

**Reporting Indicators for the Pakistan-US Science and Technology Cooperative Program**

**Name of US Principal Investigator: Mikeal Roose/Richard Lee**

**Project Title: Management of Greening by Producing Healthy Plants, Monitoring Vectors, and identification of Tolerance: no. NAS PGA-P280807**

Indicators:	Reporting Period
	7/01/2008
	- 12/31/2008
1. Total number of Pakistanis completing exchange visits to the United States on your project during the reporting period	
Number of women	0
Number of men	0
2. Total number of Pakistanis trained as a result of participation in your project during the reporting period (this figure will likely be higher than the number reported in item 1, as this item will include not only people who received training by visiting the United States but also those who received training through activities held in Pakistan.)	
Number of women	0
Number of men	0

**Narrative:**

The implementation of this project was delayed until September so this report covers only about a three month period. In Pakistan, three groves were selected for monitoring in each of four major citrus growing regions. Five trees were tagged within each grove. Each tree was monitored weekly for psylla populations using STP yellow tags. The psylla population went down significantly during the months of June, July and December, January. However, the psylla were active in the inner canopy of the trees even during the summer months. All trees were scored for symptomatic presence of Huanglongbing (HLB). There was a nine percent incidence of HLB symptoms at the beginning of the observations. Laboratory space has been organized, and necessary equipment and supplies have been ordered. Four PhD students have been recruited and research will be at a full swing during 2009. In California, fresh seed is being collected from the Citrus Variety Collection from the “core collection” of hybrids which represents about 85% of the genetic diversity which can be identified by SSR markers (Barkley, N.A., Roose, M.L., Krueger, R.R., and Federici, C.T. 2006. Assessing genetic diversity and population structure in a citrus germplasm collection utilizing simple sequence repeat markers (SSRs). *Theor. Appl. Genet* 112:1519-1531) plus seed of citrus relatives and diverse citrus varieties. (See appendix A for complete list.) This seed will be shipped to the project in Pakistan, where the seedlings will be grown in protected greenhouse conditions, then planted into the field in a replicated, random planting where they will be naturally exposed to huanglongbing (HLB) spread by the psyllid vector, *Diaphorina citri*. We anticipate shipment of seed about the first of Feb 2009, and field plantings of the seedlings (6-12 leaf stage) should occur about Oct-Dec 2009.

A Post doctoral researcher has been employed by the project in California. She has been conducting single nucleotide polymorphism studies of specific nuclear gene sequences of many of the core collection hybrids and citrus relatives to determine phylogenetic relationships. In a separate complementary project funded by the California Citrus Research Board, collections of the citrus hybrids and relatives have been made in southern Florida where HLB is now endemic. Observations are being made as to whether *D. citri* adults and/or nymphs are present (e.g. do the psyllids feed on the host and/or do they breed on the host?) and if the plants are infected with HLB using real time PCR assays.

A two meter high insect suction trap has been procured to ship to Pakistan for this project. We anticipate shipment of the trap within 30 days, and it will be established in Pakistan in the area where the seedlings described above will be planted into the field.

Because of the holidays and already scheduled travel when this project began, no exchange visits have been made yet. Lee, from California, is planning a visit in April. This will enable training in Pakistan on methods for therapy of graft transmissible pathogens in citrus germplasm. This visit to Pakistan will be followed by visits to Riverside by students and personnel from Pakistan where hands on training will be provided in methods of therapy of germplasm from graft transmissible pathogens, laboratory diagnosis of citrus pathogens, and training in greenhouse management and maintenance of pathogen-tested plants for use for budwood distribution.

#### **Conferences attended:**

M. Roose attended the International Research Conference on HLB, Dec. 1-5, 2008 held in Orlando, Florida

#### **Publications:**

Ramadugu, C., M. L. Keremane, R. F. Lee, and M. L. Roose. Phylogenetic analysis of Rutaceous plants based on single nucleotide polymorphism in nuclear gene sequences. Abstract presented at the Plant and Animal Genome Conference XVII, January 10-14, 2009, San Diego, CA.

#### **Appendix A:**

Seed collected for use in Pakistan

<b>Genus</b>	<b>Species</b>	<b>Group</b>
Aeglopsis	chevalieri	Citrus relative
Afraegle	paniculata	Citrus relative
X Citroncirus	sp.	citrumelo
Atalantia	ceylanica	Citrus relative
Balsamocitrus	dawei	Citrus relative
Fortunella	hindsii	kumquat
Citrus	aurantium	sour orange

Severinia	buxifolia	citrus relative
Citrus	celebica	papeda hybrid
Citrus	limonia	lemon hybrid
Citrus	maxima	pummelo
Citropsis	daweana	Citrus relative
Citropsis	gabunensis	Citrus relative
Citropsis	gilletiana	Citrus relative
Citropsis	schweinfurthii	Citrus relative
Citrus	amblycarpa	mandarin
Citrus	assamensis	lemon hybrid
Citrus	benikoji	tangor
Citrus	caniculata	sour orange hybrid
Citrus	ichangensis	papeda
Citrus	intermedia	Sour Orange hybrid
Citrus	keraji	mandarin
Citrus	latipes	papeda
Citrus	leiocarpa	mandarin
Citrus	lycopersicaformis	mandarin
Citrus	maderaspatana	sour orange hybrid
Citrus	nippokoreana	mandarin
Citrus	sunki	mandarin
Clausena	excavata	Citrus relative
Clausena	harmandiana	Citrus relative
Clausena	lansium	Citrus relative
Citrus	taiwanica	sour orange
Citrus	tangerina	mandarin
Citrus	davaonensis	lime
Citrus	medica	citron
Citrus	nobilis	tangor
Poncirus	trifoliata	trifoliolate
Eremocitrus	glauca	Citrus relative
Eremocitrus	glauca	Citrus relative
X		
Citromicrocitrus	sp.	Microcitrus hybrid
X		
Microcitronella	sp.	microcitrus hybrid
Citrus	jambhiri	rough lemon
Poncirus	trifoliata	trifoliolate
Fortunella	japonica	kumquat
Citrus	limon	lemon-Eureka
Glycosmis	pentaphylla	Citrus relative
Glycosmis	perakensis	Citrus relative
Glycosmis	tricanthera	Citrus relative
Citrus	aurantium	sour orange
X Citroncirus	sp.	trifoliolate hybrid
X Citroncirus	sp.	citrumelo
X Citroncirus	sp.	citrange
Citrus	hanaju	papeda
Citrus	hybrid	pummelo hybrid
Murraya	paniculata	Citrus relative
Hesperethusa	crenulata	Citrus relative

Clausena	lansium	Citrus relative
Poncirus	trifoliata	trifoliolate
Fortunella	crassifolia	kumquat
Citrus	aurantiifolia	lime
Microcitrus	australasica	Microcitrus
Microcitrus	australasica	Microcitrus
Microcitrus	australis	Microcitrus
Microcitrus	inodora	Microcitrus
Microcitrus	papuana	Microcitrus
Murraya	koenigii	Citrus relative
Fortunella	margarita	kumquat
Citrus	taiwanica	sour orange
Murraya	paniculata	Citrus Relative
Citrus	limettioides	sweet lime
Citrus	reticulata	mandarin
Citrus	sinensis	sweet orange
Pleiospermium	alatum	Citrus relative
X.		
Citrofortunella	sp.	kumquat hybrid
Citrus	maxima	pummelo
X Citroncirus	sp.	citrangle
X Citroncirus	sp.	Trifoliolate hybrid
Severinia	buxifolia	Citrus relative
Poncirus	trifoliata	trifoliolate
Citrus	reticulata	mandarin
Citrus	aurantium	sour orange
Citrus	reticulata	mandarin
Citrus x tangelo		Tangelo
X Citroncirus	sp.	citrumelo
Microcitrus	virgata	Microcitrus hybrid
Triphasia	trifolia	Citrus relative
Citrus	hybrid	lemon hybrid
X Citroncirus	sp.	trifoliolate hybrid
Citrus	aurantium	sour orange
Citrus	longispina	lime
X Citroncirus	sp.	trifoliolate hybrid
Citrus	aurantium	sour orange