Potential Differences for HLB between Florida and the Western States

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Background:

Citrus greening (huanglongbing) is thought to have originated in China in the early 1900's. Globally, greening has been regarded as one of the most important threats to commercial and sustainable citrus production. In some areas of the world where the disease is endemic, citrus trees decline within five years of planting and most never bear usable fruit. Such losses are significant, since profits are only attainable 8 to 10 years after planting. In countries where greening occurs, it is the primary limiting factor for citrus production.

Control measures are limited to the use of disease-free propagating stock, rouging of infected trees, and chemical or biological control of the vectors.

Citrus greening is caused by the fastidious, phloem-limited Gram-negative bacteria, *Liberobacter africanum* in Africa and *Liberobacter asiaticum* in Asia. There is a possible new strain of the pathogen found in Brazil in 2004. Citrus greening is also one of the worst diseases of citrus caused by a vectored pathogen. The pathogen has not been cultured on artificial media and Koch's postulates have not been completed for the organism. The pathogens at present are extremely difficult to detect and characterize, although great strides have been made in recent years with the development of detection methods based on the polymerase chain reaction (PCR) and DNA hybridization. The dynamics, epidemiology, and molecular characterization of the insect-pathogen complex are poorly understood.

1. Types of citrus grown in each area could have different symptom expression

The traditional citrus climate extends from northern California down through southern California and into the low Arizona desert. There is a break in New Mexico, a state that has high elevation with cold winters. Then the citrus belt picks up again in southern Texas and extends along the Gulf Coast and into Florida. Not all types of citrus can be grown in all parts of the citrus belt. Climatic differences within the region markedly affect fruit characteristics and quality. What can be grown in Florida cannot always be grown in California and vice versa. The warm dry days and cool nights in California develop brightly colored fruit with balanced sugar and acid and thick rinds. The warm, humid days of Florida and the Gulf Coast are usually accompanied by equally warm nights; such even temperatures promote lighter colored fruit with pronounced acidity.

Humidity and day-to-night temperature fluctuations also influence which varieties are best adapted to an area. Almost all lemons in the supermarkets come from western states because in Florida lemons do not develop enough acidity. On the other hand, some types of citrus naturally high in acids, such as tangelos, are too tart when grown in California. They reach peak quality and sweetness only in Florida or along the Gulf Coast.

All citrus and citrus relatives are potential hosts for the citrus greening pathogen. The most susceptible hosts are sweet oranges, tangelos, and mandarins. Moderately susceptible hosts include grapefruit, sour orange, lemons, Rangpur lime, and pummelos. Mexican limes and trifoliate oranges are the most tolerant. Just because a particular type of citrus is not severely affected by greening does not reduce its significance. Lemons, for example, are thought to serve as important reservoirs of inoculum for infection because their more frequent flushes of new growth make them attractive to the insect vector.

Florida: Approximately 76% of oranges produced in the US and 20% of the world crop are produced in Florida (656,600 acres). Ninety to ninety five percent of these oranges are processed for juice. Eighty one percent of the US crop and 54% of the world crop of grapefruits are also produced in Florida (144,400 acres). Only 40% of all grapefruit production is shipped fresh. Florida is the largest producer of limes in the US with 1,668 acres. All but eight acres are in Miami-Dade county and destined for the fresh marker. Florida also produces 45 acres of pummelo and 25 acres of kumquat.

California: California produces 80% if the US lemons (49,807 acres), 28% of tangerines (including mandarins, tangelos, and tangors) (8.506 acres), 21% of oranges (205, 930 acres), and 10% of grapefruit (16,462 acres). California also produces about 771 acres of limes. Approximately 82% of oranges, 70% of tangerines and grapefruit, and 50% of lemons are fresh market. Approximately 40% of all harvested citrus is for export.

Texas: Texas produces primarily grapefruit (22,000 acres) and oranges (6,000 acres). There is also small production of lemons/limes (mostly backyard production) (60 acres), tangerines (130 acres), papaya (8 acres), and pomegranates (5 acres).

Arizona: In Arizona, citrus is marketed both domestically and internationally. The majority of groves are contracted with Sunkist packinghouses. Arizona production includes lemons (13,100 acres), tangerine/tangelo (5,200 acres), oranges (5,200 acres), and grapefruits (3,600 acres)

Lousiana: Lousiana produces approximately 1,400 total acres of citrus, including naval oranges (majority) and Satsuma. All fruit is sold fresh.

2. Rootstock used – Are some more susceptible/resistant to infection?

Citrus trees are not particularly cold hardy. Rootstock selection in citrus is chosen primarily on cold tolerance and disease resistance (particularly virus resistance) needed in a particular growing area.

In some cases, the rootstock can affect symptom expression. However, the results of many studies are quite variable. Five out of 23 rough lemon rootstock selections from India induced a degree of tolerance in the sweet orange scion in greenhouse trials (Cheema et al., 1982). In another study, 100% of trees of rough lemon were infected, compared to only 25% of trees on 'Blood Red' sweet orange rootstock (Kapor et al., 1984). In South Africa the percentage of greening in Valencia oranges was higher on trifoliate orange rootstock than on Empress mandarin or Troyer citrange. Possibly the trifoliate rootstock causes an extension of the flushing period and thus extends the feeding time of the insect vector (van Vuuren and Moll, 1985). No differences were found in a Chinese study on the effects of 13 rootstocks on symptoms in Ponkan mandarin (Lin, 1963).

3. Climate – Do any factors (temperature, relative humidity, rainfall) influence symptom development, pathogen latent period, or vector establishment?

<u>The pathogen:</u> In a laboratory study, Bove et al. (1974) showed that symptoms of African citrus greening were moderate to severe at 22 to 24 °C (68 to 77 °F) and disappeared at 25 to 30 °C (78 to °F), whereas symptoms of Asian citrus greening from India and the Phillipines were expressed strongly at both temperature regimes. The Asian pathogen has been shown to displays symptoms at temperatures above 30 °C. Symptoms are also more severe with Asian form. In Africa, both the insect vector and the greening organism do not tolerate hot and dry climates. There are areas in the Western US where the African strain would not be able to cause symptoms or become established. The strain recently found in south Florida appears to be the Asian strain, which would be well adapted for temperatures occurring across the citrus belt of the US.

Little information is available on how temperature may influence the pathogen latent period (time to expression of symptoms), which ranges from 4 months to more than a year. Infected trees may be overlooked if symptoms alone are used for detection. Aubert (1990) estimated that 15% to 20% of infected plants are overlooked by nursery inspectors who rely on visual inspection. There is also little information on the effect of rainfall and relative humidity on symptom development and pathogen latent period.

<u>The vector:</u> *Trioza erytreae*, the African citrus psyllid, is also heat sensitive and only occurs at high elevations similar to *Liberobacter africanum*. This vector is currently not present in the US and, therefore, this report will concentrate on the Asian citrus psyllid, *Diaphorina citri*, which is currently present in the US.

Diaphorina citri is more prevalent in hot coastal areas. *D. citri* has a short life cycle and high fecundity. The optimum range of temperatures for population growth of *D. citri* is 25 to 28 °C. *D. citri* did not survive well at 30 °C (McFarland and Hoy, 2001). In an insectary, at 10, 15, 20, 25, 28, and 33 °C, the psyllid populations reared at 10 and 33 °C failed to develop. Adults can live for 1 to 2 months at temperature below 20 °C (68 °F). According to Aubert (1987), *D. citri* does not tolerate frost very well. However, Halbert and Manjunath (2004) state that populations of *D. citri* have overwintered in Gainesville, Florida when temperatures dropped to at least -5 °C on several nights.

Aubert (1987) also states that populations of *D. citri* do not tolerate humidity close to the saturation point because it promotes fungal epizootics, to which the nymphs are very susceptible. However, high humidity in Florida has not prevented extremely high summer populations of *D. citri* in local groves and backyards. Similarly, few *D. citri* regulatory samples sent to the Florida Dept. of Agriculture and Consumer Services, Division of Plant Industry, have cadavers resulting from fungal infection (Halbert and Manjunath, 2004).

Results from McFarland and Hoy (2001) indicate that populations of *D. citri* and two of its parasitoids increased with increasing relative humidity ranging from 7 to 97%. However, *D. citri* survived well at RH of 7 and 33%, suggesting that it could disperse and survive in the more arid conditions found in western citrus-growing regions in the United States.

Florida relies heavily on natural rainfall while the west relies on irrigation to provide moisture to the citrus crop. There is little information available on differences whether rainfall or irrigation would influence vector survival or establishment. During dry periods, adults are numerous, but nymphs are usually absent. Population fluctuations are closely correlated with the flushing rhythm of citrus trees, as eggs are laid exclusively on young flush points. In southern Florida, *D. citri* populations occur throughout the seasons on orange jessamine (*Murraya paniculata*), but there are population peaks in October through November, December, May and August, which were positively correlated to weekly minimum temperature and rainfall.

4. History of insecticide usage/potential vector control

Because psylla are sap feeders, systemic insecticides are the most effective. The vector *D. citri* is very susceptible to insecticide usage including chlorpyrifos, fenpropathrin, imidacloprid, and kaolin. The likelihood of citrus greening or the vector establishing in areas treated with insecticides is reduced but repeated applications may be necessary.

5. Tree spacing, orchard location and hurricanes; could these factors coupled with wind influence disease development?

Citrus greening disease has been shown in Thailand to be more prevalent in flat or plain fields than those surrounded by hills or wind breaks, due to the enhancement of vector dissemination by wind (Koizumi et al., 1997). This study also showed that there was tree to tree spread and orchard to orchard spread of the disease in a leeward direction. The authors demonstrated that strong wind was the most important environmental factor in the spread of citrus greening.

D. citri can fly but within a short range from leaf to leaf or twig to twig when observed in an incubator. A strong wind may disseminate the viruliferous psyllas from donor to receptor in a leeward direction, which enhances disease transmission. The distance of dissemination depends on the population of the psyllas, wind velocity, and the survival time in the air. *D. citri* has been observed disseminating over a distance of more than 500 m in a flat field and *T. erytreae* has been observed disseminating over 1500 m.

For most citrus growing areas, tree spacing varies from 20 x 20 feet to 30 x 30 feet. The spacing chosen often depends when the grower wants optimum yield. Wider spacings are used to achieve maximum production after the 15th year; while closer spacings favor optimum yield before the 15th year. Orange color develops first and is more intense at wider spacings. In some high density plantings, fruit colorings may be delayed 45 days. Wider spacings also favor earlier development of a higher sugar/acids ratio at legal maturity. The effect of tree spacing is unknown at this time, but closer tree spacing may enhance disease development.

The length of survival in air currents is also unknown, but the possibility exists for hurricane force winds to aid in the dissemination of *D. citri* in Florida and Gulf Coast areas.

6. Hand pruning and mechanically topping a common practice in lemons; is this common with other types of citrus? Could this spread the disease?

Most citrus trees do not require annual pruning. Bearing orange and grapefruit trees require little pruning, primarily topping and removal of branches for spacing between trees. Bearing lemon trees require selective pruning to strengthen the shoots and prevent crowding in the center of the tree and are typically pruned every year in California and Arizona. About 30% of the crop are hedged or topped every year.

There is no evidence at this time that the bacterium could be transmitted mechanically during pruning or mechanical topping. The disease is known to be vector transmitted, graft transmitted, transmitted experimentally via dodder, and possibly seedborne.

7. Is the bacterium/vector seedborne? What are the implications?
It is generally accepted that this pathogen is not seedborne; however there is very little information on seed transmission. Tirtawidjaja (1981) collected normal and greening-affected (very small) fruit and harvested ‘normal-looking’ seeds from each. No symptoms were observed on seedlings from seed taken from normal fruit. However, seeds derived from smaller, greening-affected fruit produced some stunted chlorotic seedlings. Three of the seedlings had the same appearance as the insect-inoculated seedlings. If seed transmission occurs in cultivars like citrange that are used for citrus rootstocks, spread could occur through liners as well as by budding.

It is also generally accepted that the vector is not transported on citrus fruit. Many specimens of *D. citri* were collected alive on fruit arriving from Abaco Island, Bahamas in Ft. Pierce, FL in March 2001. This collection confirms the ability for D. citri to travel on fruit, sometimes in large numbers. All samples were assayed for citrus greening and were found to be negative.

Over 90% of oranges in Florida are processed into orange juice. The western states occupy a fresh fruit market. Fresh market requires a much higher quality fruit than for processing (“high eye appeal”). Rind color and quality are crucial only to the fresh fruit market. Such fruit, destined for packinghouses following harvest, must have cosmetic appeal to consumers even though apparent imperfections in the outer rind usually do not adversely affect the internal quality of fruit. Greater pest management inputs are required for fresh fruit production.

A survey conducted in Reunion Island indicated that 65% of the trees were badly damaged and rendered unproductive within 7 years after planting (Aubert et al., 1996). In Thailand, citrus trees generally decline within 5 to 8 years after planting due to citrus greening (Roistacher, 1996). Roistacher (1996) showed that groves must live for a minimum of 10 years in order to make a profit. Fruit with citrus greening are small, lopsided, hard, and have a bitter flavor. Seed abortion is common. Citrus greening disease may predispose plants to other pest problems such as the citrus longhorned beetle, *Anoplophora chinensis*, and *Phytophthora* fungi. A combination of citrus greening, citrus longhorned beetle, and associated *Phytophthora* fungi are common in advanced citrus greening epidemics.

In addition to the difficulty in obtaining fruit that will meet the quality regulations for the fresh fruit market, if the bacterium is shown to be seedborne domestic and international trade could prove to be extremely difficult for the West due to the likelihood of movement citrus greening into areas that do not have the disease.

8. Elevation
The African form of citrus greening disease is restricted to cool, moist elevated 600 m to 1000 m areas, which corresponds to the range of its primary vector *T. erytreae* and citrus flushes tend to be prolonged. *D. citri,* the primary vector of the

Asian form of citrus greening, is generally found in warmer areas with low elevations. Most citrus production in the US occurs at relatively low elevation due to the danger of frost.

9. Vector presence; the vector is present in Texas, Florida, and Mexico (limited distribution) but not in Arizona or California. How does this impact sampling and or trade?

The vector *Trioza erytreae*, the African citrus psyllid, has not been found in the US. The Asian citrus psyllid, *Diaphorina citri*, was first found in Palm Beach Florida, in June 1998 in backyard plantings of *Murraya paniculata* (orange jasmine). By 2001, it had spread to 31 counties in Florida with much of the spread due to the movement of nursery plants. In the spring of 2001, the Asian citrus psyllid was accidentally introduced into the Rio Grande Valley of Texas on potted nursery stock (orange jasmine) from Florida (French et al, 2001). The Asian citrus psyllid has the potential to invade California, Lousiana, and Arizona at any time, with the most likely sources of infestation being Florida, Texas, Mexico, or Asian. There is a USDA/APHIS/PPQ record of an interception of *D. citri* from Mexico in April, 1996. There were 170 interceptions of the Asian citrus psyllid at US ports on plant material (primarily *Murraya* and citrus) from Asia during 1985 through 2003.

The establishment of *D. citri* in Florida and Texas greatly increases the possibility that the disease may become established once introduced. The West has one state with the vector but the remaining citrus producing areas are free of the vector. The west will have to consider not only movement of citrus greening but also potential movement of the vector, which may or may not harbor the bacterium, from Texas, Florida, Mexico, and Asian countries.

The greening pathogen can be detected within the vector's salivary glands. Both adult psyllids and the 4th through 5th instar nymphs are able to acquire the pathogen after feeding on a diseased plant for 30 minutes or longer. The pathogens remain latent inside the vector from between three to 20 days, after which they can be detected in the salivary gland. Inoculation feeding takes one hour or more. Once the psyllid vector acquires the pathogens, it can transmit them throughout its life span. However, it cannot transfer the virulence to its progeny via eggs.

10. Host material- . How widely distributed are these species (e.g. Do they occur in the West and East)? Are these species commonly moved from state to state. Some ornamental hosts only promote vector population increases others harbor the bacterium.

Citrus greening is restricted to Citrus and close relatives because of the narrow host range of the psyllid vectors.

Citrus greening is a disease of rutaceaous plants. The citrus greening bacterium infects citrus generally. The bacterium may persist and multiply in most *Citrus spp*. but most severe symptoms are found on oranges (*C. sinensis*), mandarins (*C. reticulata*), and tangelos (*C. reticulata* x *C. paradisi*). A range of other rutaceaous plants including ornamentals and wild species are susceptible to infection; however the severity of symptoms displayed varies. These hosts include: *Poncirus, Severinia buxifolia* (box thorn) *Limonia acidissima, Murraya spp.*, *Toddali*a, *Calodendrum capense*. The citrus greening bacterium has been experimentally transmitted, by *Cuscuta campestris* (dodder) from citrus to one non-rutaceous host *Catharanthus roseus*.

Murraya paniculata is a common ornamental used as an ornamental bush or hedge that is capable of growing from Florida to Arizona to California. This plant is found throughout the citrus belt in its native range and is often planted in the southeastern US. This plant is the preferred host of *Diaphorina citri* and can reach extremely large populations on this plant. Ahmad (1961) conducted a survey of trees in West Pakistan by spraying the citrus trees with an insecticide and collecting the psyllids on a white sheet beneath the tree. This method yielded on average 41, 561 adults per tree. This plant is not a host for the pathogen, but it can carry eggs or nymphs of the vector and its introduction to disease- and vector-free regions could be dangerous. The continuous flushes provided by orange jasmine could play an important role in maintaining high populations of this vector when the new flushes are not available in the commercial citrus groves.

*Severinia buxifolia i*s native to Florida could be planted potentially along a range includes all US citrus growing regions. The citrus greening pathogen does infect *S. buxifolia* and could serve as an important source of inoculum if planted near a citrus grove, nursery, or dooryard planting. The greening bacterium replicates as well as it does in citrus (Hung et al, 2000). *Limonia acidissima* is a transient host in which the greening pathogen exists temporarily and disappears several months later.

11. Is there commonly movement of propagating stock between states? How would illegal movement of citrus trees/budwood affect greening in the west.

Movement of infected propagating stock could be a major means of spread of citrus greening from state to state. In the past, moving citrus planting material from area to area within the United States and from outside the United States was routine. This routine movement introduced foreign diseases and pests into new areas. This type of movement is now severely restricted and budwood certification programs exist in citrus growing states. It can not be ascertained if these certified budwood programs assay for citrus greening in addition to viral, viroid, and phytoplasma diseases. Including citrus greening in the assay will be important to prevent the spread of citrus greening within states and between states.

Illegal movement of citrus trees/budwood is a practice that is apparently fairly common with home owners and dooryard citrus. Vigillance against illegal introductions must be maintained for both types of greening since the Asian psylla has been shown to transmit the African form as well. This is particularly important due to the long latent period of the pathogen (four months to more than a year) when symptoms may not be evident. The fourth and five instar nymphs and adults of *D. citri*, which can harbor the pathogen, could also be moved on these illegal introductions.

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