Holding Their Ground

To protect the global food supply, scientists want to understand—and enhance—plants' natural resistance to pathogens.

By Amanda B. Keener | February 1, 2016

Plant pathologist Jean Ristaino hunts down crop-threatening diseases all over the world. Last year, in the span of two months, she visited India, Uganda, and Taiwan to help colleagues track the fungus *Phytophthora infestans*, which infects tomatoes and potatoes and caused numerous famines in 19th-century Europe. Ristaino tracks the pathogen's modern march using farmers' online reports of outbreaks of the disease, called late blight; then she travels to those locations to collect fungal samples. In her lab at North Carolina State University in Raleigh, Ristaino's team genotypes fungi from these farms to trace their origins and monitor how *P. infestans*'s genome is changing in response to fungicide use and how it's subverting immune strategies the host plants use to defend themselves.

Just like animals, plants have to fight off pathogens looking for an unsuspecting cell to prey on. Unlike animals, however, plants don't have mobile immune cells patrolling for invaders. "Every cell has to be an immune-competent cell," says Jeff Dangl, who studies plant-microbe

© ANDRZEJ WOJCICKI/SCIENCE PHOTO LIBRARY

interactions at the University of North Carolina at Chapel Hill.

Decades of work on model plants such as *Arabidopsis thaliana* have revealed robust cellular immune pathways. First, plasma membrane receptors recognize bits of pathogen and kick-start signaling cascades that alter hormone levels and immune-gene expression. This triggers the cell to reinforce its wall and to release reactive oxygen species and nonspecific antimicrobial compounds to fight the invaders. These responses can also be ramped up and prolonged by a second immune pathway, which can lead to localized plant cell death. Some plant defense compounds even manipulate bacterial communication. The polyphenol rosmarinic acid, for example, was recently found to disrupt a quorum-sensing pathway that *Psuedomonas aeriginosa* uses to form biofilms.[1]

The molecular details of these and other pathways have yet to be worked out, however. "Mechanistically, it's still rather opaque," says Jonathan Jones, a plant immunologist at the Sainsbury Laboratory in Norwich, U.K.

Scientists are now filling in the gaps in their understanding of plant immunity, and discovering previously unsuspected roles for factors such as microbiota composition and circadian rhythms. If they can understand a plant's defenses, maybe they can engineer more-robust crops, introducing immune genes that may have been inadvertently bred out of modern varieties. Some are also looking to alter known immune receptors so that plants can recognize pathogens despite adaptations that help the invaders fly under the immune radar. Collectively, these strategies could help plant breeders keep up with economically devastating pathogens like *P. infestans*.

One-two punch

A plant's first line of defense is recognizing pathogen-associated molecular patterns (PAMPs), which may be found within proteins such as flagellin, the lipopolysaccharides of the gram-negative bacterial outer cell membranes, or the complex carbohydrates of fungal cell walls. Cell-surface pattern recognition receptors (PRRs) bind to PAMPs and activate the production of nonspecific antimicrobial compounds, such as flavonoids and alkaloids, as well as enzymes including proteases and lipases. But the PAMP response does not always go as planned, Dangl says. "Pathogens have learned ways to subvert that . . . system."

By inserting so-called effector proteins directly into a plant cell's cytoplasm, bacterial and fungal pathogens can interfere with signaling cascades downstream of PRRs, or directly target hormone pathways and transcription factors to prevent PAMP-triggered immunity. That's when the plant's second line of defense kicks into gear. The cells sense the bacterial effectors by means of other receptors, called intracellular nucleotide-binding domain, leucine-rich repeat receptors (NLRs), that trigger secondary immune cascades.

NLRs provide flexibility in the plant immune system. *Arabidopsis* only has about 150 NLR proteins—not nearly enough to cover the wide range of potential pathogen effectors the plant may encounter. But NLRs don't just recognize pathogen effectors; many recognize plant proteins targeted by those effectors.[2] For example, the bacterium *Pseudomonas syringae* produces a protease that degrades a plant protein called RIN4, which is involved in PAMP-triggered immunity. RIN4 binds to an NLR called RPS2, so when the bacterial protease results in lowered levels of RIN4, RPS2 notices the protein's absence and initiates an alarm signal.[3,4] "If the host figures out how to recognize your action as a protease activity, then you're useless," says Dangl. By recognizing damaged proteins as "modified self," one NLR can detect the presence of many effectors, which often go after the same host targets.

In the last decade, researchers have found several examples of NLRs that operate in pairs: one binds a pathogen effector and the other mediates downstream signaling. In *Arabidopsis*, for example, the NLRs RRS1 and RPS4 work together to sense effectors from several pathogens: RRS1 binds to them, while RPS4 activates the defense response. RRS1 contains a domain that looks like a member of the WRKY transcription factor protein family—a group of major immune gene regulators in plants and the targets of several bacterial effectors.[5] Subsequent research revealed that it's common for one member of an NLR pair to contain a domain borrowed from an effector target. This led some researchers to hypothesize that these extra domains can act as decoys: the effectors bind the NLR, alerting the plant's immune system to the bacterium's presence before it can wreak too much damage. Sure enough, a bacterial effector called PopP2, which acetylates WRKYs, also acetylates the WRKY domain of RRS1 to activate RPS4-mediated immunity.[6,7]

Jones says decoy NLRs can offer a helpful shortcut for identifying the signaling proteins that link immune receptors and defense-gene activation. Any decoy domain fused with an NLR is likely to be a target of a pathogen effector, and therefore likely to be involved in plant immunity.

Cataloging plant immune genes and understanding how they work are also vital to breeding and engineering crops that can stand up to rapidly mutating pathogens. Although diverse genetically modified (GM) crops are now widely sold and consumed, the vast majority of today's growers still rely on chemical pesticides. In the U.S., farmers spend an estimated $77.1 million per year on fungicide to combat late blight alone.[8] Such treatments are often too expensive for growers in the developing world, says Ristaino. So researchers are turning to genetic methods to shore up the plants' defenses. "Host resistance [is] probably the best way to reduce losses," she says.

Putting plant defense to use

The most direct way to implement knowledge of plant immune pathways in agriculture is to introduce the immune genes themselves into plants. Many wild relatives of domesticated crops still harbor so-called resistance (R) genes that defend plants against specific pathogens. Once these genes are identified, researchers can breed or engineer them into the genomes of modern fruits, vegetables, and grains.

One of the first R genes bred into crops, which codes for an NLR called R3a, came from a wild relative of the potato called *Solanum demissum*. In the early 20th century, researchers discovered that the wild potato plant was resistant to *P. infestans* and began crossing it with cultivated potato varieties to transfer that resistance into the crop.

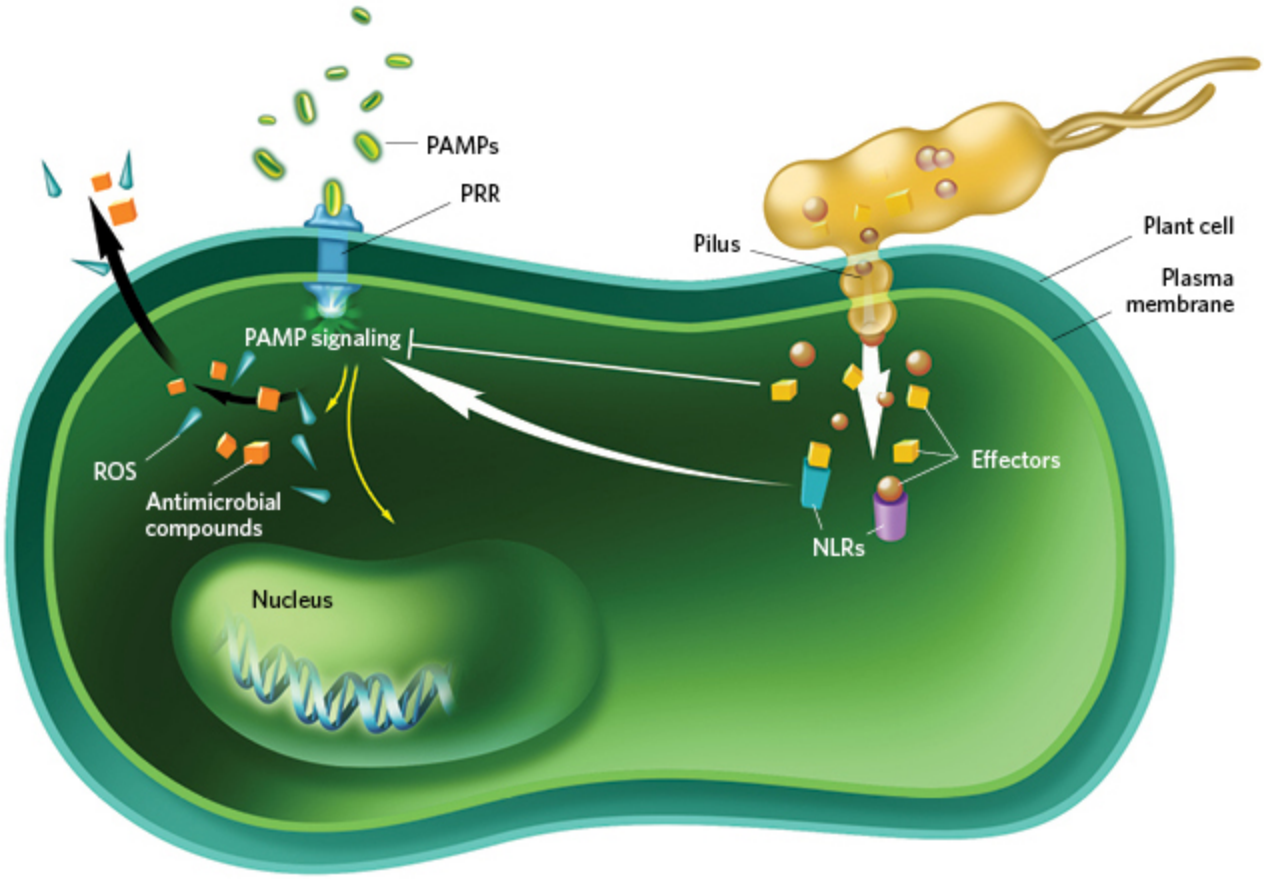
R3a recognizes a *P. infestans* effector called AVR3a, but since R3a was introduced into domestic potatoes, a fungal variant that evades R3a detection has become more prevalent. To address this issue, Sophien Kamoun of the Sainsbury Laboratory is looking to alter R3a so it can bind this stealthy effector, called AVR3a^{EM}. In 2014, his group used random mutagenesis to make a series of single amino acid changes to R3a and identified several that enabled the NLR to recognize AVR3a^{EM}.[9] The researchers also noticed that one of the mutant receptors bound an effector from a different fungal pathogen. "The really cool thing about this concept is it does open the door to engineering totally new synthetic receptors," Kamoun says.

FIGHTING BLIGHT: Strategies to fight Phytophthora infestans, an oomycete that causes late blight in tomatoes and potatoes, cost US farmers tens of millions of dollars each year.

Engineering NLRs to expand the list of effectors they can sense could be an efficient way to improve resistance to many pathogens at once. Recently, Kamoun and his colleagues applied what they learned from randomly mutating the gene that codes for R3a to selectively mutate a homologous tomato NLR gene called *I2*. They enhanced *I2*'s sensitivity to late blight and to an effector produced by a fungus that causes tomato wilt. Expressing the mutated *I2* in the leaves of the model plant *Nicotiana benthamiana* protected the leaves from late blight infection.[10]

NLRs are not the only group of receptors that researchers are mutating to enhance pathogen resistance; they also engineer effector targets. Last fall, Michigan State University plant scientist Sheng Yang He and his colleagues described a single amino acid change in a plant hormone receptor called coronatine-insensitive 1 (COI1) that protected *Arabidopsis* plants from *P. syringae* infection.[11] When the plant hormone jasmonate binds the COI1 receptor, it activates defense pathways against chewing insects at the expense of the plant's immune response to bacteria. P. syringae, which causes a disease called leaf speck on tomatoes, produces a mimic of jasmonate, called coronatine, that binds COI1 to keep antibacterial immunity repressed.[12] But a mutation in COI1 introduced by the researchers prevented binding with the bacterial mimic while maintaining normal jasmonate binding, making the plants resistant to *P. syringae* without compromising jasmonate-dependent defense against predatory insects.

By recognizing damaged proteins as "modified self," one NLR can detect the presence of many effectors, which often go after the same host targets.



HOW PLANTS FIGHT OFF PATHOGENS: Plants have two basic immune pathways. First, a pattern recognition receptor (PRR) on the plant cell's surface recognizes pathogen-associated molecular patterns (PAMPs) released by invaders and jump-start signaling pathways inside the cell that spur the production of reactive oxygen species (ROS) and antimicrobial compounds, as well as changes in gene expression and hormone levels. Second, intracellular plant protein complexes called nucleotide-binding domain, leucine-rich repeat receptors (NLRs) bind bacterial effectors and set off secondary immune cascades that boost the PAMP-triggered responses. NLR-binding can also lead to plant cell death, limiting the infection.
See full infographic: WEB | PDF

He's approach of mutating host targets to make plants less susceptible to pathogen attacks bypasses a major hurdle to breeding in new resistance genes. "There's always this trade-off," says Imre Somssich, a plant immunologist at the Max Planck Institute for Plant Breeding Research in Cologne, Germany. "If the R gene's activated constantly, you get small plants." Making plants impervious to bacterial subversion avoids the need for such heightened immune surveillance, conferring protection without compromising growth.

But to really strike a balance between plant growth and immunity, scientists need to know how the cellular pathways regulating these processes converge. Patrick Schäfer, who studies plant immunity at the University of Warwick in the U.K., is examining how immune activation by bacterial flagellin affects cell-cycle pathways in *Arabidopsis* root cells. At the moment, he says, the endocrine system appears to be the strongest link between a plant's growth and its resistance to pathogens. "It looks like the hormone pathways that are used by immunity are in part also used by growth signaling pathways," says Schäfer.

Recent work by plant researcher Xinnian Dong and her team at Duke University suggests that another way plants juggle defense and growth is through the use of internal clocks. In 2011, they unexpectedly found a correlation between the expression of immune genes and the internal circadian clock of *Arabidopsis* plants. "We were puzzled at the time," she says. "We thought these genes were just pathogen-induced, but then we found this connection to the clock."

Dong's group found that a central clock transcription factor called circadian clock-associated 1 (CCA1) activates resistance genes involved in defense against the fungus *Hyaloperonospora arabidopsidi*s first thing in the morning, when the pathogen typically releases its spores.[13] Last year, they reported that the plant's redox clock, which is driven by changes in plant cell metabolism and hormone levels, works with the circadian clock to boost plant immunity in the morning and repress it in the evening, when plants do most of their growing.[14] When the researchers perturbed the cycle by artificially inducing immunity of plants grown in the dark for a few nights in a row, the plants shriveled up and died.

The findings could help farmers who treat crops with the hormone salicylic acid to boost immunity, says Dong. "If you induce immunity at the wrong time of the day, that can cause much more damage."

BALANCING PLANT IMMUNITY: Plant immune systems must integrate a diversity of factors to successfully fight off pathogens without harming the plant. Defense-related changes in hormone signaling, for example, can interfere with plant growth. Many species power down their immune systems at night, when growing ramps up. Plant immunity also fluctuates with changes in temperature, humidity, and light exposure, and is likely dependent on a plant's microbiota below and above the soil.

See full infographic: WEB | PDF

Another important factor in a plant's resistance to pathogens is its microbiome. He's team has found that germ-free *Arabidopsis* plants express lower levels of many immune genes and exhibit impaired immune responses such as reactive oxygen species production compared to their microbe-colonized counterparts—findings that he hopes to publish this year. And Dangl's group recently reported that the *Arabidopsis* microbiome is shaped by the plant's hormones, especially salicylic acid.[15]

But how these microbial communities interact with the plant immune system is still a mystery. Just as many microbiologists would like to know how the human body tells the good microbes from the bad, those studying plant immunity are trying to understand how plants make peace with beneficial inhabitants. "All of these microbes are going to have PAMPs," says Dangl. "You have to know who your friends are."

Assisting evolution

Historically, resistance genes have been bred into crops one gene at a time. But with just one mutation that lets it bypass a new resistance gene, a pathogen can decimate a field of genetically identical crops. "Late blight has been particularly notorious for doing that," Ristaino says.

So instead of arming plants with individual genes, researchers are now looking to give plants whole suites, or "stacks," of resistance genes. Although this can be done with conventional breeding, researchers and agriscience companies are increasingly drawn to new precision gene-editing techniques such as the CRISPR/Cas9 system. Last October, scientists in South Korea demonstrated that they could make precise genetic changes to several plant species using CRISPR guide RNA and Cas9 enzymes, without leaving behind any bacterial DNA.[16] That same month, DuPont announced it would collaborate and share patents with Berkeley, California–based Caribou Biosciences to apply CRISPR technology to agricultural products in the next 5 to 10 years.

In addition to being dramatically more efficient than conventional breeding, gene editing allows researchers to introduce genes from wild varieties that won't breed with their domesticated relatives because the strains have diverged too much and their offspring are not viable. And when resistance genes are successfully bred into plants using conventional methods, they bring in a lot of unwanted extras, which then have to be painstakingly bred out. "When you're crossing you have no idea what other genes you're bringing in," says Somssich; gene editing is "much cleaner."

Like conventional breeding, however, genetic engineering methods still face the challenge of keeping up with a pathogen's rapid adaptation. "You really can't deploy stable resistance in the host unless you understand how the pathogen's evolving in response to the genes being thrown at it," Ristaino says.

But if plant scientists can predict how pathogens might evolve, as virologists do to generate a flu vaccine each year, gene-editing techniques could allow them to generate new crop varieties as quickly as the pathogens mutate, says Kamoun. "My personal vision is that we turn this into an arms race between us and the pathogen—not the plant and the pathogen."

Amanda B. Keener is a freelance science writer living in Winston-Salem, North Carolina.

References

1. A. Corral-Lugo et al., "Rosmarinic acid is a homoserine lactone mimic produced by plants that activates a bacterial quorum-sensing regulator," *Sci Signal*, 9:ra1, 2016.
2. J.D.G. Jones, J.L. Dangl, "The plant immune system," *Nature*, 444:323-29, 2006.
3. M.J. Axtell, B.J. Staskawicz, "Initiation of RPS2-specified disease resistance in *Arabidopsis* is coupled to the AvrRpt2-directed elimination of RIN4," *Cell*, 112:369-77, 2003.
4. D. Mackey et al., "*Arabidopsis* RIN4 is a target of the type III virulence effector AvrRpt2 and modulates RPS2-mediated resistance," *Cell*, 112:379-89, 2003.
5. L. Deslandes et al., "Resistance to *Ralstonia solanacearum* in *Arabidopsis thaliana* is conferred by the recessive RRS1-R gene, a member of a novel family of resistance genes," *PNAS*, 99:2404-09, 2002.
6. P.F. Sarris et al., "A plant immune receptor detects pathogen effectors that target WRKY transcription factors," *Cell*, 161:1089-100, 2015.
7. C. Le Roux et al., "A receptor pair with an integrated decoy converts pathogen disabling of transcription factors to immunity," *Cell*, 161:1074-88, 2015.
8. J.F. Guenthner et al., "The economic impact of potato late blight on US growers," *Potato Research*, 44:121-25, 2001.
9. M.E. Segretin et al., "Single amino acid mutations in the potato immune receptor R3a expand response to Phytophthora effectors," *Mol Plant Microbe Interact*, 27:624-37, 2014.
10. A. Giannakopoulou et al., "Tomato I2 immune receptor can be engineered to confer partial resistance to the oomycete Phytophthora infestans in addition to the fungus *Fusarium oxysporum*," *Mol Plant Microbe Interact*, 28:1316-29, 2015.
11. L. Zhang et al., "Host target modification as a strategy to counter pathogen hijacking of the jasmonate hormone receptor," *PNAS*, 112:14354-59, 2015.
12. J. Cui et al., "Pseudomonas syringae manipulates systemic plant defenses against pathogens and herbivores," *PNAS*, 102:1791-96, 2005.
13. W. Wang et al., "Timing of plant immune responses by a central circadian regulator," *Nature*, 470:110-14, 2011.
14. M. Zhou et al., "Redox rhythm reinforces the circadian clock to gate immune response," *Nature*, 523:472-76, 2015.
15. S.L. Lebeis et al., "Salicylic acid modulates colonization of the root microbiome by specific bacterial taxa," *Science,* 349:860-64, 2015.
16. J.W. Woo et al., "DNA-free genome editing in plants with preassembled CRISPR-Cas9 ribonucleoproteins," *Nat Biotechnol*, 33:1162-64, 2015.