Resistance and Tolerance to Huanglongbing in Citrus

Ed Stover[1,*], Gregory T. McCollum[1], Randall Driggers[1], Richard Lee[2], Robert Shatters Jr.[1], YongPing Duan[1], Mark Ritenour[3], Jose X. Chaparro[4] and David G. Hall[1]
[1]USDA/ARS, USHRL, Ft. Pierce, FL, USA
[2]USDA/ARS, National Clonal Germplasm Repository for Citrus and Dates, Riverside, CA, USA
[3]University of Florida, Indian River Research and Education Center, Ft. Pierce, FL, USA
[4]University of Florida, Dept. of Horticultural Sciences, Gainesville, FL, USA
*Corresponding author: ed.stover@ars.usda.gov

Abstract

Huanglongbing (HLB) is severely impacting Florida citrus. Productivity declines in many HLB-affected genotypes are often accompanied with thinned canopies, and reduced fruit size and quality. Development of citrus cultivars resistant or tolerant to HLB is the best long-term control solution for this endemic disease in Florida. HLB was assessed in diverse cultivars in commercial groves with high HLB incidence. 'Temple' had the least HLB symptoms and lowest Liberibacter (Las) titer, while 'Murcott' and 'Minneola' had the most symptoms and highest titers. The USDA Ft. Pierce, FL farm is managed to reveal genotype responses to HLB. Some current cultivars and hybrid seedlings demonstrate HLB resistance/tolerance, at least to the strain(s) of Las present in this location. *Citrus trifoliata* is the best documented citrus resistance source, having low Las titer even when grafted onto severely-infected rootstocks. Some cultivars and hybrids have abundant foliage symptoms, but full canopies and seemingly normal fruit set and size. In three years of a replicated trial of 'Triumph'(T), 'Jackson'(J), 'Flame'(F), and 'Marsh'(M) grapefruits or hybrids, HLB symptoms were severe in all trees and Las titers were similar. F and M were almost completely defoliated in some years while T and J had full canopies. Cumulative fruit per tree was greater for T and J (255, 220) than for F and M (29, 66). T and J fruit met commercial standards and was normal size but the fruit of F and M was unacceptable. Evidence is mounting that useful resistance/tolerance to HLB is present in cultivated citrus and sexually compatible hybrids.

Keywords: *Candidatus* Liberibacter asiaticus, grapefruit, 'Triumph', 'Jackson', 'Temple', plant disease, citrus greening

OVERVIEW OF HUANGLONGBING IN THE US

The Florida citrus industry faces many substantial threats that are most sustainably met through the development of high quality varieties with stable HLB resistance or tolerance. HLB is considered to be the most devastating of all citrus diseases (Bové, 2006) and the first HLB infected tree was discovered in Florida in 2005 (Brlansky et al., 2006). The disease is now widespread across the southern half of the Florida peninsula, is found in every citrus producing county in Florida, and has now been found in California and Texas (CDFA, 2012). Based on experiences in other areas of the world, many expect that the disease will continue to spread within the commercial citrus industry, with disease severity and associated losses escalating over time. In Florida, the bacterium *Candidatus* Liberibacter asiaticus (Las) has been associated with and is considered to be the causal agent of HLB. This phloem-limited bacterium is vectored by the Asian Citrus Psyllid (ACP) (*Diaphorina citri* Kuwayama (*Hemiptera*: *Psyllidae*)).

Trees of many citrus genotypes, such as the sweet orange (*Citrus sinensis* (L.) Osbeck) and grapefruit (*C. paradisi* Macfad.) that dominate the Florida industry, become less productive when affected by HLB, often exhibiting greatly thinned canopies and dieback. Characteristic leaf symptoms such as "blotchy mottle" are observed, with

symptoms being most readily apparent in the fall and winter months. Fruit size, appearance, and quality are also often adversely affected as the disease advances (Bové, 2006). The disease greatly debilitates trees and sometimes contributes to tree death. Losses in fruit quality and yield may render plantings nonproductive well before the death of the trees.

Development of citrus cultivars resistant to HLB is the best long-term control solution for this endemic disease in Florida.

LITERATURE REPORT ON HLB RESISTANCE IN CITRUS

Some resistance or field tolerance to HLB within citrus and citrus relatives has been described (Miyakawa, 1980; Nariani, 1982; Miyakawa and Yuan, 1990; Bhagabati, 1993; Halbert and Manjunath, 2004; Sharma et al., 2004). Compared to other tested cultivars within individual experiments, a lower susceptibility to HLB associated with Las, has been reported for limes (*C. latifolia* (Tanaka ex Yu. Tanaka) (Schwarz et al., 1973; Lange et al., 1985; Shokrollah et al., 2009), pummelos (*C. maxima* (Burm.) Merr.) (Schwarz et al., 1973; Koizumi et al., 1997), lemons (*C. limon* (L.) Burm.f.) (Schwarz et al., 1973; Cheema et al., 1982; Nariani, 1982), some mandarin types (*C. reticulata* Blanco) and hybrids, e.g. 'Ladu' and 'Som Pan' in Thailand (Koizumi et al., 1997) and various non-cultivated Citrus or species of related genera. However, reports are inconsistent, which may reflect genotypes selected, interactions between the host genotype and strains of HLB pathogens studied (Kiritani and Su, 1999), or the different methods used to evaluate disease. Reports prior to routine use of molecular diagnostic tools may also reflect confusion of apparent HLB with other diseases.

Poncirus trifoliata (proposed as *Citrus trifoliata* in a recent revision of citrus taxonomy, Bayer et al., 2009) is reported to be less susceptible to HLB than are cultivated citrus scion varieties. *C. trifoliata* and its hybrid, 'Carrizo' citrange (*C. trifoliata* × *C. sinensis*) developed less severe HLB symptoms and had among the lowest Las titers of the genotypes evaluated in a recent greenhouse study (Folimonova et al., 2009).

USDA STUDIES ON RESISTANCE TO HLB IN *C. TRIFOLIATA*

An analysis we conducted on *C. trifoliata* and some of its hybrids suggested that some of these genotypes tolerate and/or suppress Las even when grafted onto a high-titer source (Stover et al., 2010). A trial of more than 80 seedling populations from accessions of Citrus and citrus relatives (provided as seeds from the US National Clonal Germplasm Repository in Riverside, CA) has been underway for 2.5 years in an orchard with intense HLB and ACP pressure in Ft. Pierce, FL. *C. trifoliata* is among the few genotypes in the citrus gene pool that continues to show substantial resistance to HLB (Lee et al., unpublished), and *C. trifoliata* also displayed reduced colonization by ACP (Westbrook et al., 2011). Because of this continued evidence of HLB resistance in *C. trifoliata*, several trials are now underway using diverse trifoliates and their hybrids, including some advanced material with near commercial fruit quality. The hope is that molecular markers can be identified to facilitate introgression of resistance through conventional breeding and/or genes can be used to generate HLB-resistant standard cultivars using transgenic methods.

USDA SURVEY ON HLB AND LIBERIBACTER IN DIVERSE SCIONS IN COMMERCIAL GROVES

Since HLB has been widespread in the Indian River area of Florida since 2007, the decision was made to survey diverse cultivars in infected commercial groves, carefully avoiding bias in sampling, and including methods to adjust raw data based on HLB-incidence in each orchard (Stover and McCollum, 2011). 'Temple' tangor (*C. reticulata* × *C. sinensis*) showed the most consistently low incidence of HLB symptoms and low Las titers [25X lower than sweet orange and 30X lower than 'Minneola' tangelo (*C. reticulata* × *C. paradisi*)], even when comparing only infected trees. These results suggest that useful resistance to HLB, with reduced symptoms and reduced Las titers, may be found in

conventional scion cultivars. Further work is needed to assess this potential and its commercial value, and to mobilize such resistance into the range of commercial fruit types necessary to satisfy consumer demands.

USDA COMPARISON OF 'FLAME' AND 'MARSH' GRAPEFRUIT TO 'JACKSON' AND 'TRIUMPH' GRAPEFRUIT HYBRIDS

The primary grapefruit cultivars of commerce, such as Flame, Marsh, Ray Ruby, Rio Red, Ruby Red, and Star Ruby, are near-isogenic sports produced through mutations and selected for traits like low-seed-number or red color, in an iterative process building on cultivars previously selected for their beneficial mutations (Saunt, 2000; Corazza-Nunes et al., 2002). These cultivars are ultimately derived from the original grapefruit, which was likely very similar to 'Duncan'. There are cultivars (e.g., Imperial, Royal, and Triumph) with similar fruit characteristics that are also known as grapefruit, but differ markedly in genotype from the primary grapefruit cultivars (Corazza-Nunes et al., 2002) and are likely hybrids of true grapefruit.

Prior to the development of the HLB epidemic in FL, a trial of 'Triumph'(T), 'Jackson'(J), 'Flame'(F), and 'Marsh'(M) was established at the USDA Ft. Pierce farm to compare the potential for early-season fresh fruit and juice production. J is reported to be a low-seeded budsport of T that originated in South Africa (Hodgson, 1967). In 2009, it appeared that T and J may be among the genotypes with resistance or tolerance to HLB. Data were collected from the existing planting over the next three years to test the hypothesis that T and J would perform significantly better than M and F under the test conditions.

In three years of data from a replicated trial of T, J, F, and M, HLB symptoms were severe in all trees and Liberibacter titers were similar (Stover et al., 2012). However, F and M were almost completely defoliated in some years while T and J had full canopies. The cumulative fruit per tree was greater for T and J (255 and 220 kg) than for F and M (29 and 66 kg). T and J fruit met commercial standards and were normal size but the fruit from F and M were unacceptable with many being small and misshapen.

All trees displayed severe HLB blotchy mottle symptoms in the winter when they were assessed, with no significant difference between cultivars. In the spring and early summer months, when HLB symptoms are normally less pronounced, leaves with strong blotchy mottle symptoms were present in T and J but not in M and F. Most of the blotchy mottle leaves were in the interior canopy where leaves had completely abscised in M and F. However, citrus canker ratings were markedly higher in M and F than in T and J, but canker lesions were evident on all trees, and the differences in severity of canker infection did not appear to explain the differences observed in overall tree performance. Since all trees were infected with Las, it appears that T and J may have a useful level of tolerance to HLB, with substantially greater canopy health than sweet orange and grapefruit trees grown nearby with the same management and similar tree age.

SCION BREEDING FOR HLB RESISTANCE AND/OR TOLERANCE

The United States Department of Agriculture program is among the oldest sustained citrus breeding effort in the world. A large genetic base of plant material has been utilized to create unique hybrids, many of which are exposed to HLB at our Florida farms. This creates a problem, in that material is being evaluated based on compromised trees; however, it also creates an excellent opportunity to screen material for resistance or tolerance to HLB. A number of hybrids and even a few cultivars appear to have useful tolerance or resistance to HLB. A major focus of our breeding program is to characterize this resistance/tolerance and incorporate it into genotypes that also have outstanding fruit quality in a range of market phenotypes.

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