CRB Project No. 5300-11D

CITRUS RESEARCH BOARD

PROJECT PLAN - RESEARCH GRANT PROPOSAL FOR FY2010-2011

Fiscal Year: 2010-2011 **Anticipated Duration of Project:** 3 years

This Project is: x **New or** _____ **Ongoing (Year _____ of _____)**

Project Leader: Richard Lee, Ariena van Bruggen and Gregory Walker
Name
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Project Title: Characterization of the endophytic prokaryotes closely related to the bacterium associated with huanglongbing in citrus and citrus relatives

Keywords: Huanglongbing, Diaphorina citri, psyllid transmission, Liberibacter-like bacteria, host range, in vitro culture

Abstract *(limit 200 words):(clearly and succinctly state what your project is about why you are doing it and expected out come and how the industry will use these outcomes)*

The US citrus industry is threatened by huanglongbing (HLB) associated with the bacterium "Candidatus Liberibacter asiaticus" (Las), spread by the Asian citrus psyllid (ACP), *Diaphorina citri*. ACP was first found in Florida in 1998 and HLB in 2005. HLB is now in neighboring states and Mexico threatening all citrus producing states in the USA. Diagnosis of HLB is by quantitative PCR (qPCR) usually followed by conventional PCR and sequencing of clones. In Brazil, a new species of *Candidatus* Liberibacter was identified later named americanus (Lam) in addition to Las. In May 2010, the California Dept. of Food and Ag. reported a qPCR positive for HLB from *Bergera koenigii* (curry leaf) in Orange County which is neither Las nor Lam. For the past 2.5 years we have conducted surveys of citrus and citrus relatives in South Florida to identify sources of tolerance to HLB as part of a CRB project, and found "Liberibacter-like" bacteria in citrus relatives showing sequence homology of 94-95% with Las 16S rDNA. We propose to determine if these "Liberibacter-like" bacteria are pathogenic to citrus, if they are spread by psyllids, and if they could be used to cross protect against Las (provided they are not pathogenic).

Problem and its Significance*: *(include literature review)*

The "Liberibacter-like" bacteria that we are finding, mostly in citrus relatives, in South Florida could be variations of *Candidatus* Liberibacter species or true endophytes. Endophytes are micro-organisms which inhabit inner plant tissues without causing damage to the host plant nor inducing the formation of an external response structure, such as mycorriza and nodules (Azevedo & Araujo, 2007). While numerous bacteria have been identified as endophytes in plants, most belong to Pantoea and Enterobacter genera (Torres, et al. 2008). *Pantoea agglomerans*, *Enterobacter cloacae,* and *Klebsiella pneumoniae* have been reported as endophytes from citrus based on the 16S rDNA sequence (Andreote et al.

*Use as much space as necessary; attach additional pages as needed; budget info and signatures will appear on last page.

2008;Torres et al. 2008; Lacava et al. 2007). Araujo et al (2002) identified species of *Bacillus , Curtobacterium, Enterobacter, Methylobacterium, Nocardia*, and *Pantoea* genera based on fatty acid methyl ester analysis as endophytes from citrus plants which were also infected with *Xylella fastidiosa*.

With the support of a CRB project, we have made biannual surveys of citrus and citrus relatives from the landscape in South and Central Florida. Locations of citrus relatives have been identified with the help of Florida DPI Plant Inspectors, hobbyists, and University of Florida extension personnel. The plants have been GPS located, and nearly all of the plants are revisited in the fall sampling trips as this is when the symptoms of HLB are most apparent, and the bacterial titer is high as well based on our qPCR testing. All but four of the species of the 23 genera belonging to Rutaceae which are regulated by CDFA/APHIS (http://www.nurserygrowers.org/ACT_Exhibit_Z-_Regulated_Articles.pdf) have been tested from South Florida. In addition, another 14 species not regulated also have been tested (Table 1). Using the Li et al (2006) qPCR protocol, Ct values of 32 or lower are considered positive. Ct values of 33-36 are considered questionable, and Ct values of 37 and higher are considered negative. Samples found positive by qPCR are further analyzed by conventional PCR using OI1 and OI2c primers (Jagoueix et al., 1994), the amplified products are cloned and sequenced to verify homology with the 16S rDNA region of Las. Because all samples collected in Florida have to be extracted in Florida and only the DNA extracts may be returned to our laboratory in Riverside, we previously have not had the opportunity to assess the biological properties of the Liberibacter-like bacteria under controlled conditions, but observations and pictures taken of the plants sampled over this 2 ½ year period suggest many of the plants are declining (Figures 2 & 3).

Genus-Species	**# tested**	**Ct range**	**Comment**	**Genus-Species**	**# tested**	**Ct range**	**Comment**
Aegle maemelos	4	40		*Fortunella japonica*	6	30-40	**
Afaegle gabonensis	1	40		*Fortunella margarita*	10	33-40	
Afaegle paniculata	7	32-40		*Fortunella polyandra*	1	34	
Pamburus missionis	10	38-40	CDFA lists as *Atalantia missionis*	*Fortunella hybrids*	14	28-40	
Atalantia ceylonica	7	24-28	**	*Glycosmis pentaphylla*	6	36-40	**
Balsamocitrus dawei	5	35-40		*Naringi crenulata = Hesperethusa crenulata*	4	34-39	
Bergera koenigii	6	40		*Merrilia caloxylon*	NT	NT	
Calodendrum capense	4	31-39		*Microcitrus spp.*	14	32-40	
Casimiroa spp.	9	33-40	**	*Murraya paniculata*	114	30-40	
Citropsis spp.	4	34-40		*Ravenia spp.*	5	33-38	**
Clausena lanisum	10	34-40		*Poncirus trifoliate*	4	36-40	
Clymenia	NT	NT		*Severinia*	94	23-40	

polyandra				*buxifolia*			
Eremocitrus glauca	4	35-40		*Swinglea glutinosa*	16	34-40	
Eremocitrus hybrid	3	35-40		*Toddalia asiatica*	NT	NT	
Erythrochiton brasiliensis	3	34-40	**	*Toddalia lanceolata = Vepris lanceolata*	NT	NT	
Esenbeckia	1	40	**	*Zanthoxylum clava*	1	36	**
Evodia spp.	4	35-40	listed at *Tetradium spp.*	*Zanthoxylum clavehercules*	1	40	**
Feroniella spp.	4	36-40	**	*Zanthoxylum coriaceum*	6	40	**
Limonia acidissima = Feronia acidissima	NT	NT		*Zanthoxylum fagara*	11	32-40	
Fortunella crassifolia	20	28-40		*Zanthoxylum flavum*	3	37-40	**
Fortunella hindsii	3	32-40	**	*Zanthoxylum thomasianum*	3	35-40	**
Fortunella hybrid	23	34-40					

Table 1. A summary of survey results from South Florida of the 23 genera belonging to Rutaceae which are regulated by CDFA/USDA in order to prevent spread of HLB. The ** marks genera that are not included in this regulated article list that were tested in South Florida. NT = not tested, the species was not present.

Considering the samples collected and tested from Florida in Fall 2009, we find the southern areas are much more heavily infected with HLB. Amongst the 88 plants tested from Miami, 49 from Homestead and 567 from Central Florida (Polk County), it was clear that the highest incidence of HLB was still in the Southern region (Figure 1). Three plants (*Casimiroa spp., Zanthoxylum clavehercules*, and *Aegle marmelos*) in the Homestead area did not show any detectable HLB. In the Miami-Dade area, only 7 plants had Ct values of 40 (1 *Murraya paniculata*, 1 *Zanthoxylum flavum*, 1 *Evodia elleryana*, 3 *Triphasia trifolia*, and 1 *Citrus sinensis*, cv Valencia).

Since most of the citrus relatives from Florida are collected from the landscape and frequently only the leaves are available to determine the genus and species, the nomenclature is somewhat uncertain. Proper identification based on morphological characteristics and owner identification can be misleading (especially when fruit and/or blossoms are not available). We have used the sequence of part of a nuclear gene, malate dehydrogenase, to confirm the taxonomy, using the citrus relatives from our Citrus Relatives collection as reference strains. Mitochondrial and chloroplast gene/s are widely used, but these represent only maternal information and are not good for hybrids. Analysis of molecular relationship of citrus and its relatives using the sequence from a fragment of the malate dehydrogenase gene (Ramadugu et al., 2010b; Figure 4) shows a surprising good relationship with the taxonomy system of Swingle and Reese (1967).

Project Title: _Characterization of endophytic prokaryotes closely related to HLB in Citrus and citrus relatives

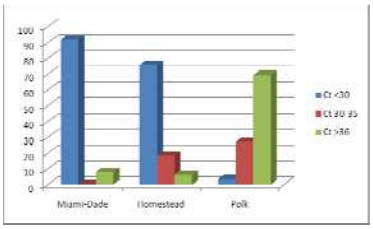

Fig. 1. Summary of testing of citrus relatives from three different areas in Florida for the presence of HLB bacteria. Bars indicate percentage of trees infected (with Ct values below 30), suspected positives for HLB (Ct 30-35) and with no detectable HLB (Ct values of above 36) are shown for each of the three areas.

Figure 2. *Murraya paniculata* samples collected from Miami-Dade area of Florida. The Ct value for the plant DNA ranged from 21-24, and the Ct value for Las ranged from 30 to 33 (937, 938, 939, 1032) with 1028 and 1029 having Ct values of 39.

Several samples of *Atalantia ceylonica* and *Severinia buxifolia* tested positive for Las with Ct values of 24-28 and 23-40, respectively (Ramadugu et al., 2010a). In addition to the 16S RNA region, six additional genomic regions of the HLB bacterium from *Atalantia* and *Severinia* were PCR amplified, cloned, and sequenced to confirm the relationship with Las. Infected trees generally showed severe decline (Figure 3).

Using universal primers (for proteobacteria) for the 16S RNA region, followed by cloning and sequencing the products, additional 16S RNA sequences were obtained from other citrus relatives which show a homology of about 92-94 percent with Las. They are in a clade slightly separated from the known species of *Candidatus* Liberibacter (Manjunath et al, 2010; Figure 5). We tentatively refer to these as new forms of Liberibacters or "Liberibacter-like", and they need to be characterized at both the biological and molecular levels. Do these presently uncharacterized Liberibacters represent variants of *Ca*. Liberibacter species? If they are pathogenic to citrus and citrus relatives, and field observations suggest they may be, are they "mild" variants which would interfere with the replication of Las? If they are closely related to Las, they may be able to protect against the expression of severe symptoms of the bacterium associated with HLB.

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Figure 3. A) *Murraya paniculata* plant declining in South Miami neighborhood (Ct value 31), the *M. paniculata* in the background is apparently healthy. B) A *Murraya paniculata* hedge near Kendall which has declined in the past year, Ct value 33. C) Curry leaf (*Bergera koenigii*) from the Homestead area, Ct values 39-40. D) *Severinia buxifolia* in a yard near Miami, Ct value 23. E & F) Leaf and tree of *Calodendrum capense* in South Miami, Ct value 31. The tree has declined between fall 2008 and fall 2009. G) Leaves from a *Severinia buxifolia* from Homestead area showing the blotchy mottle pattern typical of huanglongbing, Ct value 24.

This so-called cross protection phenomenon has been reported for Pierce's disease of grapevines (Hopkins, 2005) and is used against *Citrus tristeza virus* (Lee et al, 1994). In Brazil, the population of *Ca.* Liberibacter americanus was reduced from about 98% to less than 20% in less than four years by increasing numbers of Las (Lopes et al. 2007; Lopes et al. 2009) indicating there is competition between different *Ca.* Liberibacter species. This does provide some encouragement that there may be non-symptom causing forms of Liberibacters in nature that may be capable of dominating Las. A deeper understanding of Liberibacter-like endophytes in both citrus and citrus relatives and their possible interaction/interference with Liberibacter biology and pathology is needed.

In California, a slightly different situation has occurred as compared to Florida. Leaves of *Bergera koenigii*, curry leaf, were collected from a market in the Sacramento area, and tested positive for *Ca.* L. asiaticus. The samples were collected and tested by the Smuggling Intervention and Trade Compliance team of the USDA-APHIS. The leaves of curry leaf originated from a homeowner in Buena Park in Southern California. Following this, the CDFA collected samples from a 30 year old *B. koenigii* tree, 2 younger *B. koenigii* trees, a lemon tree and a kumquat tree. Samples of the old *Bergera*, lemon and kumquat tested positive for Las using the APHIS protocol in the CDFA lab. Samples were recollected and forwarded to the CPHST National Plant Pathogen Lab in Beltsville, MD for confirmation, where the findings of the CDFA lab were confirmed, but upon sequencing the organism was found to have only 94% homology to Las (in 16s region). These results were independently confirmed by the USDA ARS labs at Parlier, Riverside, and Ft. Detrick.

Each of the locations confirmed the results of the CPHST National Plant Pathogen Lab, and the samples were determined not to be HLB and research was encouraged on these samples and additional samples. At Riverside, we were able to graft the older *Bergera* to *Bergera koenigii* seedlings and also have established the *Bergera* on rough lemon liners. From material given to Riverside, we were able to isolate an alpha proteobacterium which appears to react identically upon PCR analysis as the original samples, e.g. it gives a positive result when tested by qPCR for Las and when sequenced has 94 percent homology with Las. In addition to this instance, we have subsequently located two additional locations where *Bergera koenigii* plants are declining, and diagnostic tests are similar to what we obtained on the Buena Park samples.

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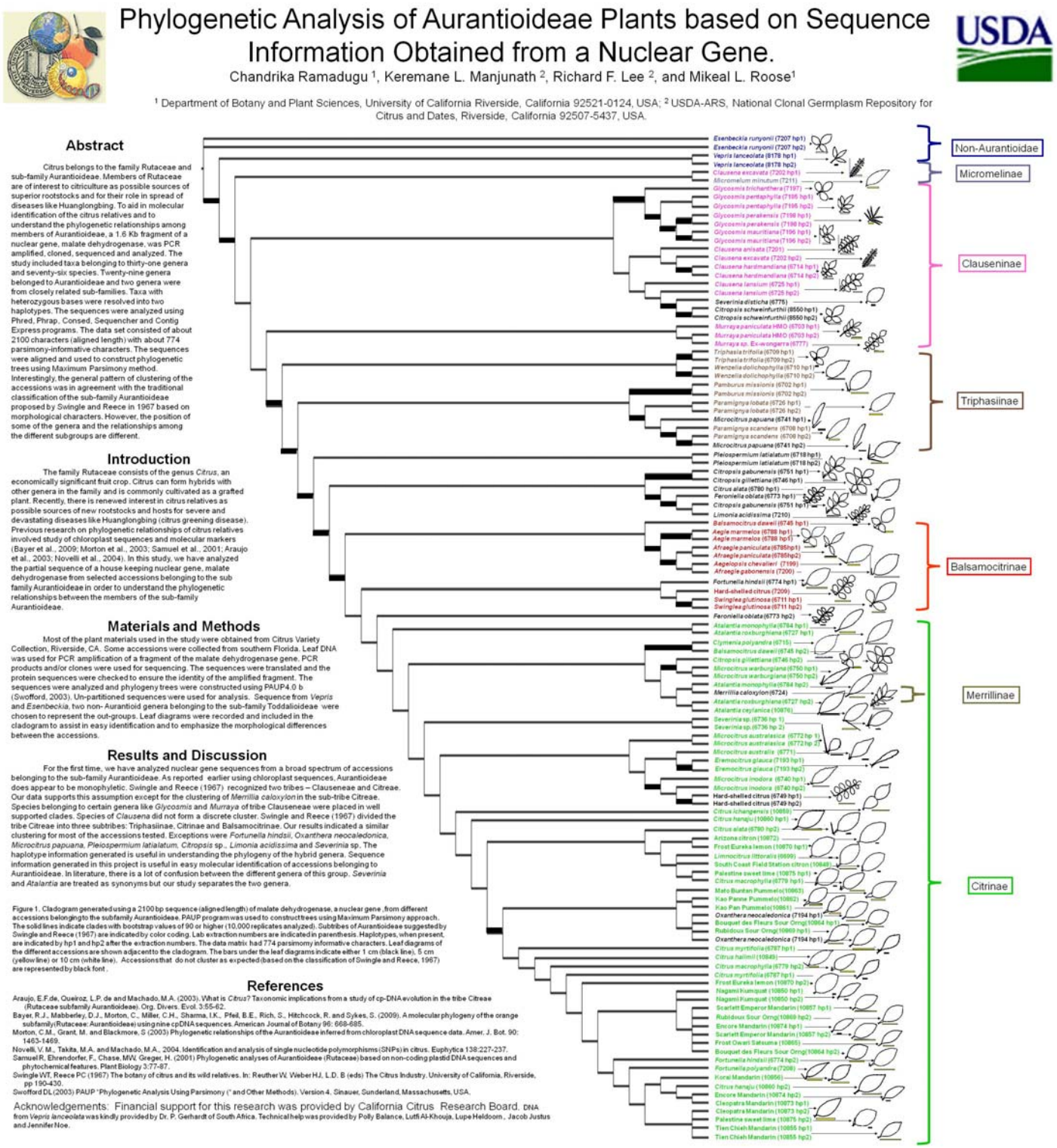

Figure 4. Phylogenetic analysis of Aurantioideae plants based on sequence information obtained from a nuclear gene, a poster presented at the 2010 Plant and Animal Genome Workshop, San Diego (Ramadugu et al, 2010).

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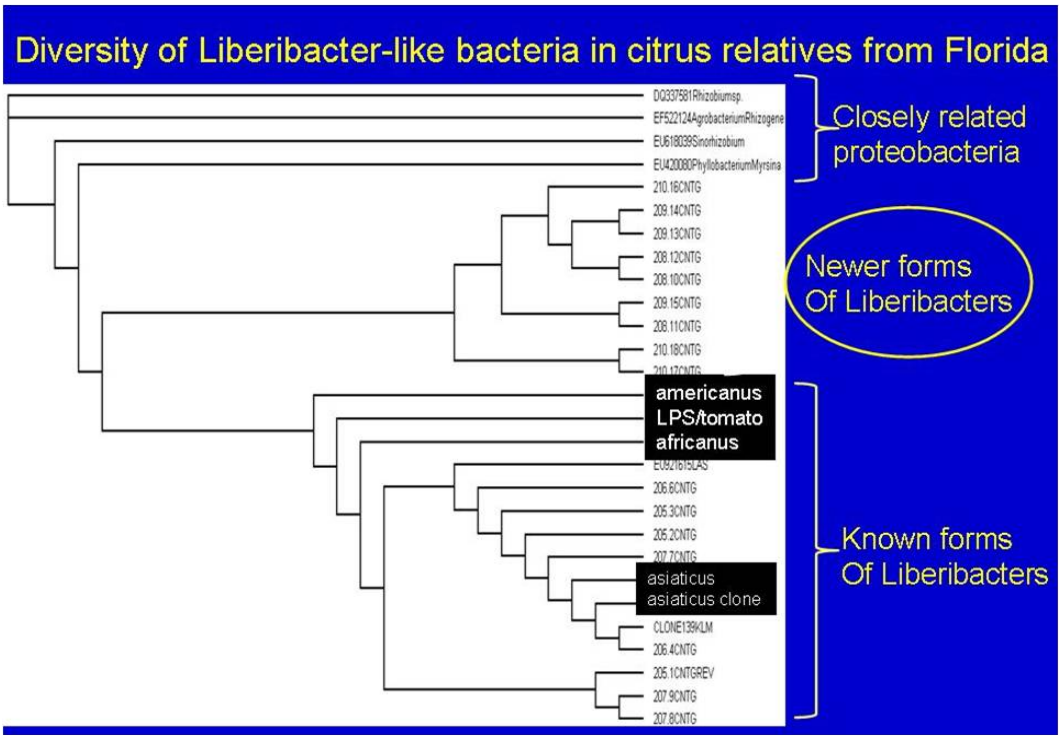

Figure 5. Phylogenetic tree showing the relationship of the "New forms" of *Candidatus* Liberibacter from various citrus relatives in Florida to the know species of *Cd.* Liberibacter.

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Objectives*: (*succinctly state each objective and milestone, ie the time expected to successfully complete an achieve each objective)*

The overall goal is to characterize the molecular and biological properties of the "Liberibacter-like" bacteria which are closely related to *Candidatus* Liberibacter species and to determine their relationship to HLB.

Specific objectives:

1) Determine if *Diaphorina citri* can vector these "Liberibacter-like" bacteria
2) Determine if these "Liberibacter-like" bacteria are pathogenic to citrus
3) Determine if these "Liberibacter-like" bacteria may be useful to ameliorate the effect of HLB

Project's Benefit to the Industry*: *(How will the industry utilize your research results or product)*

Finding bacteria closely related to *Candidatus* Liberibacter spp. in citrus relatives causes concern and more research is needed to determine if these bacteria are pathogenic to citrus and citrus relatives. Possibly they could be used to ameliorate the effect of HLB if they are ecologically and genetically closely enough related to the bacterium associated with HLB. Until recently, citrus relatives have been freely shipped throughout the US, and many of these relatives were propagated in the Homestead area of Florida. If these citrus relatives potentially contain bacteria that are pathogenic to citrus, this needs to be determined, and specific diagnostic methods need to be developed so that potential problems could be avoided in other areas. Finding Liberibacter-like bacteria in California which resulted in a false positive is an issue from the regulatory aspect. Finding these bacteria in curry leaf, lemon, and kumquat suggests that the bacteria involved have a means of spread. Is the vector the ACP, the tomato psyllid, or other insect like a plant hopper? Are these bacteria pathogenic to citrus, to curry leaf, to other citrus relatives? Proper understanding of endophytes, especially close relatives

of Liberibacters, is important for HLB management.

Research Collaboration* (*be specific*):

Dr. Lee will coordinate the project. In Florida Drs. Lee and Keremane will make collections from the citrus relatives in Florida and help Dr. van Bruggen establish in planta cultures and assist in experiments to determine the host range of the Liberibacter-like bacteria. Drs. van Bruggen and Nunes da Rocha will conduct psyllid transmission trials to citrus and citrus relatives, work on establishing *in vitro* cultures of the Liberibacter-like bacteria and aid in the molecular characterization. DNA extracts from field trees and *in planta* cultures will be sent to Drs. Lee and Keremane for molecular characterization. In California, psyllid transmissions from the *in planta* "false-positive" cultures will be conducted by Dr. Walker with Drs. Lee and Keremane testing the resultant plants to determine if the bacterial have been transmitted. Drs. Lee and Keremane will determine the graft transmissible host range for the "false-positives." Seed for citrus relatives will be provided to Dr. van Bruggen by Dr. Lee from the Repository and he will provide plant material to Dr. Walker for the psyllid transmission experiments.

Plans and Procedures* *(use this section to describe your experimental design site location(s)and elaborate on objectives and milestones)*

For the past 2 ½ years, we have conducted surveys of citrus relatives and rare citrus varieties collected from the landscape in South Florida where the ACP and HLB are endemic. On several occasions, we found plants where the qPCR assays gave low *Ct* values, indicating they were Las positive. To confirm Las, we performed conventional PCR for the 16S rDNA region using universal primers for the region, but with these samples there was low homology with the expected Las sequence (94-95%). We know from these previous surveys that we can go back to the plants and obtain similar results during the next survey period. Thus we have aliquots of the DNA from these previous survey samples available for future testing and, most importantly, we know where to locate plants which have the "Liberibacter-like" bacteria.

For this project in Florida we will collect materials from the plants which have "Liberibacter-like" bacteria. These will be established in Dr. van Bruggen's greenhouse (Biosafety level 3) in Gainesville using sideshoot and bud grafting. Additionally, *in vitro* culturing will be done from the field tissue. The "Liberibacter-like" bacteria will be cultured using protocols for alpha proteobacteria, and characterized at the molecular level by PCR, cloning, and sequencing (van Bruggen et al. 1990). Once the "Liberibacter-like" cultures are established in plants, they will be used as the source material for psyllid transmissions to citrus and to other citrus relatives to investigate if they are psyllid transmissible and determine the host range of the bacteria using psyllids for transmission. Graft transmissions will be made to determine the host range of the "Liberibacter-like" bacteria. *In planta* cultures and appropriate control plants will be monitored over time to determine if the "Liberibacter-like" bacteria are pathogenic to the plants or not. In California, we have established *in planta* cultures of the "false positive" samples from Orange County and have *in vitro* cultures of a bacterium which shows close relationships with Rhizobiaceae.

Milestones for year 1: Establish selected citrus and citrus relative seedlings in Dr. van Bruggen's greenhouse from seed collected from the Repository. Rootstock seedlings will be purchased from Florida commercial nursery sources, targeting varieties which are graft compatible with the relatives that are to be collected. A survey will be conducted by Dr. Lee in Fall 2010 (last survey supported by existing CRB grant), and collections of plants infected with the "Liberibacter-like" bacteria will be made, DNA extractions and grafting to rootstocks and greenhouse plants will be done. Dr. van Bruggen will finish building facilities for psyllid transmissions and establish her colony of *Diaphorina citri*. Recollections of plant material from the field will be made as needed due to failure of grafts by Drs. Lee and van Bruggen in the last half of year 1. Dr. Walker will begin psyllid transmission tests with different citrus relatives and citrus varieties as receptors with the "false positive" sample from Orange County with both the tomato psyllid and *D. citri*. Dr. Lee will inoculate citrus relatives and citrus varieties with the *in vitro* cultures from the "false positive."

Milestones for Year 2: Complete the molecular characterization of the bacterial isolates established *in planta*. In Dr. van

Bruggen's lab by both Dr. Van Bruggen and Lee: make *in vitro* isolations, compare molecular properties of the *in vitro* cultures with the *in planta* cultures, inoculate plants with cultures for completion of Koch's postulates, record growth and possible symptom development on *in planta* cultures. Establish trials where some hosts are inoculated with HLB, then the "Liberibacter-like" bacteria and other trials where the plants are first inoculated with the "Liberibacter-like" bacteria, then HLB later. Develop primers for better assay for HLB to prevent "false positives" Dr. Walker will continue psyllid transmission tests using California cultures. Plant inoculated in California by psyllids and cultures will be monitored to determine if HLB-like symptoms are produced and the effect on plant growth.

Milestones for Year 3: In Dr. van Bruggen's lab/greenhouse by Drs. van Bruggen and Lee: take final observations on the experiment to determine if the "Liberibacter-like" bacteria are pathogenic or not, complete the Koch's postulates trials by re-isolations and performing the molecular characterizations, complete the "cross protection" trials by running qPCR or quantitation of the relative rates of Las and the "Liberibacter-like" bacteria and recording symptoms/growth of test plants, publish research results. Drs. van Bruggen, Walker and Lee will summarize data for publication.

Other Funding Sources for this Project *(current, pending, potential; can this project be used as matching funds for other funding sources)*

Dr. Lee has submitted a proposal to the USDA ARS Post Doc program for support for a Post Doc Scientist to work on this project.

Technology Transfer* *(include any potential intellectual property issues; steps necessary for grower utilization extension/communication component):*

Progress reports will be provided as required to the CRB and posted at required intervals on http:/www.fcprac.com . Research results will also be presented at grower and professional society meetings (local, national, international), workshops, and invitational reports. A workshop will be held to transfer any new diagnostic methods to interested stakeholders and regulatory personnel. Updates will be provided at CRB and Extension meetings. Manuscripts will be written and submitted for publication in professional journals as well as local trade magazines

Budget Justification:

Technical help is requested for Drs. Lee, van Bruggen and Walker to help care for plants, collect samples from greenhouse plants, assist in preparation of samples for analyses. The first year, an extra $5000 is requested for Dr. van Bruggen to install screen cages in the quarantine facility to enable psyllid rearing and transmission experiments. Travel funds are requested for Dr. Lee and Keremane to spend time in Florida for collection of plants from the landscape, assist with grafting on plants, and to enable closer cooperation between Riverside and Gainesville. Travel funds are requested for Dr. van Bruggen to allow her to visit the Riverside labs (Repository and Walker, UCR). A small amount of supply funds are requested, most supplies will be provided by respective agency support.

CRB Project Plan – Research Grant Proposal for FY2010-2011
Project Title: Characterization of endophytic prokaryotes closely related to HLB in Citrus and citrus relatives

Project Budget

Department Account Number: *(if applicable)*_____

	Year: **2010-2011**	Year: **2011-2012**	Year: **2012-2013**
Salaries and Benefits:			
Postdocs/Research Assistants	$_____	_____	_____
SRA's	_____	_____	_____
Lab/Field Assistance	70,000	70,000	70,000
Benefits	_____	_____	_____
Supplies and Expenses:	17,000	12,000	12,000
Equipment:	_____	_____	_____
Operating Expenses and Travel:	6,000	8,000	7,000
Lindcove Recharges:	_____	_____	_____
Lindcove Packline:	_____	_____	_____
Other: _____	_____	_____	_____
_____	_____	_____	_____
ANNUAL TOTAL:	93,000	90,000	89,000

Specifics regarding contract (i.e., "split" funding to more than one PI):
Please see the following page. Year 1: van Bruggen would receive $41,000 and Walker would receive $12,000, and Lee would receive $40,000.

Signatures

Project Leader: Richard T. Lee _____ **Date:** 8 Aug 10

_____ **Date:**_____

_____ **Date:**_____

Research Leader: Richard T. Lee _____ **Date:** 8 Aug 10

*Use as much space as necessary; attach additional pages as needed – budget info. and signatures will appear on the final page.

Budget Estimate:

Budget Estimate total for 3 years: $272,000

Year 1		Personnel	Supplies	Travel	Subtotal
Lee	ARS, Riverside	$30,000	$5,000	$5,000	$40,000
van Bruggen	UF, Gainesville	$30,000	$10,000*	$1,000	$41,000
Walker	UC, Riverside	$10,000	$2,000		$12,000
Total					$93,000
Year 2					
Lee	ARS, Riverside	$30,000	$5,000	$5,000	$40,000
van Bruggen	UF, Gainesville	$30,000	$5,000	$3,000	$38,000
Walker	UC, Riverside	$10,000	$2,000		$12,000
Total					$90,000
Year 3					
Lee	ARS, Riverside	$30,000	$5,000	$5,000	$40,000
van Bruggen	UF, Gainesville	$30,000	$5,000	$2,000	$37,000
Walker	UC, Riverside	$10,000	$2,000		$12,000
Total					$89,000

*An extra $5,000 is included in the request for funds to finish construction of screening needed to maintain *Diaphorina citri* colonies.

CRB Project Plan – Research Grant Proposal for FY2010-2011
Project Title: Characterization of endophytic prokaryotes closely related to HLB in Citrus and citrus relatives

Project Budget

Department Account Number: *(if applicable)*_____

	Year: **2010-2011**	Year: **2011-2012**	Year: **2012-2013**
Salaries and Benefits:			
Postdocs/Research Assistants	$_____	_____	_____
SRA's	_____	_____	_____
Lab/Field Assistance	70,000	70,000	70,000
Benefits	_____	_____	_____
Supplies and Expenses:	17,000	12,000	12,000
Equipment:	_____	_____	_____
Operating Expenses and Travel:	6,000	8,000	7,000
Lindcove Recharges:	_____	_____	_____
Lindcove Packline:	_____	_____	_____
Other: _____	_____	_____	_____
_____	_____	_____	_____
ANNUAL TOTAL:	93,000	90,000	89,000

Specifics regarding contract (i.e., "split" funding to more than one PI):
Please see the following page. Year 1: VanBruggen would receive $41,000 and Walker would receive $12,000, and Lee would receive $40,000.

Signatures

Project Leader: _____ **Date:** _____

_____ **Date:** _____

G. P. Walker **Date:** 8/4/10

Dept. Chair: _____ **Date:** _____

Budget Estimate:
Budget Estimate total for 3 years: $272,000

Year 1		Personnel	Supplies	Travel	Subtotal
Lee	ARS, Riverside	$30,000	$5,000	$5,000	$40,000

*Use as much space as necessary; attach additional pages as needed – budget info. and signatures will appear on the final page.

CRB Project Plan – Research Grant Proposal for FY2010-2011
Project Title: Characterization of endophytic prokaryotes closely related to HLB in Citrus and citrus relatives

Project Budget

Department Account Number: *(if applicable)*_____

	Year: **2010-2011**	Year: **2011-2012**	Year: **2012-2013**
Salaries and Benefits:			
Postdocs/Research Assistants	$_____	_____	_____
SRA's	_____	_____	_____
Lab/Field Assistance	70,000	70,000	70,000
Benefits	_____	_____	_____
Supplies and Expenses:	17,000	12,000	12,000
Equipment:	_____	_____	_____
Operating Expenses and Travel:	6,000	8,000	7,000
Lindcove Recharges:	_____	_____	_____
Lindcove Packline:	_____	_____	_____
Other: _____	_____	_____	_____
_____	_____	_____	_____
ANNUAL TOTAL:	93,000	90,000	89,000

Specifics regarding contract (i.e., "split" funding to more than one PI):
Please see the following page. Year 1: VanBruggen would receive $41,000 and Walker would receive $12,000, and Lee would receive $40,000.

Signatures

Project Leader: _____ **Date:** _____

_____ **Date:** Aug 3, 2010

_____ **Date:** _____

Dept. Chair: _____ **Date:** 8-3-10

Budget Estimate:
Budget Estimate total for 3 years: $272,000

*Use as much space as necessary; attach additional pages as needed – budget info. and signatures will appear on the final page.

UF UNIVERSITY of FLORIDA

Division of Sponsored Research
http://www.research.ufl.edu/research

219 Grinter Hall
PO Box 115500
Gainesville, FL 32611-5500

Cost Sharing Commitment

TO: Division of Sponsored Research

FROM: PI: Ariena van Bruggen

UNIT: Plant Pathology

SUBJECT: Proposal Title: Characterization of the endophytic prokaryotes closely related to the bacterium associated with huanglongbing

Sponsor: Citrus Research Board

This proposal involves Voluntary Cost Sharing. The justification is given below.

Justification: 5% of PI salary plus fringe

As the Unit Leader, I have received and concur with the justification provided for the voluntary cost sharing amount that we are now obligating for this proposal. I understand that the Third Party Cost Sharing (if any) is committed independently from my Unit's resources by the letters provided and attached to this document.

Cost Sharing for Personnel	$31,725.00
Cost Sharing for Non Personnel	$0.00
Third Party Cost Sharing	$0.00
Total Commitment	$31,725.00

This Commitment is acknowledged and agreed to on August 3, 2010.

Unit Head Signature: _____
J. B. Jones

Dean's Signature: _____ FOR MRM
Mark McLellan

Name	Ariena vanBruggen					
Category	Faculty					
YR.	Avail.	Salary	% of Effort	Base Salary	Fringe Rate	Yearly Commit.
1	Yes	$160,000.00	5.00	$8,000.00	.283	$10,264.00
2	Yes	$164,800.00	5.00	$8,240.00	.283	$10,571.92
3	Yes	$169,744.00	5.00	$8,487.20	.283	$10,889.08
Total Commitment						$31,725.00

The Total Cost Sharing for Personnel is $31,725.00

The Total Cost Sharing for Other Costs is $0.00

The Total Cost Sharing for Third Parties is $0.00
