BARD

FINAL REPORT

PROJECT NO. US-339-80

**Development of Mass-Testing Procedures for PLRV
Detection in Potato Tubers and a Forecasting Model
for PLRV Infestation in Potato Fields**

**G. Tamaki (deceased), A.K. Burditt Jr., S. Marco,
P.E. Thomas**

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Title of Research Project: Development of mass testing procedures for PLRV detection in potato tubers and a forecasting model for PLRV infestation in potato field

Names of Investigators:

Principal Investigator: G. Tamaki (deceased); A. K. Burditt, Jr.

Cooperating Investigator(s): S. Marco, P. E. Thomas

Name and Address of Affiliated Institutions:

Principal Institution: USDA-ARS, Northwest Area, 809 N.E. 6th Ave., Room 204
Portland, Oregon 97232

Cooperating Institution(s): Department of Virology, The Volcani Center,
Institute of Plant Protection, P.O.B. 6, Bet-Dagan 50-200 ISRAEL

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Signatures:

Principal Investigator

Institution's Authorizing Official

A. K. Burditt, Jr.
Research Leader

N. I. James, Area Director, Northwest Area

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C. ABSTRACT

The original objectives for the Prosser location were all achieved, and these achievements are already producing much of the substantial economic and scientific benefit projected at the beginning of the project. A superior procedure to purify potato leafroll virus (PLRV) was developed, and polyclonal antisera essentially free of non-virus specific background reactions in enzyme linked immunosorbent assays (ELISA) were produced in both rabbits and sheep against several isolates of PLRV and related luteoviruses. These antisera were used to determine the etiology of leafroll disease in Western USA, to determine the universality of the ELISA for detecting all viruses that cause leafroll disease, to develop large scale tuber indexing programs in all of the potato seed certification programs of Western USA, and to supply the diagnostic needs of other aspects of the project conducted at other locations. Although various degrees of serological relationship could be demonstrated among various isolates of PLRV, tomato yellow top virus (TYTV), and beet western yellows virus (BWYV), each of these viruses were etiologically distinct. TYTV was serologically indistinguishable from PLRV but produced a distinct disease of tomato. Some virus isolates from leafroll-diseased plants produced weak reactions with BWYV antisera. However, each of two antisera produced against distinct isolates of PLRV readily detected every case of PLRV disease among thousands of diseased plants visually identified by potato seed field inspectors in seed certification programs and in breeding programs over a 3-year period in northwest USA. These antisera routinely identified PLRV-infected potatoes at all growth stages, including dormant and sprouted tuber stages. The large scale tuber indexing programs of all of the major seed certification agencies of Western USA are based upon one of these antisera. This antiserum is also being tested in Israel, and it is available in quantities sufficient to serve the needs of both nations into the foreseeable future.

Virus problems are of a crucial importance for growing seed potatoes, especially in a country with a relatively hot climate like Israel. Reducing potato leafroll (PLRV) and potato Y (PVY) viruses presents a serious challenge, since both are aphid transmitted and warm climate encourages aphid development and perpetuation of virus sources. The present work demonstrated

for the first time that it is possible to grow seed potatoes for the spring in the Golan Heights for more than one generation (section A). These seeds are not inferior to the imported ones and from the bacterial aspect much superior. The more consecutive generations we will be able to grow the higher economical advantages it brings. The main problem appears to be the relatively high reinfection rate with viruses in the generation grown in the open. The results of this work indicate that delaying plantings by two weeks (section B) and sprays of whitewash materials (section I) may reduce virus infection, which may enable growth of an additional generation which is of a crucial economical importance. Additionally, high altitude areas were shown potentially to suit seed potato growth which may extend seed production (section C).

For the first time it was demonstrated in Israel that summer grown tubers may serve as seeds for the spring (section D). This opens the option of growing seed potatoes for spring in additional areas to the Golan.

The locally grown spring tubers serve as the sole source for autumn and winter seeds. It has been shown (section E) that in spite of the high aphid activity during spring growth, adequate measures may reduce virus incidences to reasonable levels. Thus these seeds, may open the option of exporting seed-potatoes for the southern hemisphere and for winter early spring in Mediterranean countries. Preliminary work was done to find the most suitable areas for this purpose (section E,F). The possible involvement of beet western yellows virus (BWYV) with potato diseases may be of great importance for countries with high aphid populations like Israel. Section G indicates that BWYV as yet is not a serious problem in our potatoes. ELISA for PLRV and PVY assays was greatly improved and thousands of samples including seed tubers are tested in our laboratory. Monoclonal PLRV antiserum was produced and experience with this kind of work was gained. Purification of luteoviruses (section H) was improved and the serological relationship between PLRV and BWYV (section G) was investigated.

D. OBJECTIVES OF RESEARCH

The overall objective is to develop the ELISA technology, to evaluate its efficiency, and to adapt it for PLRV testing in aphids, potato plants and tubers. This entails virus purification, antiserum production, and serological assays. A superior PLRV antiserum will be produced--an antiserum free from non-virus specific background reactions in ELISA application and with the capacity to detect all variant strains of PLRV in the minute quantities present in individual aphid vectors and infected potato tubers as follows:

- a. The ELISA method will be adapted to detect PLRV in individual aphids and to determine the inoculum potential of aphid populations.
- b. Data will be collected from mass field indices of plants and aphids and thereafter used to construct a forecasting model to alert growers of the potential PLRV infection in fields.
- c. ELISA will be adapted to diagnose PLRV in all potato tissues at all growth stages, and systems developed for large scale tuber indexing programs suited to the needs of the potato industries in the United States and Israel.
- d. The universality of the ELISA test for detecting different PLRV strains with one antiserum will be studied. To this end many geographically different PLRV isolates will be tested. Additional antisera will be produced to specific strains as necessary and combined to produce the broad spectrum specificity needed.
 1. Determine the etiology of PLRV.
 2. Develop superior methods to purify PLRV, and produce antisera free of nonvirus specific background reaction in ELISA applications.
 3. Produce broad spectrum antisera that detect all strains that cause leafroll disease.
 4. Adapt ELISA technology to detect PLRV in all potato tissues at all growth stages, and develop large scale tuber indexing programs suited to the needs of the potato industries of the United States and Israel.

Research Plan:

Experiment 1: Aphid populations in relation with PLRV incidence.

Objective: Collecting data for the model of PLRV forecasting.

Secondary Objectives:

1. Screening areas suitable for growing seed potatoes for spring and autumn.
2. Studying aphid population immigration (arrival and potential PLRV transmissibility) in the areas of seed and commercially grown potatoes.
3. Survey of aphid species prevalent in these areas and their capability and efficiency to transmit PLRV.
4. Population dynamics of aphid influence by climatic conditions.

Experiment II: Adapting ELISA for mass PLRV indexing.

Objective: Developing ELISA for routine PLRV assays in potato seed tubers.

Secondary Objectives:

1. Routine use of ELISA for PLRV assays in potato foliage and/or roots.
2. Quantifying ELISA to determine virus titer in plants by correlation with known virus concentrations.
3. Investigating ELISA reaction to different PLRV strains.
4. Investigating the possibility of multiple virus assays in potato tubers.
5. Investigating variations of ELISA technique for large scale and practical use.

E. BODY OF REPORT

1. Yakima, Washington

- a. ELISA was able to detect highly significant differences in absorbance values between individual virus free and PLRV infected green peach aphids that were taken directly from infected or non-infected greenhouse grown Datura tatula plants. However, if before ELISA testing, the Datura reared aphids (from both infected and non-infected plants) were allowed a 24-hour feeding period on a non-infected Czpsella bursa-pastoris or Physalis floridzna plants, no significant difference in absorbance values between infected or non-infected aphids was detected.

In addition, we were able to show a significant host plant effect in ELISA absorbance values from individual aphid samples even without the presence of PLRV. Taking virus free aphids (4th instar) reared on non-infected Datura tatula and allowing them to feed on non-infected Capsella bursa-pastoris or Physalis floridana plants for 24 hours before ELSIA testing of the individual aphids resulted in significant higher absorbance values compared with the aphids taken directly from the Datura plant.

Adapting the present ELISA methods to reliably detect PLRV in individual aphids trapped in or near commercial potato fields does not seem feasible using the present broad spectrum antiserum that is apparently showing a positive reaction with some plant proteins. However, by exposing the field trapped aphids to the indicator plant, P. floridana, and testing the plants with ELISA two weeks later, we can still determine the inoculum potential of the aphid population.

- b. Monitoring green peach aphid in commercial potato fields. Aphid population was monitored over a 13 week period (June 6-Sept. 3) at a commercial field site in the Yakima Valley. Chinese cabbage plants growing in 6" yellow metal pots were placed around border of field to attract winged green peach aphids, (GPA) Myzus persicae. Each day all winged GPA present were returned live to the lab and new plants placed

in the field. Aphids were transferred (1/plant) to small indicator plants, Physalis floridana and allowed to feed 48 hours before fumigation with methyl bromide. Plants were observed for 3 weeks for virus symptoms.

The low initial aphid population during the first three weeks of June (110 GPA tested) were all virus free. The following three weeks (June 26-July 13, 1982) gave rise to a population peak in which 555 GPA were collected and only three of these were viruliferous (0.54%). The first viruliferous GPA was collected July 1. Aphid population declined and remained low for the remainder of the collection period (July 19-Sept. 3) averaging 42 aphids/week. Plant readings are not complete for this entire period but from July 19 through August 20, 222 aphids were collected and 25 of these were viruliferous (11.3%).

Alate green peach aphid (GPA) population was monitored over a 15 week period (June 6 - Sept. 19, 1983) at 4 commercial potato field sites in the Columbia Basin. Objective was to determine the percent of aphids that are vectors of potato leafroll virus (PLRV). Live alate GPA were collected daily from chinese cabbage plants (non-host of PLRV) growing in pots that had been painted yellow and placed around the border of each field. At the lab, the field collected aphids were transferred (1/plant) to small indicator plants, Physalis floridana and allowed to feed for 48 hours before fumigation with methyl bromide. Plants were observed for three weeks for virus symptoms.

Of the 721 total aphids tested, 48 were viruliferous (6.6%). The first winged aphids were trapped the third week of the test (June 26-27) but none were viruliferous. Total viruliferous was 6.6% (48/721) were vectors of PLRV and these all came from 2 single trapping site (Godfry farm). Volunteer potato plants growing in a nearby corn field and in a potato cull pile were assayed for PLRV and found to have a 7.5% (31/411) infection rate. The GPA population peaked during the second week of July in which 5.4% (14/257) of the aphids were viruliferous. The population declined to a low level during the last 8 weeks (Aug.-Sept.) of trapping and averaged 4.7% (9/188) PLRV infection.

Alate green peach aphids (GPA) collected from trap plants located on the perimeter of a commercial potato field. Quincy, WA area, 1983

FOUR COMMERCIAL POTATO FIELDS, QUINCY, WA AREA--1983

Test Week No.	Collection Date (ending)	Total Tested GPA	No. Viruliferous GPA	% Viruliferous GPA
1.	June 13	0	0	0
2.	June 20	0	0	0
3.	June 27	10	0	0
4.	July 4	26	3	11.5
5.	July 11	88	5	5.7
6.	July 18	257	14	5.4
7.	July 25	152	17	11.2
8.	Aug. 1	25	1	4.0
9.	Aug. 8	34	3	8.8
10.	Aug. 15	13	0	0
11.	Aug. 22	39	1	2.6
12.	Aug. 29	27	0	0
13.	Sept. 5	12	0	0
14.	Sept. 12	14	2	14.3
15.	Sept. 19	24	2	8.3
TOTALS		721	48	6.6

Alate green peach aphids (GPA) collected from trap plants located on the perimeter of a commercial potato field. Quincy, WA area, 1983

GODFRY FARM

Test Week No.	Collection Date (ending)	Total Tested GPA	No. Viruliferous GPA	% Viruliferous GPA
1.	June 13	0	0	0
2.	June 20	0	0	0
3.	June 27	4	0	0
4.	July 4	15	2	13.3
5.	July 11	10	2	20.0
6.	July 18	136	8	5.9
7.	July 25	47	7	14.9
8.	Aug. 1	4	0	0
9.	Aug. 8	16	1	6.2
10.	Aug. 15	7	0	0
11.	Aug. 22	1	0	0
12.	Aug. 29	5	0	0
13.	Sept. 5	3	0	0
14.	Sept. 12	-	-	-
15.	Sept. 19	-	-	-

TOTALS

270

20

7.4

Alate green peach aphids (GPA) collected from trap plants located on the perimeter of a commercial potato field. Quincy, WA area, 1983

RICHARDSON FARM

Test Week No.	Collection date (ending)	Total Tested GPA	No. Viruliferous GPA	% Viruliferous GPA
1.	June 13	-	-	-
2.	June 20	0	0	0
3.	June 27	0	0	0
4.	July 4	1	0	0
5.	July 11	42	3	7.1
6.	July 18	28	2	7.1
7.	July 25	34	5	14.7
8.	Aug. 1	3	0	0
9.	Aug. 8	0	0	0
10.	Aug. 15	0	0	0
11.	Aug. 22	1	0	0
12.	Aug. 29	0	0	0
13.	Sept. 5	4	0	0
14.	Sept. 12	1	0	0
15.	Sept. 19	5	0	0
	TOTALS	119	10	8.4

Alate green peach aphids (GPA) collected from trap plants located on the perimeter of a commercial potato field. Quincy, WA area, 1983

HAMMOND FARM

Test Week No.	Collection date (ending)	Total Tested GPA	No. Viruliferous GPA	% Viruliferous GPA
1.	June 13	-	-	-
2.	June 20	-	-	-
3.	June 27	6	0	0
4.	July 4	1	0	0
5.	July 11	23	0	0
6.	July 18	22	0	0
7.	July 25	7	0	0
8.	Aug. 1	7	0	0
9.	Aug. 8	4	0	0
10.	Aug. 15	0	0	0
11.	Aug. 22	1	0	0
12.	Aug. 29	3	0	0
13.	Sept. 5	1	0	0
14.	Sept. 12	5	0	0
15.	Sept. 19	9	1	11.1
TOTALS		69	1	1.4

Alate green peach aphids (GPA) collected from trap plants located on the perimeter of a commercial potato field. Quincy, WA area 1983

STETNER FARM

Test Week No.	Collection date (ending)	Total Tested GPA	No. Viruliferous GPA	% Viruliferous GPA
1.	June 13	-	-	-
2.	June 20	-	-	-
3.	June 27	0	0	0
4.	July 4	9	1	11.1
5.	July 11	13	0	0
6.	July 18	71	4	5.6
7.	July 25	64	5	7.8
8.	Aug. 1	11	1	9.1
9.	Aug. 8	14	2	14.3
10.	Aug. 15	6	0	0
11.	Aug. 22	36	1	2.8
12.	Aug. 29	19	0	0
13.	Sept. 5	4	0	0
14.	Sept. 12	8	2	25.0
15.	Sept. 19	10	1	10.0
TOTALS		265	17	6.4

c. Volunteer Potato Plants: Source of Potato Leafroll Virus

Volunteer potato plants have been implicated as the major source of potato leafroll virus (PLRV) in the Columbia Basin. The green peach aphid (GPA) is the major vector for the dissemination of the virus to the growing potato crop.

In June and July of 1983-84 volunteers were assayed for presence of the virus using ELISA. Volunteers were collected from current year potato fields (Table 1), fields which have been out of a commercial potato crop for 1-3 years (Table 2), and from potato cull piles (Table 3). Cull potatoes are those removed from storage to an open waste dump nearby and become a source of volunteer plants the next year.

PLRV was detected in 5.26% (57/1083) of the volunteer potato plant samples collected in the Columbia Basin in 1983-84.

Table 1. Volunteer Potato Plants From Commercial Fields Currently Planted to Potatoes.

Location	Year	Total Plants Sampled	No. PLRV Infected Plants	% Diseased Plants
Richardson Farm	1983	25	3	12.0%
V & I Group Farm	1984	100	10	10.0%

Table 2. Volunteer Potato Plants From Commercial Fields
Currently Planted in Crops Other Than Potatoes.

Location	Year	Current Year Crop	Years Out of Potatoes	Total Plants Samples	No. Diseased Plants	% Diseased Plants
Willow Drive Nursery	1983	Nursery Trees	3	128	0	0
Godfrey Farm	1983	Corn	1	238	19	8.0%

Table 3. Volunteer Potato Plants From Cull Piles Located Near
Commercial Potato Storages.

Location	Year	Total Plants Samples	No. Diseased Plants	% Diseased Plants
Godfrey Farm	1983	173	12	6.9%
Godfrey Farm	1984	25	0	0
B72 U97	1984	60	0	0
B75 V2	1984	30	1	3.3%
Johnson Storage	1984	30	3	10.0%
B75 U17	1984	23	0	0
B72 V103	1984	40	0	0
B72 V255	1984	20	1	5.0
Prior Land	1984	191	8	4.2%
TOTALS		592	25	4.2%

d. Effect of time of inoculation with potato leafroll virus on development of net necrosis in potato tubers

A field test was set up in which individual potato plants, under protective cages, were inoculated with PLRV (Prosser isolate #1) using viruliferous green peach aphids. Russet Burbank seed pieces were planted May 25, 1982 and inoculations made on July 2, July 28, August 16 and September 7. No single plant was inoculated more than one time and non-inoculated plants were included in the test. Tubers were harvested and examined at various times while in storage. Harvest weight and number of tubers were recorded. Replanting of selected tuber "eyes" and ELISA techniques were used to assay for PLRV. The use of the indicator plant, Physalis floridana, for weekly assay of PLRV in the field test was not reliable.

Individual potato plants (Russet Burbank) grown under cubic meter size protective field cages were inoculated at various plant growth stages with potato leafroll virus (PLRV) using viruliferous green peach aphids (GPA) reared on diseased greenhouse plants. Potato seed-pieces were planted April 29, 1983 and field inoculations made on June 10, July 5, August 1 and September 6. No single plant was inoculated more than one time and aphids used to make inoculations were killed with an insecticide spray (Naled) 48 hours following introduction to plant. Field cages remained on all test plants throughout the growing season.

There was no significant difference in tuber harvest weight, mean weight per tuber or number of tubers per plant between dates of inoculation and the non-inoculated check. Following harvest all tubers were stored under commercial type storage conditions (42-45°F; 90-95% RH). Tubers from each plant was

divided into 4 equal sub-samples, each of which were removed from storage at two month intervals and each tuber assayed for presence of PLRV (ELISA test of tuber phloem tissue). Also, based on a visual examination of internal tuber tissue, each tuber was classified:

1. CLEAN Normal, healthy appearance of internal tissue.
2. SEB Stem-end browning; shallow penetration of continuous strands of necrotic tissue into the tuber from the stem-end.
3. NET NECROSIS Shallow or deep penetration of tuber phloem necrosis from the stem-end, showing typical discontinuous necrotic tissue strands, typical of that resulting from PLRV infection.
4. OTHER NECROSIS Shallow or deep penetration of necrotic tissue from the stem-end, but differing from that typically and historically associated with PLRV. Numerous variations. Unknown cause.

The relationship between visual classification of each tuber and the presence of PLRV is shown in Table 1. PLRV was not detected (ELISA) in any tubers from the early season inoculation (June 10), the late inoculation (Sept. 6) or from tubers produced by the non-inoculated check plants. Visual internal tissue examination of tubers from these three treatment groups showed no significant differences: 85.7% (204/238) of composite tubers were classified as 'CLEAN'; 8.4% (20/238) 'SEB'; 1.7% (4/238) 'NET NECROSIS', and 4.2% (10/238) 'OTHER NECROSIS'.

The only plants that produced PLRV infected tubers (ELISA) came from four of five plants inoculated in July and one of five plants inoculated in August (Table 1). The infected plants produced a total of 79 tubers of which 69.6% (55) were infected with PLRV. Of the 55 infected tubers, 40.5% (32) produced necrotic tissue symptoms. Because the test resulted in the

production of such a small number of tubers with net necrosis, examination of sub-samples from storage at two month intervals was not able to answer the question of whether net necrosis increased during this post-harvest period.

Reliability of ELISA in detection of PLRV in the sample tubers was determined by field planting of an "eye" from each tuber and observing the resulting plant for typical secondary leafroll symptoms. In this case there was complete agreement between lab and field results.

The results here would seem to indicate that the potato plant is most capable of producing necrotic and PLRV infected tubers when initial inoculation occurs about time of full bloom. Why the young potato plants (ca 10-15 cm tall) inoculated in June did not produce PLRV infected tubers is not understood. Other workers have demonstrated a mature plant resistance factor which would help explain why no PLRV infected tubers were produced by plants inoculated in September.

If further small plot testing and natural field inoculation is found to produce similar results, the grower maybe able to reduce the amount of insecticide applied for aphid control. Currently, chemical control methods are applied from planting time to near harvest.

Table 1. Relationship between PLRV detection (ELISA) in potato tuber tissue and the visual classification of the internal tuber tissue. Tubers from potato plants inoculated with PLRV at various plant growth stages. Five plants inoculated each date.

INOCULATION DATE AND ELISA DETERMINATION	VISUAL DETERMINATION				TUBERS EXAMINED	No. PLRV INFECTED PLANTS
	CLEAN	SEB	NECROSIS			
			NET	OTHER		
JUNE 10, 1983						
PLRV NEGATIVE TUBERS	69	6	0	5	80	
PLRV POSITIVE TUBERS	0	0	0	0	0	0
JULY 5,						
PLRV NEGATIVE TUBERS	28	1	4	0	33	
PLRV POSITIVE TUBERS	21	6	18	2	47	4
AUGUST 1,						
PLRV NEGATIVE TUBERS	61	7	1	0	69	
PLRV POSITIVE TUBERS	4	1	6	0	11	1
SEPTEMBER 6,						
PLRV NEGATIVE TUBERS	70	7	2	1	80	
PLRV POSITIVE TUBERS	0	0	0	0	0	0
CONTROL (NO INOC.)						
PLRV NEGATIVE TUBERS	65	7	2	4	78	
PLRV POSITIVE TUBERS	0	0	0	0	0	0

2. Prosser, WA

- a. Superior PLRV antiserum: The ability to purify large quantities of highly pure virus is essential for development of high quality antiserum and numerous other research objectives. We developed a purification procedure using the TYTV strain of PLRV which yields as high as 1700 ug of virus/kg of infected tissue. This procedure was highly effective in purifying PLRV, TYTV, and BWYV in our laboratory, and other laboratories have applied it to purification of other luteoviruses, finding it to be much more effective than previously employed methods (S. W. Wyatt, WSU, Pullman, Washington, personal communication).

Utilizing the purified virus, antisera against several PLRV, TYTV, and BWYV isolates and strains were produced. These antisera are being utilized in a wide array of experiments associated with this project at this and the other locations. This antisera forms the basis for most potato virus testing programs conducted in the U.S. Its quality was compared with that of a world collection by the Montana State Potato Seed Certification Program and was superior to all other antisera in the collection. For greater flexibility, sensitivity, and broader virus strain specificity in ELISA applications, the antisera was prepared in both rabbits and sheep. Very large quantities of antisera were prepared to meet the testing needs of the U.S. and Israel for many years.

- b. Etiology of potato leafroll disease: At the outset of this work, BWYV had recently been reported (Duffus, J. E., Phytopathology 71: 100-106. 1981) to be an etiologic agent in leafroll disease, and our research (Hassan, S., and P. E. Thomas. Phytopathology 71:1004. 1981) had indicated that another virus, TYTV, was also related to PLRV. To develop reliable diagnostic methods for PLRV, it was necessary to determine the extent to which these viruses were related to PLRV and the extent to which they were a part of the leafroll disease syndrome. We found that TYTV was closely related to PLRV and caused a mild leafroll disease of potatoes. It had not

yet become a part of the complex of strains causing leafroll disease in Western USA, and it appeared to have a somewhat different epidemiology than that of standard PLRV. TYTV was distinctly a strain of PLRV, and it could become especially important if it should become endemic in potatoes, since it produces mild symptoms in potatoes and could not be eliminated routinely by roguing. There were varying degrees of serological relationship between BWYV and isolates of PLRV and TYTV ranging from no relationship to a weak relationship depending on the virus isolates involved. Many spur reactions were demonstrated among isolates of these viruses in agar gel double diffusion serology. However, typical isolates of BWYV from cruciferous or sugar beet plants did not infect potato. Some viruses previously isolated from potato and designated BWYV (Duffus, J. E., *Phytopathology* 71:193-196. 1981) based on host range would not reinfect potato. All of those that did reinfect potato were readily detected with each of four antisera prepared against other PLRV isolates.

- c. Broad spectrum virus strain detectability: PLRV appears to consist of a complex of strains, some of which have serological affinities with BWYV and some of which cause distinct diseases of other crops, like tomato yellow top disease. However, a major objective of this study was to prepare broad spectrum antiserum that detected all instances of PLRV disease regardless of the relationships of the causal viruses with other virus groups. Pursuant to this objective, we tested every instance of leafroll disease detected by field inspectors in the Montana, Idaho, and Oregon potato seed certification programs in 1983 and '84 and every instance of leafroll detected in an experimental USDA net to breeding program at Prosser, Washington during 1982, '83, and '84 against antisera of two PLRV isolates. In the breeding program, all tubers were winter-indexed, and this was followed by field inspection for symptoms during the growing season to determine whether any instances of virus infection escaped detection by the two antisera. Tissue from infected plants that escaped detection at the tuber stage was reindexed during the growing season. When a field sample

from a plant with symptoms failed to produce a possitive ELISA, the foliage was indexed with aphids to Physalis floridana, and if the index plant developed symptoms, its foliage and the original potato foliage were subjected to another ELISA. Thousands of individual leafroll diseased plants were tested in all, and the two antisera detected every confirmed instance of leafroll infection. In a few instances, samples received from inspectors failed to produce a possitive ELISA. In each such case either virus could not be transmitted from the sample to Physalis floridana or, if it was, the Physalis floridana index tissue produced a positive ELISA. Tuber indexing rather frequently failed to detect leafroll-infected plants, but this was usually attributable to low virus concentration or incomplete virus distribution in the tubers, since the disease plants produced by the tubers usually produced positive ELISAs. In a few cases, however, specific breeding lines were apparently somewhat resistant to virus multiplication, since diseased plants produced negative ELISAs, but the virus could be transmitted to and detected by ELISA in the index host. Some isolates clearly produced stronger ELISA reactions than others, and some produced stronger reactions with one antiserum than the other. However, both antisera detected every virus isolate that produced leafroll disease of potato.

- d. Large scale tuber indexing for PLRV: A number of massive tuber indexing programs have been initiated in the U.S. recently in direct response to the superior antisera provided by this BARD project. These include potato seed certification programs of Montana, Idaho, Oregon, Washington, Minnesota, and the Washington State University ELISA Laboratory. PLRV ELISA supplies distributed by the largest commercial supplier of plant virus diagnosis supplies in the U.S., Agdia, Inc., is based on the antisera produced in this project. The major potato breeding programs in the U.S. have adapted this antisera to monitor clones for virus contamination and as a means to select progeny for resistance to PLRV. In addition, the entomology program at Yakima, Washington, associated with this project is utilizing our antisera to detect

PLRV in aphid vectors and to develop insecticide application schedules to protect against PLRV infection. These antisera are also under evaluation in Israel for potential utilization in the various aspects of the potato industry.

3. ISRAEL - General Introduction

The potato is one of the most important crops in the world and its importance is steadily increasing in developing countries (CIP 1980). In Israel the potato is being grown for consumption in two main seasons: a. Spring where plantings takes place in February and harvest in June-July. b. Autumn with plantings in August-September and harvest after December according to weather and market conditions. From the virological aspect areas of hot climates like Israel, and many developing countries have the disadvantages of high vector populations on the one hand and a continuous growth of weeds and cultivated plants throughout the year which serve as virus inoculum sources. Therefore, incidences of viruses and especially the vector transmitted ones are obviously higher (Marco, 1981). This fact is crucial in some crops, especially those aimed at further multiplication. Hence seed potatoes which are being propagated vegetatively for several generations (Hiddema, 1972) are to be imported to Israel from countries with cooler climates.

For the spring season in Israel most of the needed seed potato are imported from Scotland, Ireland and the Netherlands. Still however, 10-20% of the spring seeds are grown locally. The single area in Israel where certified seed potatoes can be grown for the spring season is the northern Golan plateau at 800 m above sea level (Zimmerman-Gries et al., 1973). In this area temperatures are relatively low, no rainfall during summer and potato planting and harvest take place similarly to European countries (May-September). The quality of the Golan seeds is, as usual, similar to and may replace the imported ones (Levy and Marco, 1982; Zimmerman-Gries et al., 1973). However, aphid activity in Golan is high if compared to European conditions (Marco et al., 1977) and sometimes virus diseases, especially PLRV may spread to incidences of ca. 60%! (Marco and Ben Tal, 1979). In order to

reduce the probability of virus infection several measures were taken:

- a. Seed potatoes are grown in Golan for only one generation from high quality imported mother seeds.
- b. The Golan area is closed for table potato crops.
- c. Since PLRV was found by far the most widespread virus (Marco and Ben Tal, 1979), a severe regime of insecticide application is practiced in seed potato crops (Marco, 1980).
- d. Haulm killing is done according to aphid appearance in August which is monitored by Moericke and suction traps in the Golan.

These measures are probably efficient enough since no serious virus problems arose during the last several years. However, the fact that most seed potatoes for the spring and the mother seeds to Golan are to be imported every year, has several disadvantages:

- a. The cost of these seeds, including transport is high.
- b. The phytopathological status and hence the quality of the imported seeds is variable depending much upon the climate in the specific season in the exporting country.
- c. There is a danger of introduction of new diseases to Israel and especially to the Golan.

For the autumn (August plantings) import of seeds is not available since in the previously mentioned seed exporting countries, harvest takes place only in August-September. Therefore, for autumn all seed potatoes are locally grown during the spring season (June harvest) with the obvious disadvantages of a young physiological status of the seeds and the probability of high incidences of viruses especially potato leafroll virus (PLRV) (Marco, 1981). There are probably beside other factors, reduce consistent yields in autumn (Marco, 1984), as compared with yields of the same cv. in spring. Probably because of similar reasons the spring tubers are not suitable for the next spring, when the yield of Up-to-Date was found significantly lower than the imported seeds.

In the view that insecticide application succeeded in Golan, growers of seed potatoes for the autumn adopted a similar treatment which reduced PLRV incidence (Marco unpublished). Because of the early harvest of these seeds (June) these are more suitable for winter plantings used in

Mediterranean countries and for early spring plantings in countries of the southern hemisphere. Thus, improvement of these seeds may open the possibility of export to a certain niche of the international market.

In the present project the virological aspects of growing seed potatoes in Israel were investigated based on recent developments in rapid multiplication (Levy et al, 1983), improvement of ELISA and preparation of good PLRV antisera especially by our American partners and in the view of development of new methods to reduce PLRV infection (Marco, 1981).

Seed potatoes for spring:

A pilot plant of growing seed potatoes for several generations was undertaken in the Golan Heights.

- b. Aphid dynamics and PLRV epidemiology was thoroughly investigated in Golan attempting to reduce PLRV incidences and the use of insecticides.
- c. Surveys of new high altitude areas for aphid activity and the possibility of growing their seed potatoes for the spring were continued.
- d. Nonconventional attempts were undertaken to grow during summer seed potatoes for the spring season as an alternative for the imported ones.

Seed potatoes for autumn and winter

- e. Aphid populations and virus incidences were surveyed in the Negev, attempting to improve the measures undertaken in growing spring tubers as seeds for export, autumn and winter.
- f. New areas and locations were surveyed for aphid activity and virus incidence attempting to select the best choice possibility for growing autumn seed potatoes.

General investigations

- g. The possible involvement of beet western yellows virus with the potato leafroll disease was investigated in Israeli seed potatoes and the serological relation between the two viruses was checked by ELISA.
- h. Tests for improving purification and characterization of PLRV was carried out.
- i. Attempts were undertaken to reduce incidence of aphid-transmitted viruses by sprays of whitewash materials onto the plants.

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F. DESCRIPTION OF COOPERATION:

Cooperation in this project began with the fact that each of the three participating programs had different responsibilities which conformed with their respective areas of expertise, and the products of each participant were interfaced and integrated to form a uniform approach to the objectives. The antisera and ELISA methods produced at Prosser were an essential requisite for the work in progress at Yakima. These resources and our investigations of virus relationships are being shared with Israel to help solve problems in seed potato production there. This project established bases for cooperation among the participants. The cooperation initiated under this project will extend into the future and should be recognized as one of the real benefits of the project.

The Israeli investigator visited twice the laboratories at Yakima and Prosser and Dr. P. E. Thomas visited the Volcani Center, Dr. G. Tamaki and Mr. L. Fox visited Dr. Marco during his stay at the Vancouver Research Station. The information exchange was very important especially as to the application of entomological and biochemical methods to the epidemiological work. In addition valuable and excellent antisera was supplied to us by Dr. Thomas which enabled us to do the large number of tests required in this work. We express our deep sorrow for the untimely death of the excellent scientist Dr. Tamaki and wish to thank Dr. Thomas and Mr. Fox for the fruitful cooperation.

G. BENEFIT TO AGRICULTURE:

The PLRV antisera produced under this project at Prosser has had enormous impacts on the potato industry in the U.S. and on potato research conducted there. Its availability has resulted in development of mass ELISA indexing programs in the potato seed certification agencies of the major seed producing states, and most seed potatoes sold in the U.S. today have been monitored and evaluated for PLRV through the use of this antisera. Its use is expected to expand further in the near future. Concomitant with the institution of PLRV indexing programs, there has been a dramatic reduction in the incidence of leafroll disease in the Columbia Basin of the Northwest. The incidence of leafroll infection in tubers harvested in the Columbia Basin ranged from 32 - 44% in the mid 1970s. The incidence has fallen to below 10%.

The antisera produced at Prosser is also beginning to find application in reducing the losses to net necrosis which often develops during storage of tubers infected with PLRV. Lots of tubers with high incidence rates of PLRV infection, thus, most likely to develop net necrosis during storage, are identified prior to harvest by ELISA. Loss is avoided by processing these lots during the harvest season before necrosis develops, while lots of tubers with lower incidence of PLRV infection are placed in storage for processing later in the season. The necessary testing is usually performed through a public ELISA laboratory sponsored by Washington State University which utilizes the antisera produced at Prosser.

PLRV diagnostic supplies distributed by the U.S. Department of Agriculture virus diagnostic supplies in the U.S. are based upon the antisera produced in this project at Prosser.

The PLRV antisera produced under this project along with antisera produced against other potato viruses is contributing toward a revolution in approaches to potato breeding in the U.S. Virus contamination of breeding clones under evaluation has resulted in the loss of countless clones for many years. However, breeders are now able to rapidly and reliably monitor

their clones for infection and discard infected tubers before entire clones become contaminated. Furthermore, they are now able to rapidly screen large progeny populations for resistance to viruses using the new ELISA technologies. These diagnostic technologies, combined with new methods to render clones free of virus infection and in-vitro culture and maintenance methods, have made possible development of new breeding strategies that are much more efficient and effective than previous methods.

1. The results of the present work enable to establish a scheme of seed potato multiplication for several generation in the Golan Heights.
2. The system developed for virus detection and antisera production serve the whole potato and vegetable industry.
3. Methods developed to reduce aphid -transmitted viruses are being implemented in all areas of production of plant material multiplication.
4. The presented results open the possibility of growing reasonable seed potato quality for autumn, winter and for export.
5. It was demonstrated that summer grown potatoes in certain areas may serve as seed for the spring.
6. The surveys for aphid and virus incidence done provide data for detecting the best areas for seed potato production for each season. They may serve for improving many other crops which are affected by aphid transmitted viruses

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