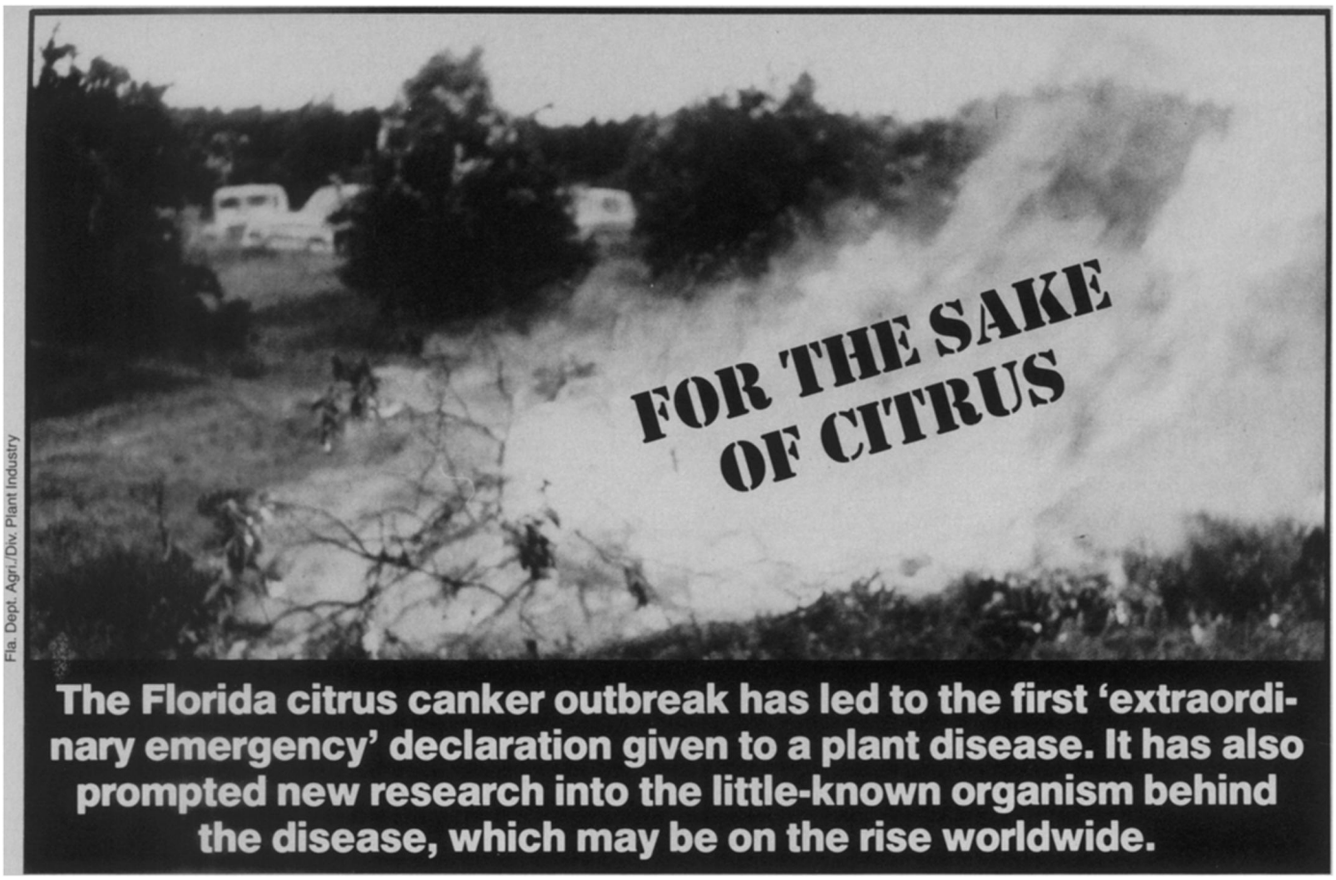


Fla. Dept. Agri./Div. Plant Industry

The Florida citrus canker outbreak has led to the first 'extraordinary emergency' declaration given to a plant disease. It has also prompted new research into the little-known organism behind the disease, which may be on the rise worldwide.

By CHRISTINE MLOT

When citrus seedlings started to turn mottled yellow and brown at an Avon Park, Fla., nursery last August, it was the first appearance of the citrus canker in Florida in over 70 years. But on a global scale, the Florida outbreak follows three others in as many years, and may be symptomatic of a spread in the worldwide distribution of the bacterial disease.

In 1983, pockets of the canker surfaced in North Yemen and Saudi Arabia; in 1982, it broke out in Mexico. It was only recently eradicated in Brazil after periodic outbreaks in the 1970s, but persists in Argentina and other South American countries. From its probable origins in India and Java, the disease has blighted nearly every humid citrus-growing region in the world.

The reason for the spread may simply be more international travel and a greater commercial exchange of fruit and plants, but the biological connection linking the outbreaks is still unclear. Differences among cells taken from the various disease pockets have prompted questions about the basic biology of the canker bacterium, how it infects and how its disease can be controlled.

To begin to answer some of these questions, Congress this fall appropriated a $1 million emergency fund to the University of Florida in Gainesville and the U.S. Department of Agriculture's (USDA's) laboratories in Orlando, Fla., and Beltsville, Md., for research on the organism and the disease that, until now, has received little attention in the United States. "Basic research is needed to give us a foundation of knowledge about the characteristics and genetics of the disease," says Walter J. Kender of the University of Florida in Lake Alfred, who is coordinating the projects of the emergency research.

The citrus canker is a bacterial infection caused by *Xanthomonas campestris* pv. *citri,* one of about 100 pathogenic variants, or pathovars (pv.), of a bacterial species that is responsible for diseases in peaches, beans, cabbages and other plants. *X. campestris* pv. *citri* specifically attacks orange, tangerine, lemon, lime and grapefruit trees, forming lesions, or cankers, on the leaves, twigs and fruits.

The lesions can quickly spread throughout a grove or region. Dispersed via orange crates, transported plants or wind-driven raindrops, the bacteria infect leaves through pores or other openings and then multiply, nurtured by warm wet weather typical of Florida's June to August rainy season. Somehow the plant cells break down, the cankerous leaves drop and the tree eventually declines. When the bacteria infect fruit, the lesions remain on the surface and the fruit is still edible, but the lesions leave openings for fungal infections that can destroy the pulp.

From studies of the international canker outbreaks, citrus experts generally recognize three related strains of *X. campestris* pv. *citri,* based on the bacteria's sphere of influence and plant preference. The Asiatic or A strain infects the largest variety of citrus trees and is widespread in Asia and Indonesia, where the disease is endemic. The B strain attacks lemons in South America, and the C strain has so far been found only in Brazil on lime trees.

But plant pathologist Edwin Civerolo of the USDA research center in Beltsville thinks that classification oversimplifies the bacteria's variation. The organism from the recent Mexico outbreak, though similar to the South American strain, doesn't infect fruit the way the other strains do, he points out, and the newest Florida strain hasn't matched up with the other strains, either.

Civerolo did much of the work showing that the Florida canker is unlike the others. Run through a battery of tests, this strain doesn't fall into any previously identified class. In serologic tests, it doesn't react with antibodies made to other strains, indicating that it has a different set of antigens. It also produces some rare fatty acids, and, while the other strains can be infected by certain viruses, the Florida strain is resistant to those viruses.

Only recently have the Beltsville researchers isolated a plasmid in the Florida bacteria. These small rings of DNA, separate from the chromosome, have been found in all the other strains, but for unknown reasons have been difficult to detect in the Florida bacteria. The newfound plasmid "lends itself to genetic analysis," says Civerolo, and could be useful in characterizing the bacteria.

The Florida disease differs from the other citrus cankers not only in biochem-

S.M. Garnsey

Facing page: Florida workers have burned about 7 million citrus seedlings in the state's effort to eradicate the disease. Officials estimate a million mature trees will now be burned after the recent finding of canker-causing bacteria in a grove that has supplied budwood for grafts onto trees at eight nurseries across the state.

Map at left: The world's citrus-growing regions, marked by white shaded areas, have seen in increase of citrus canker outbreaks in recent years. The extent of the disease, outlined in orange, now touches four continents.

istry but also in physical appearance — a factor that initially slowed state plant pathologists in identifying the disease. Instead of the usual yellow blisters (cankers) that erupt on leaves and fruit, the plants at the Avon Park nursery were blotched with smooth, flat stains.

"Collectively, all the differences are not trivial," Civerolo told SCIENCE NEWS. "I think they are going to be significant."

No one is sure where this anomalous bacterium came from. Some believe it is a remnant of the earlier Florida canker epidemic that began when bacteria were carried in on orange trees from Japan in 1910. "Wild citrus could have harbored the bacteria for all these years," says horticulturist James Ferguson of the University of Florida in Gainesville. In such a subclinical infection, the bacteria slowly build up without producing any symptoms until they reach critical numbers or some natural event sets off the full-blown disease.

But the connection to the previous outbreak may never be conclusively drawn. That outbreak, which peaked in the 1920s, was eradicated by the 1930s without preserving any bacterial cultures for scientific use. "That would be a super resource to have," says Civerolo. "It would be most interesting to know if this was a holdover from that time."

To date, the current citrus canker has turned up in eight Florida nurseries. In late November, the bacteria (but no lesions) were found on the leaf surface of two trees in a budwood grove, posing a new problem in controlling the disease in mature trees. The "treatment" for canker in supplier nurseries remains eradication, and all plants — with or without visible symptoms — are removed and burned as soon as they are located. So far, state workers have torched about 7 million seedlings.

Yet in environments like Japan's where the canker has long been established, it is managed like many other horticultural diseases. The bacteria are always present in the environment, but resistant citrus varieties are planted, antibiotics are applied during growth flushes (when the bacteria are especially virulent) and successful crops are harvested. Japanese researchers have developed resistant trees that recognize the infecting bacteria as foreign and trap them in a sticky polysaccharide before they can grow and create a canker. But such management programs are expensive and long in development — it may take 30 years to develop a resistant tree. And then growers face international market restrictions intended to prevent further spreading of the condition. That's why burning has been the immediate response in Florida.

Still, the USDA and the University of Florida have earmarked some of the emergency fund for management or curative research, along with the basic biological research. In the long run, canker-resistant tree varieties like those developed in Japan are the most effective control, according to Civerolo.

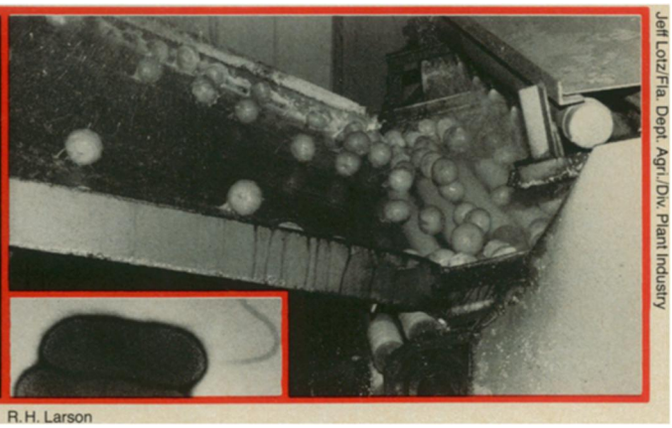
For the immediate situation, researchers will be looking at ways to disinfect soil and equipment as an adjunct to tree eradication. Right now, growers are treating oranges in weak chlorine solution as a safeguard before shipping them to out-of-state markets. And although horticulturists like to avoid spraying antibiotics that can select for resistant bacteria affecting animals, there may be other chemical controls, such as copper sprays, that can kill bacteria before they infect during spring growth.

The unexplained appearance of the Florida citrus canker, which Secretary of Agriculture John R. Block in October declared an "extraordinary emergency" to provide compensation for growers' losses, has only spurred the growing awareness of the potential threat of foreign pathogens beyond localized regions on the world's citrus production. Between 1971 and 1983, the USDA inspection service caught almost 5,800 incidents of canker-infected fruits at U.S. ports of entry. At an October USDA workshop in Beltsville on citrus foreign pathogens — scheduled before the Florida outbreak — citrus scientists discussed the possible alert needed to prevent introduction of the 53 known exotic pathogens affecting citrus and potential ways to control them. Along with the citrus canker, researchers singled out four other bacterial and fungal diseases that, if accidentally introduced, could have drastic effects on the U.S. citrus crop.

"The magnitude and severity of citrus diseases are much greater than they are on other horticultural crops," Howard Brooks of the Beltsville research center said at the workshop. Oranges may not be the country's largest cash crop, but the health of Florida's $2.5 billion industry is important for the state's economy as well as for the health of the nation, Brooks said. In 1983, Americans drank about 8 billion pounds of reconstituted orange juice, and about 58 percent of it came from Florida (Brazil supplied 35 percent), according to the USDA and the Florida Citrus Mutual in Lakeland. That vitamin C source is still intact, but its vitality may be increasingly affected by the condition of the world's citriculture. □

Before they are packed for shipment, oranges are sprayed with a chlorine solution to kill any canker-causing bacteria.

Inset: Photomicrograph of Xanthomonas campestris pv. citri.



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