CRB Project No. _____

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, Gayle Volk, John Hartung
Name

Location: Richard Lee, USDA ARS, Natl. Clonal Germplasm Repository for Citrus & Dates, Riverside, CA 92507
Gayle Volk, USDA ARS, Natl. Center for Genetic Resources Preservation, Ft. Collins, CO 80521
John Hartung, USDA ARS, Molecular Plant Pathology, Exotic Citrus Quarantine, Beltsville, MD 20705
Address *(University department, if applicable)*

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Cooperating Personnel: _____

Project Title: Development of Cryotherapy as an improved method of eliminating graft transmissible pathogens in *Citrus*

Keywords: Therapy, pathogen elimination, cryotherapy, graft-transmissible pathogens, laboratory diagnosis, biological indexing

Abstract *(limit 200 words*

There are currently two methods available for therapy of Citrus: thermotherapy and shoot tip grafting (STG). Thermotherapy does not eliminate viroids, and is inefficient at eliminating huanglongbing (HLB) and *Citrus tatterleaf virus* (CTlV). Although STG has been shown to eliminate all graft transmissible pathogens, pathogens such as viroids and CTlV are difficult. However, the major drawback to STG is the requirement for a high level of expertise since the technique is mastered only after many months of practice and requires a steady hand. A new technology, cryotherapy, has been successfully implemented in potato, sweet potato, grapevine, raspberry, and *Prunus* to eliminate pathogens that have been challenging using traditional methods. Recently HLB was eliminated from *Citrus* by cryotherapy. In cryotherapy, cells containing pathogens do not survive the exposure to liquid nitrogen thus eliminating the pathogen. Meristems up to 1 mm long (including three leaf primordia) are excised, in contrast to the meristem with one leaf primordium (about 0.1 mm long) in traditional STD. Use of larger shoot tips increases the survival rate and makes the procedures more reproducible. If proven to be easier and more reliable than traditional pathogen elimination methods, application of cryotherapy in California would help ensure availability of pathogen free materials in the threat of HLB and other exotic diseases.

Problem and its Significance*:

Elimination of graft transmissible diseases from clonally propagated crops has been a problem, especially with citrus. While citrus often produces nucellar seedlings from seed and most graft transmissible pathogens do not pass to the nucellar seedling, the juvenility traits displayed by citrus present unique problems. Juvenility in citrus often results in several years before the seedlings will come into production, excessive thorniness is displayed, the trees have vigorous, upright growth, the fruit quality is usually poorer from nucellar trees, there is a tendency toward alternate bearing, and fruit is often unequally distributed on the tree (Roistacher, 1977).

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

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Project Title: _ Development of Cryotherapy as an improved method of eliminating graft transmissible pathogen in *Citrus*

Hot water treatments were attempted with citrus beginning in the 1940s, but it was not until 1957 when Grant reported the first successful application of thermotherapy for the elimination of Citrus psorosis virus (CPsV). Grant used a temperature/light regime as is used presently on thermotherapy of citrus,42 C with a 16 hr under lights and 35 C night period for 8 hr. Experience with thermotherapy from the Repository and the Citrus Clonal Protection Program indicates that viroids and stubborn are likely to persist after 16 weeks of thermotherapy, and Citrus tatterleaf virus (CTlV) is also likely to survive. Recent trials in Florida and Brazil using the standard thermotherapy protocol was not effective at eliminating huanglongbing (HLB) from sweet orange whereas HLB appears to have been eliminated from some grapefruit (Lee, unpublished).

Shoot tip grafting approaches were applied to several horticultural crops to try to eliminate viruses from clonally propagated crops. One of the first reports was by Quark, 1970, where small meristem tips (0.1 – 0.25 mm) cultured on agar media, then later transplanted to soil. Subsequent indexing indicated they were free of virus. Murashige at UCR applied this approach to citrus, and with Bitters, pioneered the idea of grafting the shoot tip onto a small seedling growing in a test tube (Murashige 1962, 1972). Using this approach, they were able to eliminate *Citrus exocortis viroid* (CEV) from citrus and the resultant plants were identical to the parent plant (Murashige et al, 1972). Navarro, for his Ph.D. research, further developed this technology to the point where a standardized protocol was developed, and that the STG method was a reliable method for elimination of graft transmissible pathogens from citrus (Navarro, et al, 1975).

STG has been shown to eliminate most graft transmissible pathogens, with CTlV being the virus most difficult to eliminate. Often pre-conditioning with heat before STG helps in the elimination of CTlV (Navarro et al, 1991). While STG is a reliable method for therapy of citrus, it requires a very high level of expertise and lots of practice to become proficient. The procedure is performed in a transfer hood under sterile conditions and under a high magnification stereo microscope. If a meristem larger than 0.1 is used, the likelihood that the pathogen will not be eliminated is greatly increased. A procedure that is more robust would allow faster introduction of materials, although everything that is therapied has to be indexed for the target pathogens, but currently STG is used as a last resort to obtain a pathogen-tested accession because the procedure is tedious, success rate for survival low until the person performing the procedure has a lot of experience.

It has been relatively recently (1990s) that cryopreservation has been applied successfully to forest tree breeding material and the first report of cryopreservation of horticultural crops was in the mid-2000s (Wang et al, 2003, Reed, 2002). To date, cryotherapy has been reported at eliminating cucumber mosaic virus and banana streak virus from banana (Helliot et al, 2002), grapevine virus A from grapes (Wang et al, 2003), potato leaf roll virus and potato virus Y from potato (Wang et al, 2006), plum pox virus from *Prunus* (Brison et al, 1997), raspberry bushy dwarf virus from raspberry (Wang et al, 2008), huanglongbing in sweet orange (Ding et al, 2008), sweet potato little leaf phytoplasma, sweet potato chlorotic stunt virus, sweet potato feathery mottle virus from sweet potato (Wang & Valkonen, 2008a & 2008b). It is thought the mechanism of pathogen elimination is due to the uneven distribution of viruses and obligate vascular limited pathogens in the shoot tips, the freezing of these infected cells injurys the cell, and only the healthy cells (not containing the pathogens) survive in the regenerated meristem tissue resulting in the elimination of the pathogens (Wang et al, 2009). The cryotherapy approach potentially allows treatment of a large number of samples and has had a high frequency of pathogen-free regenerants (Wang et al, 2009).

With proper documentation of the ability of cryotherapy to eliminate graft transmissible pathogens of citrus, cryotherapy could become a method of choice for therapy of citrus. Because the cryotherapy protocol uses a large piece of the shoot tip meristem as compared to STG (1 mm vs. 0.1 mm), less expertise is required to get a successful graft of the therapied meristem tip onto a healthy seedling. This project will provide the documentation needed to establish cryotherapy as an acceptable method for pathogen elimination in citrus. If it is more efficient and requires less expertise that traditional STG, it would become the therapy method of choice.

Ding, F., S.X. Jin, N. Hong, Y. Zhong, Q. Cao, G.J. Yi, and G.P. Wang. 2008. Vitrification-cryopreservation, an efficient method for eliminating Candidatus Liberibacter asiaticus, the citrus huanglongbing pathogen, from in vitro adult

shoot tips. Plant Cell Reports 27:241-250.

Grant, T.J. 1957. Effect of heat treatments on tristeza and psorosis viruses in citrus. Plant Dis. Reptr. 41:232-234.

Helliot, B., B. Panis, Y. Poumay, R. Swenen, P. Lepovre, and E. Frison. 2002. Cryopreservation for the elimination of cucumber mosaic and banana streak viruses from banana (Musa spp.). Plant Cell Reprts 20:1117-1122.

Mirashige, T. W.P. Bitters, T.S. Rangan, E.M. Nauer, C.N. Roistacher, P.B. Holliday. 1972. A technique of shoot tip grafting and its utilization towards recovering virus-free citrus clones. Hort. Science 7:118-119.

Mirashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bio assays with Tobacco cultures. Physiologia Plantarum 15:473-497.

Navarro, L., E.L. Civerolo, J. Juarez, and S.M. Garnsey. 1991. Improving therapy methods for citrus germplasm exchange. *In* Proc. 11th Conf. IOCV, pp 400-408.

Navarro, L., C.N. Roistacher, and T. Mirashige. 1975. Improvement of shoot-tip grafting in vitro for virus-free citrus. J. Am. Soc. Hort. Sci.100:471-479.

Quak, F. 1970. Review of heat treatment and meristem tip culture as methods to obtain virus-free plants. Proc. Int. Hort. Congress 18:12-25.

Reed, B.M. 2002. Implementing cryopreservation for long-term germplasm preservation in vegetatively propagated species. *In* Biotechnology in Agriculture and Forestry, Cryopreservation of Plant Germplasm Vol 50, pp. 22-3.

Roistacher, C.N. 1977. Elimination of citrus pathogens in propagative budwood. 1. Budwood selection, indexing and thermotherapy. Proc. Int. Soc. Citriculture, 1977, Vol 3.Pgs. 973-987.

Wang, Q.C., W.J. Cuellar, M.L. Rajamaki, Y. Haraka, and J.P.T. Valkonen. 2008. Combined thermotherapy and cryotherapy for virus eradication: relation of virus distribution, subcellular changes, cell survival and viral RNA degradation in shoot tips to efficient production of virus-free plants. Molecular Plant Pathology 9:237-250.

Wang, Q.C., J. Laamanen, M. Uosukainen, and J.P.T. Valkonen. 2005. Cryopreservation of in vitro-grown shoot tips of raspberry (Rubus idaeus L.) by encapsulation-vitrification and encapsulation-dehyrdration. Plant Cell Reports 24:280-288.

Wang, Q., Y. Liu, Y. Xie, M. You. 2006. Cryotherapy of potato shoot tips for efficient elimination of *Potato leaf roll virus* (PLRV) and *Potato virus Y* (PVY). Potato Research 49:119-129.

Wang, Q.C., M. Mawassi, P. Li, R. Gafny, I. Sela, and E. Tanne. 2003. Elimination of *Grapevine virus A* (GVA) by cryopreservation of *in vitro*-grown shoot tips of *Vitis vinifera* L. Plant Science 165:321-237.

Wang, Q.C., B. Panis, F. Englemann, M. Lambardi, and J.P.T. Valkonen. 2009. Cryotherapy of shoot tips: a technique for pathogen eradication to produce healthy planting materials and prepare healthy plant genetic resources for cryopreservation. Annals of Appl. Biol. 154:351-363.

Wang, Q.C. and J.P.T. Valkonen. 2008a. Efficient elimination of sweetpotato little leaf phytoplasma from sweetpotato by cryotherapy of shoot tips. Plant Pathology 57:338-347.

Objectives*:

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*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 FY 2010-2011

Project Title: _ Development of Cryotherapy as an improved method of eliminating graft transmissible pathogen in *Citrus*

The primary goal of this research is to develop cryotherapy as a practical, reliable method of eliminating graft transmissible pathogens from *Citrus* and citrus relatives without inducing juvenility.
1) Develop a standard protocol for cryotherapy for the elimination of graft transmissible pathogens
2) Demonstrate the effectiveness of cryotherapy at eliminating graft transmissible pathogens that are present in California
3) Demonstrate the effectiveness of cryotherapy at eliminating graft transmissible pathogens that are exotic to California: HLB, Citrus Variegated Chlorosis, Citrus chlorotic dwarf, and Citrus yellow mosaic

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Project's Benefit to the Industry*:

It is not possible to carry on all variety improvement research under protected greenhouse conditions when a disease such as HLB is present. In Florida, if a plant has been feed upon by *Diaphorina citri*, the plant has to be considered as possibly HLB infected, although it may be months to years before symptoms appear. The citrus breeding programs at USDA ARS, Ft. Pierce and at the University of Florida, Lake Alfred will continue to operate in the field under unprotected conditions, realizing that as materials of interest are developed, the material will need to be therapied of HLB, CTV and possibly other pathogens. Although the bacterium associated with HLB has not yet been detected in California, it is likely that our situation will be similar to that of Florida in the future. In California, about 550 varieties from the Citrus Variety Collection have been propagated and moved to a protected greenhouse because they are not represented in the Repository's or CCPP's protected collection. Many of these accessions would be impossible to recollect due to political changes, changes in intellectual property laws, and because they no longer exist. The Repository is working to therapy these accessions now held in quarantine so they may be made available to the California industry. In the future, there will be a much greater demand for germplasm therapy to protect the California Citrus Industry and increase pressure to introduce desirable commercial varieties to meet market demands. We need a better, more efficient method to do this. This project will establish a standardized cryotherapy protocol and to demonstrate its reliability and effectiveness.

Research Collaboration* (*be specific*):

Dr. Volk has the expertise to develop the protocol. She will perform preliminary therapy trials on the different varieties and types of *Citrus* and citrus relatives. She will receive materials under quarantine permits following APHIS protocols, and elimination of pathogens in these materials following therapy will be monitored by laboratory testing done by Dr. Lee. The protocol development for cryopreservation, which is submitted as a separate project by Dr. Volk, will help this project by development of protocols for cryopreservation of various citrus types, e.g. lemon, lime, citranges, citrumelos, pummelos, etc.
Dr. Lee and the Repository will provide material infected with California pathogens (stubborn, psorosis, citrus concave gum, citrus viroids, citrus tatterleaf virus, citrus leaf blotch virus, citrus variegation virus, and fatal yellows, to Dr. Volk for her work on protocol development following APHIS quarantine protocols, he will perform the laboratory testing for pathogens in Volk's lab and help with the testing of exotic pathogens from Dr. Hartung's facility. Dr. Volk will work with Dr. Lee in Riverside to establish the procedure in Lee's lab in Riverside, and in Riverside, Lee will verify elimination of pathogens by both laboratory testing and biological indexing in Riverside following guidelines currently recommended by APHIS and CDFA.
Dr. Hartung has the facility which will permit the evaluation of the cryotherapy method to eliminate exotic pathogens. Preparation of materials (grafted varieties of common types of citrus will be provided by Lee from the Repository, CA. After shipment to Dr. Hartung, they will be inoculated by grafting with exotic pathogens. Dr. Volk will travel to Beltsville to perform the cryotherapy, Hartung and Lee will verify the elimination of the exotic pathogens by laboratory and biological indexing.

Plans and Procedures*

Workplan for Objective 1 (Develop a standard protocol for cryotherapy for elimination of graft transmissible pathogens)
The emphasis initially will be on therapy of sweet orange and mandarin varieties, in years 2 and 3, protocols would be developed for other varieties of citrus. A cryotherapy protocol will be developed in collaboration with Dr. Volk, Ft. Collins, CO, who will use tissue provided from the USDA ARS Repository, Riverside. The technical help in Dr. Volk's lab will work entirely on this project, under Dr. Volk's direction, as the protocol development is labor intensive. The Repository has an in planta collection of graft transmissible citrus pathogens which are endemic to California, or in some

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cases, which have been intercepted during the introduction of new varieties and which have been added to the collection. These in planta pathogens are normally used as the positive controls for biological indexing in our quarantine greenhouse. The pathogens to be used at Ft. Collins include CTV, citrus viroids, Stubborn, CTlV, and *Citrus psorosis virus* (CPsV). These pathogens all have laboratory based detection methods so that young regenerated plants in Dr. Volk's lab can be grown out for about 12 weeks, then tested by laboratory detection methods for these viruses/viroids to determine if the cryotherapy has eliminated them. Following therapy, the recovering shoots will be micrografted to Carrizo seedlings. Experimental variables for the protocol development for reliable cryotherapy will include shoot tip size, antibiotic treatments, and liquid nitrogen exposure.

Workplan for Objective 2 Demonstrate the effectiveness of cryotherapy at eliminating graft transmissible pathogens that are present in California.

Immediately at the beginning of this project, propagated plants of common sweet orange and mandarin varieties will be obtained from commercial nurseries, and inoculated with pathogens that are held in the in plant pathogen collection at the Repository. These inoculated plants will be used to validate the use of cryotherapy to eliminate graft transmissible pathogens present in California. Successful cryotherapy protocols will be transferred to Riverside where they will be applied to additional pathogens which are commonly found in California. In addition to pathogens being tested at Dr. Volk's lab, pathogens will include citrus vein enation and citrus concave gum (both of which may be detected by biological indexing only), California phytoplasmas, *Citrus leaf blotch virus*, *Citrus variegation virus*, and fatal yellows. Growouts of the regenerated shoot tips will initially be laboratory indexed at 12 weeks post-therapy (if laboratory method exists) followed by biologically indexing following CDFA protocols to ensure all traces of the pathogen were eliminated. Laboratory indexing in Dr. Volks lab will begin within six months of the start of the project, and at 12 weeks post cryotherapy at Riverside when the technology has been transferred. The technical help at Riverside will work entirely on this project to help inoculate test plants to be therapied, to prepare samples for laboratory indexing, and to help maintain biological indexing. The laboratory testing of cryotherapied plants will begin at about 9 months into the project, and the first biological indexes should be completed by the end of year 2 depending on the availability of greenhouse space. While sweet orange and mandarin varieties will be tested initially, the following year of the project will include the other varieties of citrus, such as lemon, lime, pummelo, citranges, and citromelos.

Workplan for Objective 3 Demonstrate the effectiveness of cryotherapy at eliminating graft transmissible pathogens that are exotic to California: HLB, citrus variegated chlorosis, Citrus chlorotic dwarf, and Citrus yellow mosaic

Immediately upon beginning the project, sweet orange and mandarin plants would be shipped from the Repository, Riverside to Dr. Hartung at the USDA ARS Exotic Disease Quarantine facility, Beltsville, MD. We would ship 12 plants total, the space is in high demand in the quarantine facility. The goal would be to turn the plants over quickly during the project so that other varieties could be used as hosts of the exotic diseases and subjected to elimination by cryotherapy. Once the plants are received in Beltsville, three plants each will be inoculated with HLB, Citrus Variegated Chlorosis, Citrus chlorotic dwarf, or *Citrus yellow mosaic*. Once these plants are systemically infected (as verified by PCR except for Citrus chlorotic dwarf which must be inoculated back to lemon, they will be used for cryotherapy. We estimate this to be between months 6-9 of the project. The regenerated cryotherapied shoot tips will be grown out and tested at 12 weeks, and at 12 week intervals to confirm the elimination of the targeted pathogen. Experimentation will be continued to clean up key Citrus genetic resources that are currently held in quarantine.

Other Funding Sources for this Project

Currently none, possibly the FCPRAC might consider funding part of the project when the call for new RFP comes out. The proposal, "Cryopreservation of citrus clones to increase security of critical collections," by Dr. Volk, will help the progress of this project as some of the protocol development for different varieties/cultivars will be addressed in that project.

Technology Transfer*

Presentations at grower meetings and scientific meetings. We will hold a workshop in year 3 to transfer the technology to the certification programs in other states and to interested parties.

Budget Justification:

Only the salaries of technical support staff needed to care for and maintain plants needed for this project and generated by

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CRB Project Plan – Research Grant Proposal for FY2010-2011

Project Title: _Development of Cryotherapy as an improved method of eliminating graft transmissible pathogen in *Citrus*

this project is being requested. The supply funds are requested for *in vitro* supplies, greenhouse and laboratory reagents at all three locations. The travel funds are essential but represents a minimal request considering the travel needed to transfer the technology as it is developed and to adequately test the growout plants for pathogens. Equipment funds are not requested.

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	58,000
Benefits	_____	_____	_____
Supplies and Expenses:	18,000	18,000	15,000
Equipment:	_____	_____	_____
Operating Expenses and Travel:	_____	_____	_____
Lindcove Recharges:	_____	_____	_____
Lindcove Packline:	_____	_____	_____
Other: _____	_____	_____	_____
_____	_____	_____	_____
ANNUAL TOTAL:	90,000	90,000	75,000

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

Signatures

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

_____ **Date:** 4 Aug 10

_____ **Date:** _____

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

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*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: _Development of Cryotherapy as an improved method of eliminating graft transmissible pathogen in *Citrus*

Budget Estimates: $255,000

Year 1		Personnel	Supplies	Travel	Subtotal
Lee	ARS Riverside	$30,000	$5,000	$3,000	$38,000
Volk	ARS Ft Collins	$30,000	$5,000	$3,000	$38,000
Hartung	ARS Beltsville	$10,000	$3,000	$1,000	$14,000
Total					$90,000
Year 2					
Lee	ARS Riverside	$30,000	$5,000	$3,000	$38,000
Volk	ARS Ft Collins	$30,000	$5,000	$3,000	$38,000
Hartung	ARS Beltsville	$10,000	$3,000	$1,000	$14,000
Total					$90,000
Year 3					
Lee	ARS Riverside	$25,000	$4,000	$3,000	$32,000
Volk	ARS Ft Collins	$25,000	$4,000	$3,000	$32,000
Hartung	ARS Beltsville	$8,000	$2,000	$1,000	$11,000
					$75,000

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CRB Project Plan – Research Grant Proposal for FY2010-2011

Project Title: Development of Cryotherapy as an improved method of eliminating graft transmissible pathogen in *Citrus*

Specifics regarding contract (i.e., "split" funding to more than one PI):
See specific information below. Each of the three ARS scientists will develop independent agreements with the Citrus Research Board to receive funds from this proposal.

Budget: $255,000

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Hartung	ARS Beltsville	$8,000	$2,000	$1,000	$11,000
					$75,000

For the Northern Plains (Volk portion), specific contact information is

Recipient organization for funding:
USDA, Agricultural Research Service
Northern Plains Area
2150 Centre Avenue, Building D, Suite 310
Fort Collins, CO 80526-8119
USDA EIN (Tax ID#) 72-0564834
USDA-ARS-NPA DUNS: 83=7350560

Physical Research location:
USDA-ARS
National Center for Genetic Resources Preservation
1111 South Mason St.
Fort Collins, CO 80521

Signatures

Project Leader: Richard Lee **Date:**

Gayle Volk, NCGRP Gayle Volk **Date:** 8/6/2010

John Hartung **Date:**

Research Leader: David Dierig, NCGRP (Volk portion) David Dierig **Date:** 8/6/10

Authorized Organizational Representative/Administrative Contact for Northern Plains Area (Volk portion):

Jim Quaratino James E Quaratino **Date:** 08/06/2010

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

USDA

United States Department of Agriculture
Research, Education, and Economics
Agricultural Research Service

August 6, 2010

To: Citrus Research Board

From: Gayle Volk, Plant Physiologist, NCGRP

Through: David Dierig, Research Leader, NCGRP

Through: Jim Quaratino, Authorized Organizational Representative/Administrative Contact, Northern Plains Area

I am delighted to participate in your CRB proposal entitled "Development of cryotherapy as an improved method of eliminating graft transmissible pathogens in *Citrus*". As part of this project, I understand that we will be developing and implementing cryopreservation protocols for various infected and uninfected *Citrus* cultivars. Our preliminary data for *Citrus* cryopreservation is very encouraging. This project is complementary to my CRB proposal titled "Cryopreservation of *Citrus* clones to increase security of critical collections".

We are committed to pursuing this research project. In fact, as a preliminary experiment, my technician will soon be traveling to Fort Pierce, FL to perform an initial experiment to use cryopreservation to eliminate HLB from key ARS *Citrus* breeding lines that have become infected. This week we received funding support from ARS headquarters to purchase a biosafety level 2 hood for use for pathogen research at the National Center for Genetic Resources Preservation (NCGRP). We have set aside a completely independent laboratory at NCGRP for pathogen research. This laboratory is physically isolated and is on a separate air handling system than our standard research laboratories. Independent growth chambers and cold storage facilities are also available to prevent pathogen movement.

If funded, we understand that Jim Quaratino, Northern Plains Area Office Authorized Organizational Representative/Administrative Contact will develop agreements directly with the Citrus Research Board to transfer Volk's research funds to ARS. Contact information is provided below.

Recipient organization for funding:
USDA, Agricultural Research Service
Northern Plains Area
2150 Centre Avenue, Building D, Suite 310
Fort Collins, CO 80526-8119
USDA EIN (Tax ID#) 72-0564834
USDA-ARS-NPA DUNS: 83=7350560

Physical Research location:
USDA-ARS
National Center for Genetic
Resources Preservation
1111 South Mason St.
Fort Collins, CO 80521

ars

CRB Project Plan – Research Grant Proposal for FY2010-2011

Project Title: Development of Cryotherapy as an improved method of eliminating graft transmissible pathogen in *Citrus*

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

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8/3/2010

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