



The green menace

Huanglongbing, a disease that could devastate the US citrus industry, pits national security against plant pathologists looking to battle natural outbreaks, **Ewen Callaway** reports.

In California's San Joaquin valley, it is citrus season. An open-air warehouse on the outskirts of Dennis Johnston's 600-hectare citrus grove brims with huge crates of oranges, mandarins and grapefruits. This year, his farm will ship around US\$8-million to \$10-million worth of citrus around the United States and the Pacific Rim.

Cruising through the labyrinthine dirt roads that criss-cross his farm, Johnston surveys the winter crop, passing tree after tree, each a mop of dark green dotted with baseball-size fruits.

The trees are productive and the fruit healthy, but in the back of Johnston's mind is the possibility that trees could fall sick from insect-spread infections. Tristeza virus, for instance, fells a tree every few years, he says. Johnston is vigilant. He serves on his county's pest-control board and educates other growers about plant diseases that range from minor nuisances to epidemics that could ruin the San Joaquin valley's billion-dollar citrus industry. "We have a hard time getting growers to understand the severity of these bacterial and viral infections," says the easy-going third-generation grower.

"Huanglongbing is a disease that can wipe out citrus as we know it."

— Larry Bezark

Green alert

Mention huanglongbing, and Johnston cringes. "We were being warned by the scientists and the people in the know that this is a serious, serious thing and we've got to, at all costs, keep it out of the valley and keep it out of California," he says.

Named for the colour of the infected leaves, huanglongbing is Chinese for yellow dragon disease. It is a bacterial infection that spreads from tree to tree via citrus psyllids, draining nutrients from the plant and resulting in diminutive green fruits, thinning branches and eventually death of the tree. It has devastated the citrus industry in many Asian countries, killed hundreds of thousands of trees in Brazil, and

swept through 26 Florida counties since it first arrived on US soil in 2005. It is considered the most dangerous citrus pathogen in the world.

The US government agrees. The bacterium that causes the disease, '*Candidatus Liberibacter*', comes under the same regulations that restrict research on Ebola, anthrax and other microbes that have been deemed potential agents of terror. An attack on the food supply with a plant pathogen such as '*Ca. Liberibacter*', some say, could have disastrous effects on the nation's economy. Researchers who study these 'select agents' must submit to background checks by the Federal Bureau of Investigation (FBI), install extra security outside their labs, and file mounds of paperwork.

These measures are necessary, the government says, to keep the pathogens from falling into the wrong hands. A terrorist-spread epidemic of huanglongbing, for instance, could kill millions of trees and rattle consumer confidence, says Jacqueline Fletcher, director of the National Institute for Microbial Forensics and Food and Agricultural Biosecurity in Stillwater, Oklahoma.

Other scientists, though, say that treating '*Ca. Liberibacter*' and other emerging plant pathogens as potential agents of terror leaves growers such as Johnston worse off. Slapping the select-agent status on plant pathogens inevitably means that fewer researchers can study the organisms, slowing the development of countermeasures. Compliance with the strict regulations is costly and time-consuming, researchers say. And officials in Florida contend that the bacterium's select-agent status sapped their response to the 2005 outbreak of huanglongbing, which continues to rage. Diagnostic tests have been slow to develop and researchers still haven't figured out how to grow the bacterium in the lab.

"There's no question that select-agent type

regulations are necessary for Ebola virus and 1918 influenza," says Caitilyn Allen, a microbiologist at the University of Wisconsin-Madison. "The question that needs to be considered seriously is whether these plant pathogens pose a similar risk." Indeed, the US government is now deliberating changes to the select-agent list to reflect the growing prominence of huanglongbing in Florida.

The war on agroterror

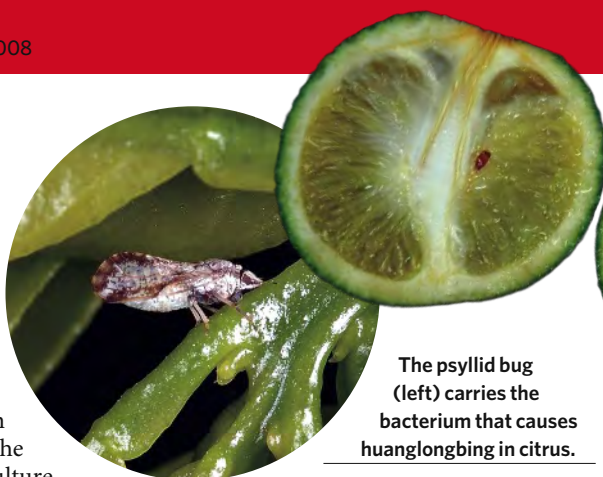
In 1996, the US government created its first list of select agents, after Larry Wayne Harris, a microbiologist and white supremacist, tried to purchase vials of the bacterium that causes bubonic plague. The initial list included pathogens in humans and animals, but not plants.

But biosecurity soon became an important issue for plant pathologists too. In 1999, the American Phytopathological Society in St Paul, Minnesota, hosted a well-attended symposium on the potential for plant pathogens to be used as weapons. That same year, the organization issued a statement recommending better surveillance of plant-disease outbreaks, eyeing the possibility of agroterror.

Those flirtations with biosecurity turned serious after letters laced with anthrax started appearing in the US mail system shortly after the terrorist attacks of 11 September 2001. Fletcher was then president-elect of the American Phytopathological Society. In February 2002, she and several other scientists briefed Congress on the potential for plant pathogens to be used by terrorists. "While there is no evidence that agriculture might be a current target of terrorism, September 11 has made us all more aware of the need to be prepared for any possibility"

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The psyllid bug (left) carries the bacterium that causes huanglongbing in citrus.



Fletcher said at the time.

Congress and President Bush listened. On 12 June that year, Bush signed the Agricultural Bioterrorism Protection Act, ordering the US Department of Agriculture (USDA) to include plant pathogens on the select-agent list. Two months later, the agency began enforcing the law, listing nine plant pathogens, including two species of 'Ca. Liberibacter' (see 'The select few').

To ensure flexibility, the USDA included only exotic pathogens — those usually found outside the United States — as select agents and mandated that they be removed from the list once they took hold in the country. The law would be updated every two years to accommodate such changes, although not until extensive consultation has been done.

Some researchers view this elevation of status as a boon. "Having the select-agent list helps us to prioritize," says Fletcher. "It also targets special funds for working on those things if they should come in." Indeed, the USDA's biodefence budget bloomed from \$200 million in 2003, the first year that numbers were made available, to \$340 million in the 2008 budget, according to the Center for Biosecurity at the University of Pittsburgh Medical Center in Baltimore, Maryland.

Heightened awareness of plant pathogens also spurred the USDA to establish a national network of laboratories to diagnose plant diseases. Before 9/11, every state ran its own plant-pathology lab, each of which had different procedures for testing diseased plants. But funding was scarce and "all these labs were languishing", Fletcher says. Although agroterrorism was the motivation behind the long-overdue network, improved diagnostics and communication between state labs will speed the response to any outbreak, she says.

"Few, if any, plant pathogens should be considered bioterror agents."

— Tim Gottwald

On 23 August 2005, plant pathologists had their worst fears realized: huanglongbing was found in the pomelo trees of two homeowners

near Miami. Ten days later, the USDA confirmed the diagnosis. In the months that followed, officials found the disease in thousands of trees in most of Florida's citrus-growing counties.

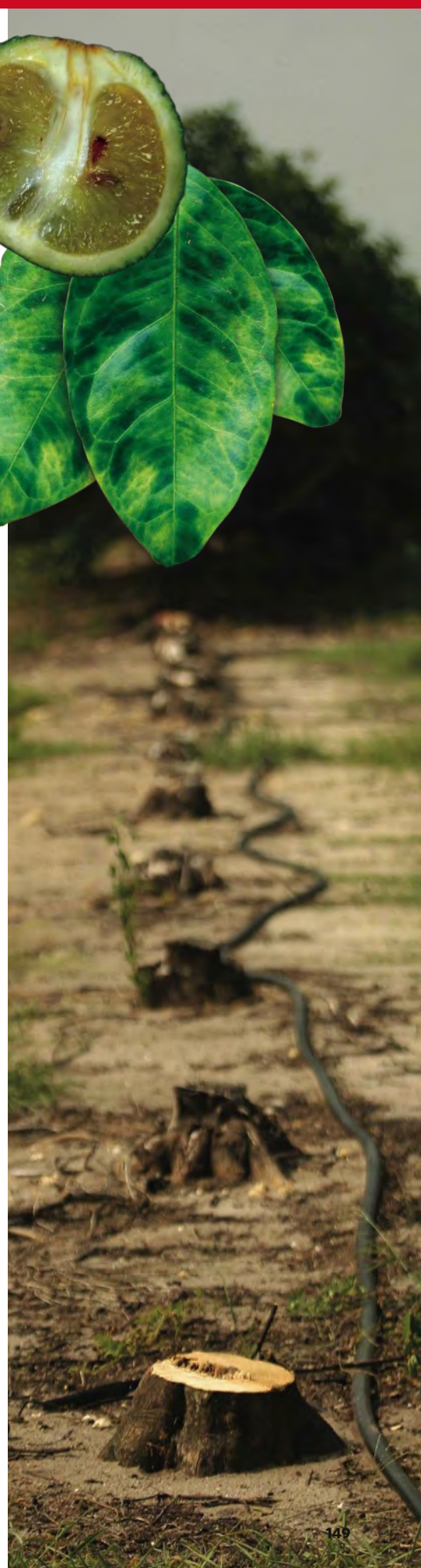
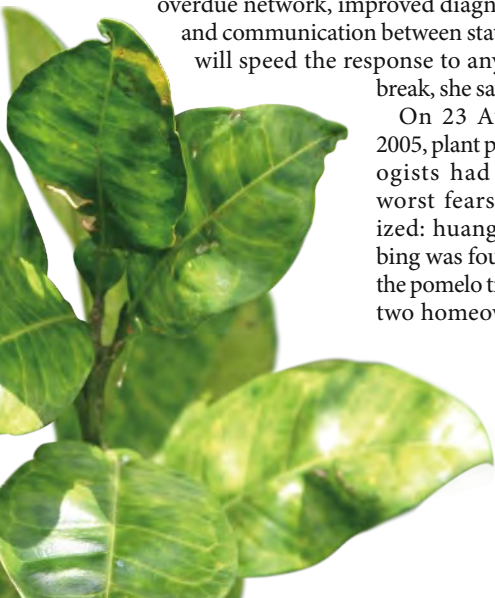
Where it came from no one knows, but historical records suggest that the disease originated in the Guangdong province in southern China or in central India in the late 1800s. It quickly sped through Asia, where one species, 'Ca. L. asiaticus', is now endemic from Japan to Pakistan. A second species, 'Ca. L. africanus' is found throughout eastern, central and southern Africa, and a third species, 'Ca. L. americanus' has been discovered in Brazil.

Yellow dragon

Citrus trees infected with huanglongbing show few symptoms for several years. Eventually, their leaves develop characteristic yellow splotches, and trees produce puny, discoloured fruit. Production drops rapidly, until the trees eventually die. Worse, infected trees churn out bacteria for the psyllid to cart elsewhere.

Given the havoc the disease has wreaked around the world, there is good reason to worry that huanglongbing will spread throughout the United States, says Phil Berger, acting director of the USDA's Center for Plant Health Science and Technology in Raleigh, North Carolina. A survey in the late 1990s estimated that 53 million trees in Asia were infected with huanglongbing, and 10 million in Africa. A systematic survey of the Reunion Islands in the 1980s found that huanglongbing had killed 65% of their citrus trees within seven years of planting. And in parts of northern Thailand, the bacterium kills at least one-tenth of tangerine trees every year. In the state of São Paulo in Brazil, an estimated 800,000 trees have been lost since an outbreak hit in 2004.

As the citrus psyllid moves west from Florida — it has turned up in Texas and Mexico — officials and growers are preparing for the worst. "The threat is enormous," says Larry Bezark, of the California Department of Food and Agriculture in Sacramento. Bezark spearheads the state's response to huanglongbing — scouring farms, nurseries and even ports for psyllids and infected plants. "This is a disease that can wipe out citrus as we know it," he says.



THE SELECT FEW

Microbe	Disease	Select agent status
' <i>Candidatus Liberibacter africanus</i> '	Huanglongbing	Current
' <i>Ca. L. asiaticus</i> '	Huanglongbing	Current
<i>Peronosclerospora philippinensis</i>	Philippine downy mildew of corn	Current
<i>Ralstonia solanacearum</i> , race 3, biovar. 2	Brown rot of potato	Current
<i>Sclerophthora rayssiae</i> var. <i>zeae</i>	Brown stripe downy mildew of corn	Current
<i>Synchytrium endobioticum</i>	Potato wart disease	Current
<i>Xanthomonas oryzae</i> pathovar. <i>oryzicola</i>	Rice leaf streak	Current
<i>Xylella fastidiosa</i> (citrus variegated chlorosis strain)	Citrus variegated chlorosis	Current
<i>Phakospora pachyrhizi</i>	Asian soya bean rust	Removed 2005
Plum Pox potyvirus	Plum pox	Removed 2005
' <i>Ca. L. americanus</i> '	Huanglongbing	Proposed 2007
<i>X. oryzae</i> pathovar. <i>oryzae</i>	Rice leaf streak	Proposed 2007
<i>Phoma glycinicola</i>	Ref leaf blotch of soya bean	Proposed 2007
<i>Phytophthora kernoviae</i>	Related to sudden oak death	Proposed 2007
<i>Rathayibacter toxicus</i>	Gumming disease in ryegrass	Proposed 2007

But while California prepares for an outbreak, researchers and officials in Florida feel handcuffed by the pathogen's continued status as a select agent, even though it is now widespread in Florida. "We had our hands tied," says Wayne Dixon, chief of plant pathology at the Florida Department of Agriculture and Commerce in Gainesville. Whenever a sample from a tree turns up positive, the select-agent law mandates that the lab notify the USDA and destroy the sample within a week. This complicates efforts to perform additional diagnostics, says Dixon, who supports listing some exotic plant pathogens as select agents but not '*Ca. Liberibacter asiaticus*'.

To comply with the select-agent listing, researchers must register with the federal government, install added security to their labs such as video monitors and fingerprint-accessed doors, and submit to background checks. Foreign researchers have an especially hard time getting approved to work on select agents. The penalties for violating the law are harsh — up to \$250,000 and five years in jail.

Species swap

In August 2007, the USDA proposed updating the select-agent list to remove the asiaticus species found in Florida, while adding the Brazilian *americanus* species to the list.

It is now reviewing the comments it received on its proposals, which are overwhelmingly in favour of lifting the select-agent status of the asiaticus species — and

hopes to issue a final rule later this year, says Michael Firko, a USDA official who is leading the agency's efforts.

While the proposal to remove the asiaticus strain from the select-agent list seems to be headed for approval, several other plant pathogens may be added, including diseases of soya, rye, woody trees and shrubs, and rice.

The proposed addition of a strain of the rice pathogen *Xanthomonas oryzae* has left some microbiologists scratching their heads. The cause of leaf streak in rice, *X. oryzae* has done significant damage to crops in Asia and Africa.

"It doesn't make sense to have such enormous restrictions when it's already outside our window."

— Eric Triplett

Yet the pathogen is unlikely to take hold in the United States because of different farming practices and a hostile climate, says Leach, who studies the bacterium.

The added cost of complying with the select agent rule may force some researchers to abandon their work on *X. oryzae*. "I stay up late at night worrying about this stuff," says Pam Ronald, a geneticist at the University of California in Davis who studies the rice pathogen.

Firko contends that the select-agent status of '*Ca. Liberibacter asiaticus*' hasn't hampered research. "We have not refused registration to anybody for this agent, and I don't think we have refused any experiment that people want to do."

Classifying '*Ca. L. asiaticus*' as a potential agent of bioterror is paradoxical, says Eric Triplett, a microbiologist at the University of Florida in Gainesville. "It doesn't make sense to have such enormous restrictions on laboratories and confine it to strict BSL-3 conditions when it's already outside our window." To study the disease, Triplett and his lab members must



Are the complex rules and regulations governing select agents stymying work to study and monitor the spread of huanglongbing (HLB)?

travel to a facility 225 kilometres away that has been approved specifically for work on select agents. The microbiologist would like a lab of his own to work on huanglongbing, but the government has yet to approve it.

"Few, if any, plant pathogens should be considered bioterror agents," says Tim Gottwald, a plant pathologist at the research wing of the USDA in Fort Pierce, Florida, who studies huanglongbing. Gottwald estimates that he spent \$50,000 upgrading his lab to comply with the select-agent rules. More troubling, he says, are delays in getting scientists approved to work on the disease. "I have 12 to 14 people somewhere in the FBI black hole," he says.

The pathogen's select-agent status may also scare away promising young scientists because the restrictions slow down the progress of research, says Triplett. "It's very hard to persuade a post-doc or grad student to work on a project when it's going to hamper [his] career," he says.

More than 3,000 kilometres away, Johnston has his own worries as he scans his grove at the end of a long day. He stops at a navel tree and plucks a shoulder-high fruit, then slices the orange open with a couple of quick motions of a pocket knife. Its juice squirts out and the tangy perfume fills the air for a second. Huanglongbing would reduce the sweet fruit to a withered, bitter gall.

Meanwhile, Johnston says that he hasn't even heard of the select-agent rule. "I am much more concerned that the US government, through inaction, will allow this stuff to move from Florida to Texas to California." When asked about agroterror, he laughs and points out that those wishing to do harm could pick a better weapon than huanglongbing. "Are they going to try to kill orange trees rather than people? I doubt it."

Ewen Callaway is a science journalist in Washington DC.

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