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BARD

FINAL REPORT

PROJECT NO. US-1036-85

**Isolation and Characterization of Closterovirus-Like
Particles Associated with Leaf Roll Diseased Grape-
vines from Different Geographical Regions**

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Appendix VI

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לפרוייקט היילואה
בית-דגן

Title

Isolation and characterization of closterovirus-like particles associated with
leafroll diseased grapevines from different geographic regions.

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Abstract

Techniques were developed for routine purification of long closterovirus particles (ca. 1800nm) associated with leafroll diseased grapevines, isolated virions were characterized, and polyclonal and monoclonal antibodies were produced to several isolates. Virions had higher molecular weights (36,000-40,000) than expected for closteroviruses. Serological diversity of leafroll isolates were observed and a system for their designation was proposed. Antibodies were used in ELISA tests to routinely detect virus antigens in grapevines, including screening large numbers of grapevines. Data indicate that serologically distinct types of the long closteroviruses exist in different geographic regions. The closterovirus particles were transmitted from grapevines to grapevines via mealybugs with subsequent development of leafroll symptoms on test grapevines. ELISA tests showed that antigens of other viruses (ca. 800nm closterovirus and a potyvirus) can be detected in leafroll diseased grapevines, suggesting a possible complexity of the leafroll syndrome. The techniques and serological reagents developed are being used in numerous laboratories for diagnosis of leafroll by ELISA and for further investigations of the leafroll disease.

Objectives

1. Isolate the closterovirus-like particles associated with leafroll disease from different geographic regions.
2. Develop polyclonal and monoclonal antibodies to these particles.
3. Develop rapid serological tests for practical diagnosis of leafroll.
4. Serologically compare leafroll isolates from different geographic regions.
5. Reproduce the leafroll disease by mechanical transmission of isolated virus preparations to grapevines.

INTRODUCTION

Leafroll is a major virus disease of grapevines on a worldwide basis (Goheen, 1988). Despite its economic importance, definitive evidence on the etiology of leafroll is still lacking. The literature has reported on the association of closterovirus-like (e.g. Namba et al., 1979; Engelbrecht and Kasdorf, 1985; and Milne et al., 1984), isometric (Castellano et al., 1983), and potyvirus (Tanne et al., 1977) particles had been associated with the leafroll disease. The closterovirus-like particles generally fell into short (ca. 800nm) category which was referred to as GVA (Milne et al., 1984) and into a long (ca. 1800-2000nm) category (Gugerli et al., 1984; and Zee et al., 1987), which we refer to in this report as long closteroviruses.

Work done on this grant (BARD U.S.: 1036-85) included the development of purification methods, characterization, and development of rapid detection methods for the long closteroviruses associated with leafroll. We focused on the long closteroviruses because our observations and previous published reports showed a consistent association of these particles with the disease (Gugerli et al., 1984; Zee et al., 1987; and Namba et al., 1979). This report summarizes work that we did on the long closteroviruses. Additionally, we report on ELISA and dot-blot hybridization experiments with a previously described grapevine potyvirus and on the spread of corky bark disease under vineyard conditions and transmission by mealybugs.

MATERIALS AND METHODS:

Virus isolates. The primary grapevine leafroll (GLRV) isolates used in characterization studies originated from New

York (NY-1), California (CA-4, CA-6), and Arkansas. Other isolates that were also characterized or tested in surveys originated from Israel, Cyprus, and other countries as Germany and Uruguay. Additionally, a grapevine potyvirus (GPV) previously described by Tanne et al. (1977) was used in serological and nucleic acid hybridization experiments.

Closterovirus purification. Purification methods were developed using an isolate of leafroll from a row of Pinot noir vines growing in the Finger Lakes region of New York (Zee et al., 1987). The basic techniques were those described by Gugerli et al. (1984) and Zee et al. (1987). Various parameters as pH, buffers, type of tissue, stability of partially purified virus, and Cs₂SO₄ gradients were tested in an attempt to improve the purification procedure. The recommended procedure is described by Hu et al. (1990c).

Characterization of isolated virions. Particle length was measured from crude leaf extracts or from partially purified virus preparations after being trapped with homologous antibodies (Hu et al., 1990c). Western blotting was used to measure coat protein molecular weight (MW) of purified virions. The procedure was described by Hu et al. (1990a,c). DSRNA isolation was described by Hu et al. (1990c).

Immunosorbent electron microscopy (ISEM). Various ISEM techniques were used to determine mixed infection in grapevines and to characterize virus particles. The basic techniques were decoration and gold labelling of virions as described by Hu et al. (1990a,c).

Serology. Monoclonal and/or polyclonal antibodies were produced to three leafroll isolates (NY-1, CA-4, and CA-6). The techniques for producing polyclonal and monoclonal antibodies have been described by Zee et al. (1987) and Hu et al. (1990a,b). Similarly, enzyme-linked immunosorbent assay (ELISA) techniques were described by Zee et al. (1987) and Hu et al. (1990b,c).

Epidemiology and mealybug transmission. Transmission studies on leafroll and corky bark diseases were done with mealybugs (Planococcus ficus and P. longispinus) as described by Tanne et al. (1990a) and Tanne and Raccach (1989). The spread of corky bark as it relates to temporal and spatial distribution was done as described by Tanne et al. (1990b).

RESULTS

Purification of GLRV. An improved purification procedure was developed and used routinely to purify several

serologically distinct GLRV isolates (Hu et al., 1990c; Boscia et al., 1990). The improvements basically included 'settling' of partially purified preparations prior to Cs₂SO₄ gradient centrifugation, use of the sucrose-Cs₂SO₄ gradient procedure described by Bar Joseph et al. (1985), and the use of phloem tissue from bark or petiole. Although, our experience indicated that the leafroll purification procedure could be utilized for all virus isolates regardless of origin, we found significant differences in yield of virus particles among isolates. For example, much higher yields were obtained from NY-1 as compared to CA-4 (Hu et al., 1990c). Consistent and relatively higher yields were obtained from phloem tissue of bark or petioles as compared to leaf lamina material.

Physical properties of leafroll isolates. Low particle yields and host contaminants prevented us from determining the MW of the coat protein using conventional staining of SDS (sodium dodecyl sulfate) degraded virus preparations. However, western blotting using polyclonal or monoclonal antibodies showed that the MW of the coat protein of two serologically distinct isolates were 36,000-40,000. Refinements of the western blotting technique allowed the use of small amounts of tissue (5g). This technique was applied to a number of leafroll isolates (Hu et al., 1990a,c; and Boscia et al., 1990).

Cytopathology of leafroll diseased grapevines. Investigations by Kim et al. (1989) showed the association of unusual cellular structures associated with a Reisling grapevine infected with leafroll. Flexuous rod-shape particles were consistently found in phloem cells. In phloem parenchyma cells, the particles were associated with clusters of membranous vesicles containing fibrils characteristic of closterovirus infections. Unlike other known closteroviruses, however, the fibril-containing vesicles originated from modified mitochondria.

Particle measurements (ca. 1800nm) and dsRNA patterns were similar for two serologically different isolates (Hu et al., 1990b,c; and Boscia et al., 1990). Invariably, a high MW weight dsRNA band (ca. 10 x 10⁶) was detected along with lower MW ones.

Serology. A major objective of the grant was to develop rapid detection methods through the use of serology, primarily ELISA. We produced antisera to three serologically distinct isolates that belong to type II, III, and IV groups (see below for designation of leafroll types). Fortunately, high titered antisera could be produced even though very low concentrations of antigens were used to immunize the animals (Zee et al., 1987; Hu et al., 1990c; and Boscia et al.,

1990). Absorbing the antisera with healthy antigens effectively removed host reaction. Thus, ELISA tests using monoclonal (Hu et al., 1990a) or polyclonal antibodies were routinely used for detecting these particles in grapevines.

ELISA and ISEM tests showed that NY-1, CA-4, and CA-6 isolates were serologically distinct (Hu et al., 1990a,b,c).. Further work using decoration and gold labelling experiments with monoclonal antibodies unequivocally showed that mixed infections with serologically distinct particles were prevalent (Hu et al., 1990a). Also, Hu et al. (1990b) showed that antibodies to serologically distinct isolates could be combined in a single ELISA test and still effectively detect the isolates in grapevines. Based on our work and those already published (Gugerli et al., 1984; Zee et al., 1987), we (Hu et al., 1990c) proposed a designation of serologically distinct leafroll isolates into types I, II, III, and IV (Hu et al., 1990c). NY-1, CA-4, and CA-6 leafroll isolates were designated as types III, IV, and II, respectively.

Monoclonal antibodies were produced to the NY-1 isolate in an effort to obtain antibodies that react with the other serologically distinct leafroll isolates (Hu et al., 1990a). However, the monoclonal antibodies that were screened reacted only to type III isolates.

Virus distribution in grapevines. A detailed analysis of virus distribution in a NY-1 infected Pinot noir was done to determine the best type of tissues for testing by ELISA (Teliz et al., 1987). For example, it was shown that viral antigens were first detected in flower clusters 15 days after budbreak and that basal leaves were not reliable for detection until bloom. This work helped us to determine the best time and tissue types for sampling and for virus purification.

Transmission of leafroll and corky bark by mealybugs. One of our objectives were to mechanically transmit the closterovirus particles to grapevines in order to obtain unequivocal proof that these particles can cause leafroll symptoms. However, we failed in numerous attempts to mechanically transmit the virus to grapevines or other herbaceous hosts.

Work on transmission of leafroll via mealybugs were more fruitful (Tanne et al., 1990a). Transmission of the long closterovirus particles to healthy grapevines was demonstrated by using ELISA to detect viral antigens (the leafroll isolate reacted with NY-1 antibodies) and ISEM to 'trap' and 'decorate' particles. Importantly, ELISA positive test plants developed symptoms of leafroll.

Similar work was done with corky bark. Teliz et al. (1980) had provided evidence for natural spread of corky bark in vineyards growing in the Aguascalientes region of Mexico. Unlike leafroll, however, closterovirus particles were not yet associated with the disease when the experiments were done. Transmission work with P. ficus by Tanne and Raccach (1989) showed that the disease could be transmitted from infected corky bark to LN-33, the indicator plant for corky bark.

Spread of corky bark in Israel. Observations by Tanne since 1981 showed that corky bark was spreading in a "Thompson seedless" vineyard growing in the Lackhish region at the southeastern part of Israel. Spread of the disease within the vineyard was recorded yearly from 1983-87. Spatio-temporal analysis of the data indicated that corky bark is vector borne (Tanne et al., 1990b).

Large scale detection of leafroll by ELISA. Preliminary survey work had shown that the predominant isolate in Uruguay was of the NY-1 type, as judged by ELISA reactions. In fact, other isolates were not detected in vineyards that were surveyed in 1989. Given this circumstance, personnel in Sanidad Vegetal of Uruguay initiated a "Pilot" certification program in which ELISA would be used as the detection technique for leafroll [Gonsalves, 1989; 1990, Report to Organization of American States (not included in this BARD report)]. Briefly, growers identified vines that they wanted to use for propagation of rootstocks or scions, and personnel in Sanidad Vegetal subsequently tested them by ELISA using NY-1 antisera. Vines that tested negative were used for propagation. In the second year, samples were taken from propagants of mother vines that had previously tested ELISA negative to determine if any "positive" vines were missed by ELISA. Growers entering the program paid a fee for each grapevine entered into the program.

Over 36,000 grapevines were ELISA tested in a three month period. Leafroll was detected in 3.31% of the tested grapevines which included a range of scion and rootstocks (Table 1). Moreover, the virus was detected among a range of varieties and rootstocks. Follow up tests on nursery plants that were propagated from ELISA negative vines showed that the initial ELISA test was reliable because none of the tested propagants were ELISA positive (data not shown).

Serological variability of leafroll isolates from different geographic regions. In Germany, about 95% of the leafroll positive vines tested ELISA positive to type I (Gugerli et al., 1984) antisera while the remaining 5% tested positive to NY-1 type III antibodies (Stellmach and Gonsalves, unpublished data). In Cyprus, type III was detected in about one third of the leafroll positive vines

that were tested (Ioannou and Gonsalves, 1990). In Uruguay, numerous tests showed that vineyards affected with leafroll invariably had type III closteroviruses [Gonsalves, 1989; 1990, Report to Organization of American States (not included in this BARD report)]. Using antibodies to types II and III, comparisons with leafroll positive samples from California showed that 16% tested positive for type II, 36% for type III, and 7% for both types II and III (Boscia et al., 1990).

Assaying for leafroll using antibodies or nucleic acid probes to GVA, GPV, and GLRV. The etiology of the leafroll disease has remained unclear due to the inability of investigators to reproduce the disease by rub inoculation of virus preparations to grapevines. Although work in this grant on mealybug transmission (Tanne et al., 1990a) suggested that the long closteroviruses were indeed closely associated with the leafroll disease, it did not exclude the possibility that leafroll is a complex that may be caused singly or by combinations of different virus particle types (e.g. GVA, long closterovirus particles, and the grapevine potyvirus). Thus, a number of leafroll positive vines were tested by ELISA using antibodies to these viruses. Many of the leafroll diseased vines (Tanne et al. 1989) contained antigens to one or more of the virus types. The presence GPV in diseased vines was further corroborated by dot-blot hybridization tests using radiolabelled GPV-cDNA.

DISCUSSION

Our general approach in this work was to first develop routine purification methods for grapevine leafroll, produce antibodies to various isolates from different geographic regions, develop rapid serological tests for diagnosis of leafroll, use these tests to compare leafroll isolates from different geographic regions, and to reproduce the leafroll disease by mechanical transmission. All of our objectives were achieved, with the exception of the last one on mechanical transmission. However, this was covered in a different way by using mealybugs to show transmission of the NY-1 type closterovirus particles from grapevines to grapevines, and the subsequent development of symptoms on plants that tested positive for the NY-1 type particles. Additionally, work was continued on the grapevine potyvirus by Tanne's group. This work showed the possible complexity of the leafroll disease (Tanne et al., 1989) and added more working tools (cDNA probes) for the characterization of the grapevine potyvirus.

Production of polyclonal and monoclonal antibodies was deemed important in our project because of the uses they would have in further characterization of the long closteroviruses (ca. 1800nm) and the development of rapid

assay methods. Although virus yields from grapevines were very low, we were fortunate in that the particles were highly antigenic and thus the antisera we produced were useful in ELISA tests. Given these observations, we conclude that useful antibodies to these long closteroviruses can be routinely produced.

The serological diversity of the leafroll isolates poses a problem in using ELISA for broad spectrum detection of leafroll. There are at least four serologically distinct groups (Hu et al., 1990c) and further work may find more. Development of a "bank" of antisera that covers the range of serological types would be useful. We showed that antibodies to serologically distinct isolates can be effectively combined and used in a single ELISA test. This aspect is especially useful in quarantine work where broad spectrum detection is desirable.

Development of monoclonal antibodies have the distinct advantage in that a continual supply of antibodies is theoretically available. Another important advantage is the potential to select monoclonals that react to serologically diverse isolates of leafroll. However, we did not succeed in selecting monoclonals that reacted with the other types of leafroll. We will continue to screen other monoclonals in an effort to detect broader reacting ones.

For the first time, the MW of the coat proteins of the types II and III closteroviruses associated with leafroll were determined. Their MW (36,000-40,000) were significantly higher than those reported for other closteroviruses. It would be of interest to determine the coat protein MW of particles of leafroll types I and IV.

We believe that it is necessary to obtain a consensus designation for the serological types of closteroviruses in order to minimize redundancy in the literature and to enable different laboratories to correlate serological data. We proposed a designation of the serologically distinct long closteroviruses as types I, II, III, and IV (Hu et al., 1990c). However, this designation should be agreed upon or debated in an organized forum. This could be done at the meeting of the International Council for the Study of Virus and Virus-like Diseases of Grapevines which will be held in Greece in September 1990.

Practical application of rapid detection methods for leafroll was another important goal of this grant. The work in Uruguay clearly demonstrates that the ELISA test can be used for screening a large number of grapevines in a relatively short time. However, careful considerations must be given to the prevalence of serologically distinct types of leafroll. Large scale tests with only one type of antisera

was possible in Uruguay because initial surveys showed that the great majority of the vineyards were infected with only type III isolates. Many laboratories have requested and successfully used the antisera we produced during this grant period. It is reasonable to predict that antisera will be produced to more isolates of these closteroviruses and thus broaden the practical application for detecting leafroll by ELISA.

At the start of this grant, we acknowledged that leafroll could be caused by a number of agents. Work was concentrated on the long closteroviruses because more and more evidence indicated that these were very closely associated to the leafroll disease. Survey work and mealybug transmission experiments strengthened this association. However, the possibility that leafroll symptoms could be caused by one or more virus agents needed to be considered even though it was not in the original grant proposal. The possible components of this complex syndrome include GVA, the closteroviruses we worked on in this grant, and the grapevine potyvirus originally described by Tanne *et al.* (1977). In this work, Tanne *et al.* (1989) detected antigens to one or more of these viruses in leafroll infected plants. Additionally, radiolabelled cDNA made to the potyvirus RNA reacted with extracts that tested positive for antigens of GPV by ELISA. Clearly, more work is needed on the question of the complexity of the leafroll syndrome. The information and reagents developed from our work should help towards answering this important question.

In summary, major advances were made on the isolation, characterization, antibody production, and the rapid detection of the long closteroviruses (ca. 1800nm) associated with leafroll disease. Products from this work have been used in large scale practical applications. And, progress was made towards answering the question of the complexity of the grapevine leafroll syndrome.

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Table 1.

PROGRAMA PILOTO DE CERTIFICACION DE PLANTAS DE VID
CONVENIO IICA/MGAP/DSV/DIGRA

DETALLES DE LOS ANALISIS REALIZADOS POR VARIEDAD

CULTIVARES	CERTIFICADA			BASE			DESCONOCIDA			TOTAL		
	Pl. A	Pl. P.	% I.	Pl. A	Pl. P.	% I.	Pl. A	Pl. P.	% I.	Pl. A	Pl. P.	% I.
ACHERZULOBA	27	0	0.00	0	0	0.00	0	0	0.00	27	0	0.00
ACHEROYETA	36	0	0.00	0	0	0.00	0	0	0.00	36	0	0.00
ACHERULOBA	85	7	8.23	687	22	3.20	0	0	0.00	772	29	3.75
ASERRI	0	0	0.00	509	28	5.50	0	0	0.00	509	28	5.50
BLACK SEEDLESS	0	0	0.00	0	0	0.00	3	0	0.00	3	0	0.00
CABERNET FRANC	131	1	0.76	474	19	4.00	0	0	0.00	605	20	3.30
CARDINAL	240	1	1.66	0	0	0.00	267	12	4.16	527	16	3.03
CABERNET SAUVIGNON	215	1	1.86	748	35	4.67	49	7	14.28	1012	46	4.54
CHARDONNAY	1352	58	4.29	0	0	0.00	0	0	0.00	1352	58	4.29
CHENIN BLANCHE	15	0	0.00	0	0	0.00	0	0	0.00	15	0	0.00
DANLAS	81	0	0.00	0	0	0.00	0	0	0.00	81	0	0.00
DONIBANE	0	0	0.00	118	0	0.00	0	0	0.00	118	0	0.00
EGIODOLA	0	0	0.00	620	5	0.80	0	0	0.00	620	5	0.80
EMPERATRIZ	0	0	0.00	0	0	0.00	3	0	0.00	3	0	0.00
FLANE SEEDLESS	0	0	0.00	0	0	0.00	3	0	0.00	3	0	0.00
FOLLE NOIRE	0	0	0.00	40	0	0.00	0	0	0.00	40	0	0.00
GEUNTRAMER	574	28	4.87	0	0	0.00	0	0	0.00	574	28	4.87
GROSS- HANSENG	17	0	0.00	0	0	0.00	0	0	0.00	17	0	0.00
ITALIA	84	7	8.33	0	0	0.00	0	0	0.00	84	7	8.33
JURANCON	0	0	0.00	133	3	2.25	0	0	0.00	133	3	2.25
MALVINA	0	0	0.00	0	0	0.00	3	0	0.00	3	0	0.00
MERLOT	545	7	1.28	1055	8	0.73	16	12	66.66	1652	27	1.63
MOSCATEL HAMBURGO	244	0	0.00	1563	11	0.80	343	6	1.74	1956	17	0.86
MOSCATUEL	0	0	0.00	0	0	0.00	3	0	0.00	3	0	0.00
PASIGA	0	0	0.00	0	0	0.00	3	0	0.00	3	0	0.00
PERDEA	0	0	0.00	380	5	1.31	0	0	0.00	380	5	1.31
PERLETTE	63	6	7.22	0	0	0.00	0	0	0.00	63	6	7.22
PERLON	0	0	0.00	0	0	0.00	3	0	0.00	3	0	0.00
PINOT BLANC	0	0	0.00	0	0	0.00	180	5	2.77	180	5	2.77
RIESLING	78	0	0.00	0	0	0.00	0	0	0.00	78	0	0.00
RIESLING RENANO	0	0	0.00	124	1	0.80	0	0	0.00	124	1	0.80
SAUVIGNON BLANC	69	2	2.89	196	2	1.02	0	0	0.00	265	4	1.50
SAUVIGNON	125	0	0.00	0	0	0.00	0	0	0.00	125	0	0.00
SEMILLON	14	2	14.28	0	0	0.00	0	0	0.00	14	2	14.28
SYRAH	20	6	30.00	0	0	0.00	0	0	0.00	20	6	30.00
TAMNAT	135	2	1.48	628	12	1.91	0	0	0.00	763	14	1.83
TREBBIANO ROMAGNOL	17	0	0.00	0	0	0.00	0	0	0.00	17	0	0.00
UGNI BLANC	171	0	0.00	701	11	1.56	0	0	0.00	872	11	1.26
TOTALES:	4358	134	3.07	7816	162	2.07	898	42	4.67	13072	338	2.58

origin {
 Certificado = Certification category from France
 Base = Level above certification
 Desconocida = Do not know origin

Pl. A = Plantas analizadas

Pl. P. = Plantas con resultado positivo

% I. = % de infestacion

Table 1-cont'd

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PORTAINJERTOS	CERTIFICADA			BASE			DESCONOCIDA			TOTAL		
	Pl. A	Pl. P.	Z I.	Pl. A	Pl. P.	Z I.	Pl. A	Pl. P.	Z I.	Pl. A	Pl. P.	Z I.
1616-6	4	1	25.00 :	0	0	0.00 :	0	0	0.00 :	4	1	25.00 :
196-17	17	0	0.00 :	0	0	0.00 :	0	0	0.00 :	17	0	0.00 :
3309 COUDERC	0	0	0.00 :	92	0	0.00 :	0	0	0.00 :	92	0	0.00 :
5.88	0	0	0.00 :	41	4	9.75 :	6	2	33.33 :	47	6	12.76 :
1103 PAULSEN	0	0	0.00 :	258	52	20.15 :	0	0	0.00 :	258	52	20.15 :
R-99	0	0	0.00 :	1552	36	2.32 :	0	0	0.00 :	1552	36	2.32 :
R 110	0	0	0.00 :	3355	274	8.16 :	7	3	42.85 :	3362	277	8.23 :
RUPESTRIS DU LOT	29	0	0.00 :	0	0	0.00 :	0	0	0.00 :	29	0	0.00 :
SO4 6	145	4	2.75 :	0	0	0.00 :	0	0	0.00 :	145	4	2.75 :
SO4	2960	22	0.74 :	14585	452	3.09 :	40	0	0.00 :	17585	474	2.69 :
T.5C	0	0	0.00 :	212	17	8.01 :	0	0	0.00 :	212	17	8.01 :
TOTALES:	3155	27	0.85 :	20095	835	4.15 :	53	5	9.43 :	23303	667	3.72 :

Pl. A = Plantas analizadas

Pl. P. = Plantas con resultado positivo

Z I. = Z de infestacion

TOTALES GENERALES
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TOTAL DE PLANTAS ANALIZADAS DE ORIGEN CERTIFICADA:	7513	
TOTAL DE PLANTAS CON RESULTADO POSITIVO:	161	
PORCENTAJE DE INFESTACION:	2.14	
TOTAL DE PLANTAS ANALIZADAS DE ORIGEN BASE:	27911	
TOTAL DE PLANTAS CON RESULTADO POSITIVO:	997	
PORCENTAJE DE INFESTACION:	3.57	
TOTAL DE PLANTAS ANALIZADAS DE ORIGEN DESCONOCIDO:	951	
TOTAL DE PLANTAS CON RESULTADO POSITIVO:	47	
PORCENTAJE DE INFESTACION:	4.94	
TOTAL DE PLANTAS ANALIZADAS :	36375	36,375
TOTAL DE PLANTAS CON RESULTADO POSITIVO:	1205	1,205
PORCENTAJE DE INFESTACION:	3.31	3.31

Description of Cooperation:

Dr. Edna Tanne spent 5 months at Geneva working on this grant project. She helped significantly in the work on determining the distribution of leafroll in grapevines. As reagents were produced in Geneva, such as antibodies to leafroll, they were sent to Israel for use in various experiments. An example is the use of the NY-1 antisera to determine the transmission of leafroll by mealybugs. Antisera was produced in Geneva and transmission work was done in Israel. Importantly, each laboratory did what they were most effective in doing. For example, the work with determining the complexity of leafroll was done in Israel where numerous isolates of leafroll are available and where the grapevine potyvirus was first described and worked on. At Geneva, we concentrated on purification, serology, monoclonal antibody, and virus characterization because of our group had much experience in this type of work and we had the technical help and facilities for monoclonal antibody production.

Evaluation of Research Achievements.

We achieved all of the research goals (as stated in the original objectives) with the exception of the mechanical transmission of purified preparations of the long closteroviruses to healthy grapevines. Nevertheless, we demonstrated that these particles could be transmitted by mealybugs from infected grapevines to healthy ones. Therefore, in an indirect way, we developed etiological evidence to suggest that the long closteroviruses do cause leafroll symptoms in grapevines.

Our work on the development of serological reagents that could be used in rapid detection assays such as ELISA have had significant impact towards benefitting agriculture. Some of the benefits are: 1) The antisera were used in a pilot certification program in Uruguay. The antisera and the dsRNA isolation technique are being used at the USDA Geneva Grapevine Clonal Repository to screen their germplasm. Furthermore, infected vines are being subjected to therapy treatments to eliminate the virus and the antisera is being used to quickly monitor the treated material. 2) Development of the antisera will allow the direct comparison of standard indexing technique with ELISA. This is especially useful in quarantine programs, since ELISA may help to shorten the quarantine process. In fact, experiments to compare both techniques are underway at Geneva and at the Foundation Plant Materials Service at Davis, California. 3) Grapevines can now be rapidly tested in various countries to determine the prevalence of leafroll in their vineyards or clean stock programs. Also, the serological diversity of leafroll various countries can now be quickly determined.

And 4) Development of large quantities of antibodies have encouraged private companies (for example, Bioreba in Switzerland) to include these antibodies in their commercial ELISA kits. Many of these kits are now being used by growers and nurserymen.

Although leafroll is primarily spread through the distribution of infected materials, the finding that mealybugs can transmit leafroll suggests other means for the spread of the disease. Thus, control of mealybugs may be an important cultural practice for preventing natural spread of the disease.

Indexing for grape viruses, especially for certification or quarantine purposes, is done primarily with grape indicators. Efforts to improve on this method have been going on for many years, mainly by development of serological detection techniques. Serological tests have been developed for nearly all of the grapevine NEPO viruses and other grapevine viruses that are mechanically transmitted. However, serological detection methods needed to be developed for three important virus diseases: leafroll, corky bark, and stem pitting. Our work in this grant helped in developing serological detection techniques for leafroll. Our efforts on corky bark is being supported by a new BARD grant. Preliminary data suggest that techniques developed for leafroll can be applied to corky bark.

List of Publications

A. Published, In Press

- Boscia, D., J.S. Hu, D. Golino, and D. Gonsalves. 1990. Characterization of grape leafroll associated closterovirus (GLRaV) type II and comparison with GLRaV type III. *Phytopathology* 90: 117 (abstract).
- Hu, J.S., D. Boscia and D. Gonsalves. 1990. Use of monoclonal antibodies to characterize grapevine leafroll associated closteroviruses. *Phytopathology* 80: (in press).
- Hu, J. S., D. Boscia, J. Ma, V. Mittak, and D. Gonsalves. 1989. Use of monoclonal antibodies in the study of closteroviruses associated with grape leafroll disease. *Phytopathology* 79: 1189. (abstract).
- Hu, J.S. and D. Gonsalves. 1988. Biochemical and serological characterization of closterovirus-like particles associated with grapevine leafroll disease. *Phytopathology* 78: 1568 (abstract).
- Hu, J.S., D. Gonsalves and D. Teliz. 1990. Characterization of closterovirus-like particles associated with grapevine leafroll disease. *J. Phytopathology* 128: 1-14.
- Hu, J. S., D. Teliz, and D. Gonsalves. 1989. Detection of closterovirus-like particles from crude plant extracts with immunosorbent electron microscopy. *Phytoparasitica* 17: 76. (abstract).
- Kim, K.S., D. Gonsalves, D. Teliz and K.W. Lee. 1989. Ultrastructure and mitochondrial vesiculation associated with closteroviruslike particles in leafroll-diseased grapevines. *Phytopathology* 79: 357-360.
- Tanne, E., Y. Ben-Dov and B. Raccah. 1989. Transmission of the corky-bark disease by the mealybug *Planococcus ficus*. *Phytoparasitica* 17: 55.
- Tanne, e., Y. Ben-Dov and B. Raccah. 1989. Transmission of closterovirus-like particles by mealybugs (*Pseudococcidea*) in Israel. *Phytoparasitica* 17: 63. (abstract).
- Tanne, E., L. Naveh and I. Sela. 1989. Molecular-hybridization evidence for the presence of a potyvirus in leafroll-infected grapevines. *Phytoparatsitica* 17: 69. (abstract).
- Tanne, E., L. Naveh and I. Sela. 1989. Serological and molecular evidence for the complexity of the leafroll disease of grapevine. *Plant Pathology* 38: 183-189.
- Teliz, D. D. Gonsalves, J.S. Hu and D. Hummer. 1989. Detection of grapevine leafroll associated closterovirus in recently infected tissues and spread of the disease in Mexico. *Phytoparasitica* 17: 68. (abstract).
- Teliz, D., E. Tanne, D. Gonsalves, and F. Zee. 1987. Field serological detection of viral antigens associated with grapevine leafroll disease. *Plant Dis.* 71: 704-709.
- Teliz, D., E. Tanne, D. Gonsalves, and F. Zee. 1989. Field serological detection of viral antigens associated with grapevine leafroll disease. *Phytoparasitica* 17: 68. (abstract).

B. Submitted, in preparation (copies enclosed)

- Boscia, D., J.S. Hu, D. Golino, and D. Gonsalves. 1990. Characterization of grapevine leafroll associated closterovirus (GLRaV) type II and comparison with GLRaV type III. (ms prepared for submission to Plant Dis.).
- Hu, J.S., D. Gonsalves, D. Boscia, and D. Golino. 1990. Comparison of Rapid detection assays for grapevine leafroll associated closteroviruses. (ms prepared for submission to Vitis).
- Hu, J.S., D. Gonsalves, D. Boscia and S. Namba. 1990. Production and application of monoclonal antibodies against grapevine leafroll disease associated closteroviruses. (abstr). 10th Meeting of International Council for Study of Virus and Virus-like Diseases of Grapevines, to be held in Greece, Sept. 1990.
- Hu, J.S., D. Gonsalves and D. Teliz. 1990. Characterization of grapevine leafroll disease associated closteroviruses. (abstr). 10th Meeting of International Council for Study of Virus and Virus-like Diseases of Grapevines, to be held in Greece, Sept. 1990.
- Hu, J.S., M. Wang, M. Maixmer, and D. Gonsalves. 1990. Mechanical transmission and characterization of a closterovirus from a grapevine leafroll diseased grapevine. (abstr). 10th Meeting of International Council for Study of Virus and Virus-like Diseases of Grapevines, to be held in Greece, Sept. 1990.
- Ioannou, N. and D. Gonsalves. 1990. Grapevine leafroll disease in Cyprus: Serological detection of a closterovirus in relation to the reaction of different indicator grape varieties. (abstr). 10th Meeting of International Council for Study of Virus and Virus-like Diseases of Grapevines, to be held in Greece, Sept. 1990.
- Tanne, E., Y. Ben-Dov and B. Raccach. 1990a. Transmission of closterolike particles associated with grapevine leafroll by mealybugs (Pseudococcidae) in Israel. (ms in preparation).
- Tanne, E., R. Marcus, E. Dubizky and B. Raccach. 1990b. Analysis of temporal and spatial distribution and spatio-temporal development of corky bark in grapes. (ms submitted to Ann. Appl. Biol.).

הספריה לתרבות
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