Scientists identify pheromone from insect that transmits citrus greening (HLB)

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The Asian citrus psyllid Diaphorina citri is only 2 mm long, yet it strikes terror into the hearts of citrus growers in Brazil, China and the United States. This is because it acts as a vector for the bacteria that causes Huanglongbing (HLB), commonly known as citrus greening. HLB originated in Asia and now affects most citrus groves worldwide. The disease is devastating for the citrus industry because infected trees must be eradicated.

To prevent the insect from re-infecting a grove after contaminated trees have been eradicated, growers are typically advised to monitor pest populations, locate them and apply agrochemicals, but until now, there has been no suitable technology for this task.

Researchers at the National Institute of Science & Technology have now identified and synthesized D. citri's sex pheromone for use as bait in traps to lure the insects and kill them before mating. Their findings were recently published in *Scientific Reports*.

"The discovery and synthesis of this sex pheromone should make monitoring and controlling the insect more efficient so that the incidence of HLB can be reduced," said José Roberto Postali Parra, a professor at ESALQ-USP and co-principal investigator for INCT Semiochemicals in Agriculture.

The researchers had been trying since 2009 to identify D. citri's sex pheromone for use in baited traps. They began by studying the insect's

behavior and trying to extract semiochemicals, chemical substances involved in communication, including sex pheromones. However, isolating semiochemicals was made difficult by the insect's complex behavior as well as its biology.

"We realized from the word go that it would be very hard to identify the Asian citrus psyllid's sex pheromone because its behavior is complex and semiochemicals are present only in very small quantities," said José Maurício Simões Bento, also a professor at ESALQ-USP and the other co-principal investigator for INCT Semiochemicals in Agriculture.

To surmount these barriers, the researchers redoubled their efforts by partnering with Walter Leal, a professor at UC Davis, and colleagues in Fundecitrus's R&D department. Initially, they developed a novel system to breed Asian citrus psyllids in the laboratory, establishing a colony on orange jasmine (Murraya paniculata). Temperature, luminosity and humidity mimicked the conditions found in most citrus groves in Brazil. The adults that emerged were collected and separated by sex for analysis of mating behavior.

Mating activity began four days after emergence and peaked after seven days. Virgin and mated females released a pheromone that attracted virgin males. Based on these findings, the researchers proceeded to isolate and identify the sex pheromone's constituents. Chemical analysis of the compounds they isolated showed that the main component of the sex pheromone released by virgin females was lignoceryl acetate.

To find out whether this substance could be used to attract insects in the field, the researchers conducted tests in citrus groves located in the region of Araraquara, São Paulo State, where HLB is widespread. Different doses of lignoceryl acetate were used as bait in yellow sticky traps, commonly used to monitor populations of Asian citrus psyllid.

The number of insects caught in traps baited with lignoceryl acetate did not differ from the number caught in control traps during the first weeks of the field tests, but significantly more males were caught with the sex pheromone-derived substance than in control traps at 35 and 42 days, after which the lure became ineffectual. Analysis of volatile compounds detected small amounts of acetic acid in bait left in the field, but not in newly set traps, suggesting that lignoceryl acetate, although chemically stable, might undergo slow degradation and that acetic acid might be the product of this degradation and act as an attractant for males.

To test this hypothesis, the authors of the study analyzed the volatile compounds released by the insects during peak mating activity. The results clearly confirmed the presence of acetic acid.

Electroantennography (EAG), a technique for measuring the behavior of insect antennae in response to a given odor, along with analysis by olfactometry, showed that males were attracted by acetic acid. The researchers also used field tests to prove that traps baited with acetic acid captured significantly more male insects that control traps without the substance.

Surprisingly, traps baited with acetic acid also captured significantly more females than control traps, possibly owing to physical stimuli emitted by captured males, the researchers believe. "We now plan to estimate the number of traps needed per area in citrus groves and work out a correlation with the insects attracted by traps in order to verify the reduction in the incidence of the disease and map infected sites," Bento said.

The idea, he added, is to make sure the bait lasts at least 30 days in the field, attracting insects to sticky traps so that growers can obtain weekly samples of captures to quantify and evaluate the presence of the pest. "Our aim is to enable citrus growers to use this pheromone to control the

disease at the lowest possible cost," he said.

In Brazil, where HLB first appeared in 2004, some 46.2 million citrus trees (or 26% of the existing stock) have since been eradicated. In São Paulo State, the country's largest producer of oranges, 17% of orange groves have been affected.

In Florida (USA), Brazil's main competitor in orange production, HLB first appeared in 2005 and currently affects 80%-90% of the state's citrus groves. As a result, growers are expected to produce 68.7 million boxes of oranges this year, down from 150 million in 2017 and 240 million 15 years ago. The US Department of Agriculture (USDA) forecasts production of 27 million boxes of oranges by Florida in 2025. "HLB is the most important disease affecting the world's citrus industry today. Infected citrus plants can't be cured and must be eradicated," Parra said.

More information: Odimar Z. Zanardi et al, Putative sex pheromone of the Asian citrus psyllid, Diaphorina citri, breaks down into an attractant, *Scientific Reports* (2018). DOI: 10.1038/s41598-017-18986-4

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