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RESEARCH

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The infection process and activation of disease resistance in turfgrasses

Compounds that help plants activate their natural defense systems can promote plant health.

Plants possess many disease-resistance mechanisms that are triggered following pathogen attack. Under plant maintenance systems such as intensively managed turfgrass, plants can become highly stressed, and their resistance mechanisms may be insufficient to guard against disease outbreaks without significant loss. However, certain compounds can be applied to pre-trigger resistance responses against plant pathogens. These compounds generally have little or no direct activity against fungal pathogens, but they can activate a plant's defense mechanisms before pathogen attack, resulting in greater subsequent resistance.

Induced defense mechanisms

Induced (also known as *acquired* or *activated*) *resistance* is a physiological state in which environmental, chemical or biological stimuli pre-trigger a part of the plant's defenses against ensuing pathogen attack. During induced resistance, a plant defense activator is recognized by the plant, which triggers the recognition-signaling pathway. The signaling pathway(s) eventually promotes the expression of defense genes that result in the expression of defense mechanisms, such as antimicrobial proteins. The enhanced resistance is expressed locally at the site of infection, and in some cases, systemically throughout the plant.

These types of activated defense mechanisms are considered nonspecific (that is, not directed against a particular pest or disease) and are associated with ancient responses in plants against a variety of stresses, including abiotic stresses such as drought and heat, as well as biotic stresses such as diseases and insect pests. To date, we know of two general mechanisms by which disease resistance is induced in plants, and these are called "ISR" and "SAR" (but we'll get to those later).

Pathogen plan of attack

Figure 1. A pathogen (ninja mushroom) contemplates how to get into a plant (castle wall). Illustrations courtesy of Tom Hsiang

How do pathogens infect plants?

Let's review the process by which attacking agents (pathogens) infect plants. The first barrier the invading organism faces is the outer wall of a plant, coated by a waxy layer called the cuticle. This is what makes plants shiny. Each pathogen has its own method of attempting to break through a plant's outer defenses (Figure 1). Some pathogens attempt to sneak in through natural openings such as stomates (Figure 2). Others, such as the anthracnose fungus, try to bash their way in

Sneaking into a plant

Figure 2. A pathogen sneaks into a plant through natural openings or wounds.

Forced entry

Figure 3. A pathogen bashes its way into a plant.

Escaping plant defenses

Figure 4. A pathogen escapes the defense chemicals of a plant cell and grows into neighboring plant cells.

with the use of specialized penetration structures (Figure 3).

When a pathogen attacks a plant — by seeking entry into the plant or into plant cells to feed on them — a plant cell will perceive that it is under siege and respond with a barrage of defensive chemicals. The critical part of plant defense is how quickly the cell is able to detect the presence of the pathogen and respond to that pathogen. If the response is too slow, then the pathogen is able to escape the counterattack and grow beyond the defenses of the first invaded cells, and then progress into adjacent cells (Figure 4). However, if the response is rapid, the cell may be able to fight off the attack. One plant defense strategy is to sacrifice an infected cell (Figure 5) and, in doing so, attempt to kill the invader (Figure 6). Activated resistance is a tactic that combats plant disease by enhancing the ability of the plant cell to fight back more quickly. This is the basis of a new class of substances called *plant defense activators.*

Research

Background

Some earlier research revealed that salicylic acid, which is the active ingredient in aspirin, can trigger defense responses in plants. This chemical can lead to the accumulation of defense chemicals in plants that are known as pathogenesis-related proteins, which are involved in disease resistance. However, direct application of salicylic acid to plants resulted in phytotoxicity, and this led researchers to develop chemicals that show effects against disease but are less dangerous to the plant. One of these is called acibenzolar-S-methyl, also known as BTH, and it is the active ingredient in Actigard (Syngenta). Earlier research has examined the effects of BTH against dollar spot disease in turf (4). Another group of chemicals that are suspected of inducing disease resistance in plants are the phosphonate compounds, and an informative review for turf managers can be found in the literature (3). We have been investigating the activity and mode of action of several new plant defense-activating compounds on turfgrasses, and present here a summary of some of our research findings, using Civitas as an example.

Examining plant disease resistance with Civitas

Over the past decade, we have been testing a compound called Civitas (Petro-Canada). It is a mixture of food-grade synthetic isopar-

affins and a food-grade emulsifier. At room temperature, it is a clear, colorless liquid composed of molecules with 16 to 36 carbons. The formulation has been registered in the United States (February 2009) and Canada (January 2011) and is being investigated in other parts of the world. Label rates are 1.2% to 25% in 0.98 to 4.9 gallons of water per 1,000 square feet (4 to 20 liters/100 square meters) of grass surface.

One of the features of a resistance-inducing compound is that it should have a weak or no direct effect on the pathogen. We tested 16 different turfgrass pathogens on culture media amended with 0% to 20% Civitas and found that there was only slight inhibition even at the highest rate. (Note that for field applications of Civitas, the label rate is only 5%.) However, even this inhibition disappeared after 10 days, as growth rates after that time were similar between media with and without Civitas.

Civitas was sprayed on turfgrass in the field and was found to have significant activity against various turfgrass diseases, with almost full disease suppression in some cases (Figure 7). We also conducted tests on turfgrass grown in the laboratory in plastic containers, and found that, even when the compound was directly applied to soil without contacting the leaves, the leaves became more resistant to several fungal pathogens (Figure 8) and thus the diseased area could be reduced by 20% to 40% (1). This activity gave us the idea that the compound was activating a form of systemic resistance in the plant.

The next step was to figure out how this resistance was being induced. As mentioned previously, there are two general mechanisms by which disease resistance is known to be induced in plants, ISR (Induced Systemic Resistance) and SAR (Systemically Acquired Resistance). There are probably other mechanisms, but these two are the best characterized at this point. They have not been well characterized in turfgrass plants, however. In other types of plants, particular genes are known to be associated with these pathways.

We looked for these genes, which were first found in grass plants such as rice or wheat, in creeping bentgrass (*Agrostis stolonifera*). After quite a bit of searching, we found possible counterparts (the scientific term is "homologs") to these genes in creeping bentgrass. We used molecular biology techniques to assess whether these genes were producing

Self-destruction of a plant cell

Figure 5. A plant cell destroys itself in an attempt to kill the invading pathogen.

Fighting off the invader

Figure 6. A plant can successfully fight off an attacking pathogen, but sometimes must sacrifice some plant cells.

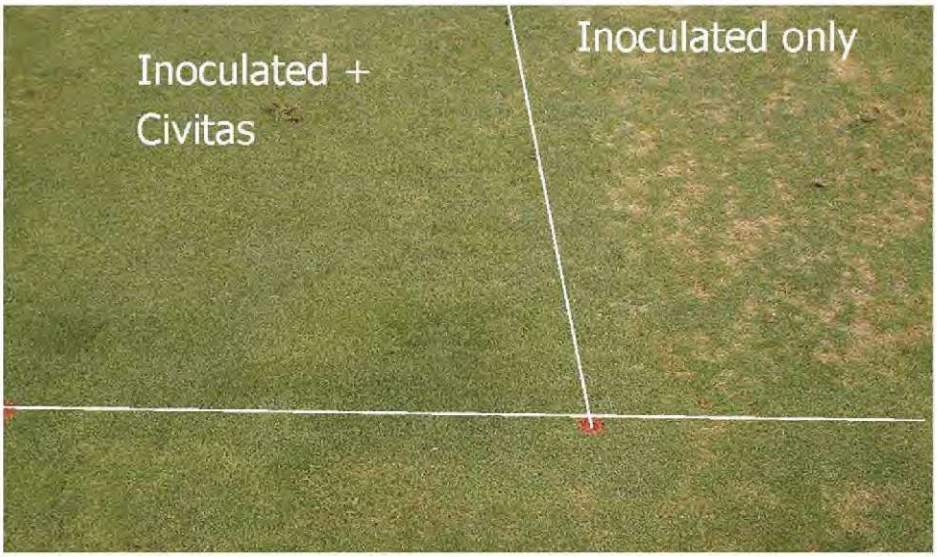

Figure 7. Field trial with 5% Civitas applied in water at 2.45 gallons/1,000 square feet (10 liters/100 square meters). The plots were inoculated with dollar spot fungus in July. This photo was taken a month later. Photos by Tom Hsiang

Figure 8. Civitas was applied to the soil of pots containing 2-week-old creeping bentgrass plants. The soil was inoculated with dollar spot fungus seven days later. This photo, taken five days after the inoculation, shows much more fungal activity and disease in the inoculated non-treated control.

proteins, particularly after they had received applications of known and previously characterized defense activators that caused ISR or SAR. By carefully examining the patterns of ribonucleic acid (RNA) production following Civitas application and then fungal pathogen inoculation, we concluded that Civitas shows effects on expression of genes similar to effects caused by ISR compounds.

Conclusions

Our conclusions on the activity of the defense activator Civitas are as follows: (i) It has minor direct effects on fungi; (ii) it has suppressive effects against diseases in the lab and field; (iii) it primes plant defense response genes for greater and faster expression after infection; and (iv) its mode of action is ISR, based on gene expression analysis when compared with gene expression after application of a known ISR or SAR activator. A more detailed scientific explanation of ISR and the evidence of its activity in plants is available in the literature (1,2).

The search for defense activators to control disease and even other stresses is an increasingly active area of research. In some ways, this research parallels the development of human medicine, where we seek to decrease disease by providing supplements to strengthen the host, as well as by making whatever environmental changes we can to reduce stress and improve the overall health of the host.

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The RESEARCH SAYS

- With induced resistance, stimuli (natural or artificial) can pre-trigger part of a plant's natural defenses against pathogen attack.
- Some commercially available products have ingredients that can activate natural plant defenses but have little or no effect when applied directly to the pathogen.
- Turfgrass showed systemic resistance to fungal pathogens in field and lab studies and had enhanced resistance after product was applied.