained unclear.

New work by researchers at the University of Wisconsin-Madison shows that fungi exposed to fludioxonil experience a spike in the concentration of a reactive stress molecule that triggers a biochemical cascade in fungi that leads to cell death.

The researchers created mutant strains of yeast resistant to the fungicide, which provided insight into how fungi sense the damage caused by fludioxonil and commit themselves to a metabolic pathway from which they cannot recover. Understanding this mechanism can help researchers assess the role fludioxonil plays in the agricultural system and may better illuminate how drugs kill fungal pathogens and how fungi develop resistance to anti-fungal chemicals.

The research is published March 25 in the journal *Scientific Reports*. The work was led by Tristan Brandhorst and Iain Kean in the lab of Bruce Klein, a professor of pediatrics, internal medicine and medical microbiology and immunology at UW-Madison and the UW School of Medicine and Public Health.

Since its introduction in 1993 by the progenitor of the agrochemical company Syngenta, fludioxonil was believed to directly target a protein in fungal cells known as a hybrid histidine kinase, or HHK for short. Syngenta hypothesized that fludioxonil bound directly to HHK in order to activate a biochemical pathway that causes fungal cells to

inadvertently kill themselves.

"HHK is a little bit unusual in that it's highly conserved throughout the fungal kingdom, and it's not present in humans," says Klein. "It also offered an opportunity for clarifying a drug target that might be selective for fungal microbes and therefore not toxic in humans."

So Klein's lab set out to understand how fludioxonil attacked HHK. But in 2016, they reported that, although fludioxonil requires the HHK protein to kill fungi, the pesticide and protein do not directly interact, leaving fludioxonil's true mechanism of action up in the air. In the current study, the researchers resolved to test alternative possibilities for how fludioxonil works.

They found that fludioxonil caused a form of cellular stress in fungi called oxidative stress. Oxidative stress is fairly common and is caused by a combination of the oxygen in the air and cell damage from stressors like ultraviolet light. The researchers speculated that HHK acted as a sensor that was triggered by oxidative stress to promote cell death.

Surprisingly, when Klein's team exposed fungi to various forms of oxidative stress, cells remained healthy. Clearly, while the pesticide produced oxidative stress, this stress alone wasn't enough to trigger cell death through HHK.

While looking for evidence of oxidative stress damage, Kean supplied the fungal cells with dimedone, a chemical that can alleviate a different type of cellular stress called aldehydic stress. Aldehydic stress is caused by aldehydes, such as the preservative formaldehyde, which are highly reactive. With dimedone around to suppress aldehydic stress, the fungi became resistant to fludioxonil, suggesting that aldehydic stress might be the missing link between fludioxonil and HHK.

Looking for aldehydes triggered by fludioxonil, the researchers identified a spike in the aldehyde methylglyoxal, a particularly reactive chemical that can damage both DNA and cellular proteins. Methylglyoxal is normally formed in small amounts as cells break down sugars, but cells possess catalytic enzymes to break it down before it becomes a problem. Klein's lab found that fludioxonil inhibited one of the enzymes involved in metabolizing sugars in a way that causes it to release extra methylglyoxal, which in turn activates the lethal HHK cascade.

"The take home lesson is that fludioxonil is multifactorial. It's not compromising cells by one solitary mechanism. It has potential to damage cells in a variety of ways," says Brandhorst. "And aldehydic stress is particularly problematic because its damage is hard to detect."

During the study, the researchers modified HHK to make it resistant to fludioxonil. HHK has several sulfur-containing amino acids within its protein structure, and these amino acids can use the sensitive sulfur atoms to sense and respond to environmental conditions like aldehydes. When the researchers removed the sulfur, HHK no longer responded to fludioxonil and the cells became resistant, pointing to the importance of these sensitive sulfur atoms in the detection of the aldehydes induced by fludioxonil.

The researchers note that the ability of fludioxonil to act on a sugar-metabolizing enzyme common to all cells, and to produce the damaging compound methylglyoxal, may mean that the pesticide has more potential to harm non-fungal cells than previously thought. Although fludioxonil has been deemed safe for use, the authors of the current study suggest that the effects this widely used pesticide has upon animals be re-examined.

"There's more to be studied here," says Klein.

Provided by University of Wisconsin-Madison