Project Number: WCC-020

Requested Duration: October 1, 2001 to September 30, 2004

Title: Virus and virus-like diseases of fruit trees, small fruits and grapevines.

Description and justification:

Diseases caused by viruses and virus-like organisms continue to be important to tree fruit and berry industries in the United States and Canada. The impact of these pathogens can be significant and often limit the economic and biological viability of fruit production. WCC-020 facilitates a reduction in the impact of disease on this sector of agriculture by providing a forum for information exchange at annual meetings and by establishing contacts that encourage communication throughout the year. The discovery of *Plum pox virus* in North America in 1999 had an immediate impact on the tree fruit and nursery industries. Globally, this aphid-transmitted virus is the most economically important virus of stone fruits. The diagnosis of Plum pox virus was hastened because WCC-020 provided a network of researchers and specialists that were able to make the preliminary diagnosis and insure that the necessary testing was conducted to confirm this diagnosis. WCC-020 continues to foster experts that provided leadership and advice in developing testing and management strategies. Pollen-borne ilarviruses and nematode-transmitted nepoviruses cause significant decreases in orchard, raspberry, blueberry and vineyard production throughout the United States. Citrus tristeza virus (CTV) is a major limitation to citrus production worldwide and efforts to control this disease in California and Arizona have been critical to maintaining the viability of this fruit tree industry in the western United States. Grapevine leafroll virus, corky bark, rugose wood, Rupestris stem-pitting associated virus and other graft-transmissible pathogens contribute to the decline of grapevines, decrease vegetative growth and fruit yields. Raspberry bushy dwarf virus causes serious yield and quality losses in *Rubus* spp. and *Blueberry scorch carlavirus* is becoming a major disease problem in blueberry in the Pacific Northwest and in the Northeast. WCC-020 also promotes the exchange of information on diseases caused by isolates of the bacteria Xylella on citrus (variegated chlorosis), coffee (leaf scorch), peach (phony), almond (leaf scorch), and grape (Pierce's disease). Phytoplasmas that cause diseases such as Western X disease, pear decline, and peach yellow leafroll seriously reduce the production of stone and pome fruits in the western region of the United States. Although bacterial in nature, phytoplasmas and *Xylella* spp. have many features in common with viruses such as systemic infection, vector transmission, and graft transmission.

WCC-020 is a unique network that encourages interaction among regulatory, research, and extension personnel. Through this group, WCC-020 has been effective in providing information and solutions to disease problems and transferring technologies between states and across provincial lines. Many of our members are the sole person involved with diseases of fruit trees, small fruits or grapevines in their state. WCC-020 has fostered the exchange of new and recent information on research findings and regulatory concerns and helps researchers keep current with rapid developments in detection technology. For example, a cooperative project involving L. Skrzeczkowski (Washington State University (WSU)), K. Eastwell (WSU), W. Howell (National Research Support Project-5 (NRSP-5)), B. Kirkpatrick (University of California, Davis (UCDavis)) and J. Foster (USDA-APHIS, MD) was used to develop new information regarding detection of phytoplasma in fruit tree samples. These data ultimately resulted in significant revision of quarantine regulations and has enhanced the introduction of foreign material into the US. Cooperative research between J. Uyemoto (USDA-ARS, CA) and A. Rowhani (Foundation Plant Material Service, UCDavis (FPMS)) led to the discovery and characterization of a new closterovirus in grape causing decline and death of grafted

plants. Consultation and cooperation between K. Eastwell (WSU) and A. Rowhani (FPMS) led to the identification of *Cherry leafroll virus* in commercial cherry orchards of Washington State.

Advances in detection and virus treatment technologies during the late 1980's and the 1990's have been impressive and extremely useful. Immunological assays (i.e. ELISA), nucleic acid hybridization assays and amplification of virus, viroid and phytoplasma nucleic acids using polymerase chain reaction (PCR) have been developed for disease detection and to understand relationships between organisms. The dependence of quarantine and certification programs and research programs on these techniques has increased dramatically. Together, these technologies have been critical to developing our knowledge of fruit diseases and their epidemiology. Detection tools are at the heart of clean nursery stock programs that have been implemented by several states and British Columbia. These programs have greatly improved the quality of orchards that use certified pathogen-tested nursery stock and contribute to the reduction in new diseases entering regions. The advice and information generated from group meetings has been important to programs that provide foundation grade material for clean stock programs (i.e. NRSP-5 at WSU, FPMS at UCDavis and the Canadian Food Inspection Agency (CFIA) at the Centre for Plant Health, B.C.) in designing rapid and accurate diagnostic techniques. New insights into the etiology of several diseases of fruit trees and grapevines have been essential in formulating quarantine regulations based on the most recent research findings.

Objectives:

1) Promote and improve communication and cooperation among entomologists, plant pathologists, horticulturists, and other professionals concerned about plant health to determine the vectors of virus and virus-like diseases and to investigate the role of vector biology in the epidemiology of diseases.

2) Encourage, facilitate, and speed work on the cause and control of newly detected diseases and disorders by increasing contacts and communication on newly discovered problems likely to be caused by viruses or virus-like agents.

3) Facilitate rapid adoption and proper use of newly developed techniques and information that aid in the characterization and detection of virus and virus-like plant pathogens.

4) Evaluate regional fruit tree inventories of operational clean stock programs with horticulturists to select superior clones for use by industry.

5) Provide a source of research information and service to quarantine and certification agencies, to germplasm repositories, experiment station and government administrative agencies nationwide and the tree fruit, small fruit and grapevine industries.

Expected outcomes and impacts:

Since its inception, WCC-020 has a proven record of collaborative research efforts among its committee members. We anticipate that the generation of new information on the cause and management of fruit tree virus and virus-like diseases will continue to be the primary product resulting from the collaborative interactions of WCC-020 members. More specifically, the outcome of our collaborative investigations will include, but is not limited to, advances in the following areas during the next three years:

1) Identification and characterization of the phytopathogenic viruses, viroids, and phytoplasmas that impact fruit tree, small fruit and grapevine production in North America and Hawaii. Pathogen isolates will be exchanged as necessary and with properly authorized permits. Newly developed molecular diagnostic reagents and procedures that allow better characterization of diverse fruit tree, small fruit and grapevine pathogens will also be distributed. 2) Develop and evaluate techniques to produce and maintain pathogen-free planting materials. Provide scientific expertise to stakeholders, as well as state and federal programs that disseminate planting materials.

3) Develop, optimize, and disseminate standard detection protocols to WCC-020 members, clean stock programs such as NRSP-5; FPMS (CA); Oregon Nursery Services, as well as state and federal (APHIS and CFIA) regulatory agencies. Compare the accuracy and reliability of rapid pathogen detection/identification techniques with graft-indexing protocols currently used in clean stock and regulatory programs. The successful implementation and acceptance of rapid pathogen detection systems will save commercial nurseries and governmental agencies considerable money, space and time. In addition to providing protection against foreign, exotic pathogens, new diagnostic capabilities will expedite the introduction of new planting materials and keep American and Canadian growers competitive in the world marketplace.

4) Identify and develop control programs for fruit tree, small fruit and grapevine viruses and phytoplasmas vectored by insects and nematodes. WCC-020 membership has always, and continues, to include entomologists, nematologists and plant pathologists. Productive and significant research on the biology of virus vectors requires interdisciplinary collaboration. Collaborative interactions between WCC-020 members will assess the impact of new vectors on the disease threat of *Xylella fastidiosa* in the Western region. Another project will determine the ability of *Xiphinema thornei* and *X. utahense* to transmit *Cherry raspleaf virus*. Several WCC-020 members from the United States and Canada (Eastwell, Halbrendt, Levy, Scott, Thompson, Uyemoto, Welliver) are participating in a new coordinated effort to development disease and plant management strategies to limit the spread and impact of *Plum pox virus* in North America.

5) Continue to conduct collaborative research on pathogens that impact common crops grown in geographically diverse areas. Disease management strategies developed for one type of pathogen or crop in one geographical area are often applicable to managing the disease in other areas. Collaborative efforts would thus save considerable time and resources by completing the research in a timely fashion and avoiding unnecessary duplication of research efforts.

Educational plan:

Annual meetings and written progress reports provide important forums for WCC-020 members to become acquainted with the most recent research accomplishments and emerging disease situations. Minutes of annual meetings are displayed at the Western Association of Agricultural Experiment Station Director's website (http://www.colostate.edu/Orgs/WAAESD/). Several WCC-020 members, including Howell, Kirkpatrick, Uyemoto, Rowhani, and Thompson, are participants in clean stock programs (NRSP-5; FPMS, CFIA) or federal regulatory agencies (APHIS, CFIA). Information presented at WCC-020 meetings is therefore rapidly assimilated and, where feasible, quickly integrated into these programs. Other state and federal regulatory personnel often attend WCC-020 meetings. The results of our collaborations are often transferred to clientele via Cooperative Extension Services, and WCC-020 members regularly publish the results of collaborative research efforts in refereed discipline oriented and popular press publications (see Publications in Appendix).

Signatures:

Chair, Western Directors Association

<u>July 18, 2001</u> Date

ACCOMPLISHMENTS (1998-2000):

The associations forged between members of the WCC-020 have enabled us to quickly respond to significant virus disease situations. In 1999, *Plum pox virus* (PPV), the most economically important virus disease of stone fruits globally, was discovered in Pennsylvania and thereafter in Ontario. The educational role and historical links established and nurtured by WCC-020 facilitated the coordinated effort of researchers and agricultural professionals:

- 1. PPV was confirmed in Canada and the USA. Cooperation between researchers, industry and regulatory agencies is needed to combat this pathogen, the most serious virus in stone fruit. WCC-020 provided a solid foundation for information exchange and cooperation among stakeholders and government agencies.
- 2. The "North American Forum on *Plum pox virus*" was organized and involved the participation of more than one hundred professionals from throughout North America, including WCC-020 members.
- 3. PPV survey covering PA fruit tree nurseries and commercial stone fruit production blocks was conducted. This has delineated the extent of PPV movement in PA.
- 4. Conducted assays within the Pennsylvania PPV quarantine area to determine if weeds or native tree species are potential sources of the virus.
- 5. Peach and nectarine trees obtained from nurseries in eastern & central U.S. as part of the variety trial plantings at OMRC and RMRC were sampled in October and sent to APHIS to be tested for PPV infection. All were negative for PPV.
- 6. All mother trees in nursery certification programs of Washington, California and Oregon were tested and verified free of PPV. All potential host plants originating in PA and planted in commercial orchards in Washington were tested free of PPV.

Members of WCC-020 continue to provide leadership in development and implementation of new diagnostic methods. Recent advances in the detection of phytoplasmas have enabled the development of a revised importation protocol that allows for the safe introduction of propagation material from foreign sources and allows this material to be expanded within a matter of months:

- 7. Members of WCC-020 reviewed and provided significant input during the revision of the protocols for testing pome and stone fruit introductions in quarantine at Beltsville, MD and Prosser, WA. These protocols were implemented in January 1998.
- 8. The detection of phytoplasma has been further improved by the development of new PCR primers that are more selective for these pathogens. It was discovered that other commonly used primers would amplify sequences from saprophytic and endophytic bacteria that inhabit woody plant specimens.
- 9. Determined etiology of almond kernel shriveled disease as caused by peach yellow leaf roll phytoplasma.
- 10. Cloning and sequencing the 16S rDNA of phytoplasmas associated with red suture, little peach, and peach rosette diseases showed them to be closely related to each other and to the phytoplasma associated with Western X-disease.

The virus associated with Rupestris stem pitting disease in grapevines has been characterized. The knowledge of the genome has allowed the development of rapid and reliable diagnostic methods. New information provided by this new technology has led to thorough review of quarantine and certification procedures throughout grape growing States. Surveys are underway in Washington and California that will provide baseline data needed to make appropriate disease management decisions:

- 11. *Rupestris stem pitting associated virus* was sequenced and its association with Rupestris stem pitting disease was confirmed.
- 12. Virus strain diversity among different sources was studied by cloning and sequencing the coat protein gene of 17 different isolates collected worldwide and comparing their sequences. The phylogenetic analysis showed that the 17 isolates separated into 3 distinct groups with about 79% similarity between the groups. Subsequently, specific PCR primers were developed to identify individual isolates.
- 13. RT-PCR assays for grapevine *Rupestris stem pitting associated virus* were developed. This replaces biological indexing on Rupestris St. George that took up to two years to complete.
- 14. *Rupestris stem pitting associated virus* is phloem-limited and all attempts to purify virions have failed. Even so, specific polyclonal antiserum was produced to the coat protein. The coat protein gene was amplified and cloned in an expression vector in *E. coli*. The expressed protein in *E. coli* was purified and used to immunize a rabbit. The antiserum titer was 1:5000 as determined by Western blot. Although ineffective in ELISA, the antiserum detected *Rupestris stem pitting associated virus* in grape extracts by dot immunoassay and Western blot.

Members of WCC-020 have been instrumental in discovering and gathering biological information on *Xylella fastidiosa*, a fastidious bacterium that can infect a wide range of crop and ornamental species and causes significant agricultural and urban losses. Much of this information is being utilized to create important management tools for the disease caused by this pathogen. The strain that causes Pierce's disease in grapevines has had a particularly devastating impact on major grape production regions of the South:

- 15. Demonstrated that pruning can be useful in regenerating healthy vines from grapevines with Pierce's disease.
- 16. Determined the fate of the pathogenic bacterium *Xylella fastidiosa* in over 30 species of plants that are important for vectors of Pierce's disease or for vegetation management of Pierce's disease.
- 17. Developed experimental evidence that management of riparian vegetation can reduce populations of the Pierce's disease vector, *Graphocephala atropunctata* (blue-green sharpshooter), pathogen reservoirs, and improve the environmental quality of riparian habitats. Developed guidelines for growers to implement riparian management methods with approval of appropriate governmental agencies.
- 18. Identified the minimum temperature requirements of *G. atropunctata* for flight and how this corresponds to sticky trap catches in monitoring vector activity.

Several known economically important viruses of fruit trees and grapevines have been characterized. This data has led the increased arsenal of molecular biology tests that are available for virus detection. This strategy will speed the rate of disease diagnosis and improve management decisions:

- 19. Cloned and sequenced *Cherry green ring mottle virus* and developed rapid RT-PCR assays for its rapid detection (also molecular strains), which heretofore require graft-indexing onto 'Kwanzan' Japanese flowering cherry and 2 to 3 months incubation.
- 20. About two-thirds of the grapevine leafroll associated virus–3 genome was sequenced and recombinant antibodies to the coat protein developed and shown to be effective in ELISA.
- 21. The genome of grapevine leafroll associated virus-2 was sequenced and recombinant antibodies to the coat protein developed and shown to be effective in ELISA.
- 22. The causal agent of little cherry disease was characterized. This has lead to the development of routine diagnostics to replace to a three-year woody indexing procedure and greatly enhanced the little cherry disease control program in British Columbia.

Several new virus disorders have been recognized in grapevines and fruit trees. These new disorders may help account for production levels less than optimal. In situations where the cause is an unknown pathogen, disease control measures are frequently hampered. Determining the cause of these diseases depended heavily on cooperation between members of WCC-020. The availability of the expertise of group members and their cooperation has allowed and encouraged closer scrutiny of many disease situations and development of appropriate corrective plans:

- 23. Discovery of a new closterovirus (Redglobe virus) causing death of grafted grape plants.
- 24. A virus was Redglobe table grapes was characterized. Partial sequence information revealed that the Redglobe virus was approximately 74% homologous to *Grapevine leafroll associated virus-2* (GLRaV-2). The Redglobe virus particles also reacted weakly with GLRaV-2 antiserum. The source vines of Redglobe were repeatedly indexed on Cabernet franc, the standard indicator for leafroll disease. After two years, no symptoms were evident even though the Redglobe virus was detected by RT-PCR in the indicator plants. Redglobe virus appears to be important economically in commercial vineyards. We have detected the virus in four wine grape varieties in 22 samples collected from poor growing, declining young grapevines.
- 25. Discovery of a second graft-transmissible agent causing young grapevine decline on rootstocks 3309C, Freedom, and 101-14Mtg.
- 26. A new and severe disease of sweet cherry has been spreading rapidly through an orchard. A foveavirus has been identified, partially sequenced and sequence data used to develop a sensitive RT-PCR assay. This test is being used to aid in controlling and eradicating the virus from the affected orchard and to identify alternate hosts.
- 27. The *Prunus* isolate of *Cherry leafroll virus* (CLRV) was detected in the US sweet cherry industry for the first time. The impact of this virus on cherry production has just been realized in the US. Survey results show that CLRV, in combination with ilarviruses, can be devastating. As a result, CLRV testing is conducted annually in the Washington State certification program.

The basic biology of fruit tree viruses and their vectors is being examined through the cooperative efforts of WCC-020 members. Understanding basic biology of virus-plant and virus-vector interactions help develop rational disease control regimens:

28. In Colorado, *Cherry raspleaf virus* (CRLV) is endemic and causes significant tree losses in cherry orchards. As a result of discussion initiated at a WCC-020 annual meeting in Grand Junction, replicated trials were established in 1998 at two locations to compare susceptibility of

'Mahaleb' and 'Colt' cherry and M-9 apple rootstocks to field acquisition of CRLV. No evidence of infection has been detected as of May 2000 and this trial will continue.

- 29. Soils samples collected from five western Colorado orchards with known spread of *Cherry raspleaf virus* in them (causing both cherry rasp leaf disease in cherry and flat apple disease in apple) contained high numbers of *Xiphinema* nematodes. When examined by Italian nematode systematist Franco Lamberti, these were found to be "mostly *X. thornei* mixed with a lesser number of *X. utahense.*" Neither of these species is a known vector of soil borne viruses and further studies are needed on this aspect.
- 30. The *Prunus* quarantine for peach mosaic in Mesa County, CO was modified (effective May 30, 1998) to allow importation of yellow-fleshed, freestone nectarines varieties and white-fleshed, freestone peach and nectarine cultivars which express peach mosaic symptoms when infected.
- 31. In Washington, orchards with significant incidence of sweet cherry trees infected with *Cherry leafroll nepovirus* were surveyed for nematodes. Significant populations of *Xiphinema revesi* were found. The role of *Xiphinema* spp. in transmission of *Cherry leafroll virus* is controversial; the possible role of *X. revesi* in virus transmission is being examined
- 32. The complete genome of an isolate of *Prunus necrotic ringspot virus* (PNRSV) and *American plum line pattern* (APLPV), and the sequence of the RNA 2 of an isolate of *Prune dwarf virus* (PDV) were determined. Research indicated that PNRSV and *Apple mosaic virus* (ApMV) could be distinguished at the molecular level because of differences in their respective coat proteins. A RT-PCR system for the detection of APLPV was developed and this detected the virus (confirmed by sequencing of PCR product) in material that was previously considered free of APLPV. The RNA 5 observed in some isolates of PNRSV was shown to be a copy of the 3' UTR region of the RNA-3. Other data suggested that the corresponding molecule observed in some isolates of ApMV originated in the same manner.
- 33. In South Carolina, bloom delay in peach associated with graft-transmissible agents present in the germplasm 'Tao Tao 5' could be correlated with the presence of *Peach latent mosaic viroid*. No correlation between *Apple chlorotic leaf spot virus* (ACLSV), also present in 'Ta Tao 5', and bloom delay could be detected. The interaction between PNSRV and PDV in peach caused up to 60% loss in yield. Effects of viral infection were clearly influenced by the rootstock ('Lovell' or 'Nemaguard') and the scion variety being examined (GF305, 'Elberta', or 'Garnet Beauty'). In trees of GF305 and 'Elberta' infected with both viruses, Northern blots indicate that the amount of RNA-3 of PDV is reduced 17-fold compared to trees infected by PDV alone. This interaction at the molecular level is unlike previously characterized synergistic viral diseases and presents a unique opportunity for study of the interaction between viruses and the host plant at the molecular level.

Results of contemporary virus research programs have been translated into changes in quarantine and certification programs. Continued advancements and refinements in virus diagnostics have enhanced efforts to restrict the movement of disease domestically and internationally. Much of the adaptive work has been facilitated by cooperation between WCC-020 members and their collaborations with other tree fruit and grape specialists:

34. The PA Department of Agriculture virology laboratory has added two new diagnostic techniques to its arsenal. Light microscope examination of stained virus inclusions and polymerase chain

reaction for detection of virus and phytoplasma in plant tissues were successfully incorporated into their program. Both techniques allow for diagnosis of pests for which they had no standard test available.

- 35. RT-PCR has provided the most sensitive means currently available for detecting viruses in woody plants. The efficacy of RT-PCR was evaluated and compared to ELISA and a non-radioactive molecular hybridization (using digoxygenin-labeled RNA probe) on *Prunus necrotic ringspot virus*. When purified virus preparations were used, the detection threshold of the RT-PCR technique was 1.28 pg/ml, whereas by molecular hybridization and ELISA, the thresholds were 0.8 and 4 ng/ml respectively. With crude extracts, the sensitivity of non-isotopic molecular hybridization was 25 times higher than ELISA and 625 times lower than RT-PCR.
- 36. Enzyme inhibitors present in plant tissue extracts, especially abundant in woody plants, interfere with RT-PCR tests. A simple extraction procedure was developed in a simple one tube RT-PCR assay. This procedure successfully detected several grapevine and fruit tree viruses including: *Grapevine leafroll associated viruses* (GLRaV) types 1 through 5, *Grapevine virus A* (GVA), *Grapevine virus B* (GVB), *Grapevine virus D* (GVD), *Rupestris stem pitting associated virus* (GRSPaV), *Grapevine fanleaf virus* (GFLV), *Grapevine fleck virus* (GFKV), *Arabis mosaic virus* (ArMV), *Prunus necrotic ringspot virus* (PNRSV), and *Prune dwarf virus* (PDV). This simplified extraction protocol and one-tube RT-PCR have allowed large sample numbers to be processed in a rapid, cost effective manner. In addition, an indicator dye was included in the RT-PCR cocktail so that the final reaction mixture can be loaded directly onto a gel for electrophoreses.
- 37. Some members of the *Closterovirus*, *Vitivirus* and *Trichovirus* genera were screened by RT-PCR using degenerate primers. PCR primers, targeted to conserved sequences of the HSP70 homologue (Closteroviruses) or to the RNA dependent RNA polymerase gene (Tricho- and Vitiviruses), amplified the expected size fragments from total RNA or dsRNA preparations. The amplified products were cloned, sequenced, and phylogenetically analyzed. Results supported the relatedness of VA, GVB, and GVD in the genus *Vitivirus*. Similarly, presence of a HSP70 homologue in *Grapevine leafroll associated viruses* agreed with their inclusion in the genus *Closterovirus*. New reagents have been developed to assist in generating new sequence data of unknown viruses associated with grapevine leafroll and rugose wood diseases
- 38. A rapid cDNA cloning procedure for plant RNA viruses was developed. In this protocol, cDNA synthesis reagents and random hexamers were used to make cDNAs from viral dsRNAs. The cDNAs were mixed with Taq DNA polymerase to add an A-residue at the 3' end of cDNAs which facilitated its cloning using a TA cloning kit. This step eliminated use of adapters and linkers, reduced several cloning steps and thereby minimized cDNA losses.
- 39. With *Grapevine fanleaf virus* (GFLV), the genetic variation among different virus isolates was examined by RT-PCR and restriction fragment length polymorphism (RFLP). A 1,500 bp fragment was amplified from the 3' end of RNA2. The specific fragment was recovered from ten isolates collected from vineyards in different regions of California and four sources in the UC Davis Clonal Virus Collection. In these experiments, the AvaII digestion generated multiple fragment sizes of varying quantities and produced banding patterns distinct per viral source. Results indicate that GFLV infections are a complex mixture of closely related genomes (quasispecies). In further studies, each virus isolate was passaged in *Chenopodium quinoa* and

Nicotiana occidentalis and then examined by RFLP. The resulting RFLPs suggested rapid evolution in the type and titer of quasispecies members after passage in alternate hosts. RFLP analysis showed that the adaptation stabilized after a number of passages. In a different experiment it was found that inoculation of varietal isolates to closely related grape varieties gave rise to infection more rapidly than inoculation to distantly related varieties.

The development of technology for the genetic transformation of fruit crops has greatly accelerated. Indeed, this strategy has been used to produce virus-resistant papaya and will provide a valuable tool in many other aspects of virus research:

- 40. Transgenic papaya 'Rainbow' and 'SunUp' that are resistant to *Papaya ringspot virus* were commercialized for Hawaii in 1998. The virus-resistant 'Rainbow' is now widely planted in Hawaii.
- 41. An approach was developed to obtain transgenic plants with multiple-virus resistance by linking short segments of viral coat proteins to a DNA silencer.
- 42. Effective transformation method for papaya was developed.
- 43. Effective transformation method for grapevine rootstocks was developed.

In addition to research and development, WCC-020 plays a lead role in education and dissemination of information. This information is utilized by graduate students and by various agencies to improve their ability to function effectively. The type of information that is transmitted includes new advances in virology and diagnostic strategies as well as providing a resource for contacts for consultation:

- 44. WCC-020 members have been engaged in contemporary research activities, and provided report on new viruses in North America, and virus distribution. Annual meetings were held in each year of the WCC-020 current petition in different locations of Canada, Mexico and the United States. Participation from numerous research institutes and government agencies permitted rapid and effective dissemination of information to organizations including: United States Department of Agriculture (ARS and APHIS); Canadian Food Inspection Agency (CFIA); North American Plant Protection Organization (NAPPO) Tree Fruit Panel; Universities from all fruit producing States; States Department of Agriculture; National Research Support Project 5 (NRSP-5); Foundation Plant Material Service, CA (FPMS).
- 45. International collaborative research on phytoplasmas, *Little cherry virus*, *Peach latent mosaic viroid*, an uncharacterized virus from plum, and *Plum pox virus* have evolved from WCC-020 contacts. These efforts enhanced virus-testing and certification programs in the United States, and Canada. WCC-020 members have had great impact on testing methodology by providing advice and diagnostic reagents.
- 46. WCC-020 assists the National Clonal Germplasm Repositories to collect, maintain, evaluate and distribute healthy and virus-free plant material. Representatives from Corvallis, Geneva, Davis and Beltsville programs are active participants in WCC-020.

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LIST OF ATTENDEES

Attendees at the 1999 meeting in Clemson, South Carolina:

Eastwell, Ken	Washington State University, Prosser, WA	
Foster, Joe	USDA-APHIS, PPQ, Beltsville, MD	
Green, Margaret	Canadian Food Inspection Agency, Centre for Plant Health, Sidney, BC	
Gumpf, David	University of California, Riverside, CA	
Hadidi, Ahmed	USDA-ARS, Beltsville, MD	
Heutte, Tom	USDA-ARS, PGQO, Beltsville, MD	
Hillman, Brad	Rutgers University, Rutgers, NJ	
Howell, Bill	National Research Support Project - 5, WSU, Prosser, WA	
Hu, John	University of Hawaii, Manoa, HI	
Hughes, Pam	Clemson University, Clemson, SC	
Jones, Alan	Michigan State University, East Lansing, MI	
Kinard, Gary	USDA-ARS, PGQO, Beltsville, MD	
Kirkpatrick, Bruce	University of California, Davis, CA	
Larsen, Harold,	Colorado State University - OMRC, Grand Junction CO	
Michelutti, Roberto	Agriculture & Agri-Food Canada, Haroow, ON	
Miller, WalkerClemson University, Clemson, SC		
Nelson, Merritt	University of Arizona, Tuscon, AZ	
Podleckis, ED	USDA-APHIS, PPQ, Beltsville, MD	
Reighard, Greg	University of Clemson, Clemson, SC	
Curt Rom	University of Arkansas, Fayetteville, AR	
Rowhani, Adib	Foundation Plant Material Service, UCDavis, Davis, CA	
Scott, Simon	Clemson University, Clemson, SC	
Skrzeczkowski, Lech	Washington State University, Prosser, WA	
Thompson, Dan	Canadian Food Inspection Agency, Centre for Plant Health, Sidney, BC	
Welliver, Ruth	Pennsylvania Department of Agriculture, Harrisburg, PA	

Attendees at the 2000 meeting in Troutdale, OR:

Clark, Bev	Oregon Department of Agriculture, Salem, OR
Eastwell, Ken	Washington State University, Prosser, WA
Foster, Joe	USDA-APHIS, PPQ, Beltsville, MD
Guerra, Lauri	Washington State Department of Agriculture, Prosser, WA
Heutte, Tom	USDA-ARS, PGQO, Beltsville, MD
Howell, Bill	National Research Support Project - 5, WSU, Prosser, WA
Kinard, Gary	USDA-ARS, PGQO, Beltsville, MD
Larsen, Harold,	Colorado State University - OMRC, Grand Junction CO
Martin, Robert	USDA-ARS, Corvallis, OR
Michelutti, Roberto	Agriculture & Agri-Food Canada, Haroow, ON
Milbrath, Gene	Oregon Department of Agriculture, Salem, OR
Nelson, Merritt	University of Arizona, Tuscon, AZ
Postman, Joseph	USDA-ARS, NCGR, Corvallis, OR
Rowhani, Adib	Foundation Plant Material Service, UCDavis, Davis, CA
Skrzeczkowski, Lech	Washington State University, Prosser, WA
Traeger, Matt	Oregon Department of Agriculture, Salem, OR

Third meeting of current petition is scheduled for Kearneysville, WV on June 11-13, 2001.