Plant transformation in the laboratory: Embryos are extracted from seeds and maintained on prepared culture media (unflavored gelatin fortified with essential nutrients). A bacterium, Agrobacterium, *is typically used to insert the target gene into the tender plant tissue. Marker genes for fluorescence or antibiotic activity are used to determine whether the gene was successfully inserted. Hundreds of transformation events are necessary to obtain only a handful of viable genetically modified seedlings.*

Photo credit: Maria Oliveira, Ph.D., USDA-ARS

GENETIC ENGINEERING TO PROTECT CITRUS FROM HLB

Carrie Teiken, Peggy Lemaux, Beth Grafton-Cardwell and Neil McRoberts

This past summer, the Citrus Research Board (CRB) and University of California Cooperative Extension hosted citrus grower seminars in Exeter, Riverside and Santa Paula, California. A range of topics was covered – including export challenges due to plant disease, strategies for dealing with water shortages, labor issues facing the California citrus industry, and the potential for using genetically engineered organisms to control the deadly citrus disease, huanglongbing (HLB).

A cure for HLB has not been identified, and all citrus varieties are susceptible to the disease. This is an issue of extreme importance for California citrus growers. Although to date only one HLB-infected tree has been identified in California, the Asian citrus psyllid (ACP), the insect that vectors the bacteria causing HLB, has spread throughout Southern California, is working its way up the coast and has been found in small numbers in the San Joaquin Valley, where 75 percent of commercial citrus is grown.

An important way to stop the spread of HLB is to stop the ACP; however, that is easier said than done. Natural enemies, such as parasites and predators, can reduce psyllid populations, but they do not eliminate the entire pest population; and so the disease continues to spread. Continuous broad-spectrum insecticide treatments can reduce psyllids to very low levels. However, even these treatments do not completely eliminate psyllids, and they are not economically and environmentally sustainable. Lastly, there are limited choices and problems with efficacy of insecticides for organic growers. Long-term solutions are needed, and these may include engineering a citrus tree that can withstand the pathogen and/or a psyllid that cannot transmit the disease. The industry is now faced with the decision as to whether or not an engineering solution should be employed to save California citrus.

ADDRESSING GE SOLUTIONS

Peggy Lemaux, Ph.D., spoke at the Santa Paula and Exeter grower seminars and addressed the topic of engineering citrus or ACP during her presentation, "Food fights in the marketplace: is there a path forward for citrus to address HLB disease." Genetically engineered (GE) crops (also called GMOs or genetically modified organisms) are already being grown commercially in the U.S. with crops like alfalfa, canola, corn, cotton, soybean, papaya and sugar beet; and GE acreages for most of these are above 90 percent.

Although widely grown, GE crops have not been widely accepted in California, leading to county-based bans on growth and propagation of such crops. In California and other states, there have been efforts to pass laws that would require labels on foods containing an engineered ingredient. Using the term "genetic modification" to describe these newly-engineered crops adds to the confusion, because classical breeding (which has long been used to alter the genetic information in crop varieties) also results in modification of the genetic material of the plant. GE crops are modified using some of the same mechanisms used during breeding to change traits of a crop, but the modifications are performed in the laboratory and then reintroduced into the plant.

Currently, genetic engineering for HLB resistance is focused on a number of approaches: GE citrus trees that are resistant to the bacterium, GE citrus trees that kill ACP when it feeds on the tree, and GE ACPs that are unable to vector the bacterium, '*Candidatus* liberibacter asiaticus' (CLas), that is closely associated with HLB. These technologies not only have the potential to save the citrus industry, but also will help growers reduce the number of pesticide applications used to control ACP, thereby reducing costs and increasing profits. Cutting back on insecticides will help growers maintain an integrated pest management program for all citrus pests and reduce pesticide resistance, secondary pest outbreaks and risks to the environment and workers.

However, GE organisms are often met with grower and general public apprehension. Concerns range from export issues (because some countries don't accept engineered crops), impacts on non-target organisms, movement of engineered genes to unintended crops and allergenicity caused by introduced genes. Yet, GE approaches will quite possibly be a component of the long-term solution for the HLB crisis.

CREATING A "nuPsyllid"

The federally-funded USDA National Institute of Food and Agriculture-Cooperative Agriculture Pest Survey's "nuPsyllid" project is a multiple research laboratory effort to engineer ACP and create a "nuPsyllid" that would replace the wild type ACP with a population that cannot transmit the HLB-associated bacterium HLB. The "nuPsyllid" non-vector then would be released into the ACP population, much like the release of *Tamarixia*, the parasitic wasp, and eliminate the wild ACP population.

Three methods currently are being studied to potentially modify the ACP. Bryce Falk, Ph.D., at the University of California Davis, is identifying naturally occurring ACP viruses. He then plans to genetically modify one of the psyllid viruses so that it will disrupt an essential function of the ACP, causing the psyllid to die or be unable to transmit CLas. Kirsten Pelz-Stelinski, Ph.D., at the University of Florida, is studying strains of *Wolbachia*, a bacterium that occurs naturally inside the body of many different types of insects. She plans to infect ACP with natural, foreign or altered *Wolbachia* to reduce the ACP's ability to transmit the bacteria. The third ACP modification is being investigated by Bruce Hay, Ph.D., at CalTech. Hay is working on creating a modified ACP that has a genetic element containing a toxin that kills the HLB-associated bacteria.

Several members working on the "nuPsyllid" project, including Neil McRoberts, Ph.D., University of California Davis Assistant Professor of Plant Pathology; Elizabeth Grafton-Cardwell, Ph.D., Director of the Lindcove Research and Extension Center and University of California Riverside IPM Specialist; and Carrie Teiken, University of California Davis Plant Pathology graduate student, are involved with investigating the socio-ecological consequences of engineering ACP. If a "nuPsyllid" engineering approach is successful, there likely will be reluctance to accept the altered psyllid, within both urban and grower communities, due to a variety of concerns. These concerns include the movement of introduced genes to other insects, consumer acceptance of oranges exposed to "nuPsyllid," potential damage to the crop by released psyllids and regulatory issues for organic citrus production. Therefore, the "nuPsyllid" Socio-economics and Modeling Team is evaluating how to effectively disseminate information on genetic engineering approaches to the citrus industry and provide them with an understanding of the potential long-term benefits and risks of the project.

SURVEYING THE INDUSTRY

The team began this evaluation task by individually surveying growers, pest control advisors and others who attended the March 2014 Citrus Showcase in Visalia, California, hosted by California Citrus Mutual with special presentations by the CRB. Attendees at the citrus grower seminars in Exeter and Santa Paula were given a similar survey, but were able to answer the questions with 'clickers' (handheld electronic transmitters). The clicker survey posed multiple-choice questions projected on a screen. Each participant then submitted their answers using the clicker, beaming a signal to the presentation computer, which collected the participants' answers and produced a chart that showed immediately how many participants chose each answer. The results of the survey were anonymous. A total of 259 responses were recorded: 46 at the Visalia Citrus Showcase, 42 in Santa Paula and 171 in Exeter.

Survey questions included information on citrus acreage grown or managed, age of participant, and their opinion on using genetic engineering to prevent HLB from spreading in California citrus. The survey also asked which type of engineering approach growers preferred: GE citrus trees that resist the disease, GE trees that kill the ACP when they feed, released GE ACPs that don't spread HLB, or none of the above. The last question asked growers to select what they believe is the biggest impediment to using GE approaches to manage ACP and HLB. Choices included grower acceptance, public acceptance, government approval or "I don't know."

KEY SURVEY FINDINGS:

• **What size are citrus farms?** The majority who were surveyed at the Visalia Showcase (63 percent) and the Santa Paula meeting (76 percent) farmed less than 100 acres of citrus. In Exeter, there were similar proportions of growers with less than 100 acres (39 percent) and those with more than 500 acres (37 percent). The remainder (24 percent) farmed between 100–500 acres **(Figure 1)**.

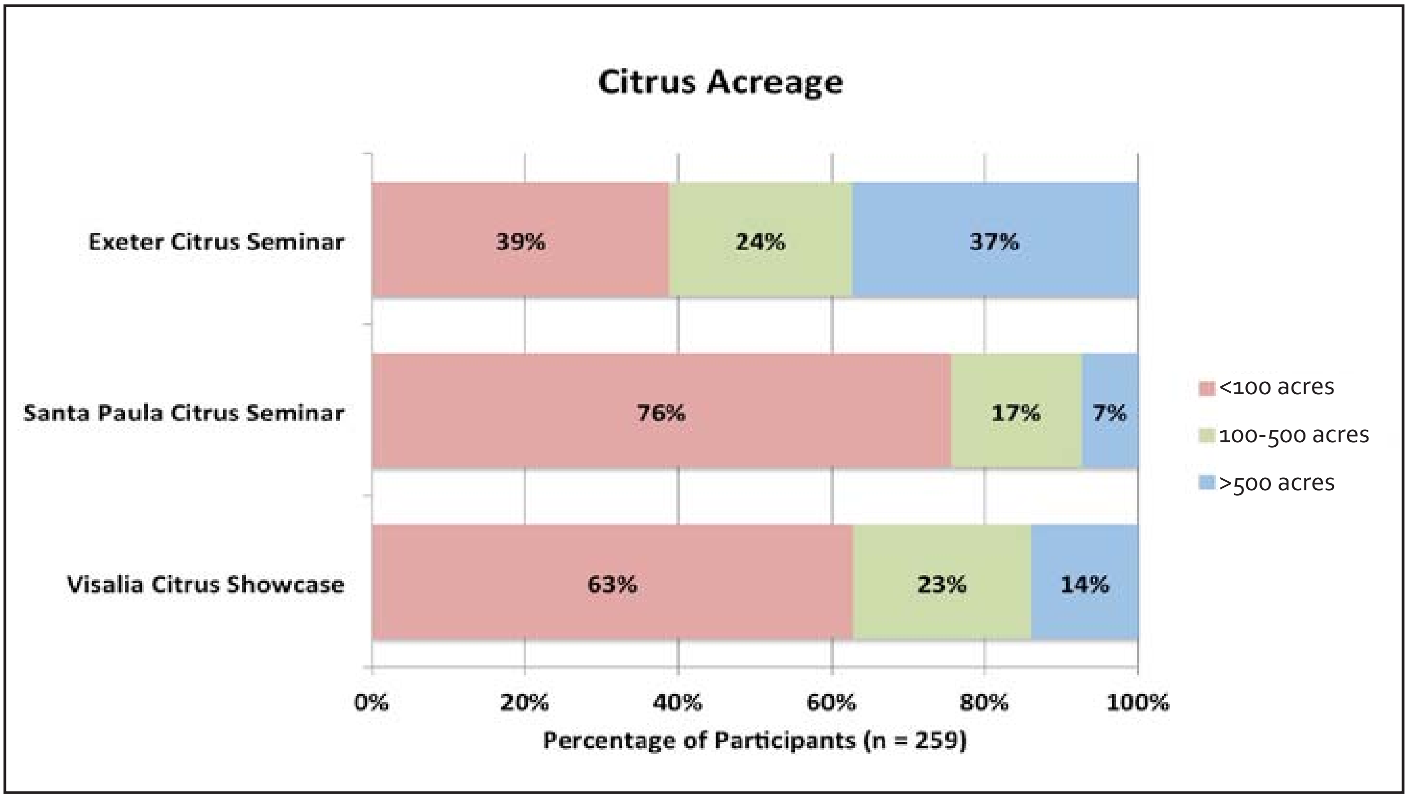

Figure 1: Number of acres of citrus grown.

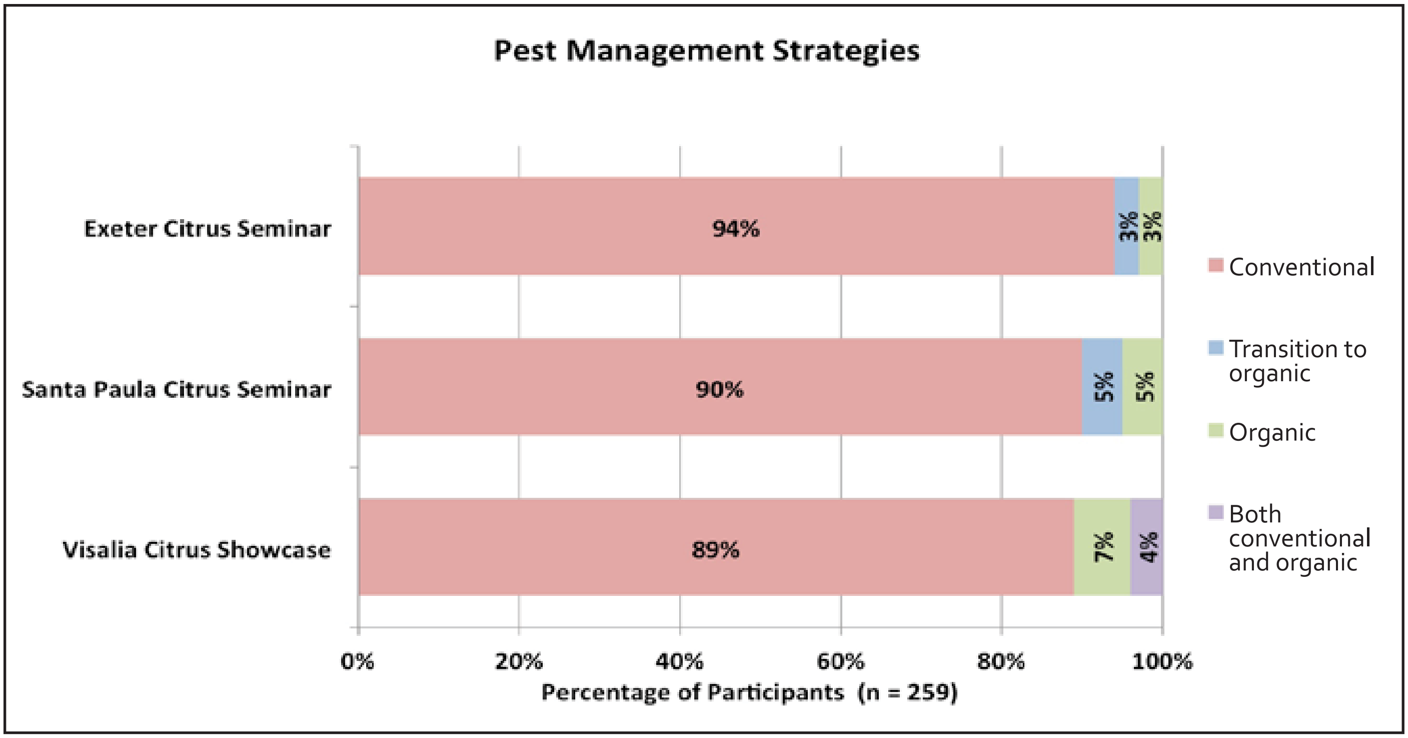

Figure 2: Proportion of growers practicing conventional, transitional and organic pest management strategies.

• **What types of citrus growers?** In all three locations, 89-94 percent of growers utilized a conventional pest management strategy of synthetic insecticides and herbicides; the remainder were organic growers or growers transitioning to organic **(Figure 2)**.

• **Thoughts on engineering?** Most of the survey participants were either strongly (65 percent) or cautiously (25 percent) in favor of a GE approach for controlling HLB. A low percentage (six percent) were indifferent or were completely against (six percent) GE approaches **(Figure 3)**.

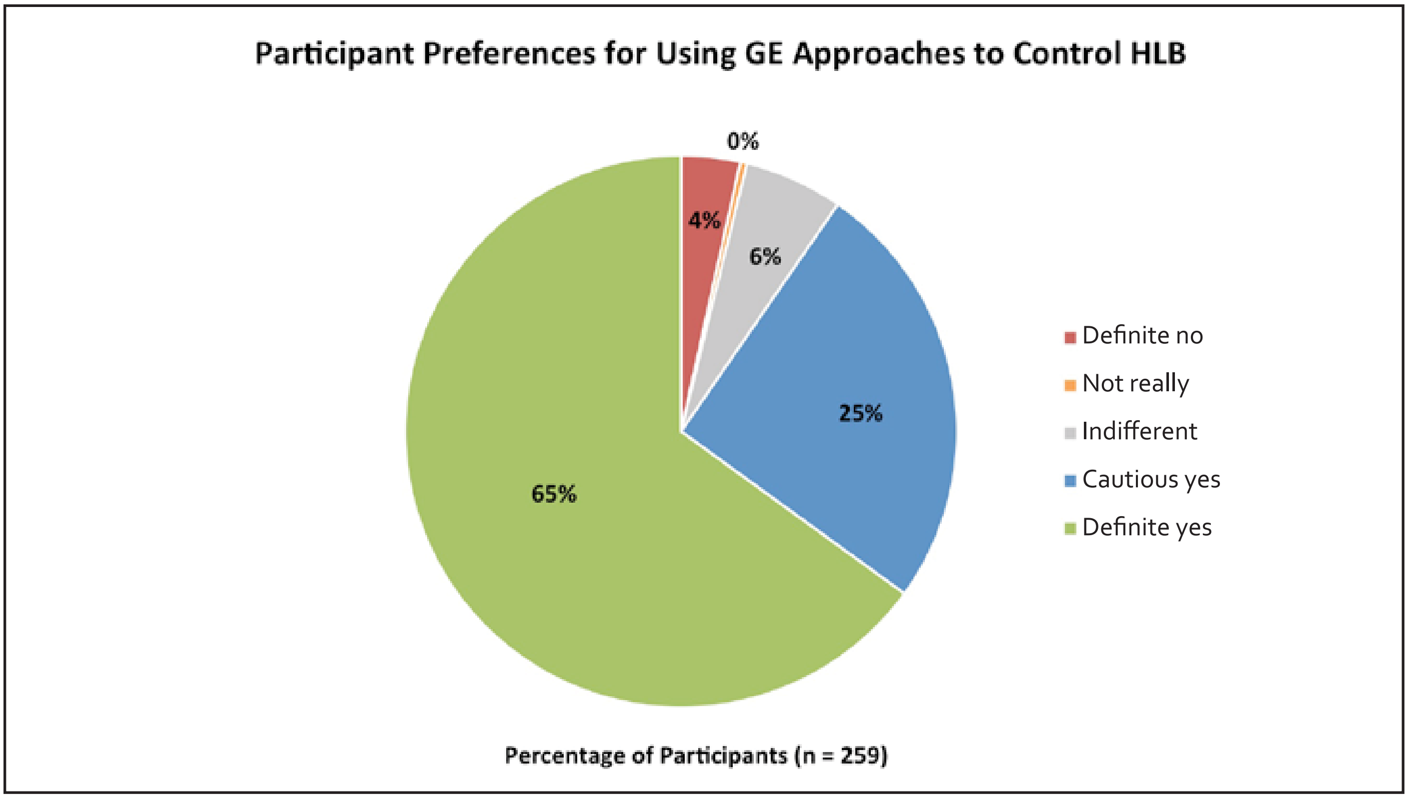

Figure 3: Participant preferences for using GE technology to prevent HLB from spreading in California.

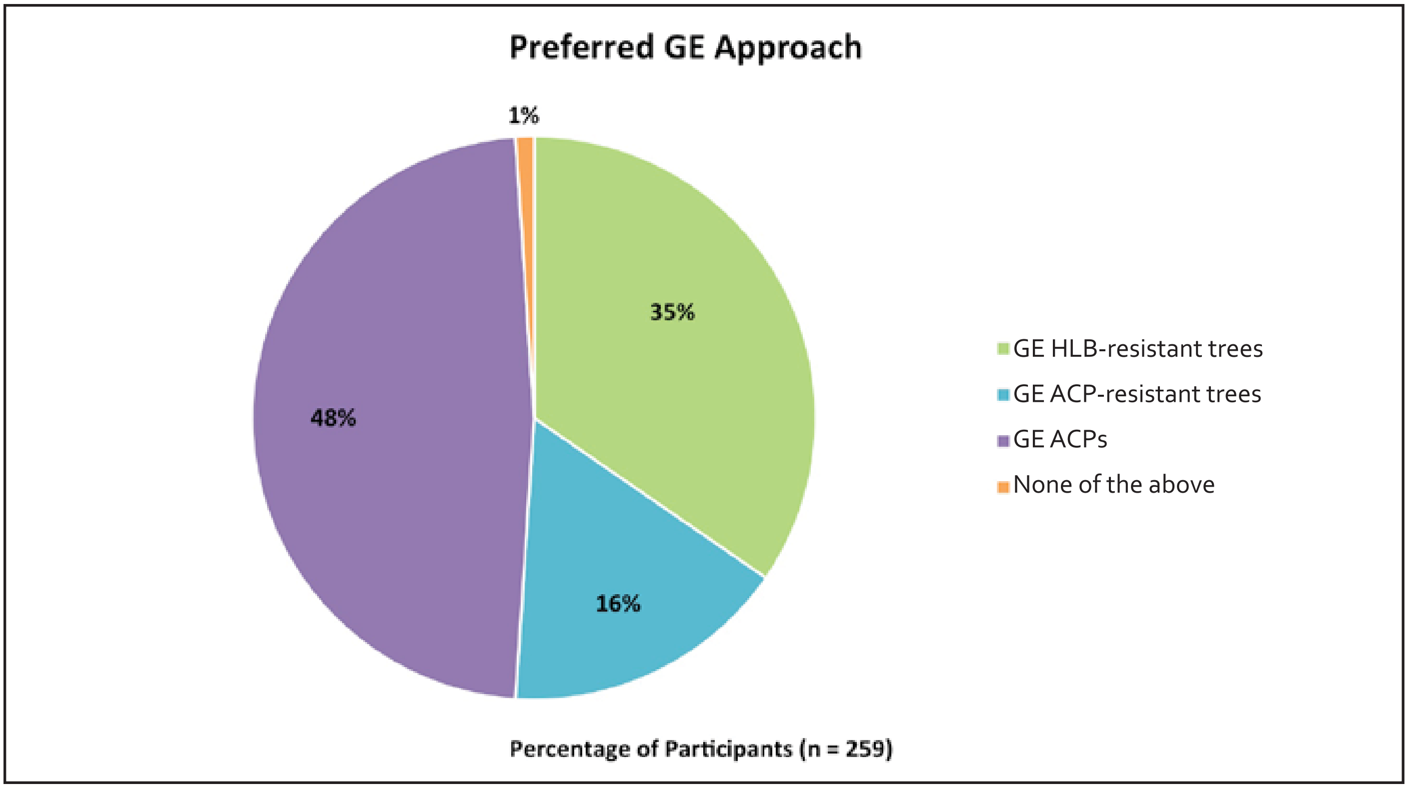

Figure 4: Preferred GE approach to control HLB.

• **What engineering approach?** Participants were evenly split between GE ACPs (48 percent) and GE trees (51 percent) as preferable for controlling HLB **(Figure 4)**.

Between the two techniques for GE trees, HLB-resistant trees were preferred (35 percent) over ACP-resistant trees (16 percent) **(Figure 4)**.

Growers with more than 500 acres of trees preferred GE trees (57 percent) to GE ACPs (43 percent); growers with fewer than 100 acres of trees preferred the opposite, GE ACPs (61 percent) over GE trees (39 percent) **(Figure 5)**.

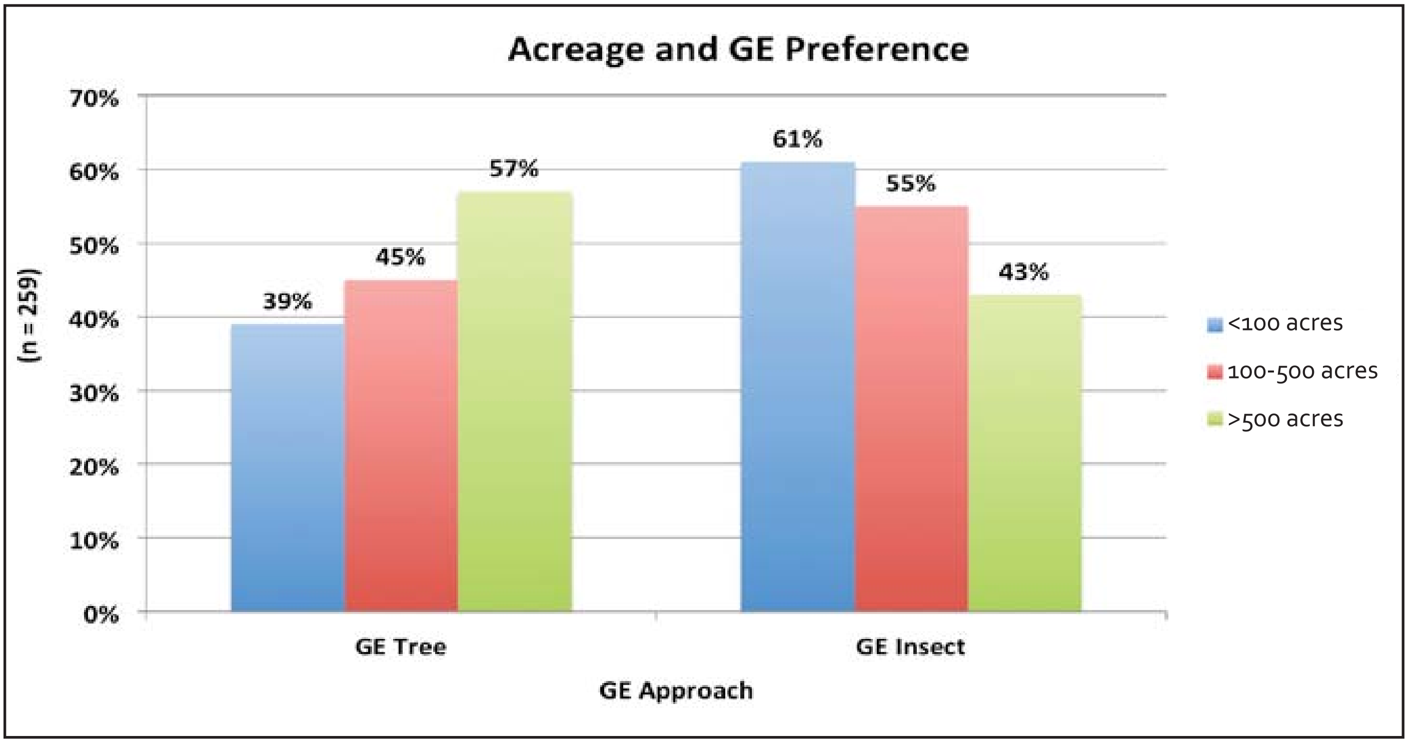

Figure 5: Grower acreage and preferred GE approach to control HLB.

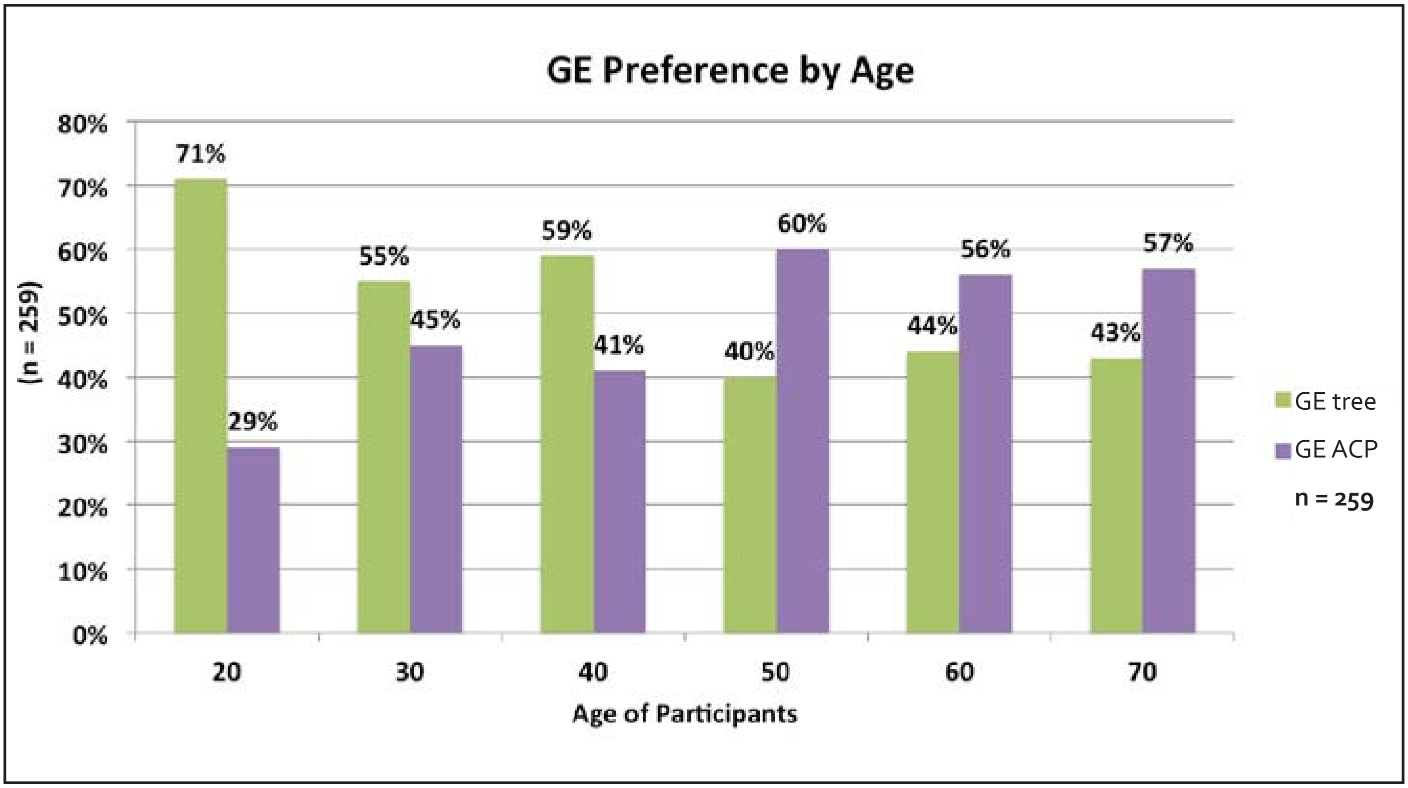

Figure 6: Age of survey participants and preferred GE approach to control HLB.

Participants aged 40 and younger had a stronger preference for GE trees (62 percent), while those over 50 preferred GE ACPs (58 percent) to control HLB **(Figure 6)**.

• **What would the impediment be?** Most attendees believed that public acceptance (56 percent) would be the biggest impediment to adoption of genetic engineering of either the tree or the psyllid, followed by government approval (33 percent). A small percentage thought grower acceptance would be an impediment (six percent), and some did not know (five percent) **(Figure 7)**.

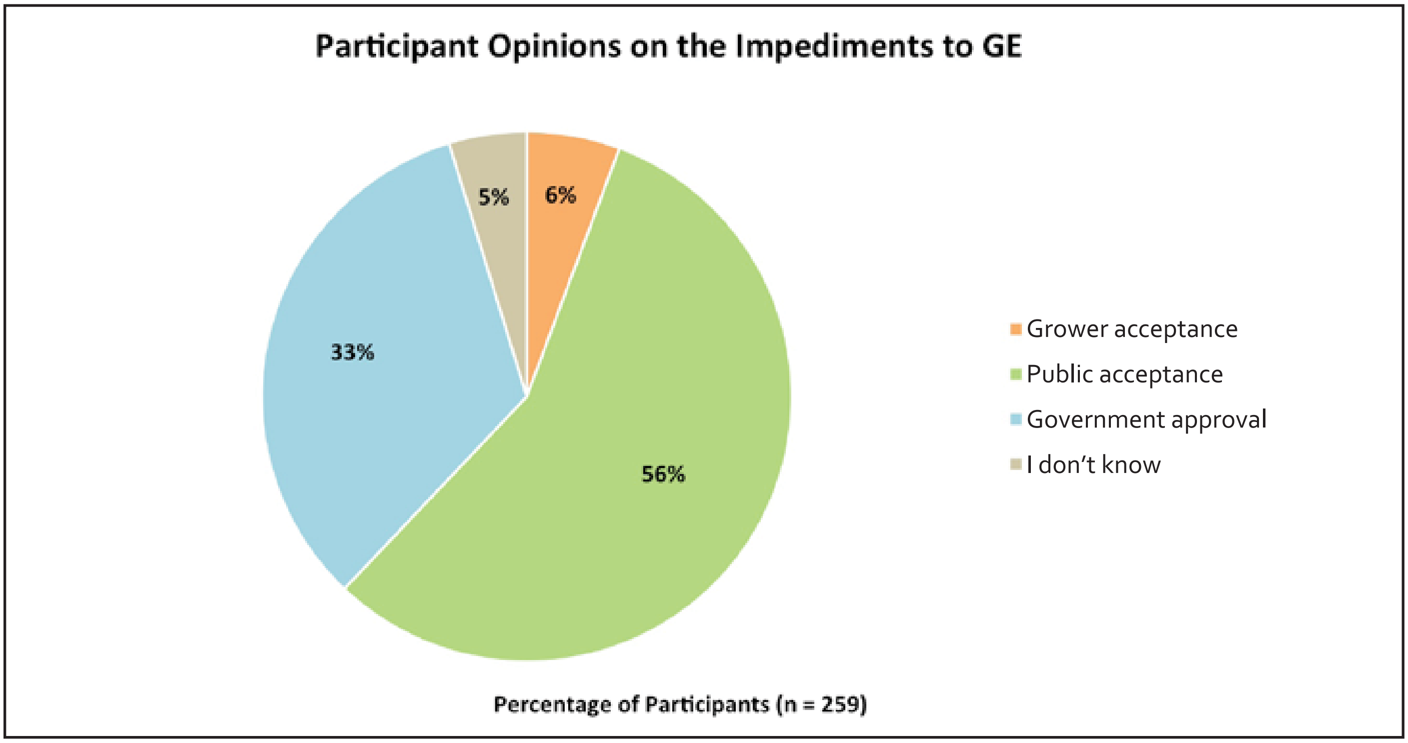

Figure 7: Participant opinions on the impediments to adoption of GE approaches to control HLB.

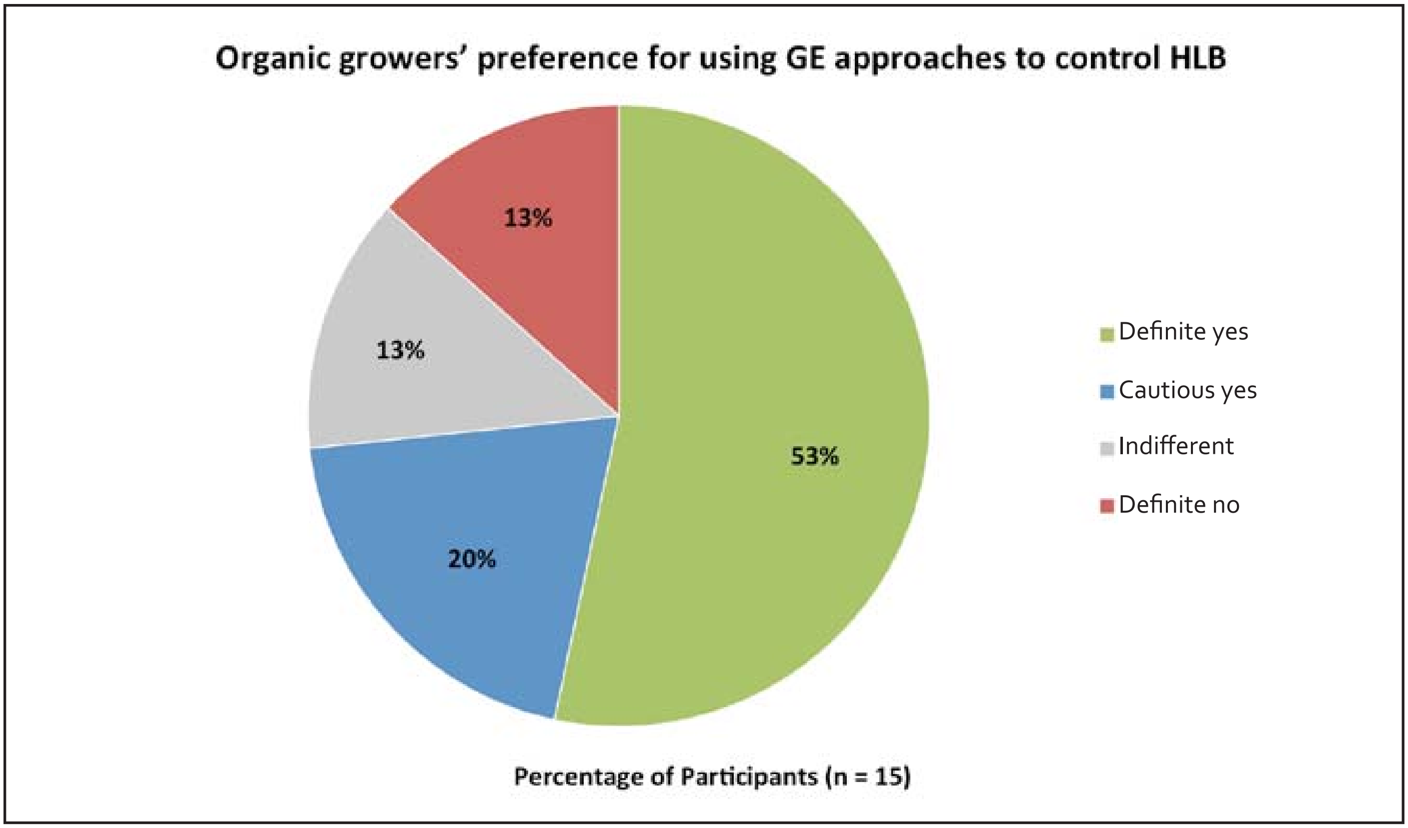

Figure 8: Organic growers' preferences for using GE technology to prevent HLB from spreading in California.

• **What did organic/transitional growers think?** While there were only 15 organic/transitional growers who participated in the survey, the majority either definitely or cautiously supported GE approaches to control HLB (73.3 percent). A small number completely rejected GE (13.3 percent), while some were indifferent (13.3 percent) **(Figure 8)**. Those who supported GE were split between GE trees (54 percent) and GE ACPs (46 percent).

Overall, the majority of those surveyed are in support of GE approaches to control HLB. Interestingly, preference for the GE approaches varied strongly based on age and acreage. When the Exeter audience was questioned about why they chose one GE approach over another, the older participants pointed out that they don't have time to replant citrus and reap the benefits of full production; and they, therefore, preferred modification of the psyllid. The younger participants felt that a GE tree would be a more permanent solution. Small growers preferred a transformed ACP solution, because replanting would have a negative impact on their income.

Although only 16 organic/transitional growers participated in the survey, the results showed most were in favor of using GE approaches to control HLB. One GE supporter at the Visalia meeting asked if GE would hinder one's status as an organic grower. We cannot answer that question at this point, because GE insects have not been released for agricultural purposes in the United States, and the regulatory process and consequences for the organic industry have not yet been determined.

IDENTIFYING POTENTIAL ISSUES

Many participants recognized that there are potential issues associated with GE technology that will need to be addressed. The majority felt that public acceptance would be the most difficult hurdle, followed by government approval. Only a few participants thought that growers would not support GE approaches, which was strongly substantiated by the grower survey responses. Several participants who completed the survey in Visalia also mentioned concerns about the safety of GE citrus for human consumption and its impact on the price of fruit. In Exeter, one participant was concerned that there would be fewer citrus varieties, and that the industry could lose some of the tastiest varieties since it takes time to engineer each variety and obtain regulatory approval to release into commercial production. Another expressed concern about having a monoculture of GE trees and the potential for the whole system to "crash and burn."

All GE technologies are under development at this time: transforming the plant itself to kill the bacterium or psyllid;

introducing a virus into the plant that carries an anti-bacterial gene; altering the psyllid itself so that it cannot transmit the bacteria; or introducing an organism into the psyllid to block its activity as a vector. If one or more of the GE approaches being researched is successful, one of the greatest challenges for the citrus industry will be to address the general public and regulatory concerns surrounding the technology. In all probability, both modified ACP and modified trees will be introduced along with other management tactics for a systems approach to addressing the devastating effects of HLB.

We are at the beginning of thinking about how to best deploy such technologies, and this is an on-going conversation in which the views of the industry are a crucial part. The recently announced investment in research to combat HLB by the federal government is likely to accelerate the pace at which new technologies are developed. The University of California extension and outreach team will be working hard to help with the education and implementation processes and we strongly encourage the active involvement of the grower community.

Carrie Teiken is a graduate student in the Department of Plant Pathology at the University of California Davis; Peggy Lemaux, Ph.D. is a cooperative extension specialist in plant and microbial biology at the University of California Berkeley; Beth Grafton-Cardwell, Ph.D. is the director of the Lindcove Research and Extension Center and a University of California Riverside integrated pest management specialist; and Neil McRoberts, Ph.D., is an assistant professor of plant pathology at the University of California Davis.