PLANT BIOLOGY

THE END OF ORANGE JUICE

A devastating disease is killing citrus trees from Florida to California

By Anna Kuchment

Photographs by Grant Delin

GROWING SCOURGE: An invasive insect known as the Asian citrus psyllid is spreading deadly bacteria through the world's citrus groves, leaving fruit misshapen and unripe.

One day

in 2005, just before Hurricane Katrina blew through Florida and devastated New Orleans, Susan Halbert stood before a pomelo tree on a farm outside Miami. Something about this tree did not look right. It seemed undernourished: its leaves were sparse, and its melon-size citrus fruit was lopsided. Yet all the other plants in the garden were thriving, and the woman who took care of them had carefully tended the pomelo with a fresh layer of fertilizer. "She clearly knew how to grow plants," says Halbert, an entomologist for the Florida Department of Agriculture and Consumer Services (FDACS).

Halbert scrutinized the tree like a detective at a crime scene, mentally ticking off every condition she could think of. She ruled out root rot, which is caused by a fungus, because the tree showed none of the characteristic signs of decay. Next, she considered a viral disease known as citrus tristeza—Spanish and Portuguese for "sadness"—which affects trees that have been grafted. (Citrus growers often raise trees not from seeds but by inserting a branch from one tree into the bark of another.) The pomelo, however, had not been grafted. Eventually Halbert got to the bottom of her list to the most devastating disease of citrus plants in the world—huanglongbing, Chinese for "yellow dragon disease."

Huanglongbing, which is also called HLB or citrus greening, had been spreading slowly through India, China, Indonesia and South Africa. Just the year before, it had turned up in Brazil. It kills trees by gumming up their circulatory systems and leaving deformed, bitter fruit. It is the work of bacteria that hide in the salivary glands of a tiny winged insect called the Asian citrus psyllid, which injects the germ into plants as it sips sap from their leaves. There is no known cure—no pesticide that kills psyllids in large enough numbers, no effective treatment for the disease.

Apprehensively, Halbert snipped off a few tree branches and took them back to her laboratory for testing. Within a few days her suspicion was confirmed. Citrus greening had made landfall at the heart of America's orange juice industry.

Halbert sounded the alarm. Scientists and growers responded by throwing every resource into containing the disease. They uprooted infected plants, sprayed copious amounts of pesticides, encased entire nurseries in protective screens and imported wasps from Asia to prey on the psyllids. Researchers began injecting antibiotics into tree trunks and looking for resistant genes to splice into orange trees. But huanglongbing continues to spread. In the past eight years it has infected more than half of Florida's citrus trees and, between 2006 and 2011, cost the state $4.54 billion and more than 8,200 jobs. "Five years from now, there may be no more Florida orange juice," says J. Glenn Morris, director of the Emerging Pathogens Institute at the University of Florida.

Since Halbert's discovery, the disease has traversed Georgia, South Carolina, Louisiana and Texas. Last spring it was found in Los Angeles. In November inspectors found the first psyllids—which can herald the arrival of the disease—in California's commercial orange groves. The disease could cripple the U.S. citrus industry unless scientists find a way to stop it.

MEET THE PSYLLID

TO REACH HALBERT'S OFFICE in Gainesville, Fla., visitors pass a reception area with glass cases of yellow jacket colonies and live tarantulas. Just across the hall is the Florida State Collection of Arthropods: some nine million insects, each dried and mounted inside slim wood drawers that fill several rooms of tall metal cabinets. As an entomologist in the FDACS Division of Plant Industry, Halbert uses the collection to help identify the dozens of insects that state inspectors pick off produce and potted plants, drop into glass vials of alcohol, and mail to her in small, yellow business envelopes, which pile up in a tray on her desk.

Halbert, who is in her 60s and wears her hair in a low, braided bun, could pass for a kindly librarian. She is militant, however, when it comes to battling bugs that threaten Florida's multibillion-dollar produce industry. Halbert is one of eight entomologists for the state and one of two of its experts on Hemiptera—

IN BRIEF

A gnat-sized insect known as the Asian citrus psyllid has been spreading a deadly plant disease through America's citrus groves. Early attempts to contain the disease, known as huanglongbing, have failed, and it has become a major threat to the U.S. citrus industry.

Huanglongbing is caused by bacteria in the genus *Candidatus Liberibacter,* which Asian citrus psyllids carry in their salivary glands. The bacteria infiltrate plants' circulatory systems, which results in blockages that disrupt the flow of nutrients from leaves to roots.

To slow huanglongbing, scientists have imported wasps from Asia to prey on the psyllids, among many other approaches. The best long-term solution may be genetic modification, which faces a long and costly road to regulatory approval and public acceptance.

insects such as aphids, leafhoppers and psyllids that suck juice from plants. Her main responsibility is alerting growers and regulatory agencies to the arrival of any new pests. "It's my job to keep things on my radar, to know what the bad actors are out there," Halbert says.

Greening has been on Halbert's radar since the mid-1990s, when she first heard about the disease's devastation from some colleagues in South Africa. In June 1998 she was inspecting citrus trees in Palm Beach County, when she became the first person to spot a psyllid in the U.S. She recognized it by its characteristic stance: it sticks its behind up in the air at a 45-degree angle.

ON THE LOOKOUT: **Asian citrus psyllids sit at a 45-degree angle (*1*). Psyllid nymphs excrete honeydew (*2*). A salivary toxin that even healthy psyllids emit can deform leaves (*3*). Trees at a field-test site grow inside protective psyllid-proof screens (*4*).**

In retrospect, Halbert should have felt more alarmed by this discovery, she says. Yet Brazil had lived with psyllids since the 1940s and by 1998 had not had a single case of greening. So Halbert and her colleagues decided to watch and wait. They returned to Palm Beach with a team of inspectors and fanned out by car in all directions to see how far the pests had spread. Armed with plastic sticks and white plastic trays, Halbert and her colleagues literally beat the bushes in search of psyllids: they hunted for shrubs and trees in the citrus family, whacked them with their batons and counted how many psyllids fell onto their trays. Psyllid numbers, they found, grew sparser as they headed away from Palm Beach County and soon petered out. The infestation seemed to be limited to a 60-mile stretch of coastline, and none of the bugs they examined tested positive for huanglongbing.

That was a good sign. Yet Halbert and her co-workers stayed alert for symptoms of greening, regularly testing psyllids and trees. In 2005 she decided to undertake more wide-ranging surveys, branching out to ethnic neighborhoods whose populations hailed from greening-infested regions. And that is how she found the diseased pomelo tree in Miami, which grew in the backyard of a woman from Taiwan. "It was another really bad day," Halbert says.

By the time Halbert confirmed the presence of the disease, it had already spread with devastating speed, in large part because of the psyllid's amazing fecundity. Each female lays up to 800 eggs in her one-month life span, resulting in populations on a single orange tree that can exceed 40,000 bugs. With that many insects hopping and flying around, even pesticides with a kill rate of 99 percent leave plenty of survivors. And it turned out one of the Asian citrus psyllid's favorite plants to feast on was a wildly popular shrub, orange jasmine, that was produced in Miami, sold in nurseries and major discount stores across Florida, and shipped widely, giving the psyllids an easy means of travel.

View to a Kill

Huanglongbing (HLB) is one of the most devastating diseases of citrus plants. Small winged insects, Asian citrus psyllids, transmit the bacteria that cause huanglongbing as they drink sap from the leaves of trees. Inspectors initially found Asian citrus psyllids in the U.S. in 1998 and first detected huanglongbing in 2005. Both were first found in Florida.

The Vector: Asian Citrus Psyllids

Psyllids lay eggs on shoots and leaves as they emerge from buds. Newly hatched, wingless nymphs feed exclusively on this soft growth as they develop into adults. As psyllids of all life stages drink sap from the plant's leaves, they can transmit HLB from their salivary glands. Infected plants can also transmit HLB to psyllids.

Asian citrus psyllid

Parasitic wasp

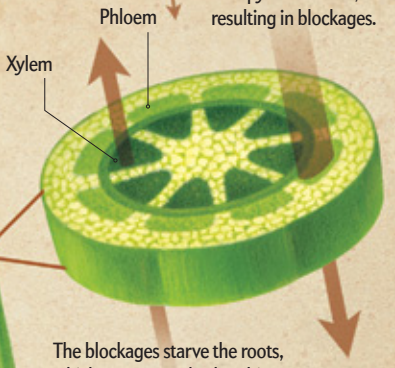
An Attempt to Fight Back

Because Asian citrus psyllids have no native specialized predators in the U.S., entomologists have brought in tiny parasitic wasps from Asia, *Tamarixia radiata*, to help keep psyllid populations in check. Lab studies suggest that each wasp can kill hundreds of psyllids by drinking their blood and laying eggs on them. The eggs develop into larvae that eventually eat the psyllids from the outside in.

The Disease: Huanglongbing

Most scientists believe huanglongbing is caused by three bacteria in the genus *Candidatus Liberibacter*, although researchers have yet to conclusively prove the relation. The three are: *Candidatus Liberibacter asiaticus*, which is the most prevalent and is found in the U.S.; *Candidatus Liberibacter africanus*, found primarily in South Africa; and *Candidatus Liberibacter americanus*, found primarily in Brazil.

The bacteria infect the phloem, the circulatory system that transports sugars from the tree canopy to the roots, resulting in blockages.



The blockages starve the roots, which stop properly absorbing nutrients from the soil to send back up to the tree canopy, a circulatory system known as the xylem.

The Result: Dying Plants

HLB can masquerade as a nutrient deficiency. Symptoms include mottled, yellowish leaves and fruit that is lopsided, small, green and bitter and that drops prematurely. The disease weakens plants and makes them more likely to succumb to drought and other stressors.

Illustration by Cherie Sinnen

Florida's deadly hurricane seasons may also have been a factor. Winds from Katrina and other tropical storms may have blown psyllids farther than they could travel on their own. The storms may have also weakened trees and made them more susceptible to infections.

And then there is the fact that as an invasive species, the Asian citrus psyllid has no native, specialized predators in the U.S., allowing them to proliferate quickly. This situation set entomologists in search of an insect that could wreak havoc with psyllids the way psyllids had wreaked havoc with orchards.

SEND IN THE WASPS

ON A HOT DAY LAST SUMMER Mark and Christina Hoddle packed up a rented white Ford sedan and made the one-hour drive from their home in Riverside, Calif., to Los Angeles. A small blue Rubbermaid cooler sat on the backseat. Inside it was an ice pack and half a dozen vials containing wasps feasting on small drops of honey. As Mark drove, Christina flipped through a sheaf of papers with data on the research sites they would visit.

California is the U.S.'s second-largest citrus producer after Florida. While the vast majority of Florida's oranges are squeezed into juice, California provides most of the oranges that Americans eat whole. When the first Asian citrus psyllid was spotted in San Diego County in 2008, the state's priority became keeping the insects away from the commercial groves to the north, in California's Central Valley. Officials began spraying insecticides in San Diego, but soon the psyllids spread to Los Angeles and continued moving up the coast. They needed a new plan.

The Hoddles are entomologists at the University of California, Riverside, and experts on invasive species. Soon after the first Asian citrus psyllid was detected in the state, Mark read a 1927 paper by scientists in Punjab. The authors described the effects of greening in stark terms ("It is not an uncommon sight to see once valuable orchards reduced to unproductive plantations of dried skeletons of trees") and reported on a species of local, parasitic wasp that could kill 95 percent of Asian citrus psyllids. "Could these wasps thrive in California?" Mark wondered.

It made sense that the Asian citrus psyllid's natural enemy would live in South Asia, which is the insect's likely birthplace. When, where and how huanglongbing, psyllids and citrus all met up, however, is still an open question. The genus *Citrus* was long thought to have evolved in China, but recent research by Andrew Beattie of the University of Western Sydney in Australia and his colleagues suggests it first appeared in Australasia some 35 million years ago and spread to Asia. The genus of the bacteria that are thought to cause huanglongbing, *Candidatus Liberibacter* ("*Candidatus*" indicates that scientists do not know for sure because the bacteria have never been cultured), may have evolved in Africa and jumped to citrus trees from a citrus relative only within the past 500 years, judging by the virulence of the disease. (If citrus and huanglongbing had come together earlier, citrus would have either developed resistance by now or died off.)

Beattie suspects this jump took place in Africa, when a citrus psyllid transferred the bacteria to an imported orange or mandarin tree that was then shipped to India as part of the colonial trade. Human cultivation has played a role as well. Psyllids lay their eggs on the tender shoots of budding trees, which are easier for nymphs to feed on. Thanks to irrigation and the use of fertilizer, citrus trees grow and bud rapidly, creating a tempting salad bar for psyllids of all ages.

For the wasps, known as *Tamarixia radiata,* to survive in California's Central Valley, where most of the state's citrus growers are based, the climates of the two regions would have to be similar. Mark entered data into his climate-matching software and discovered that Punjab's and California's citrus regions both had hot, dry summers and cool, foggy winters—an excellent match. He then discovered, seemingly by kismet, that the vice chancellor of the major agricultural university in Punjab was a graduate of U.C. Riverside. "Suddenly, these doors were open that I thought would be incredibly difficult to walk through," says Mark, who is originally from New Zealand. In early 2011 he and Christina headed to Pakistan to learn everything they could about *T. radiata*.

Importing wasps from Pakistan in the post-9/11 era is no simple task. Mark, working with the California Department of Food and Agriculture, secured a permit from the USDA and set up a wasp-rearing operation under quarantine to ensure that the incoming insects were disease-free. He and his postdoctoral student also spent months testing *T. radiata*'s host range—pitting it against native psyllids on their native plants and against beneficial insects that attack noxious weeds—to make sure it would not prey on California's local flora and fauna or disrupt weed biocontrol efforts. Finally, he and Christina set up a complex sequence of cages inside a series of quarantine labs at U.C. Riverside to allow the wasps to multiply on Asian citrus psyllids infesting small citrus plants.

Since December 2011 the Hoddles have released thousands of *T. radiata* wasps at more than 100 sites in Los Angeles, Riverside, Orange County and San Bernardino County. On this summer day they were visiting release sites in Los Angeles to check on the parasites' progress. "This is urban warfare," Mark said from the driver's seat. Despite the heat, he and Christina were dressed in long-sleeved shirts and long pants—the sun-protective clothing they wear in the field.

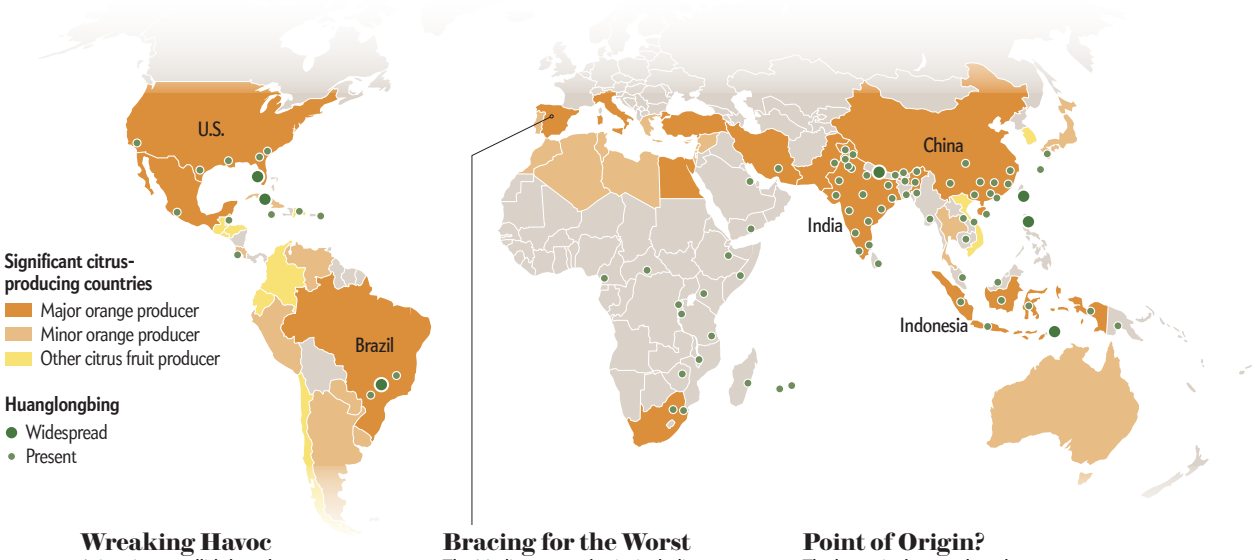
Although the Asian citrus psyllid was first spotted in San Diego, it seems to have moved fastest through Los Angeles. Backyard lemon and lime trees are very popular here, and many people bring them in across the border from Mexico or smuggle cuttings inside their suitcases when flying back from Asia. The branch of an infected lemon tree can easily be grafted onto a lime or pomelo tree, and the plant will produce both varieties of fruit. Once psyllids arrived in Los Angeles, they reproduced feverishly on these backyard trees, just as they did in Florida.

The California Department of Food and Agriculture had started spraying pesticides in Los Angeles to control the psyllids and prevent them from spreading, but the effort rapidly proved futile. One need only glance at census data to see what went wrong. Of the more than three million houses in Los Angeles, about 40 percent had at least one citrus tree in 2010. That means about 1.2 million properties need to be treated. Sprays last only one week to several months and then need to be reapplied. By last October the state had sprayed 46,941 properties, or 4 percent, at a cost of $4.7 million, or $100 per property. "You can see why this quickly became unfeasible," Mark says. Once the state suspended its pesticide campaign in Los Angeles, it was safe for the Hoddles and their wasps to move in.

The Hoddles pulled into a hotel parking lot in Los Angeles's

It Gets Around: How a Deadly Global Traveler Arrived in the U.S.

"From the time citrus was first recognized as an edible fruit with positive nutritional qualities, it has moved with man, and so too have its pests and diseases," wrote Tim R. Gottwald of the USDA in a 2010 review article. That is the story of huanglongbing, also known as citrus greening, which was first recorded in India in the 1700s and now threatens every citrus-producing region of the world.



Wreaking Havoc

Asian citrus psyllids have been present in Brazil, the world's largest orange producer, since the 1940s, but inspectors did not detect HLB there until 2004. Psyllids may have spread to Florida via the Caribbean and to California via Mexico.

Bracing for the Worst

The Mediterranean basin, including major citrus producers Spain, Italy and Turkey, is one of the few regions that remains free of psyllids and of HLB.

Point of Origin?

The bacteria that are thought to cause HLB, from the genus *Candidatus Liberibacter,* may have evolved in Africa but most likely moved into citrus only within the past 500 years, thanks to psyllids and the global trade in oranges and their relatives.

working-class Mexican neighborhood of Azusa and grabbed their cooler. They had found the site by examining state data on the number of psyllids caught in sticky yellow traps set all over Los Angeles. This garden had a particularly high count. Christina headed over to a curry bush (a relative of citrus) at the edge of the parking lot. She examined its shiny green leaves and immediately spotted large clusters of Asian citrus psyllids at every stage of their life cycle. At the tips of the branches, littering the newly sprung buds, were yellowish-orange eggs, whitish wingless nymphs, and brown mottled adults hopping and crawling about. Three weeks earlier the Hoddles had released a few dozen wasps on some lemon trees on the far side of the lot, and they wanted to see if the wasps had made their way to this shrub; if they did, it would show that the wasps were beginning to proliferate on their own.

The news was good: although the wasps, no larger than the size of half a chocolate sprinkle, were hard to spot, they had left death and destruction in their wake. A female wasp kills a psyllid essentially by laying an egg under its belly. Once that egg hatches, the wasp larva—which looks like a pudgy maggot—will start scraping away at the nymph's underbelly, eating its insides over the course of a week until all that is left is a shell. "It's a husk of its former self," Mark says. The wasp larva seals itself inside the shell by spinning silk that attaches the shell firmly to the leaf. It then weaves its cocoon, pops out as an adult and chews a small hole at the top of the psyllid husk to escape. Christina and Mark observed several hollowed-out psyllid husks on the curry plant.

The wasps also destroy psyllids in another way—something Mark calls a "bonus kill." A female wasp stands on the back of a psyllid, stabs it repeatedly with her egg-laying tube and then drinks its blood. "The trauma of being stabbed multiple times and then having your blood drunk is sufficient to kill those Asian citrus psyllid nymphs," he says. Using this powerful combination of killing techniques, a single wasp can kill hundreds of psyllids. Before leaving, the Hoddles hung a few more of their vials on the curry plant and opened the lids to allow the wasps to fly out and begin their hunt.

As of last December, the Hoddles estimate that the wasps they have released have become established at about 40 percent of their release sites in California and are fanning out to new psyllid-infested neighborhoods, sometimes several miles

SOURCES: "CITRUS FRUIT: FRESH AND PROCESSED: ANNUAL STATISTICS 2012," BY U.N. FOOD AND AGRICULTURE ORGANIZATION (*citrus production*); "CITRUS HUANGLONGBING (GREENING) DISEASE," IN *INVASIVE SPECIES COMPENDIUM*, BY CENTER FOR AGRICULTURAL BIOSCIENCE INTERNATIONAL, 2013 *www.cabi.org/isc* (*HLB disease*)

Map by XNR Productions

away. The wasps will not solve the problem, however. "It's not going to be a silver-bullet solution," Mark says. "I think if we can get a 30 percent kill, that will lower the population pressure in these urban areas and will reduce the rate at which things are spreading out."

THE BEST PATH FORWARD

FLORIDA'S BIOLOGICAL-CONTROL EFFORTS predate California's. The state released its first batch of parasitic wasps in 1999, and starting later this year, it plans to release millions more from Pakistan, Vietnam and China in urban areas where the state has stopped spraying pesticides. (Florida's climate, unlike that of California's Central Valley, is better matched to Vietnam.)

Some of Florida's growers have embarked on another, more controversial approach: helping trees live with the disease. *C. Liberibacter* invades a plant's circulatory system, which then blocks the passage of sugar and other nutrients from its leaves to its roots. "If roots suffer, they're not going to be able to efficiently absorb and move micronutrients and other substances from the soil up into the leaves, so now we have a compounding effect," says Philip Stansly, an entomologist at the University of Florida's Institute of Food and Agricultural Sciences.

The genus *Citrus* was long thought to have evolved in China, but recent research suggests it first appeared in Australasia some 35 million years ago and spread to Asia.

In response, many Florida growers started feeding extra nutrients to the trees through leaf sprays. "I compare it to AIDS," says Tim Willis, a third-generation citrus grower and manager at McKinnon Corporation in Winter Garden, Fla., which operates an orchard. "They keep humans alive now for years with a devastating disease. Why can't we do the same thing to an orange tree?" Even before the arrival of psyllids in Florida, Willis and Maury Boyd, president of McKinnon, had put their trees on what Stansly calls a "Cadillac" nutrition program—they were feeding their plants all the manganese, zinc and boron they could. With the arrival of HLB, plant disease experts advised growers to pull up any tree that was infected. Yet by the time the disease was discovered in Florida, it was so widespread that pulling up infected trees may have run Boyd and Willis out of business. "These trees have taken care of me my whole life," Willis says. "They've put my son through college. You can't just give up."

So McKinnon stepped up its nutrition program and joined with other growers to implement a system of regular, coordinated pesticide sprays. When Boyd and Willis refused to pull up their trees, experts told them their plants would be dead within five years. But seven years later they are still here, and they say their yield is undiminished.

Last November, Willis drove his pickup truck through his groves on a routine survey. The trees were lush and hung with large, ripe yellow Hamlin oranges, an early variety harvested in late fall and early winter. Although nearly 100 percent of his trees had huanglongbing, only a few leaves and trees bore the disease's classic hallmarks: mottled leaves and green, prematurely dropped fruit littering the ground. Still, no one knows how long his good fortune will last. "When we planted a tree, we used to think that tree would be there for generations," Willis says. "A lot of people now think, 'If I get 10, 15 years out of it, it's going to be good.'"

So far published studies have failed to show that nutrition programs like Boyd's can impart any benefit. "You can't fertilize your way out of this," says Tim R. Gottwald, an epidemiologist at the U.S. Department of Agriculture. He and several colleagues have published controlled studies showing that enhanced nutrition programs have no effect on tree health, fruit quality or yield. In fact, Gottwald argues, they can be detrimental because they mask symptoms and turn trees into Typhoid Marys.

Neighboring grower Southern Gardens Citrus, which supplies orange juice to the major brands, took a different path. Rick Kress, president of Southern Gardens, says that the company is replacing more than 650,000 infected trees—one quarter of its stock—with clean nursery trees that have been grown inside psyllid-proof screens. Workers continue to monitor groves for signs of greening, although trees can harbor *C. Liberibacter* for months or years before the bacteria start showing up in lab tests or causing visible symptoms, which makes the disease difficult to eradicate. While Kress has reduced the infection rate of his trees, his costs are up 40 to 50 percent.

Scientists are desperately seeking new approaches. Some studies have shown that feeding penicillin to infected orange trees through their roots and via trunk injections can help them outgrow their symptoms and develop stronger roots. In 2011 Jim Graham of the University of Florida found that the bactericide copper sulfate has a similar effect. Copper sulfate might pass EPA regulations more easily than penicillin because it is not used to treat humans. But plants would need to receive injections for the rest of their lives at a potentially prohibitive cost.

The best long-term prospects may lie with genetic modification. Erik Mirkov, who is a plant pathologist at Texas A&M University, has transferred two genes from spinach into citrus trees, thereby conferring resistance to huanglongbing. Researchers at Cornell University are developing citrus trees that would repel Asian citrus psyllids, and the two technologies may eventually be combined. Both projects are being funded by Southern Gardens, which has spent $6 million on research to stop citrus greening. But genetically modified produce faces a long and expensive path to regulatory approval and public acceptance. Many worry it will not arrive in time to save the industry. Says Halbert, "We need something we haven't thought of before." SA

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MORE TO EXPLORE

Oranges. John McPhee. Farrar, Straus and Giroux, 1967.

Current Epidemiological Understanding of Citrus Huanglongbing. Tim R. Gottwald in *Annual Review of Phytopathology*, Vol. 48, pages 119–139; September 2010.

SCIENTIFIC AMERICAN ONLINE

View a slide show of images and a video of Christina and Mark Hoddle releasing parasitic wasps at ScientificAmerican.com/mar2013/citrus