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Investigations of the Effect of Guava as a Possible Tool in the Control/Management of Huanglongbing

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ABSTRACT. The farmers of the Mekong Delta of Vietnam have discovered a unique interaction between citrus and guava, *Psidium guajava* L., that extends the commercial life of citrus plantings plagued by huanglongbing (HLB). Due to HLB, the normal life of citrus plantings in the region is 2-4 yr. However, when interplanted with white guava, plantings can survive for up to 15 yr. Vietnamese/Japanese/Australian collaborative research projects have demonstrated that psyllid vector populations are reduced to near non-detectable levels in interplantings compared to citrus monocultures. The Florida research project described here is an attempt to adapt and determine the feasibility of this discovery to Western citriculture conditions. White guava, determined to be genetically similar to that grown in Vietnam, and pink guava have since been tested in greenhouse studies in Florida. High adult mortality rates occurred when psyllids were confined to guava in no-choice situations, with 95% mortality occurring within 6-9 days. Mortality rates of adults confined to tomato or cotton were similar while near 100% survival was observed on citrus. Adults generally found citrus faster when citrus was caged alone than when it was caged with guava, and lower numbers of adults were consistently observed on citrus over time in cages with both citrus and guava. The effect may be due to volatile compounds produced by guava that are deleterious to psyllids. Mortality rates of adult psyllids in cages with citrus and guava were significantly greater than in cages with citrus alone in one experiment but not another. The results of the two experiments were similar with respect to mortality rates of adults in cages with just citrus. The effect of various volatile extracts on psyllid behavior and survival are under investigation. In addition, large-scale replicated field plots have been established in multiple locations in Florida to examine the dynamics of HLB epidemics in citrus/guava interplantings versus citrus monocultures. Mekong farmers often use spray oils (agricultural and horticultural mineral oils) in lieu of insecticides in addition to guava to inhibit psyllid populations. This same strategy is presently under investigation in Florida.

Florida HLB History and present Situation:

Huanglongbing (HLB) was first discovered in Florida by a Florida Department of Agriculture and Consumer Services scientist on August 24, 2005, during a routine survey (18). The first discovery was in a pummelo tree in a commercial tropical fruit planting in Florida City, south of Metropolitan Miami, and immediately thereafter in the residential communities within 65 km. Presumptive PCR-positive samples were confirmed by the USDA, APHIS diagnostic lab in Beltsville on September 2, 2005, and determined to be '*Candidatus* Liberibacter asiaticus', the causal agent of Asian HLB. The discovery immediately prompted a survey to delimit the infection which soon confirmed the northward extent of the disease along the Atlantic coast of Florida to Fort Pierce, 193 km from the point of discovery and northwest and inland to the closest commercial citrus industry planting 80 km from the point of discovery.

Over the following 2.5 yr since discovery, state-wide spot surveys of the entire Florida commercial citrus industry combined with grower submitted suspect samples have shown that the disease now encompasses more than two-thirds of the Florida peninsula and almost the entire Florida commercial industry. There appears to be a disease gradient across the Florida citrus industry, that is, the disease incidence is higher and symptoms more severe in the southern portion of the state where HLB was first discovered. Disease gradient decreases to the north, however the disease is increasing rapidly even in the northern extents of the Florida citrus industry.

It is assumed that HLB arrived in the southernmost commercial citrus planting consisting of ~4900 ha in Hendree County via psyllid vector dissemination and transmission. This is further suspected because the distribution of HLB symptomatic trees is more intense in the southeastern portion of this planting, proximal to the residential area and separated from it by a citrus-free void, i.e., the Everglades area. Although not conclusive, this distribution of HLB on both sides of the citrus-free Everglades may be further evidence that long distance movement of HLB is achieved by psyllid vectors and may have been facilitated by hurricanes and tropical storms that have affected Florida recently. Some of the individual blocks in this southernmost commercial citrus region are now as much as 80% infected. Fruit drop and losses of crop quality have also been seen in some areas.

Intensive insecticide treatments to control psyllid vectors were immediately put in place by commercial producers as was removal of HLB-infected trees to lower inoculum. One of the most problematic characteristics of HLB epidemics is the latency between vector transmission and symptom expression, which can range from a few months to multiple years, depending on tree age, cultivar, and tree health. Thus, when a few infected trees are seen, there are many more symptomless trees in the planting and surrounding area that are infected, but visually not detectable (19). In almost all cases worldwide, by the time the initial detection of the disease is confirmed, it is already too widespread either to eradicate or contain due to this latency, and therefore eradication is rarely considered. That is, when the disease is discovered, it is probably much more advanced than indicated by the number of symptomatic trees that are recognized. The experience in Florida has been similar. Although psyllid populations are nearly eliminated in some commercial plantings, and massive elimination of HLB-symptomatic trees is ongoing, reduced spread of HLB has not been realized. These conventional HLB control strategies originally defined by the UNDP FAO Project for citrus rehabilitation in Southeast Asia (6, 32) are currently providing little, if any, prospect of adequate disease control.

The disease is also regional in aspect. The incidence of HLB infections within the surrounding region greatly affects the probability and efficacy of slowing the epidemic (18). A commercial producer can be very diligent with respect to vector control and rouging of infected trees, but if surrounding plantings are not as rigorously managed or large numbers of HLB-infected residential trees remain in the area, the planting may be overwhelmed with infections from surrounding infected trees and high vector populations. In a few cases in both Florida and Brazil, groups of growers have joined forces in an attempt to conduct areawide spraying for the Asian citrus psyllid. It remains to be seen how effective such area-wide psyllid control attempts will be.

Without a doubt, HLB is the most serious disease of citrus worldwide (7, 10, 13, 14, 19). The commercial citrus industries in Florida and Brazil that are now dealing with this devastating disease are facing the toughest challenge they have yet faced. The implications of HLB on production, longevity, markets and trade are severe and have resulted in numerous rapid changes including costly eradication, vector control, and nursery certification programs. No citrus industry worldwide has ever effectively controlled this disease and in many prior citrus growing areas, continued commercial production of citrus becomes economically marginal to infeasible. In both the US and Brazil, grave concerns for the potential impact of HLB and survival of the commercial citrus industries have also led to immediate increases in research funding directed at HLB and an international fervor to develop superior control/mitigation strategies and resistance. In the interim, any control measure that has promise should be investigated, in hopes of prolonging survival of the citrus industry until resistance can be achieved.

The realization of the effect of guava on HLB

Perhaps the first international presentation of the possible effect of guava (*Psidium guajava* L.) as a possible control strategy for HLB was made at the *International Workshop for Prevention of Citrus Greening Disease in Severely Infected Areas*, held in Ishigaki, Japan, in December 2006, sponsored by Japanese International Research Center for Agricultural Sciences (JIRCAS). At this meeting, Dr. Nguyen Van Hoa, from the Vietnamese Southern Fruit Research Institute, described collaborative research projects between researchers at JIRCAS, SOFRI, and the University of Western Sydney (UWS), New South Wales, Australia, the latter through funding provided by the Australian Centre for International Agricultural Research (ACIAR) (9). The intentions of the experiments were to demonstrate insecticide, mineral oil, and farmer practices on suppression of populations of the Asian citrus psyllid in replicated field plots in Vietnam. In one SOFRI experiment, two commercial citrus plantings were selected near Cai Be, in the Mekong Delta region of South Vietnam. The first site was approximately 1,500 m^2 in area and was planted in October 2003 with 102 disease-free King orange (*C.* × *aurantium* L.) trees in a 2.5 m x 2.5 m pattern as is common in the region. The second location was similar in size and located approximately 0.5 km from the first. On this site, the land owner had planted white guava, cultivar Xaly, in a 2.5 m x 2.5 m on the land the previous year, and requested that the citrus be interplanted between the guava which is a common practice in the region to provide income that would be unaffected by the research trial. The research team agreed to allow the guava to remain and in October 2003 established this second citrus plot with 102 King mandarin trees planted on the same planting pattern but interspersed with the existing guava plants.

Dr. Hoa reported that the research team monitored psyllid populations monthly over the next year, but quickly became concerned that the test would fail because no psyllids were found in the citrus/guava interplanted plot, while high psyllid populations were seen in the citrus monoculture plot. Indeed over the next year only two adult psyllids were discovered in the citrus/guava interplanting whereas hundreds were detected in the citrus monoculture planting.

Psyllid nymph populations were similarly depressed. Within the monoculture planting, HLB symptoms began to appear within 5 mo, and by the end of the first year, the citrus monoculture plot expressed approximately 32% HLB incidence, whereas the citrus/guava interplanting had no trees expressing HLB symptoms.

In the Mekong region of South Vietnam, the incidence of HLB is very high, and most citrus plantings succumb to the disease within 2-4 yr (17). However, some Vietnamese farmers in the region have adopted a practice of interplanting citrus with cultivars of white guava and have observed much improved longevity of the citrus plantings, in some cases up to 15 yr to date and have observed little HLB. The combination of the scientific study reported in Ishigaki and the anecdotal accounts of increased citrus survival when interplanted with guava prompted two of the USDA, ARS authors of this paper and three Florida citrus industry representatives to travel to Vietnam in May, 2007, to meet with the Vietnamese/Japanese/Australian research team to observe this trial and other guava interplantings versus citrus monocultures in the area. SOFRI generously hosted the US group during their visit. During the excursion, all members of the US group observed first hand the effect of guava/citrus interplanting on psyllid population suppression and mitigation of HLB symptoms. We also found no evidence of psyllids in citrus/guava interplantings. However in nearby citrus monocultures Asian citrus psyllids were prevalent.

The assumption is that the guava plants most likely give off volatile compounds that affect the Asian citrus psyllid. However, the existence of these presumptive volatiles has not yet been fully demonstrated or characterized although related research is underway in several laboratories. Therefore, it is unknown if individual volatiles are repelling, confusing, etc. the citrus psyllids, or if there are other modes of action of the guava in these interplantings. Interestingly, the protective effect of guava for citrus is not completely unheard of in the US. The effect is described in a 1966 publication on companion plants and how to use them (22). Preliminary studies conducted by SOFRI and JIRCAS researchers have indicated that hexane extractions of guava leaves repel psyllids from visiting citrus sprayed with the extracts. They believe the hexane is extracting terpenoid compounds from the guava leaves that act as a repellent to the psyllids (Do Hong Tuan et al., pers. comm.). Preliminary caged studies conducted in Florida have demonstrated increased mortality when psyllids are caged with guava alone or both guava and citrus versus citrus alone (20, 21). Trials with whole plant extracted oils in four-armed olfactometer tests have demonstrated that host plant volatile oils significantly attract adult female psyllids, whereas guava extracted oils repel them (11, and Cen Yijing, pers. comm.). Interestingly the volatile oils had no significant affect on psyllid males in these experiments. However, responses of both sexes in Y-tube olfactometer tests were the same, both being repelled by volatiles from fresh guava leaves (31).

Plant intercropping strategy

The practice of plant intercropping to control plant pests is by no means a new concept or strategy (8, 15, 23, 24, 30). In fact, humans have used this strategy for thousands of years. The use of plant monocultures in modern agriculture has many benefits for high production, quality control, and sustainable and predictable yields, but also shifts the reliance of pest-control almost totally to the use of agrochemicals and pesticides and away from the benefits of natural biocontrol achieved by intercropping systems. Over the past few years the scientific literature has become rich with the rediscovery of the beneficial use of integrated pest management by incorporating multiple cropping systems and agroecosystem diversity for elevated pest control (1, 3, 5, 12, 16, 25). There are many examples throughout the literature of the synergistic effect of crop species diversity on reducing both insect and disease damage to agronomic crops. This is especially true for the use of multiple crop systems for insect pest control. Some authors have recently explored the effect of crop mixtures on repellency or confusion of a number of insect pest species. Andersson (2) provides an excellent review of insect repellency by non host species. A number of studies have pointed to the reduced need of insecticides in multi-crop systems (2, 26). Common examples of this mitigating influence is the use of marigold, catnip, garlic and other companion plants in home gardens to discourage infestations of homopterous, hemipterous, coleopterous, and lepidopterous garden insect pests (4). Throughout Southeast Asia today, monocultures of plant species are common in crops such as rice, but the landscape is still lush with a multitude of intercrop combinations (27, 28). The combination of citrus, an old-world species, and guava, a Western hemisphere species introduced into Southeast Asia, is probably relatively recent in human agronomic history. The beneficial effects of this new combination of plant crop species has apparently only been noted over the last two decades by the keen observation of Vietnamese farmers. Although a brief mention was made of combining guava with citrus to repel insect pests in 1966 (21).

US guava/citrus greenhouse investigations

Hall et al. (19) reported on a series of greenhouse studies conducted to assess biology and behavior of adult psyllids in cages containing citrus alone, guava alone, and citrus with guava. High adult mortality rates occurred when psyllids were confined to guava in no-choice situations, with 95% mortality occurring within 6-9 days. However, survival of adults was also reduced to a similar magnitude when they were confined to cotton or tomato, included as non-citrus, neutral plant species, whereas, nearly 100% survival was observed when adults were confined on citrus. Adult psyllids introduced into cages generally moved to citrus faster when citrus was alone than when citrus was with either guava or cotton, and greater numbers of adults were consistently observed on citrus over time in cages with just citrus. This may have been in part due to differences in total plant surface areas in cages with citrus alone or citrus with another plant. However, in one study, decreased numbers of adults on citrus caged with guava was attributed to the presence of guava. Mortality rates of adults was increased in cages containing both citrus and guava in one study but not another, which may have been related to differences between the two studies with respect to air temperatures. While significant reductions in infestations of adults sometimes occurred in cages containing both citrus and guava, the levels of reduction were less dramatic than anticipated. Thus, cage studies as conducted may be inadequate for assessing the effects of guava on the psyllid (20). Guava volatiles may be too concentrated in such studies to achieve effects that occur in the field. Also, the spectrum and concentrations of guava volatiles might change as a plant matures. Verifying the Vietnamese guava effect is currently thought to be dependent on field studies.

US guava/citrus interplanting trials

The USDA, ARS has established a cooperative project with the JIRCAS, SOFRI, and WSU researchers combined with Florida commercial citrus growers, University of Florida scientists and USDA, APHIS researchers, to attempt to bring the guava/citrus interplanting technology to Florida and Texas and test it under western citriculture conditions, exposing it to the pressures of the current Florida HLB epidemic. The project is call SAGE, for Southeast Asian Guava Effect, and is an attempt is to determine if guava can be integrated with other control methods to extend the commercial life of citrus plantings in Florida. To date, two large guava/citrus interplanting trials are under way in commercial plantings in Florida and several smaller field research trials are underway on USDA, ARS and USDA, APHIS facilities in Florida and Texas (Fig. 1). One of the main constraints to establishing any plots in Florida was the lack of guava plants. The team had to first propagate nearly 19,000 guava plants to establish these trial plantings. This has been accomplished and interplanted with citrus in late summer 2009, as is the procedure used in Vietnam. This is to assure that the guava plants are producing the presumptive repellant volatile compounds prior to exposing the citrus to possible infection. This typically may require that the guava grow for approximately 1 yr prior to interplanting with citrus. To date, no HLB has been detected visually or via PCR in the interplanting.

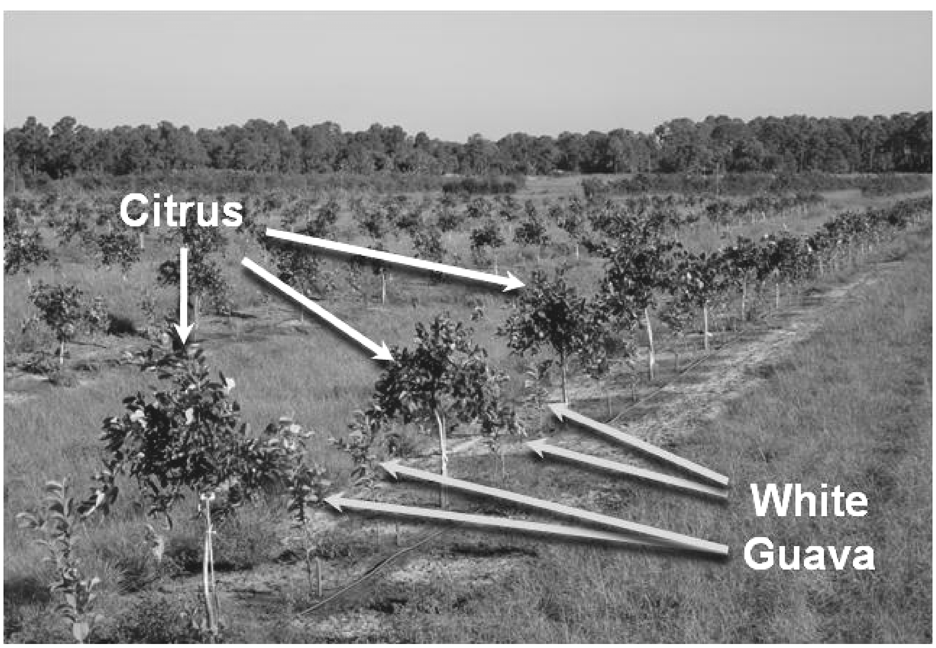

Fig. 1. Example of a new Valencia sweet orange/white guava interplanting in a USDA-ARS experimental plot in Fort Pierce, Florida.

There are many questions and concerns relative to the use of guava as a psyllid deterrent and much that is unknown. For example, plant density is a profound concern. In Vietnam and throughout Southeast Asia citrus plantings are at a much higher density than are used in Western citriculture (Fig. 2). Both the distance between trees within row and the distance between rows are much reduced in Vietnam compared to the majority of commercial citrus planting in the Western world. Presumably, as the density of guava plants decreases within an area, so will the concentration of volatiles produced that inhibit psyllids, potentially jeopardizing effectiveness. The minimum density of guava plants per unit area to be effective is unknown.

Fig. 2. Intercropping in Mekong Delta region of South Vietnam. Guava and citrus intercropped on raised beds with occasional coconut and banana and bordered by water coconut along ditch banks (as illustrated). The form of intercropping varies, and some instances cajuput (*Melaleuca cajuputi* Powell) is planted on the sides of beds to reduce the risk of sunburn damage to fruit.

Air movement through the planting will likely influence the concentration of volatiles. In Vietnam, citrus plantings are immersed in a sea of tropical plant vegetation. In a majority of cases, there is no significant break in the canopy of citrus plantings and the surrounding canopy of other vegetation. That is, Vietnamese citrus plantings have a built-in windbreak on all sides that often extends in all directions. This may be very important for buildup and retention of guava volatiles within the citrus plantings. In many western citriculture situations, there are significant breaks between the edge of citrus cultivation and the nearest vegetation or obstruction to air movement. In Florida, geographical features such as roadways, right of ways, canals, ponds, parking areas, equipment staging areas, etc. provide many voids in the citrus landscape that provide a means for significant airflow and air drainage away from citrus plantings. Such drainage will likely continually reduce the concentration of guava volatiles when intercropping with citrus. Therefore for guava to be a viable HLB mitigation strategy, it may be necessary to incorporate windbreaks within high density citrus plantings.

Another concern is whether species and cultivars of guava differ in effectiveness. It is interesting to note guava is a tree of the tropical Americas that is currently grown in the tropics and subtropics around the world. In Vietnam, the species commonly used is *P. guajava*, and the cultivars are referred to as white Vietnamese or Thailand (Thai) guava. Although the same species, the fruit of white guava cultivars are considerably different from the red or pink guavas that are most familiar in the West (Fig. 3). Whereas the flesh of red or pink guava fruit is soft and very sweet to the taste, white guava fruit have a much more firm flesh that is crisp, bland to the taste and much less sweet. In Vietnam, the flesh of the white guava is often sliced and eaten by dipping the slices in a mixture of salt and ground red pepper (Fig. 4). A few established commercial plantings of white guava, referred to as 'Thai' guava, do exist in the United States especially in South Florida. These were used as a source of the propagating material for our tests of the guava effect to control psyllids. Over 19,000 individual plants were air-layered from one commercial white guava planting.

Fig. 3. White guava fruit being grown commercially in south Florida. Value of each fruit is quite high thus fruit are individually covered with a styrene netting and clear plastic bags to protect them from scaring and insect damage.

Fig. 4. Examples of commercially grown white guava fruit. Coin on top of left fruit is ca. 2.5 cm in diameter.

Genetic similarity within guava, other Myrtaceous plants, and moving forward:

There are a number of US guava accessions in germplasm collections in Florida, Hawaii, and other locations. A study was conducted to determine the diversity and similarity of these accessions to the White guava cultivars common to Vietnam. SSR analysis was conducted on all readily accessible US guava accessions, as well as three varieties collected in Vietnamese citrus/guava orchards. Several common guava varieties in Florida are in the same analytical clusters as the Vietnamese cultivars (29). *P. guajava* is in the plant family Myrtaceae. Many members of this family are known to produce volatile compounds. It is interesting to contemplate that other species in this family may also produce volatiles that would inhibit insects and specifically psyllids. It is possible that other volatile producing plant species may be more easily integrated into western citriculture practices compared to guava. Considerable exploration and work would have to be done to determine if other plants would be more useful and appropriate in citrus plantings. One of the unfortunate side effects of guava as a companion plant is that it also attracts various fruit fly species, including some that are under quarantine and/or control. We are simultaneously taking steps to generate a fruitless white guava, to avoid the fruit fly issues. An additional problem with using guava is lack of cold tolerance which may limit potential application anywhere that temperatures below 4C° (40F°) are common, such as the northern extent of the present Florida citrus industry.

The first phase of the study on citrus/guava interplantings is aimed at proof of concept. We need to determine if the same protective effect that occurs in south Vietnam can be reproduced in western citrus plantings. The first objective is a test under Florida commercial citrus conditions to determine if the protective method is valid and worthy of further study and refinement. This will be done under field conditions in south Florida where comparative epidemiological studies will determine the occurrence and increase of HLB in citrus monoculture versus citrus/guava interplantings. Only if this first phase is successful will we move into testing alternative planting patterns, planting densities, orientations, etc. However, simultaneously with this first phase, we will attempt to isolate the guava volatile fractions and test their effect on psyllid behavior. If this is successful, we will explore other plant species for inhibitory volatile production. An obvious extension of this work is to isolate and either extract or manufacture inhibitory volatiles that can simply be applied to citrus orchards without the need to interplant guava. The advantage of the guava plant is that it grows quite rapidly under warm conditions, continually producing volatile oils that may protect interplanted citrus around-the-clock. Alternative application or dispersal mechanisms would have to be developed to continuously release these presumptive insect inhibitory volatiles in the absence of guava plants.

It is unknown whether interplanting guava in Western citrus plantings will be effective against HLB. The seriousness of the HLB epidemic in our citrus industry demands that all possible solutions or mediations to disease be explored. With the favorable reports from Vietnam, it would be irresponsible to not explore the potential benefits of guava and other plants to protect citrus against HLB. Use of such plants should also include their evaluation as managed ground-cover plants in interrow spaces (M. Stewart, pers. comm.) in conjunction with windbreaks.

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