PN ABZ 168 8.359

FINAL REPORT

Covering Period: January 1, 1990 to December 31, 1993

Submitted to the Office of the Science Advisor
U.S. Agency for International Development

Development of Monoclonal Antibodies for Rapid, Accurate Indexing of Sweet Potato for Mycoplasma, 8.359.


Principal Investigator: Robert E. Davis
Grantee Institution: Agricultural Research Service, USDA

Collaborator: Sylvia Green
Institution: Asian Vegetable Research and Development Center


Project Number: 8.359

AID Grant Project Officer: Wayne Ching

Project Duration: January 1, 1990, to December 31, 1993

# TABLE OF CONTENTS


| | Pages |
|---|---|
| Executive Summary | 3 |
| Research Objectives | 3 - 5 |
| Methods and Results | 5 - 13 |
| Impact, Relevance, and Technology Transfer | 13 - 14 |
| Project Activities/Outputs | 14 |
| Project Productivity | 15 |
| Future Work | 15 |
| Literature Cited | 15 - 16 |

## EXECUTIVE SUMMARY

The goal of this project was to develop means for rapid and sensitive detection of mycoplasmalike organisms (MLOs) causing witches' broom disease in sweet potato, to apply these means to investigate the relationship between the sweet potato and other MLOs, and to investigate their use in indexing sweet potato for MLO infection. Results included a) development of useful methods for indexing sweet potato (and other crops) for MLO infection, b) knowledge of the relationship of sweet potato witches' broom disease with disease in peanuts, c) recognition of the sweet potato MLO as representative of a new genomic group also containing MLOs associated with diseases in cactus and peanuts, d) training of AVRDC personnel and transfer of the monoclonal antibody and DNA-based technologies, e) awareness in AVRDC and LDCs of the association between diseases in two important crop plants, f) design of non-radioactive methods suitable for transfer to international centers dedicated to production of disease-free planting stocks and germplasms, and g) a continuing committment to collaborative and cooperative research and technology transfer.

As a result of this project, AVRDC personnel acquired skills and knowledge which can be utilized in LDCs in the development of monoclonal antibodies and in the development and utilization of DNA-based tools for pathogen detection and identification. These skills and knowledge specifically included, but were not limited to, the isolation of sieve cells from diseased plants for production of antibodies and the cloning of MLO-specific DNA fragments, methodology for using cloned DNA probes in DNA-DNA hybridizations, methods for Southern hybridizations and restriction fragment length polymorphism (RFLP) analyses of DNA, use of polymerase chain reactions, and the interpretation of results from these several lines of experimentation. The approaches and methods used and developed do not employ radioactivity; thus, health and environmental impacts of radioactivity are avoided, and the methods should be beneficial in regions where use of radioactivity is not possible. In addition, the results obtained in the project provided new knowledge and an awareness that disease in sweet potato was linked to diseases in a cactus and in peanuts. The findings underscore the possibility that the disease in sweet potato could spread to these other plants if appropriate measures are not taken to index germplasms and planting stocks. Ideally, regional and international movement of sweet potato vegetative propagules should involve only pathogen-tested materials. Considering the importance of sweet potato and of peanut in many countries, including LDCs, the new knowledge and awareness assume particular significance in the context of international development.

## RESEARCH OBJECTIVES

Sweet potato is an increasingly important food crop in less developed countries (LDCs) around the world. Efforts directed toward genetic improvement of the crop will necessitate the regional and international movement of germplasm and planting stock, but this vegetatively propagated crop is affected by a serious witches' broom disease caused by a

mycoplasmalike organism (MLO). No rapid, sensitive, accurate means has been available for detecting the presence of this MLO in infected hosts. Such a method is needed to facilitate the movement of important germplasm, to augment the development of disease-free planting stock, and to enhance the assessment of breeding stock for disease resistance. This project was carried out to develop these means, to apply them to investigate the relationship between the sweet potato and other MLOs, and to investigate their use in indexing sweet potato for MLO infection. The aim was to provide the capacity for avoiding spread of the disease to regions and countries including LDCs where it does not now occur.

Sweet potato witches' broom (SPWB) disease is a severe problem of most major commercial varieties in sweet potato-growing areas around the world (4, 9). The disease is caused by a MLO that can be transmitted through Cuscuta spp. to healthy plants. A natural insect vector (Orosius orientalis Matsumura) is known (10, 11), but other insects may also be capable of spreading the pathogen. Since the MLO pathogen cannot be isolated in pure culture, procedures other than in vitro culture must be used for pathogen detection, but most previously used methods for MLO detection suffer from lack of specificity, low sensitivity, inability to identify the detected organism, or lack of rapidity. Thus, such methods have not come into general use for indexing in either developed countries or LDCs. The development of means to avoid spread of the disease is a new contribution that is beneficial for both economic development and human nutrition in LDCs.

This project succeeded in developing rapid and sensitive DNA-based methods for detection of the sweet potato pathogen. In the present project, a relationship was found to exist between the MLOs involved in witches' broom diseases of sweet potato and peanut. This finding has been confirmed by recent work with monoclonal antibodies developed against the SPWB MLO (8). Thus, two approaches - use of DNA-based tools and use of monoclonal antibodies - are now possible for assessing the MLO infection status of sweet potato and for indexing germplasm and planting stocks. Although the two approaches have not been compared directly for sensitivity of SPWB MLO detection, DNA-based methods (and particularly the use of polymerase chain reactions [PCR] such as those developed in the present project) are likely to be more sensitive for detection of the pathogen. Sensitivity is a particularly critical issue, because vegetative propagules moved internationally will most likely be derived from symptomless plants, in which the titer of MLOs can be very low.

Recognition in this project of the SPWB MLO as representative of a new MLO genomic cluster and group, and the identification of cactus and peanut witches' broom disease MLOs as members of the same cluster and group, is consistent with other work in which these three MLOs have recently been classified in a single group based on partial sequencing of the 16S rRNA gene (Gundersen, D., I.-M. Lee, S. Rehner, R.W. Hammond, D. Kingsbury, and R.E. Davis; unpublished data).

Innovative aspects of this project include the isolation of sieve cells from diseased plants as the means to obtain preparations enriched for MLO cellular components, the cloning and selection of DNA probes

specific for the SPWB MLO and its close relatives, recognition of unique RFLP "fingerprints" of SPWB MLO DNA, creative design of SPWB MLO-specific polymerase chain reactions (PCR), use of methods for increased sensitivity and convenience of PCR product detection, and use of sensitive, non-radioactive chemiluminescent signal detection methodology.

The Agricultural Research Service, USDA, and the AVRDC are acknowledged for providing technical support, secretarial and contract support, financial accounting support, space, facilities, and portions of the equipment and supplies used in this project.

## METHODS AND RESULTS

Control MLO strains were maintained by grafting in a white-flowered vegetatively propagated clone of periwinkle (Catharanthus roseus). Plants of sweet potato exhibiting symptoms of the witches' broom disease were collected in Asia and the South Pacific region. Plants of periwinkle (C. roseus) containing a MLO strain derived from diseased sweet potato (and transmitted to periwinkle by means of the parasitic plant, Cuscuta sp.) were maintained by grafting in a greenhouse; this plant material served as source of MLO immunogens and of MLO DNA for the research. Host origin, geographical origin, and associated diseases for MLOs used in this study are listed in Table 1.

Table 1. Mycoplasmalike organisms (MLOs) used in this study: host origin, geographical location, and associated disease.

| MLO strain | Origin | | |
|---|---|---|---|
| | Host | Disease | Location |
| Test MLOs: | | | |
| SPWB | Sweet potato | Sweet potato witches' broom | Asia |
| PnWB | Peanut | Peanut witches' broom | Asia |
| RBCWB | Cactus | Red bird cactus witches' broom | Asia |
| Control MLOs: | | | |
| AY | Periwinkle | Maryland aster yellows | Maryland |
| BB | Tomato | Tomato big bud | Arkansas |
| AshY | Ash | Ash yellows | New York State |
| EY | Elm | Elm yellows | New York State |
| WX | Peach | Western X-disease | California |
| PWB | Potato | Potato witches' broom | Canada |
| VR | Periwinkle | Beet leafhopper-transmitted virescence | California |
| APU | Apple | Apple proliferation | Italy |
| MPY | Periwinkle | Malaysian periwinkle yellows | Malaysia |
| PPWB | Pigeon pea | Pigeon pea witches' broom | Florida |

Monoclonal antibodies

Hybridoma technology as employed previously (2, 3) was used to obtain monoclonal antibodies that could be used for investigating relatedness of the sweet potato witches' broom (SPWB) MLO to other MLOs and detection of the MLO in sweet potato. Mice were immunized using phloem tissues isolated from plants of C. roseus (5) infected by the sweet potato MLO. The mice were subsequently used as source of spleen cells for formation of hybridomas which were screened for production of antibodies that react with extracts from sweet potato MLO-infected but not from healthy plants. Hybridoma cell culture fluid was used in ELISA tests for this screening process. Several hybridoma cell lines producing MLO-specific antibodies were identified among several hundred tested; these cell lines were cloned, and antibody isotypes, produced by cell lines viable after freezing, were determined. The antigen for testing antibody production by hybridomas consisted of extracts of sieve cells isolated from infected periwinkle (C. roseus) plants. In the next step of monoclonal antibody testing, we desired to more closely simulate conditions expected in the field use of monoclonal antibodies. Therefore, relatively crude extracts of infected plants, rather than preparations from isolated sieve cells, were used as test antigen preparations. In these latter tests, it was not possible to distinguish diseased plants from healthy plants. Further testing has failed to indicate useful specificity of the antibodies.

DNA-BASED APPROACHES

Work to develop and test rapid, sensitive, and accurate means for detection of the SPWB MLO in sweet potato progressed well through research using DNA-based technologies. In this part of the work, DNA hybridization probes were developed, RFLP "fingerprints" of pathogen DNA were obtained, the 16S ribosomal (r) RNA gene of the SPWB MLO was analyzed, and polymerase chain reactions (PCR) were designed for specific amplification of SPWB MLO DNA. The advantages of these new tools and procedures included high sensitivity and the use non-radioactively labeled DNA sequences.

Cloning of SPWB MLO DNA and development of hybridization probes. Fragments of DNA from the SPWB MLO were cloned, using previous methods (1), in order to develop molecular hybridization probes for detection and identification of the pathogen in its hosts. DNA was extracted from sieve cells (isolated from SPWB MLO-infected periwinkle plants) and was cloned in Escherichia coli. Recombinant DNA molecules were labeled nonradioactively and used as probes to screen for those that hybridized with DNA from SPWB-diseased periwinkle plants but not with DNA from healthy plants. Repeated screening resulted in the identification of 11 recombinants that hybridized with DNA from SPWB MLO-infected periwinkle plants but not with DNA from healthy periwinkle plants. Sizes of the cloned SPWB MLO DNA inserts in the recombinants are shown in Table 2.

Table 2. Cloned sweet potato witches broom (SPWB) MLO probes.

| Probe designation | Approx. insert size (Kbp) |
|---|---|
| SPWB2 | 1550 |
| SPWB5 | 2800 |
| SPWB6 | 1050 |
| SPWB7 | 1700 |
| SPWB29 | 1600 |
| SPWB31 | 1700 |
| SPWB38 | 2700 |
| SPWB121 | 2300 |
| SPWB125 | 1200 |
| SPWB129 | 800 |
| SPWB133 | 2400 |

The cloned DNA probes were then used according to previously described methods (1) in dot hybridizations against DNA extracted from plants experimentally infected singly by one or another of an array of different MLOs. Results are summarized in Table 3. The data indicated genetic relatedness between the SPWB MLO and MLOs associated with witches' broom diseases of cactus and peanut. One cloned SPWB MLO DNA probe hybridized with DNA of periwinkle plants singly infected by either SPWB MLO or the cactus MLO but did not hybridize with DNA from plants infected by the peanut MLO. This probe therefore provided a means for distinguishing the sweet potato/cactus-infecting MLO(s) from the peanut-infecting MLO strain, although data were not obtained to indicate whether the MLO strain from peanut was representative of strains infecting that crop plant. All other cloned SPWB MLO DNA probes hybridized with DNA from periwinkle plants singly infected by SPWB and the peanut and cactus MLOs. None hybridized with DNA from healthy periwinkle plants or periwinkle plants infected by other MLOs. These results clearly distinguished the sweet potato, peanut, and cactus MLOs as a genomic cluster of closely related strains.

Table 3. Specificity of biotinylated cloned sweet potato witches' broom MLO DNA probes in dot hybridizations against nucleic acid preparations extracted from MLO-infected plants of periwinkle (Catharanthus roseus).

| MLO strain | Hybridization with cloned SPWB DNA probe indicated[1] | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2 | 5 | 6 | 7 | 29 | 31 | 38 | 121 | 125 | 129 | 133 |
| SPWB, RBCWB | + | + | + | + | + | + | + | + | + | + | + |
| PnWB | + | + | + | + | + | + | + | + | + | + | - |
| AY, BB, AshY, EY, WX, PWB, VR, APU, MPY, PPWB | - | - | - | - | - | - | - | - | - | - | - |

[1] [illegible] indicated; see Table 2 for full probe designation.

RFLP analysis of total chromosomal DNA. Restriction fragment length polymorphism (RFLP) analysis of total SPWB MLO chromosomal DNA was carried out as described (1) using non-radioactively labeled cloned SPWB MLO DNA probes that had proven to be specific for hybridization with SPWB, peanut witches' broom, and redbird cactus witches' broom MLOs. Results are depicted in Figure 1.

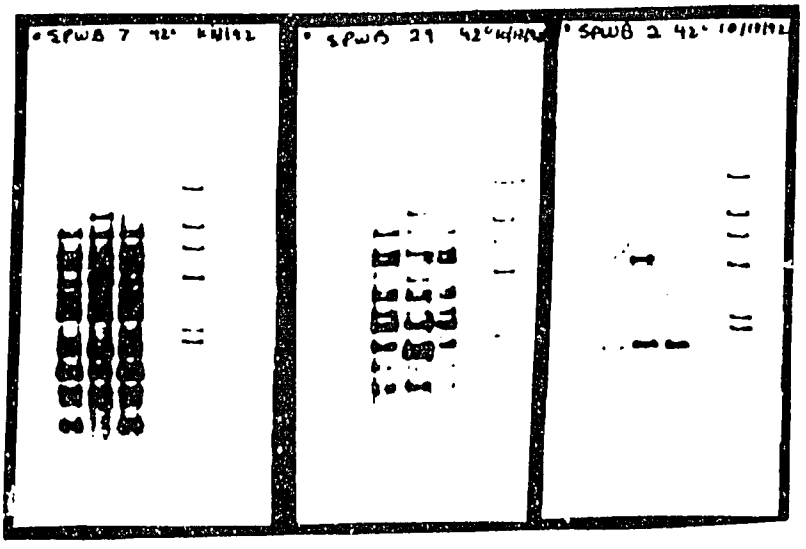

Figure 1. RFLP analysis of chromosomal DNA of mycoplasmalike organisms (MLOs) associated with witches' broom diseases in sweet potato and peanut. Panels depict results from Southern hybridizations of DNAs digested with EcoRI against cloned DNA probes 7, 29, and 2 (panels left to right, respectively). In each panel, lanes contained DNA extracted (lanes from left to right) from plants of Catharanthus roseus singly infected by the cactus, peanut, and sweet potato MLOs, from healthy C. roseus plants. Last lane contained HindIII digest of lambda DNA.

---

DNA from SPWB MLO gave a RFLP pattern that was distinct from that of DNA from MLOs associated with diseases in plants including elm, ash, tomato, clover, peach, carrot, potato, and other species in North America; and Paulownia sp., tomato, chrysanthemum, and other species in Asia. The RFLP patterns of DNA from SPWB MLO were closely similar or nearly identical to those of DNA from MLOs associated with witches' broom diseases in red bird cactus and peanut in Asia and the South Pacific region. These data confirmed our earlier finding that the SPWB MLO probably represents a distinct MLO genomic cluster, or group, which also includes the MLOs from red bird cactus witches' broom and peanut witches' broom diseases. Furthermore, these data indicated that the red bird cactus and peanut were alternate plant hosts of the SPWB MLO. The RFLP "fingerprints" provided identifications against which RFLP patterns of DNA from unknown MLOs could be compared. These "fingerprints" will be valuable in future searches for alternate host plants in nature, as well as for potential insect vectors active in spreading the diseases in cactus, peanut, and sweet potato.

Polymerase chain reactions. Several cloned SPWB MLO DNA fragments

were selected for nucleotide sequence determination on the basis of their sizes and strengths of hybridization signals with DNA from experimentally inoculated periwinkle plants. Oligonucleotide primers were then designed based on the sequences and tested in polymerase chain reactions (PCR) for specific amplification of MLO DNA by a method described (7). Several primer pairs were found useful in PCR for detection of SPWB MLO and were found to prime specific amplification of SPWB MLO DNA and DNA of the closely related peanut and cactus MLOs (data not shown).

MLO detection in naturally diseased plants. To test detection of MLOs in naturally diseased, field-collected plants, probes consisting of cloned DNA fragments of the SPWB MLO were used initially in dot hybridizations against nucleic acid extracted from field collected plants including both sweet potato and peanut. Clearly positive signals of MLO detection were obtained, indicating potential usefulness of the probe in practical applications (Figure 2).

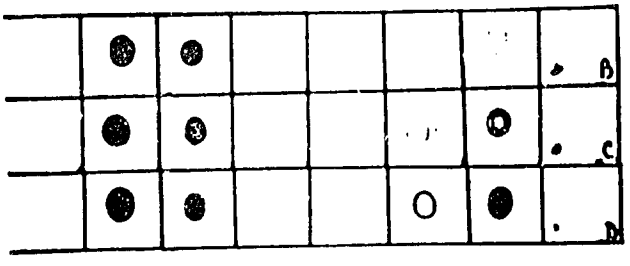


Figure 2. Dot hybridizations of biotinylated cloned DNA probes to nucleic acid extracted from naturally diseased plants of sweet potato and peanut. Nucleic acid samples in vertical rows were, from left to right, healthy periwinkle (Catharanthus roseus), SPWB MLO-infected periwinkle plant 1, SPWB MLO-infected periwinkle plant 2, healthy sweet potato plant 1, healthy sweet potato plant 2, SPWB-diseased sweet potato, and witches'broom-diseased peanut. Horizontal rows B, C, and D, were hybridized against probes 29, 121, and 129, respectively.

Subsequently, polymerase chain reaction (PCR) amplification of MLO DNA was investigated for detection of MLO infection in naturally diseased plants of sweet potato. Oligonucleotide primers were designed on the basis of the nucleotide sequence determined for cloned SPWB MLO DNA probe SP121. Use of these primers in PCR resulted in the amplification of MLO-specific DNA from templates extracted from naturally diseased field-collected plants of sweet potato and peanut (Figure 3). The results indicated potential usefulness of PCR using these primers for monitoring MLO infections in sweet potato and peanut plants in the field and in germplasm collections.

Figure 3. DNA sequence amplification primed in polymerase chain reactions by oligonucleotide pair SPWBF1/R1. First and last lanes contain kb ladder standard. Remaining lanes contain, left to right, PCR products from template DNAs derived from healthy periwinkle, SPWB MLO-infected periwinkle, healthy sweet potato plants 1 through 5, witches' broom diseased sweet potato plants 1 through 2, witches' broom diseased peanut plant 1, control reaction devoid of template, recombinant plasmid containing cloned SPWB MLO DNA.

Enhanced sensitivity of pathogen detection. Although PCR appeared to be a highly sensitive method for detection of target SPWB MLO DNA sequences, work was done in an attempt to increase the sensitivity of the assay, if possible. For this purpose, a target SPWB MLO DNA sequence was amplified by PCR from template DNA extracted from diseased plants. The reaction products were spotted onto a nitrocellulose membrane and hybridized the membrane against a labeled probe consisting of an internal DNA sequence. The internal sequence was synthesized in a PCR in which the target DNA consisted of known SPWB MLO DNA and the primers were nested within the primers used in the original PCR. The internal probe sequence was labeled with digoxigenin, and probe hybridization was detected by chemiluminescent signal. Results are depicted in Figure 4. This method gave very sensitive detection of SPWB MLO DNA and obviated the need for determining MLO detection by analysis of PCR products by gel electrophoresis.

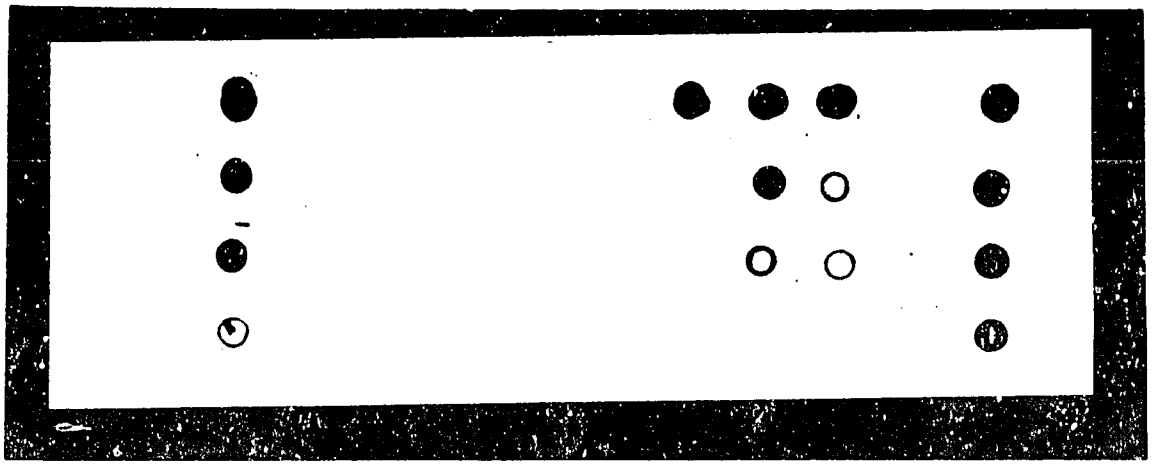

Figure 4. Dot hybridizations of a digoxigenin-labeled internal sequence probe against products of polymerase chain reactions (PCR) containing primer pair SPWBF1/R1 and template DNAs extracted from healthy periwinkle (first vertical column), periwinkle infected by sweet potato witches' broom MLO (second vertical column), healthy sweet potato plants 1 through 5 (columns 3 through 7), SPWB-diseased sweet potato plants 1 and 2 (columns 8 and 9), witches' broom diseased peanut (column 10). Column 11 contained products from PCR devoid of template DNA. Column 11 contained control DNA of cloned fragment SPWB121. PCR products were applied in 3 ul per spot at (top row to bottom row, respectively) undiluted, 10-, 100-, and 1000-fold dilutions.

A separate approach to increase sensitivity of MLO detection involved amplification of SPWB MLO 16S rDNA (using MLO-universal primer pair r16SF2/R2; ref. 6) and the detection of specific amplified DNA by hybridization against an internal 16S rDNA probe termed probe 758/1232 (B.D. Mogen, R.E. Davis, J. Prince, and E.L. Dally, unpublished data). This procedure, results from which are shown in Figure 5, also gave sensitive detection of SPWB MLO DNA.

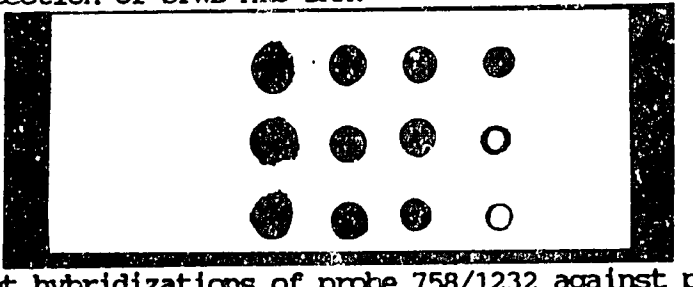

Figure 5. Dot hybridizations of probe 758/1232 against products of polymerase chain reactions (PCR) containing primer pair R16F2/R2 and template DNAs extracted from healthy periwinkle (botom horizontal row), or periwinkle infected by aster yellows MLO (top row), peanut witches' broom MLO (second row), or sweet potato witches' broom MLO (third row). PCR products were applied to nitrocellulose, in 3 ul per spot, as undiluted samples or samples diluted ten-, 100-, or 1000-fold (vertical columns left to right).

These results illustrate that SPWB MLO detection is feasible by use of a sensitive and convenient format involving PCR followed by detection of PCR-amplified MLO target DNA through hybridization with a labeled MLO-specific sequence that is internal to the primers used for the PCR. The internal sequence both verifies that a DNA fragment homologous to known SPWB MLO DNA has been amplified in the PCR, and it provides (when used as a labeled probe) the means for a visual signal indicating presence of SPWB MLO DNA in the PCR product. This format for testing the infection status of sweet potatoes and other plant material should fit well with procedures used currently in diagnostic laboratories and should be well suited to routine diagnostic applications.

Analysis of 16S rRNA gene. For analysis of the SPWB MLO 16S rRNA gene, we used restriction analyses of products from polymerase chain reaction (PCR) amplification of a 1.2 Kb sequence from the 16S rRNA gene. Although the results (see Figure 6) from this approach indicated that the SPWB MLO was distinct from MLOs from North America, Europe, and several MLOs from Asia, the SPWB MLO was closely related to a peanut witches' broom MLO and a cactus witches' broom disease MLO, both of which had been collected from naturally infected plants in Asia. The results clearly defined a new genomic cluster containing the peanut and cactus disease MLOs. Importantly, the data confirmed the finding in our previous results that the sweet potato, peanut, and cactus MLOs were members of a single genomic cluster, and our suggestion of sweet potato, peanut, and red bird cactus as sources of cross-infecting MLO inoculum. These conclusions provide significant understanding toward controlling the spread of MLO disease in sweet potato and other crops.

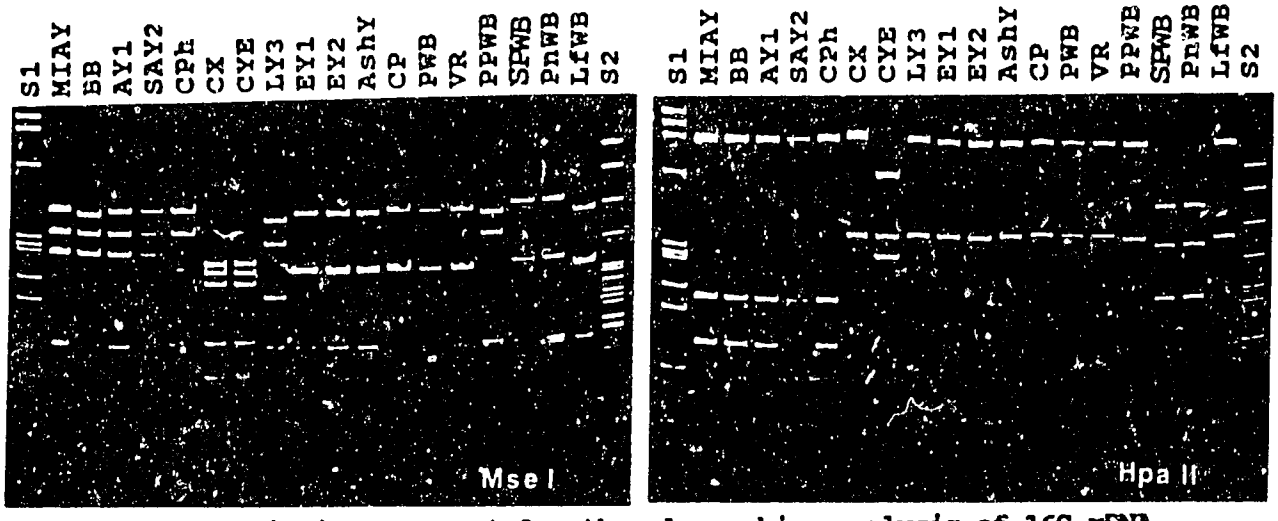


Figure 6. Restriction fragment length polymorphism analysis of 16S rDNA amplified by polymerase chain reaction using the primer pair R61F2/R2 from MLO strains associated with witches' broom diseases in sweet potato (SPWB) and peanut (PnWB). Control DNAs were from the following MLO strains: Aster yellows group strains MIAY, BB, AY1, SAY2, and CPh; X-disease group strains CX and CYE; coconut lethal yellowing strain LY3; elm yellows group strains EY1 and EY2; Ash yellows group strain AshY; clover phyllody (CP), potato witches' broom (PWB), and beet leafhopper-transmitted virescence (VR) MLOs; pigeon pean witches' broom strain PPWB), and Loufa witches' broom group strain LfWB. S1, PhiX174 RF DNA HaeIII digest. S2, pBR322 DNA MspI digest. PCR products were digested with MseI or HpaII and separated by electrophoresis in a 5% polyacrylamide gel.

The detection of infection through use of PCR, in which the target was 16S rDNA, enables universal detection of all MLOs, on the basis of our parallel work (6); this test conceivably could be used in cases where MLOs other than SPWB MLO-related strains might be suspected in sweet potato (or peanut). Thus, 16S rDNA amplification could be used as an adjunct to the more specific SPWB MLO detection test using a target sequence such as SF121. In the latter case, the detected MLO is automatically identified as a SPWB MLO strain -- and a member of the unique sweet potato witches' broom MLO strain cluster.

## IMPACT, RELEVANCE, AND TECHNOLOGY TRANSFER

Several methods, using cloned SPWB MLO DNA hybridization probes, MLO-universal PCR, and SPWB-specific PCR, yielded detection of the MLO in sweet potato. The sensitivity of the methods should make it possible to index sweet potato (and other crop plants such as peanut) for infection by the SPWB MLO and closely related strains and to investigate possible cross inoculation of sweet potato, peanut, and other plants in the field. In particular, the high sensitivity of the PCR makes it feasible to consider investigation of wild host plants as reservoirs of the sweet potato MLO, and to search for potential insect vectors carrying the MLO in nature. The use of these tools has significant implications for understanding and controlling spread of disease in sweet potato and other crops.

During the course of this project, the AVRDC was able to purchase specialized equipment consisting of a low temperature freezer and an ELISA plate reader, using funds made available under the grant. This equipment is and will be used by AVRDC for the preservation of antibodies and other critical materials and for tests of pathogen detection and identification, respectively. This equipment enhances the capacity of AVRDC both to apply the new methods and technologies in research and problem solving and to transfer the technologies to laboratories in LDCs.

Scientific capabilities were improved through transfer of technologies to AVRDC. Ms. E. Cho, from the Asian Vegetable Research and Development Center (AVRDC) traveled to the Beltsville laboratories for a period of training from October through December 1992. During that time, Ms. Cho gained training in our methods for handling and testing hybridomas and monoclonal antibodies and learned and gained experience with methods for extraction of nucleic acids from plants, labeling cloned DNA probes, DNA-DNA hybridizations, and use of the polymerase chain reactions for amplification of target pathogen DNA to achieve MLO detection in infected hosts. This technology is now in place for broader applications within AVRDC and transfer to other international centers and LDCs.

The development of the methods in this project now make possible larger scale trials of their utility in indexing of germplasms and planting stocks in phytosanitary/plant quarantine and disease-free planting stock programs. The International Center for Potatoes in Peru has been contacted and has indicated interest in applying these

technologies in its program of sweet potato indexing; we expect that the Center will be able to begin during CY 1994. Application by the Center would enhance LDC capabilities and introduce the methodology on a broad practical scale. In addition, the Center's program for training personnel from LDCs will be a vehicle for further dissemination of the technology among LDCs in which local programs for germplasm indexing, production of disease-free planting stock, and facilitation of disease resistance breeding could be implemented. Indexing of plant materials, for which the overall project was originally conceived, is expected to be readily practiced in LDCs by the methods elucidated in this project, since the methods exhibit the needed sensitivity and accuracy but do not involve use of radioactive materials.

## PROJECT ACTIVITIES/OUTPUTS

Training:

Travel by an AVRDC collaborator took place during the latter half of CY 1992. The collaborator spent a period of three months' training in the Beltsville laboratories and learned methodologies for separation of sieve cell tissues from diseased plants, production of monoclonal antibodies against MLO pathogens, use of cloned DNA hybridization probes, RFLP analyses of DNA, Southern hybridizations, dot hybridizations, and polymerase chain reaction amplification of specific DNA sequences. The collaborator also learned interpretation of results from these approaches and their relevance to the problems of disease in sweet potato and other crops in LDCs.

Meetings:

American Phytopathological Society, Annual Meeting, August 1994. (To present results of the project.)

Publications:

Davis, R.E., E.L. Dally, J.P. Prince, I.-M. Lee, E. Cho, and S. Green. 1994. Delineation of a genomic cluster containing sweet potato witches' broom and peanut witches' broom mycoplasmalike orgnisms (MLOs). To be submitted to Phytopathology.

Davis, R.E., E.L. Dally, J.P. Prince, I.-M. Lee, E. Cho, and S. Green. 1994. Detection and determination of genetic relatedness among mycoplasmalike organisms (MLOs) associated with diseases in sweet potato and peanut. (Abstract for meeting of the American Phytopathological Society, 1994).

Patents: Patentability of some of the methodology developed in the course of this project is under study.

## PROJECT PRODUCTIVITY

The project did achieve its major goal of developing means for rapid and sensitive detection of mycoplasmalike organisms (MLOs) causing witches' broom disease in sweet potato. However, this goal was met by an experimental approach that differed from that originally proposed. The development of monoclonal antibodies, in which uncertainties concerning specificity of reactivity were encountered, was largely supplanted by the accomplishments achieved through use of DNA-based technologies. The greater sensitivity of these latter technologies and their broad applicability are factors influencing our expectation that DNA-based technologies are likely to supplant use of antibodies in many applications in LDCs, especially since the DNA-based methods can be effectively utilized without the hazards of radioactivity.

## FUTURE WORK

The development of sensitive means for detection and identification of the MLO pathogens causing diseases in sweet potato and peanut crops in Asia and the South Pacific provides the potential for investigating the identities of insect vectors that are active in the spread of these diseases. A search for vectors may be carried out by AVRDC scientists in collaboration with other LDC laboratories. In addition, these means provide the possibility to determine relationships between disease in sweet potato in Asia and a possibly similar sweet potato disease that has been observed in South America. In collaboration with scientists at the University of Brasilia, Brazil, the Beltsville laboratory has detected the presence of MLOs in sweet potato in South America; plans have been made to determine whether the MLOs in South American sweet potatoes are related to the Asian/Pacific pathogens. In addition, if the South American MLOs are related to the Asian sweet potato/peanut MLO, then the tools and methods developed in this project will be used in work to detect potential insect vectors in South America. This latter work will be done in collaboration with LDC scientists in South America, and LDCs in South America will gain additional capacity for research on MLO pathogens.

Although results were not highly encouraging in the development of monoclonal antibodies, plans were made for continued testing of these antibodies using antigen preparations to be carried out by the AVRDC following completion of this project. If tests yield positive results, the antibodies will then be tested for detection of the MLO in sweet potato.

## LITERATURE CITED

1. Davis, R.E., W.A. Sinclair, I.M. Lee, and E.L. Dally. Cloned DNA probes specific for detection of a mycoplasmalike organism associated with ash yellows. Mol. Plant-Microbe Interact. 5:163-169.

2. Hsu, H.T., J. Aebig, and W.F. Rochow. 1984. Differences among

monoclonal antibodies to barley yellow dwarf viruses. Phytopathology 74:600-605.

3. Hsu, H.T., and R.H. Lawson. 1985. Comparison of mouse monoclonal antibodies and polyclonal antibodies of chicken egg yolk and rabbit for assay of carnation etched ring virus. Phytopathology 75:778-783.

4. Lawson, R.H., R.P. Kahn, S. Hearon, and F.F. Smith. ,1070. The association of mycoplasmalike bodies with sweet potato little leaf disease. Phytopathology 1016.

5. Lee, I.M., and R.E. Davis. 1083. Phloem-limited prokaryotes in sieve elements isolated by enzyme treatment of diseased plant tissues. Phytopathology 73:1540-1543.



6. Lee, I.M., R.W. Hammond, R.E. Davis, and D.E. Gundersen. 1993. Universal amplification and analysis of pathogen 16S rDNA for classification and identification of mycoplasmalike organisms. Phytopathology 83:834-842.

7. Schaff, D., I.M. Lee, and R.E. Davis. 1992. Sensitive detection and identification of mycoplasma-like organisms in plants by polymerase chain reactions. Biochem. Biophys. Res. Comm. 186:1503-1509.

8. Shen, W.C., and C.P. Lin. 1993. Production of monoclonal antibodies against a mycoplasmalike organism associzted with sweetpotato witches' broom. Phytopathology 83:671-675.

9. Yang, T.L. 1969. Studies on witches' broom of sweet otato in Taiwan. J. Taiwan Agric. Res. 18(3):50-60.

10. Yang, I.L. 1979. Transmission of sweet potato witches' broom by dodder (Cuscuta chinensis Lam.) J. Agric. Res. China 28(1):23-28.

11. Yang, I.L. 1982. Transmission of sweet potato witches' broom by Orosius orientalis in Taiwan. J. Agric. Res. China 31(2):169-172.