

## Enzyme-Linked Immunosorbent Assay to Detect Potato Leafroll Virus in Potato Tubers and Viruliferous Aphids

R. G. CLARKE, Assistant Professor, Department of Entomology, Oregon State University, Corvallis, OR 97331; R. H. CONVERSE, Research Plant Pathologist, Agricultural Research, SEA, U.S. Department of Agriculture, and Oregon State University; and M. KOJIMA, Associate Professor, Faculty of Agriculture, Niigata University, Niigata, Japan

### ABSTRACT

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Enzyme-linked immunosorbent assay (ELISA) with alkaline phosphatase successfully detected potato leafroll virus (PLRV) in naturally infected Russet Burbank potato tubers. Statistically significant differences in  $A_{405}$  values of ELISA reactions could be detected between known infected and healthy tuber samples. A multiple-eye sampling scheme was devised to detect PLRV in potato tubers by the ELISA method. PLRV was also detected in viruliferous *Myzus persicae* by the ELISA method.

In the United States potato tubers are frequently infected with potato viruses M, S, X, and Y and potato leafroll virus (PLRV) (1). In most U.S. seed-potato certification programs, the standard method for determining tuberborne PLRV infection in seed lots is to grow random samples of seed pieces and record the number of plants with foliar leafroll symptoms. This process requires about 6 wk.

European workers showed that PLRV could be detected in potato tubers and in leaves and roots, using enzyme-linked immunosorbent assay (ELISA) (2,7). This serologic technique (3) has been used for a number of viruses that, like PLRV, occur in very low or variable concentrations in plant material (3-5,10,12). Successful detection of PLRV in potato tubers by ELISA would therefore provide an attractive alternative diagnostic method for seed certification to that currently used.

This article reports the first detection of PLRV in aphid vectors and presents a multiple-eye sampling method for screen-

ing dormant tubers for this phloem-limited virus.

### MATERIALS AND METHODS

Anti-PLRV globulin obtained from antiserum produced by M. Kojima (9) was conjugated to alkaline phosphatase, Type VII (Sigma Chem. Co., St. Louis, MO) using the methods of Clark and Adams (3). ELISA tests were of the double antibody sandwich type (13) in protein-binding polystyrene Microelisa plates (Dynatech Laboratories, Alexandria, VA). Preliminary studies led to the adoption of coating globulin concentration of 1  $\mu\text{g}/\text{ml}$  and conjugated globulin at a 1:500 dilution. Coating globulin and tuber extracts were incubated overnight at 5 C and the conjugated globulin was incubated for 4 hr at 37 C. Substrate (*p*-nitrophenyl phosphate, Sigma No. 104) was added to plates at a concentration of 1  $\mu\text{g}/\text{ml}$  in diethanolamine, sodium carbonate buffer, pH 9.8, and incubated for 1 hr at room temperature. The reaction was then halted by adding 50  $\mu\text{l}$  of 3 M NaOH to each well.

Quantitative measurements of generated *p*-nitrophenol were made by determining absorbance at 405 nm ( $A_{405}$ ) in a Beckman Model 25 Spectrophotometer (Beckman Instruments Co., Fullerton, CA) using an 80- $\mu\text{l}$  flow cell connected to a variable peristaltic pump (Harvard Apparatus Co., Millis, MA). Readings of ELISA reactions to PLRV were found from dilution studies of infected potato leaves to be linear ( $r^2 = 0.97$ ) in the 0-2  $A_{405}$  range. The mean healthy absorbance reading plus two standard deviations was established as the threshold for determining PLRV infections (13). Tuber absorbance readings in excess of this

threshold were scored as positive for PLRV.

Potato tuber samples were ground 1:2 (w/v) with mortar and pestle in an extraction buffer consisting of phosphate-buffered saline, pH 7.4, plus 0.05% Tween 20, 2% polyvinyl pyrrolidone (40,000 mol wt, Sigma) and 0.2% egg albumin. The resulting solutions were squeezed through two layers of Kimwipes and centrifuged at 3,000 *g* for 5 min to sediment the larger particulate material. Known healthy tuber samples as well as buffer checks were included in each plate.

**PLRV standards.** A group of Russet Burbank potato tubers grown at Klamath Falls, OR, were individually eye-indexed for PLRV content according to standard greenhouse testing procedures (8). From this group, eight PLRV-infected tubers and six tubers indexed as free of PLRV were chosen as reference standards for ELISA tests. Because PLRV was shown to be unevenly distributed in tubers (8), three sample sites were tested from each tuber. Three 1-g ELISA samples, hereafter designated as stem-end, bud-end, and middle-eye, were taken from each tuber and prepared for ELISA testing. Each sample was dispersed into three wells randomly assigned on Microelisa plates. Anatomically, the ELISA within-tuber samples each contained periderm, corky epidermis, vascular ring, outer medulla, and inner medulla (pith). Specifically, the middle-eye sample also contained a single bud (eye), the bud-end eye contained a cluster of several buds, and the stem-end contained no bud tissue. The middle-eye was taken from the area of the tuber about equidistant from the stem-end and bud-ends of the tuber. Leaves from plants grown from seed pieces from each of these tubers were also evaluated for PLRV content by ELISA after dilution 1:5 (w/v) in extraction buffer.

**Field testing.** Three groups of 20 field run Russet Burbank potato tubers from Hermiston, OR, were evaluated for PLRV by ELISA. Because of the variation among sample sites from standard tubers, all buds of a given tuber in this test were removed with a 4-mm diameter cork borer to a depth of 8 mm, polled, and triturated 1:2 (w/v) in extraction buffer. This procedure will be referred to as multiple-eye sampling.

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**Table 1.** ELISA absorbance readings for Russet Burbank potato tubers

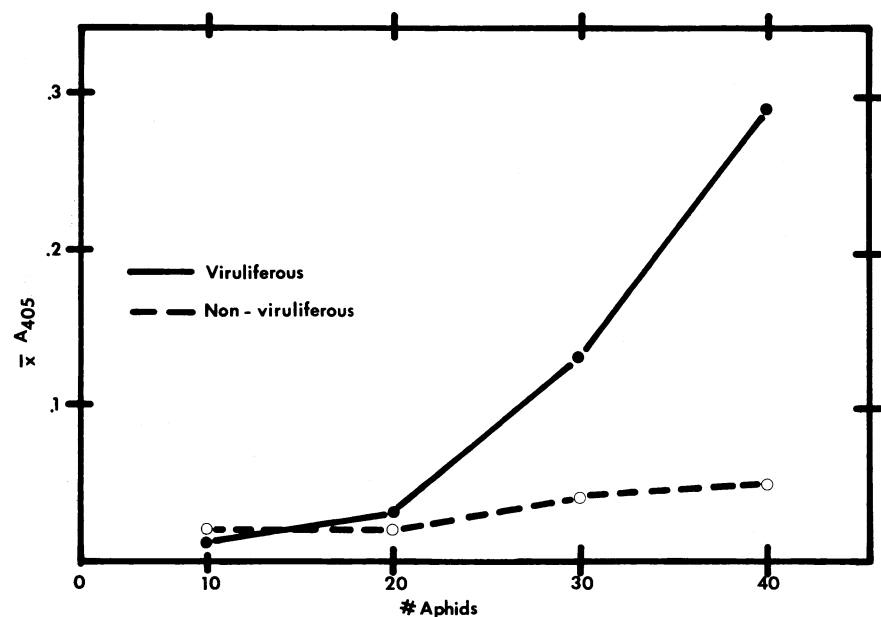
Virus condition	Number of tubers	A <sub>405</sub> for leaves	Mean A <sub>405</sub> for within-tuber sites			
			Stem-end	Bud-end eye	Middle-eye	Overall <sup>a</sup>
PLRV-infected	8	0.98 ± 0.28	0.54 ± 0.32	0.79 ± 0.66	0.63 ± 0.39	0.68 ± 0.28
Noninfected	6	0.02 ± 0.03	0.33 ± 0.08	0.29 ± 0.06	0.33 ± 0.07	0.32 ± 0.03

<sup>a</sup>Difference between means was significant ( $P = 0.05$ ) using  $t'$  statistic.

**Table 2.** ELISA absorbance readings for potato leafroll virus from Russet Burbank potato tubers

Sample designation <sup>a</sup>	Number of tubers	Range of A <sub>405</sub> values of ELISA reactions	ELISA reading
Infected	10	0.09–0.60	0.28 ± 0.19
Noninfected	10	0.05–0.08	0.07 ± 0.01
Healthy standard	5	0.05–0.07	0.06 ± 0.01
Sample 2			
Infected	7	0.16–0.59	0.36 ± 0.19
Noninfected	13	0.08–0.14	0.12 ± 0.02
Healthy standard	5	0.07–0.13	0.08 ± 0.03
Sample 3			
Infected	6	0.21–0.59	0.36 ± 0.13
Noninfected	14	0.08–0.14	0.17 ± 0.02
Healthy standard	5	0.14–0.20	0.16 ± 0.02

<sup>a</sup>Each sample consisted of 20 tubers. The A<sub>405</sub> values of ELISA reactions to these tubers were rated as positive for potato leafroll virus if they exceeded a threshold value equal to the mean absorbance reading of the healthy standard for that plate plus twice its standard deviation.



**Fig. 1.** Mean absorbance values (A<sub>405</sub>) of tritirates of various-sized samples of adult *Myzus persicae* allowed 3-day access to potato leafroll virus-infected *Physalis floridana*. Nonviruliferous aphids were included as controls.

Three groups of five tubers from the Oregon Potato Certification Program, known to be free from PLRV, were similarly sampled. ELISA absorbance readings were obtained from duplicate wells.

**Aphid testing.** Apterous adult green peach aphids *Myzus persicae* (Sulzer) were allowed 3-day access to PLRV-infected *Physalis floridana* plants. Ninety

percent of single *M. persicae*, given 3-day access to PLRV-infected *Physalis* plants, were able to transmit the virus (R. G. Clarke, unpublished). Groups of 10, 20, 30, and 40 aphids were then tritirated with glass rods in 0.3 ml of extraction buffer per group in the depression wells of Corning spot plates. Equal volumes of Amberlite XAD-4 (Rohm and Haas Co., Philadelphia, PA) were mixed with the

tritirates to reduce background absorbance (W. D. Loomis, personal communication). The homogenates were centrifuged at 3,000 g for 5 min and the supernatant fluids were placed in single ELISA wells. Nonviruliferous aphids given similar treatment were used as controls.

## RESULTS

**PLRV standards.** A<sub>405</sub> values for ELISA reactions to healthy Russet Burbank potato leaves averaged 0.02 (Table 1). Leaf samples from plants grown from all eight PLRV-infected tubers exceeded the threshold value of 0.08 (mean of infected potato leaves, 0.98). ELISA readings varied considerably among sample sites from individual tubers. Pooling all readings from a given tuber decreased this variation (Table 1). The threshold value calculated from the six noninfected standard tubers (0.38) was exceeded by mean readings from the eight PLRV-infected tubers. The difference between the average A<sub>405</sub> value from tubers known to be infected (0.68) and healthy tubers (0.32), when evaluated by means of the  $t'$  statistic (11), was significant at the 5% level of probability. Middle-eye samples from a separate group of 14 Russet Burbank healthy tubers from the Oregon Potato Certification Program averaged A<sub>405</sub> 0.24 ± 0.03.

**Field testing.** Three samples of 20 Russet Burbank tubers from a commercial field were tested for the presence of PLRV by multiple-eye sampling and determining the ELISA absorbance of the tritirate (Table 2). An average of 38% of the field tubers sampled was infected with PLRV. In each sample, mean absorbance values of infected tubers significantly exceeded mean absorbance values of virus-free standard tubers and of noninfected tubers ( $P = 0.01$ ).

**Aphid testing.** PLRV was readily detectable in aphids that fed on PLRV-infected *Physalis* plants when at least 30 aphids were included in sample tritirates. A<sub>405</sub> values for ELISA reactions to tritirates of aphids given 3-day access to PLRV-infected *Physalis* plants increased rapidly when the number of aphids per sample exceeded 20 (Fig. 1). Tritirates of 40 viruliferous aphids had a mean absorbance value of 0.29 ± 0.13, compared with 0.05 ± 0.021 for the comparable healthy group (threshold value = 0.09). ELISA absorbance values for PLRV of tritirates of nonviruliferous *M. persicae* aphids were unaffected by numbers of aphids per sample (mean A<sub>405</sub> = 0.03 ± 0.019).

## DISCUSSION

Serologic detection of PLRV in the United States by means of antiserum prepared against a foreign isolate of PLRV has not been previously reported. It is now clear that PLRV isolates from Germany (2) and Oregon react in the ELISA test with an antiserum (9) produced against a Japanese isolate of this virus.

Our data demonstrated that healthy potato tubers had a relatively high background absorbance in the ELISA system for PLRV detection. The technique was nevertheless successful for detecting PLRV in tubers, in the range of virus concentrations for which the response in  $A_{405}$  values of ELISA reactions was linear. Multiple-eye sampling compensated for the variation in PLRV content in a given tuber. Under these conditions, statistical procedures were used to define threshold values for positive determinations. We believe the ELISA method will be useful for rapid estimation of the number of PLRV-infected potato tubers in commercial samples.

The ELISA test has been used to detect cucumber mosaic virus in *Aphis gossypii*

(6). Successful detection of a persistent plant virus in an aphid vector by the ELISA method has not previously been reported. Our data show a dosage-response between the amount of PLRV detected by the ELISA test and the number of viruliferous aphids sampled.

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