CITRUS RESEARCH BOARD

PROJECT PLAN - RESEARCH GRANT PROPOSAL FOR FY2010-2011

Fiscal Year: 2010-2011Anticipated Duration of Project: Three years

This Project is: _____ New or XX Ongoing (Year 2 of 3)

Project Leader: Mark Hoddle Name Location: Dept. of Entomology, University of California, Riverside, CA 92521 Address (University department, if applicable) Mailing Address (if different):

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Co-PI: Kris Godfrey, CDFA, Biocontrol Program, 3288 Meadowview Road, Sacramento, CA, Phone: 916-262-1185, Fax 916-262-2059; Email: kgodfrey@cdfa.ca.gov

Cooperating Personnel: Ru Nguyen, Florida Dept. of Agriculture and Consumer Services, Division of Plant Industry, 1911 SW 34 Street, Gainesville, FL; Phone: 352-372-3505; Email: nguyenr@doacs.state.fl.us

Project Title: Host Specificity Testing of *Tamarixia radiata* Sourced from the Punjab of Pakistan for Classical Biological Control of Asian Citrus Psyllid in California

Keywords: Asian Citrus psyllid, Tamarixia radiata, Biological Control

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*)

A classical biological control program against Asian citrus psyllid (ACP) is underway in California. The goal is to permanently reduce population densities of this pest which vectors Huanglongbing (HLB), a lethal bacterial disease of citrus. *Tamarixia radiata* from the Punjab region of Pakistan, is an ACP parasitoid of interest to California (CA) because it is from an area with a very good climatic match with the major citrus producing areas of CA, and it may be able to suppress ACP densities in areas where pesticide use is not possible (e.g. organic farms). Before this parasitoid can be established in CA host specificity testing and preparation of an Environment Assessment Report (EAR) must be completed for review by the USDA-APHIS. This project is partially supported by a CDFA Specialty Crops Grant and work on safety tests in quarantine on native CA psyllids and weed biocontrol agents is underway. Continuation of funding from the CRB is requested for a second year to complete the safety tests, prepare and submit the EAR, and move *Tamarixia* out of quarantine for mass rearing, release, and establishment in CA.

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

Asian citrus psyllid (ACP), *Diaphornia citri* Kuwayama (Hemiptera: Psyllidae), was first detected in California on August 27, 2008, when it was trapped in backyard citrus in southwestern San Diego County. As of June 2010, populations of this were known from San Diego, Imperial, Los Angeles, and Orange Counties in California. ACP now has a secure foothold in southern California after invading from Mexico. This pest is viewed as a serious threat to California citrus production because of its ability to acquire and transmit bacteria which cause the disease, Huanglongbing (HLB), a malady lethal to citrus.

The use of classical biological control agents against this pest is potentially one tool that can be incorporated into future IPM programs that will be needed for sustainable ACP management in California. Host-specific parasitoids that attack the nymphal stages of ACP are perhaps the most efficacious natural enemies available. The establishment of host-specific parasitoids for the suppression of ACP populations, especially in areas where insecticide use will be limited (e.g., organic growers, backyard citrus plantings, and urban ornamentals that can support ACP) could be extremely beneficial.

Around nine parasitoids of ACP are known from the home range of this pest in the Punjab region of Pakistan and India, and some are attacked by hyperparasitoids (Husain and Nath 1927). *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae) is considered the most important parasitoid in the Punjab where the parasitoid guild collectively parasitized 95% of ACP (Husain and Nath 1927). In orchards with high parasitism ACP is not considered an important pest (Husain and Nath 1927). *T. radiata* and *Diaphorencyrtus aligarhensis* (Shaffee et al.) (Hymenoptera: Encyrtidae), have been used most frequently against ACP in classical biocontrol programs. Published studies suggest *T. radiata* is the more efficacious parasitoid. Biological control programs against ACP in Florida with *Tamarixia* from southeast Asia has provided variable control of ACP during the year and across different locations with average parasitism rates ranging from ~20% to 56% (Tsai et al. 2002, Skelly and Hoy 2004, Qureshi et al. 2009). Recently published cage exclusion studies clearly demonstrated that natural enemies play an important role in limiting ACP population growth (Qureshi and Stansly 2009).

A new strain of *Tamarixia* has been imported into Florida and Texas from the Punjab region of Pakistan. *Tamarixia* currently in quarantine in Florida and Texas from the Punjab are of most interest for establishment in California because of excellent climate match to California's major citrus producing regions. These parasitoids have not been released in Texas because of the need to complete host specificity testing and the preparation of an Environment Assessment Report (EAR) as required by USDA-APHIS. The EAR has been completed for Florida and the USDA-APHIS has issued a FONSI (Finding of No Significant Impact) for this parasitoid in Florida. Similarly, before parasitoids can be released into California for classical biological control of ACP, USDA-APHIS requires that host specificity testing be conducted in quarantine to evaluate the risk this natural enemy poses to native California psyllids and psyllids that are used as weed biocontrol agents. Data from these tests are used to prepare the EAR. If safety evaluations are not completed, and an EAR is not provided for review by APHIS, APHIS will prohibit deliberate releases of the Punjabi strain of *T. radiata* in California. Consequently, the purpose of this proposal is to request continuing support to complete the host specificity testing phase of *T. radiata* from the Punjab in quarantine at UCR and prepare the EAR for USDA-APHIS.

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

This proposal has <u>one</u> objective: To complete the required host specificity and safety evaluations for the Punjabi strain of *T. radiata* in quarantine at UC Riverside. The goal of this work is the rapid completion of safety tests and preparation of the Environmental Assessment Report for USDA-APHIS thus clearing the way for release, establishment, and evaluation of the Punjabi strain of *T. radiata* for classical biological control of ACP in citrus production and urban areas in California.

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

The anticipated benefits from this classical biological control project with *T. radiata* will be to conventional and organic citrus growers in CA and homeowners in urban areas with backyard citrus and ornamentals that ACP can infest. All citrus growers will benefit from some level biological control because of an anticipated decrease in ACP densities throughout infested production areas and adjacent urban reservoirs. This density reduction has the potential to reduce the cost and number of insecticide applications required to manage ACP, thereby reducing the disruption to other insect IPM programs and lowering the risk of environmental and/or human health concerns. Successful integration of biological control into area-wide management programs may also prove very cost effective for all growers in the longer-term. It is anticipated that host specificity testing will be completed during the first 12-18 months of this three year project, with submission of the petition to field release *T. radiata* to USDA, APHIS by the middle of the second year of the project (April-May 2011). We anticipate that the petition will be accepted, and the remainder of year two and all of the third year of the project can be used to begin mass rearing, releases establishment, and evaluation of *T. radiata* in California.

Research Collaboration* (*be specific*):

Dr. Hoddle will supervise the activities conducted in the Quarantine Facility at UCR. Dr. Godfrey will assist in the collection of native psyllids and weed biocontrol agents for host testing, production of native plants on which to rear the test psyllids, and later Dr. Godfrey will coordinate mass production of *T. radiata* at CDFA facilities, and assist in the release and evaluation studies after USDA-APHIS has granted permission to release *T. radiata* from quarantine.

Dr. Ru Nguyen, Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Gainesville Florida, has considerable experience in the biological control of ACP, especially with parasitoids. He is currently rearing the Punjabi strain of *Tamarixia* in Florida and will continue to make pupae available to us for this proposed work (see letter of collaboration) in accordance with the USDA-APHIS permits that have been applied for to work on *T. radiata* in quarantine at UC Riverside.

Dr. Nguyen's work is being covered by the CDFA Specialty Crops program funding (see funding support statements below).

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

(A) <u>Host Specificity Tests</u>: Selection of psyllid species for safety testing have been developed along four lines: (1) native species with high relative abundance in close proximity to citrus orchards^(see †for species below); (2) Taxonomic relatedness to ACP^(see ‡ below for species); (3) weed biocontrol agents^(see !! below for species), and (4) common exotic pest species^(see △ for species below). Host specificity testing for the Punjabi strain of *T. radiata* will require colonies of native California psyllid species(1 and 2 above), psyllids used as weed biocontrol agents, and some common exotic pest psyllid species. From the literature and consultation with psyllid specialists at CDFA and Dr. Diana Percy we will test at least five native California psyllid species ({1} *Ceanothia ceanothi*[†] [Host plant = *Ceanothus* sp.], {2} *Dichlidophelbia fremontiae*[‡] **[*Fremontia californica*], {3} *Calophya nigrella*[‡] **[*Rhus ovata*], {4} *Aphalaroida* spp. [‡] [*Prosopsis* and *Acacia* sp.], {5} *Neophyllura* spp. [‡] [*Arctostaphlos* spp.]); the broom psyllid "
(a weed biocontrol agent [*Arytainilla spartiophila*]); and two pest psyllids potato (^{△ **} *Bactericera cockerelli*), and olive (^{△ □} *Euphyllura olivina*) for host suitability to *T. radiata*. Currently, in quarantine we have colonies of species () or have ready access to field sites with test subjects from which we can take nymphs for testing ([□]).

The current taxonomic standing of California psyllids is very confused because no revisionary work on this fauna has

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been undertaken for over 20 years. The names presented above were taken from literature sources that were compiled in 1980's (Gill, 1989; Hodgkinson 1988), or earlier (Heslop-Harrison, 1961; Journet and Vickery, 1979) and have been revised by Dr. Diana Percy as part of her CRB project on preparing a current checklist for psyllid species in California. Dr. Percy's revision and preparation of the California psyllid checklist will eliminate uncertainty over accepted names of psyllid species that may cause unnecessary problems when the EAR is prepared and reviewed by APHIS. This will be especially problematic if the same species are being referred to by different names, or genus designations are challenged. The taxonomic revision of the psyllids in California and the preparation of a new checklist using modern taxonomic placements will ensure every group vested in ACP biocontrol is on the same page when reviewing lists of psyllid species and interpreting the results of host specificity tests.

Host specificity testing will consist of static choice and no choice tests, and sequential testing alternating between host and non-host species to confirm parasitoid competency and account for time effects and host exposure experiences on parasitism rates (see Appendix One and Two for experimental designs for no choice and choice experiments). The function of these tests is to define the acceptable behavioral and physiological host range of *Tamarixia* and to ascertain how likely it is that this parasitoid can attack and reproduce on non-target psyllids when it has either: (1) no ACP around to attack and only one species of non-target to parasitize, (2) prior exposure to ACP in which is has oviposited then exposure to non-target species, (3) it has a choice between several non-target species to attack, but no ACP are available, and (4) it has a choice between ACP and several non-target species that are all presented simultaneously for attack. Data from these studies will provide information on how many different psyllid species *Tamarixia* is likely to attack; if non-targets are available in the presence of ACP, the strength of *Tamarixia*'s preference for attacking ACP when it has other hosts to attack, and if prior attacks and host feeding experiences on ACP increase or decrease the likelihood that *Tamarixia* will attack non-targets should it find them. Every experiment that is conducted will be simultaneously paired with sets of control psyllids not exposed to Tamarixia which are treated in an identical manner to psyllids exposed to parasitoids. Naturally-occurring mortality in these control groups will act as a baseline against which psyllid mortality rates in the presence of *Tamarixia* will be compared. The tests we propose to conduct for *Tamarixia* have been designed following a review of case studies and review articles compiled by Van Driesche and Reardon (2004).

The key data to be collected from these studies are: (1) rates of naturally-occurring mortality in control psyllid groups not exposed to *Tamarixia*. (2) Parasitism rates by *Tamarixia* on different host species. (3) Comparison of host mortality rates resulting from host feeding across host species which will be compared to naturally occurring mortality rates observed in the absence of *T. radiata*. (4) Evaluation of the fitness (i.e., sex ratio, fecundity, and longevity) of all parasitoid offspring from hosts from which adult *Tamarixia* emerged. All fitness measures will be statistically compared across psyllid hosts. Collectively these data will indicate the relative preferences and suitability of different species of psyllid hosts for attack by *Tamarixia*, subsequent reproduction, and the likelihood of population growth and maintenance on target and non-target psyllid species.

(B) Source of Tamarixia for Testing: The Punjabi strain Tamarixia to be used in host specificity tests will be mass reared and shipped as pupae from quarantine cultures being maintained in Florida by Dr. Nguyen. <u>Currently, APHIS will not grant</u> permits for *Tamarixia* colonies in California. All *Tamarixia* shipped from Florida consists of pupae detached from leaf material reared in laboratory colonies of ACP known to be free of HLB. Emerged *Tamarixia* adults, will be honey water fed, and are of a standardized age when choice and no choice experiments are conducted in Quarantine at UC Riverside.

(C) Data Collection, Analysis, and Preparation of the Environmental Assessment Report: All data on host preferences, attack rates, reproductive outputs, and offspring fitness characteristics will be statistically analyzed and used to prepare the EAR that will be presented to USDA-APHIS for review. We anticipate that these data will be of sufficient quality and importance to be published in peer-reviewed scientific journals.

(D) Release from Quarantine: Following issuance of approvals for release from quarantine, CDFA personnel at the Mt. Rubidoux Facility will assist with the mass rearing, distribution, and impact monitoring in California for *T. radiata* at a secure facility maintained by CDFA, but located away from UCR and/or the Citrus Germplasm Repository. The CDFA Sacramento Facility will assist in host plant production should supply problems develop. Dr. Godfrey will coordinate the mass efforts and assist in the mass release and establishment of *Tamarixia* and subsequent evaluation studies.

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<u>Time Line for Achieving Objectives:</u>

	2010		2011				Î	2012			
Objective	Fall	Winter	Spring	Summer	Fall	Winter		Spring	Summer	Fall	Winter
Establishment and/or											
maintenance of colonies											
of test psyllids and/or											
ACP for experiments	****	****	****	****	****	****					
Test/study plant											
maintenance	****	****	****	****	****	****					
Importation of <i>Tamarixia</i>											
from Florida for testing	****	****	****	****							
Host specificity testing	****	****	****	****							
Data analysis and											
preparation of EAR for APHIS and NAPPO				****	****						
				* * * *	* * * *						
<i>Tamarixia</i> culture											
management, mass											
rearing, release,											
establishment, and											
evaluation								****	****	****	****
Outreach to stakeholders	****	****	****	****	****	****		****	****	****	****

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

CDFA Specialty Crops Grant, submitted April 2009. Year 1: \$110,264; Year 2: \$83,461**; Year 3: \$89,965**; Total = \$282,740. This grant is split between UCR, CDFA, and DPI in Florida.

**Funding to the ACP biocontrol program at UCR from the Specialty Crops Program will be \$31,400 in 2011, and \$33,000 in 2012. The remainder of the funds are supporting the CDFA's contributions and Dr. Nguyen's production and shipment of the Punjabi strain of *Tamarixia* to UCR from Florida.

Facilities:

California Department of Food and Agriculture - Sacramento

- North B Street Greenhouse 200 sq.ft. bench space and a propagation bench, and other facilities for plant propagation and growth.
- Meadowview Greenhouse 100 sq. ft. bench space; dissecting microscope; computer, printer, and software; GPS unit; camera

California Department of Food and Agriculture – Riverside - Mt. Rubidoux Facility

Greenhouse – 100 sq. ft. of bench space; dissecting microscope; computer, printer, and software; laboratory bench space – 50 sq. ft.; three growth chambers; freezer and refrigerator; GPS unit; camera

University of California - Riverside

- Access to one greenhouse room, 1 laboratory room, and two rearing rooms in the University of California Riverside Level II Quarantine Facility
- Retrofitted and ACP secure greenhouse space at UCR supported by CHRP Funds to CDFA will be available for ACP rearing for this project.

Computer facilities and software; refrigerator and freezer; dissecting and compound microscopes; camera and software for viewing and photography of insects under microscope

Florida Division of Plant Industry – Gainesville

Access to Florida Level II Quarantine Facility – one greenhouse room, one laboratory room, and one insect rearing room. Computer facilities and software. Refrigerator and freezer. Dissecting and compound microscopes.

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

Every opportunity will be taken to keep stakeholders informed on the progress being made with this project. We will make regular updates on websites: e.g., <u>www.biocontrol.ucr.edu</u>, <u>www.cisr.ucr.edu</u>, <u>www.citrusent.uckac.edu</u>, <u>www.cdfa.ca.gov</u>, <u>www.citrusresearch.com</u>, <u>http://ucanr.org/sites/kaccitrusentomology</u> and <u>www.ipm.ucdavis.edu</u>.

Project participants will orally present the results of this work at citrus grower, CAPCA, AAIE, and professional meetings. Field days demonstrating natural enemy impact in the field will be organized through the California Citrus Research Board, UCCE, and CDFA. Articles will be written for publication in citrus newsletters, trade magazines. Media Relations and Strategic Communications will be used to advertise milestone accomplishments to the public through local news media.

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Budget Justification:

This project requires additional financial support to supplement the Specialty Crops Funding. Salary support is needed for the Dr. Raju Pandey, the post-graduate researcher and support personnel to conduct this proposed work. Year two of this project will focus on host specificity testing and preparation of the Environmental Assessment Report for APHIS. After the EAR is completed the remainder of Year 2 will focus on mass rearing, release and establishment, and impact monitoring of *Tamarixia* on ACP.

Salaries and Benefits:

<u>Total Salary and benefit support from Specialty Crops grant = \$29,400</u> Values requested below for salary support from CRB supplement the partial funding from the Specialty Crops Program.

Post Graduate Researcher III, Dr. Raju Pandey, to conduct ACP natural enemy studies in Quarantine at 100% time (50% salary support from Specialty Crops and 50% support from CRB) at \$3,300/month with benefits at 39%.

Lab Assistant III, Ruth Vega, at 50% time for 12 months at \$2,561/month and benefits at 57%. Museum Preparator, Vladimir Berezoski – 20% time for 12 months at \$3,144/month and benefits at 40%.

Total salary requested from CRB = \$42,730, Total Benefits = \$19,506, Grand Total = \$62,235

Supplies and Expenses:

Supplies funding from Specialty Crops Grant = \$500

Values below are requested from CRB to supplement funding from the Specialty Crops Program.

Host plants for maintaining psyllid colonies, potting mix, pest control supplies, museum supplies for curating specimens; supplies for mass rearing *T. radiata*; lab supplies etc. Total estimated at \$5,000. *Total requested from CRB* = \$4,500

Travel:

Travel Funds from Specialty Crops Grant to support this project = \$1,500

Daily rental for truck to move plants from production areas to Quarantine and to collect native psyllids from wilderness areas. The rental truck will be needed three times per week @\$65 per day for 8 months/yr (= \$6,240). Mileage is estimated at \$0.55 per mile for ~ 600 miles/month (= \$2,640). Total vehicle expenses = \$8,800. *Total requested from CRB* = \$7,380.

Travel to attend two professional meetings a year to present the results of this research. *Total requested from CRB* = *\$6,000*

TOTAL BUDGET REQUEST: \$80,115

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	Project	Budget	
Department Account Number: (if a	pplicable)		
	Year: 2010-2011	Year: 2011-2012	Year: 2012-2013
Salaries and Benefits:			
Postdocs/Research Assistants	\$19,818 \$20	,809	
SRA's			
Lab/Field Assistance	\$22,912	\$24,057	
Benefits	\$19,506	\$20,481	
Supplies and Expenses:	\$4,500	\$4,725	
Equipment:			
Operating Expenses and Travel:	\$13,380	\$14,049	
Lindcove Recharges:			
Lindcove Packline:			
Other:			
ANNUAL TOTAL:	\$80,115	\$84,121	

Specifics regarding contract (i.e., "split" funding to more than one PI):

Signatures

Date: July 15 2010

Project Leader: Kris Do dfrey

Date: July 15 2010

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	Date:
Dept. Chair:	 Date:

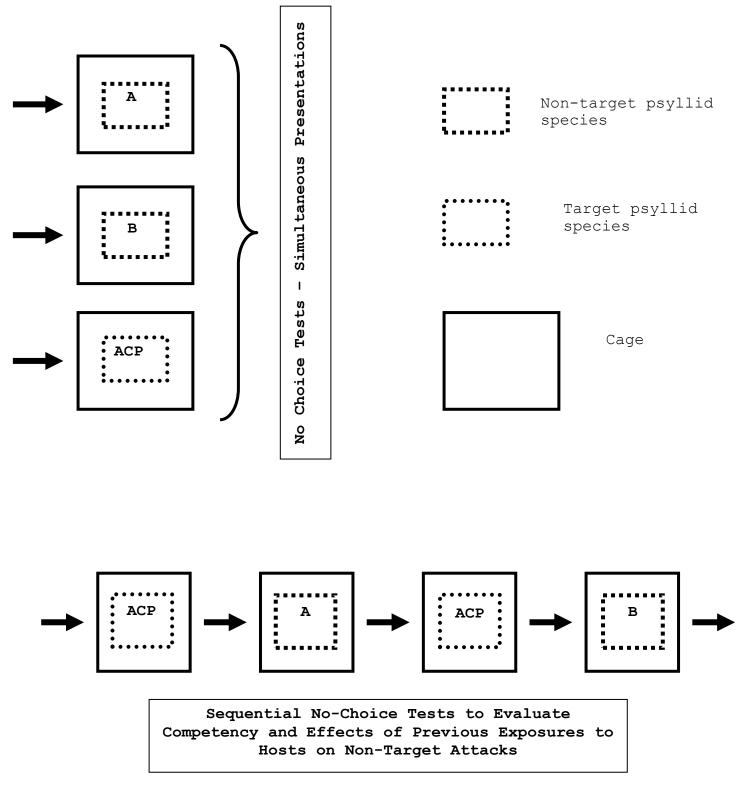
Project Title: Host Specificity Testing of *Tamarixia radiata* Sourced from the Punjab of Pakistan for Classical Biological Control of Asian Citrus Psyllid in California

Literature Reviewed for Preparation of this Proposal:

- Gill, R. J. 1989. The Psyllidae of California: a preliminary checklist. California Plant Pest and Disease Report 8: 21-28.
- Halbert, S. and K. Manjunath. 2004. Asian citrus psyllids (Sternorrhyncha: Psyllidae) and greening disease of citrus: a literature review and assessment of risk in Florida. Florida Entomol. 87: 330-353.
- Hall, D., M. Hentz, and R. Adair. 2008. Population ecology and phenology of *Diaphorina citri* (Hemiptera: Psyllidae) in two Florida citrus groves. Environ. Entomol. 37: 914-924.
- Heslop-Harrison, G. 1961. The Arytainini of the sub-family Psyllinae. Hemiptera-Homoptera, family Psyllidae 2. Annals of the Magazine of Natural History 13: 417-439.
- Hodkinson, I. D. 1988. The nearctic psylloidea (Insecta: Homoptera) an annotated checklist. Journal of Natural History 22: 1179-1243.
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- Van Driesche, R. G. and R. Reardon (eds.) 2004. Assessing Host Ranges for Parasitoids and Predators Used for Classical Biological Control: A Guide to Best Practice. Forest Health Technology Enterprise Team, Morgantown, West Virginia FHTET-2004-03. 243pp.
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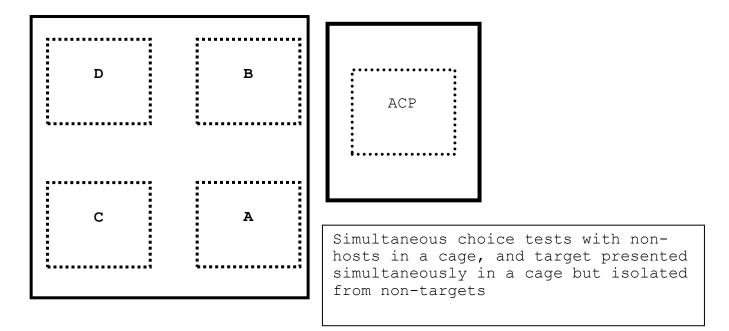
Appendix One: Possible experimental designs for conducting no-choice tests for *Tamarixia* as part of the host-specificity testing work to be conducted in quarantine at UC Riverside.



*Use as much space as necessary; attach additional pages as needed – budget info. and signatures will appear on the final page. *Not for publication without the express written consent of the project leader. Before quoting or reproducing any information in whole or extracted in any form, contact the project leader responsible.*

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Appendix Two: Possible experimental designs for conducting choice tests for *Tamarixia* as part of the host-specificity testing work to be conducted in quarantine at UC Riverside.



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Target offered simultaneously with nontarget psyllid species.

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Florida Department of Agriculture and Consumer Services CHARLES H. BRONSON, Commissioner The Capitol • Tallahassee, FL 32399-0800 www.doacs.state.fl.us

July 28 2010 Please Respond to:

Ru Nguyen, Ph.D. Biological Scientist IV Division of Plant Industry P. O. box 147100 Gainesville, Florida 32614

Dear Members of the Citrus Research Board,

I am writing this letter to acknowledge the fact that I am a willing collaborator on a grant entitled <u>"Host Specificity Testing of Tamarixia radiata Sourced from the Punjab of Pakistan for Classical Biological Control of Asian Citrus Psyllid in California</u>" that is being submitted by Dr. Mark Hoddle (Dept. of Entomology, University of California Riverside) and Dr. Kris Godfrey (Biological Control Program, California Department of Food and Agriculture) for possible funding.

My role in this project will be to supply regular weekly shipments of *T. radiata* pupae to the Insectary and Quarantine Facility at UC Riverside. These parasitoid pupae will be used to complete the mandatory host specificity testing required by USDA-APHIS so an Environmental Assessment report can be prepared. This report will document the host-specificity of *T. radiata* and its proclivity to attack and reproduce on native California psyllids, and several exotic pest psyllids as well, including weed biocontrol agents.

I am aware of the research proposal that is being submitted by Hoddle and Godfrey, and I am confident that my lab can supply the *T. radiata* pupae that will be needed for this project.

If there are any questions pertaining to this letter, please do not hesitate to contact me.

Sincerely,

CHARLES H. BRONSON COMMISSIONER OF AGRICULTURE

nguyuka

Ru Nguyen, Ph.D. Phone: (352) 372-3505 E-mail: <u>nguyenr@doacs.state.fl.us</u>



Florida Agriculture and Forest Products Over \$100 Billion for Florida's Economy