Incidence, Distribution, and Association of *Spongospora subterranea* and *Potato mop-top virus* in Costa Rica

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ABSTRACT

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A survey was conducted in 39 potato (*Solanum tuberosum*) fields in Costa Rica to determine incidence and association of *Spongospora subterranea* f. sp. *subterranea* and *Potato mop-top pomovirus* (PMTV). The fields were located in Costa Rica's two major potato-production regions and were further characterized by their altitude. In all, 633 paired samples of leaf tissue and corresponding tubers were collected, assessed visually for disease, and subsequently assayed by enzyme-linked immunosorbent assay (ELISA). *S. subterranea* presence in tuber tissue was tested by double-antibody sandwich (DAS)-ELISA and PMTV presence in leaf and tuber tissues was tested by triple-antibody sandwich (TAS)-ELISA. Moreover, soil samples were collected from 10 fields surveyed and were evaluated for both pathogens via ELISA and bioassay. The incidence of both diseases ranged from 0 to 100% within individual fields, with incidences lower than 40% occurring in more than 70% of the fields. Higher incidences were found in fields located at higher altitudes. Of the 633 paired samples, 179 and 146 were positive for PMTV and *S. subterranea*, respectively, according to ELISA in either the foliage or tubers. A low correlation was found for PMTV visual symptoms and ELISA test results. Only 14 of the 81 foliar samples testing positive for PMTV had visual symptoms; the remaining 67 samples were asymptomatic. Conversely, comparison of visual evaluation with detection of *S. subterranea* by ELISA on tubers showed that 70% of the results were coincident. *S. subterranea* was detected in 4 of 10 soil samples tested by ELISA. Soilborne PMTV was detected by ELISA in roots of bait plants sown in these soil samples. Co-occurrence of both pathogens was detected in 64 samples. A significant but low degree of association for vector and virus was determined, and data suggests that *S. subterranea* is participating in the transmission of PMTV in Costa Rica in low frequency.

Additional keywords: epidemiology

Powdery scab of potato (*Solanum tuberosum* L.) is caused by the plasmodiophorid *Spongospora subterranea* (Wallr.) Lagerh. f. sp. *subterranea* J.A. Toml. (17). *S. subterranea* is also a vector of *Potato mop-top virus* (PMTV; 2,14). The diseases are recognized as important threats to potato production in Northern Europe, China, Japan, South America, and Australia (12,36), and the presence of PMTV was recently confirmed in North America (39). Both diseases have been detected in Costa Rica

(23,34), where no information on their epidemiology exists.

Powdery scab and PMTV are mainly blemish diseases of potato tubers. The symptoms of powdery scab are occasionally difficult to recognize and may be confused with common scab, caused by *Streptomyces* spp. *S. subterranea* causes lesions on tuber skin, cankers, and tuber deformation (8,13,17), and has been shown to affect plant growth and yield (9,13). PMTV causes "spraing" of potato, which is characterized by the presence of rust-colored necrotic arcs and patterns in the flesh or the skin of the tubers (4). However, PMTV is erratically distributed through the plant and asymptomatic infection of the tubers is common in some cultivars (11,27,36).

S. subterranea and PMTV can be found in soil or in potato tubers used as seed. In the soil, the virus survives for long periods of time in association with *S. subterranea* resting spores (14,15). Therefore, dissemination of both pathogens within or between fields can occur through the movement of infested soil or infected tubers

(14,15). Studies using artificially spiked soil have shown that *S. subterranea* can be detected directly by enzyme-linked immunosorbent assay (ELISA) with a detection limit of 100 sporosori/g of soil (22,38). However, the vector and virus can be detected with greater efficiency in soil using a bioassay with bait plants (19,21,32,38). The bioassay has been coupled with ELISA and polymerase chain reaction (PCR) techniques for quantitative detection of both pathogens (1,32); however, it has been suggested that ELISA testing of soil samples may be more useful than the bioassay for predicting disease intensity on tubers (38).

No chemical treatment is available for effective control of *S. subterranea* or PMTV. The most efficient measure for prevention of disease is avoidance; that is, to plant clean seed into uninfested soil (20). Therefore, knowledge of vector and virus presence in soil is a crucial component to disease management (6). Moreover, the relative importance of each source of inoculum in the development of the disease in the field is unknown, and it is difficult to study this aspect of their epidemiology due to the complexities and uncertainties of the pathogen's biology (6). The objectives of this research were to (i) determine the incidence of *S. subterranea* and PMTV in commercial potato-growing regions in Costa Rica and (ii) determine the magnitude of association between *S. subterranea* and PMTV.

MATERIALS AND METHODS

Field survey and sample collection.

Thirty-nine potato fields were sampled throughout Costa Rica's main potato-growing areas, Cartago (cultivated area estimated at 2,400 ha) and Zarcero (approximately 270 ha). Samples were collected throughout 2002 and the first six months of 2003. In all, 25 fields were sampled in Cartago, identified as Car-1 to Car-25, and 13 fields were sampled in Zarcero, labeled Zar-1 to Zar-13. A field (RR-1) dedicated to tuber seed production in Rancho Redondo area was also sampled. The Cartago area was stratified according to altitude into a zone below 1,800 m above sea level (m.a.s.l.), a zone between 1,800 and 2,500 m.a.s.l., and a zone above 2,500 m.a.s.l. The altitude of Zar-

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cero extends from 1,500 to 2,100 m.a.s.l. The majority of sampled fields were planted with either cv. Granola or cv. Floresta (clone CIP386040.9). Nevertheless, some growers had mixed plantations or had other cultivars during the survey. Growers obtained their seed from local seed producers or they used their own seed produced from the previous crop.

Individual fields were variable in size, with most being less than one-half of a hectare. In all, 10 or 20 plants were sampled in each field according to field size with one exception, where 15 plants were sampled in Car-10. Fields were divided into two (10 samples), four (20 samples), or three (15 samples) subplots and, from each subplot, five plant samples were chosen in the same "zig-zag" pattern. Several leaves were taken randomly from each plant during bloom and two or three tubers from the same plant were collected at harvest. Due to some losses, the final number of paired samples of leaf tissue and corresponding tubers collected was 633. Incidence of PMTV or *S. subterranea* at each field was calculated based on ELISA results (tests described below).

Soil samples were collected at 10 of the surveyed fields. Soil was collected from around each plant sampled at the corresponding field and mixed together to get a composite field sample. These 10 soil samples were left to air dry at room temperature in the laboratory (approximately 20°C) for a minimum of 10 weeks where they were tested for *S. subterranea* by ELISA and used subsequently as inoculum in a modified bioassay to test for PMTV (19).

Evaluation of foliar and tuber tissue samples. *Visual rating of foliar tissue.* The 633 foliar samples were visually inspected for the presence of PMTV-like symptoms (aucuba, chevrons, and calico spots arranged in "V" pattern) prior to ELISA testing for the virus as described below.

Visual rating of tuber tissue. Tuber samples were stored in paper bags upon arrival at the laboratory and kept between 18 and 22°C for approximately a month before evaluation (27). To rate tubers, each sample was lightly washed under running tap water and dried overnight on a paper towel. Tuber samples were visually evaluated for the presence of powdery-scab-like lesions. Observations were scored per plant (sample) as "positive" for tubers with powdery scab lesions, "negative" for tubers with no apparent lesions or deformities, or "doubtful" for tubers in which lesions and deformities existed but appeared different than powdery scab. Afterward, tuber samples were tested for the presence of *S. subterranea* and PMTV.

ELISA testing for PMTV. The presence of PMTV in potato leaves and tuber samples was tested by triple-antibody sandwich (TAS)-ELISA using specific commercial antibodies (Neogen Europe Ltd.,

Auchincruive, Scotland, UK) following the manufacturer's instructions. Foliar tissue samples were ground 1:5 (wt/vol) in general extraction buffer (0.010 M NaSO₃, 0.001 M KH₂PO₄, 0.008 M Na₂HPO₄, 0.136 M NaCl, 0.002 M KCl, 2% polyvinyl-pyrrolidone [PVP; wt/vol], 0.05% Tween 20 [vol/vol], and 0.2% egg albumin [wt/vol], pH 7.4). For tuber analysis, one or two flesh tissue cubes (0.5 × 0.5 × 0.5 cm) were taken from the midregion of each tuber and ground 1:1 (wt/vol; 24) in general extraction buffer in a plastic sample bag lined with mesh (Agdia Inc., Elkhart, IN). No new shoot growth was present on the tubers at the time of testing. Samples were tested in duplicate and all plates included certified negative and positive controls. Plates were read after 60 min in a Dynex MRX microplate reader at 405 nm (Dynex Technologies, Chantilly, VA).

ELISA testing for S. subterranea. *S. subterranea* presence on tubers was tested by double-antibody sandwich (DAS)-ELISA using commercial monoclonal antibodies specific for *S. subterranea* sporosori (BIOREBA AG, Reinach, Switzerland) according to the manufacturer's instructions. For each sample, tuber skin tissue was peeled off at two different sites on each tuber (average weight, 0.10 g). When typical or suspicious powdery-scab lesions were present, these areas were chosen for analysis. The skin tissue was ground 1:10 (wt/vol) in tuber extraction buffer (0.020 M Tris, 0.136 M NaCl, 0.002 M KCl, 2% PVP [wt/vol], 0.05% Tween 20 [vol/vol], and 1% egg albumin [wt/vol], pH 7.4). Samples were tested as described above, but read after 120 min.

Samples tested for either PMTV or *S. subterranea* with ELISA absorbance values higher than the mean of the negative controls plus three standard deviations were considered positive (29).

Data analysis. For each pathogen, a χ^2 test was used to determine whether the proportion of positive samples between Cartago and Zarco and among the different altitude zones at Cartago differed significantly (40). A two-by-two and three-by-two table, respectively, were constructed for these analyses.

A χ^2 test of independence was used to test for association between the apparent visual foliar symptoms of PMTV and its corresponding detection by ELISA (40); the Yates continuity correction factor was applied to this two-by-two table. The same analysis was used to test for association between disease ratings of powdery scab obtained from visual evaluation of tubers and its detection by ELISA; a three-by-two table was constructed for this analysis. Cramer's V^2 was used to measure the strength of association for each test (10). Cramer's V^2 is a χ^2 -based measure of nominal association and, like most indices of association, gives a value from 0 to 1. The index is preferred to other measures

because it corrects for table size; however, the maximum value of 1 can only be achieved when row and column marginals are equal. V^2 is calculated as χ^2/nm , where n is the sample size and m is the smaller of (rows - 1) or (columns - 1).

Detection of *S. subterranea* and PMTV in soil samples. For each soil sample, 0.3 g of soil was homogenized in 1.0 ml of tuber extraction buffer in a microcentrifuge tube for approximately 30 s. The macerate was left to settle for 15 to 30 min and the supernatant was used for detection of *S. subterranea* sporosori by ELISA as described above. Autoclaved soil from a field where potato was never planted served as a negative control and a commercial *S. subterranea*-positive control (BIOREBA AG) were included in all plates.

The presence of soilborne PMTV was tested in the soil samples by a bioassay. Tomato (*Lycopersicon esculentum* Mill. cv. Supermarmande), *Nicotiana benthamiana* Domin., and *N. tabacum* L. var. Xanthi were used as bait plants. Tomato seed were planted in plastic nursery trays filled with sterilized hydroponic media (50% quartz sand/50% grounded coconut fiber). The seeds were watered with a threefold dilution of a modified Hoagland's nutrient solution containing 1.48 mM NH₄H₂PO₄, 6.34 mM Ca(NO₃)₂, 5.44 mM KNO₃, 2.04 mM MgSO₄, 0.05 mM H₃BO₃, 0.001 mM CuSO₄, 0.008 mM MnSO₄, 0.003 mM ZnSO₄, 0.00005 mM NaMoO₄, and 0.012 mM Fe-EDTA, adjusted to pH 5.2 and electric conductance of 2 dSm⁻¹. The seed were kept in a Percival E-30B growth chamber (Percival Scientific, Inc., Perry, IA) with a 24-h light period at 22°C for 7 days. Afterward, the growth conditions were adjusted to 16 h of light at 18°C and 8 h of darkness at 15°C for an additional 6 weeks. *N. benthamiana* and *N. tabacum* L. var. Xanthi seed were sown in hydroponic media and placed in a growth chamber set for 16 h of light at 18°C and 8 h of darkness at 15°C for 1 week. Thereafter, plantlets were kept in the laboratory at approximately 20°C for 4 weeks.

To perform the bioassay, bait plants were transferred to plastic pots filled with 150 g of the soil samples. There were three plants per pot, three pots per soil sample, and one pot for each plant species. Bait plants planted in disinfested soil were used as a negative control. Plants were maintained in the laboratory at approximately 20°C for 3 months. They were then removed from their pots and the roots were washed under tap water and frozen until ELISA testing 1 week later. To perform the ELISA, the entire root system of the bait plant was ground in 1 ml of general extraction buffer (the same used for leaf tissue) and samples were tested for PMTV in duplicate as described above for leaf tissue.

Statistical analysis of the association between PMTV and *S. subterranea*. The

association between PMTV and *S. subterranea* was determined with the Jaccard index of similarity (J) for the 633 paired foliar and tuber samples (31). In addition, the index was calculated for subsets of the data in separate analyses by region (i.e., Cartago and Zarcero) as well as by altitude within the Cartago region. The values of the index ranged from 0 to unity, where values close to 1 are suggestive of a high degree of association and values close to 0 represent dissociation. The Jaccard index was calculated using: $J = a/(a + b + c)$, where a represents the number of samples where both the virus and vector occurred, b represents the number of samples where the virus occurred but the vector was absent, and c represents the number of samples where the vector occurred but the virus was absent. The Jaccard similarity index does not make use of the number of samples where neither the virus nor its vector occurred. Therefore, it represents the probability of both the virus and vector being present in the same sample within the set of samples where virus or vector are found.

To test the null hypothesis of independence, a Z statistic was calculated using the procedure described by Turechek (31). In short, randomizations of the data were used to calculate a value, $\bar{J}_{ran}$, which

represents the expected value of the index if the virus and its vector were distributed independently given their observed incidences (i.e., under the null hypothesis of independence). A nonparametric estimate of the standard error of the Jaccard index, s_j , was obtained using the jackknife procedure (18).

To test whether the observed Jaccard index was significantly different from the value calculated under the assumption of independence, a normal distribution was assumed for the index estimated by J (the observed Jaccard value) and standard error estimated by the jackknifed value, s_j . A standard normal statistic was calculated using:

$$Z = \frac{J - \bar{J}_{ran}}{s_j} \quad (1)$$

Treating Z as a two-sided test, values of $Z > 1.96$ indicate significant positive association and values of $Z < -1.96$ indicated significant negative association or dissociation at $P = 0.05$.

Association was also assessed using the χ^2 test of independence and its magnitude was determined with Cramer's V^2 as described above. These were calculated for comparison with the Jaccard index because these statistics use different information to measure association.

RESULTS

Field survey. *S. subterranea* was detected by ELISA in at least one tuber sample in 28 of the 39 potato fields surveyed: in 16 out of 25 fields at Cartago, in 11 out of 13 fields at Zarcero, and in the potato plantation at Rancho Redondo (Table 1). PMTV was detected in at least one leaf or tuber sample in 35 fields: 24 at Cartago and 11 fields at Zarcero (Table 1). *S. subterranea* and PMTV incidences were below 40% in more than 70% of the fields, but incidences of 100% were found for both diseases in single but separate fields.

The proportion of the total number of *S. subterranea*-positive samples (Table 2) was similar at Cartago (80/394) and Zarcero (54/225) ($P = 0.3104$). However, there was a significant difference between the proportion of PMTV-positive samples between Cartago (134/394) and Zarcero (45/225) ($P = 0.0002$). Disease incidence for both *S. subterranea* and PMTV at Cartago showed a tendency to vary with altitude, with disease incidence being greater at higher altitudes. This can be seen at the field level (Table 1) and among the individual paired samples (Table 2). The highest incidence of PMTV and *S. subterranea* was found at altitudes greater than 2,500 m.a.s.l. at Cartago. When the proportions of the total number of positive

Table 1. Number of potato fields within each incidence class testing positive by enzyme-linked immunosorbent assay to *Spongospora subterranea* (S) and to *Potato mop-top pomovirus* (P) in relation to the region of production and elevation in Costa Rica^a

Class	Cartago													
	Elevation ^b													
	>2,500 (7)		2,500–1,800 (9)		<1,800 (9)		All (25)		Zarcero (13)		RR ^c (1)		Total (39)	
	S	P	S	P	S	P	S	P	S	P	S	P	S	P
0.00	0	0	3	0	6	1	9	1	2	2	0	1	11	4
0.01–0.20	0	1	4	4	3	5	7	10	6	7	0	0	13	17
0.21–0.40	3	0	0	2	0	3	3	5	3	2	0	0	6	7
0.41–0.60	1	2	1	1	0	0	2	3	0	2	0	0	2	5
0.61–0.80	2	1	0	1	0	0	2	2	1	0	0	0	3	2
0.81–1.00	1	3	1	1	0	0	2	4	1	0	1	0	4	4

^a Tubers of 10, 15, or 20 plant samples per field were tested depending on the field size. Number in parentheses = total per elevation or region.

^b Elevation is measured in meters above sea level.

^c Rancho Redondo.

Table 2. Association of *Potato mop-top pomovirus* (PMTV) and *Spongospora subterranea* based on enzyme-linked immunosorbent assay test results and calculated with the Jaccard index and Cramer's V^2 for 633 paired samples (N = number of samples) of foliar and tuber tissue collected at 39 different potato fields in Costa Rica

Locality ^d	N	PMTV/ <i>S. subterranea</i> ^a				Jaccard analysis ^b				χ^2 Analysis ^c			
		+/+	+/-	-/+	-/-	J	s_j	$\bar{J}_{ran}$	P	χ^2	P	V^2	
Cartago area	394	54	80	26	234	0.338	0.038	0.146	<0.001	50.16	<0.001	0.1273	
>2,500 m.a.s.l.	80	29	24	16	11	0.420	0.060	0.438	0.382	0.150	0.6995	0.0018	
1,800–2,500 m.a.s.l.	149	22	31	8	88	0.361	0.062	0.150	0.001	23.37	<0.001	0.1569	
<1,800 m.a.s.l.	165	3	25	2	135	0.100	0.056	0.021	0.151	6.776	0.035	0.0410	
Zarcero area	225	10	35	44	136	0.112	0.034	0.122	0.382	0.097	0.755	0.0004	
RR locality	14	0	0	12	2	0.000	...	...	...	...	...	...	
Total	633	64	115	82	372	0.240	0.027	0.146	<0.001	22.65	<0.001	0.0358	

^a Contingency table values indicating positive (+) or negative (–) test results.

^b J = Jaccard similarity index; s_j = jack-knifed estimate of the standard error of J ; $\bar{J}_{ran}$ = expected value of the Jaccard index if the occurrence of the two organisms is independent; P = probability that J is significantly different than $\bar{J}_{ran}$ according to Z test; ... indicates no association analysis performed because there was no PMTV detection.

^c Cramer's V^2 ranges from 0 to 1, where 1 represents perfect association; ... indicates no association analysis performed because there was no PMTV detection.

^d Cartago area subdivided by altitude (m.a.s.l. = meters above sea level); RR = Rancho Redondo.

samples were compared for the different Cartago altitudes (Table 2), there was a statistical difference ($P < 0.001$) between each altitude for either disease.

Detection of PMTV in potato leaves and tubers. The detection of PMTV by ELISA varied among the different tissues tested on the same plant. Of the 179 plants that tested positive for PMTV, 63 samples tested positive in their foliage, 98 samples were positive in tuber tissue, and only 18 samples tested positive for PMTV in both foliar and tuber tissue simultaneously. Of the 81 (63 + 18) foliar samples testing positive for PMTV, only 14 had visual symptoms; the remaining 67 samples were asymptomatic (Table 3). However, there were an additional 36 samples that had PMTV-like symptoms but tested negative by ELISA (Table 3). The χ^2 test of association indicated a lack of independence between visual symptoms and ELISA test results for PMTV ($\chi^2 = 11.26$, $df = 1$, $P = 0.0008$).

Detection of *S. subterranea* in potato tubers. For powdery scab, 56 samples were rated as positive, 454 as negative, and 123 as doubtful according to visual evaluation of tuber symptoms (Table 4). Comparison of visual evaluation with detection of *S. subterranea* by ELISA indicated that 70% of the results were coincident; that is, of the 510 samples that could be visually classified as *S. subterranea* positive or negative, only 61 samples were incorrectly classified when compared with ELISA results (Table 4). From these 61 samples, 47 (7.4% of the 633 samples) were visu-

ally rated as negative, with no apparent lesion on the tubers, but tested positive by ELISA. Conversely, 14 samples were visually rated as positive but negative by ELISA. The 123 samples rated as doubtful represented 19.43% of the total and, from these, 57 were found ELISA positive and 66 were found to be ELISA negative. The χ^2 test of association indicated a lack of independence between visual symptoms and ELISA test results for *S. subterranea* ($\chi^2 = 164.02$, $df = 2$, $P < 0.0001$). Cramer's V^2 was 0.2591, indicating a low degree of association.

Detection of *S. subterranea* and PMTV in soil samples. ELISA was performed to determine pathogen presence in 10 soil samples collected as part of the survey. Of the 10 samples, 4 tested positive for *S. subterranea*. These same four soil samples also tested positive for PMTV in the bioassay (Table 5). There was no apparent association between the detection of *S. subterranea* in the soil samples and the incidence of *S. subterranea* or PMTV in the plants sampled from these fields (*data not shown*).

Soilborne PMTV was detected in at least one of the three bait plant species in 8 of the 10 field soil samples assayed (Table 5). Soilborne PMTV was not detected in bait plants from soil samples Car-F2 and Car-F15 and only a low absorbance value was detected in *N. tabacum* for soil sample Car-F11. For several soil samples, *N. benthamiana* produced higher PMTV titer than did tomato or *N. tabacum*.

Association between PMTV and *S. subterranea*. The value of the Jaccard index of association for the 633 paired samples was 0.245, indicating a low but significant level of association ($\alpha = 0.05$) according to the randomization test (Table 2). Similarly, the χ^2 test of independence indicated significant association, and the

magnitude of association was low according to Cramer's V^2 , which is in agreement with the results obtained with the Jaccard index. When the data set was stratified by elevation, results indicated that the diseases were associated at altitudes between 1,800 and 2,500 m.a.s.l., and the results were mixed at elevations below 1,800 m.a.s.l. In contrast, the two diseases occurred independently (i.e., they were not associated) at altitudes greater than 2,500 m.a.s.l. at the Cartago and Zarcero areas according to both tests.

DISCUSSION

S. subterranea and PMTV were found to occur throughout the two potato-growing regions surveyed. The incidence of each disease in most plantations was typically less than 20% (Table 1) but incidences of 100% were detected in two fields. In Cartago, the number of samples with one or both of the pathogens increased with altitude (Tables 1 and 2). At a higher altitude lower temperatures, higher humidity, and longer periods of soil wetness may be contributing factors to the increase of disease because such conditions are favorable for the development of the vector (33,35) and virus (5,11).

In general, the data showed that where more PMTV occurred, more *S. subterranea* was detected. However, the degree of association between PMTV and *S. subterranea* in potato plants, as determined by the Jaccard index and Cramer's V^2 , varied with altitude and production region (Table 2). The indices calculated for the complete data set and the Cartago mid-level-altitude data set showed a significant level of association ($P < 0.001$) between the virus and its vector. However, the magnitude of associations detected for these data sets were generally low, agreeing with previous findings (Table 2) (25,30). Temperature and

Table 3. Contingency table for the relationship between leaf visual symptoms and enzyme-linked immunosorbent assay (ELISA) test results for *Potato mop-top pomovirus* (PMTV)

ELISA ^b	Visual symptoms ^a		Total
	+	-	
+	14	67	81
-	36	516	552
Total	50	583	633

^a Visual symptoms observed (+) or no visual symptoms (-).

^b ELISA test result confirm presence of PMTV (+) or no PMTV (-).

Table 4. Contingency table for the relationship between visual symptoms and enzyme-linked immunosorbent assay (ELISA) test results for powdery scab of potato, caused by *Spongospora subterranea*

ELISA ^b	Visual symptoms ^a			Total
	+	-	?	
+	42	47	57	146
-	14	407	66	487
Total	56	454	123	633

^a Visual symptoms observed (+), no visual symptoms (-), or visual symptoms inconclusive (?).

^b ELISA test result confirmed presence of *S. subterranea* (+) or no *S. subterranea* (-).

Table 5. Detection by enzyme-linked immunosorbent assay (ELISA) of *Spongospora subterranea* in field soil samples and *Potato mop-top pomovirus* (PMTV) in the roots of three bait plant species sown in field soil samples and in disinfested control soil^a

Soil sample	ELISA for ^b			
	PMTV			
	<i>S. subterranea</i>	<i>L. esculentum</i>	<i>N. tabacum</i>	<i>N. benthamiana</i>
Control soil	-	0.036 (-)	0.040 (-)	0.038 (-)
Car-F2	-	0.035 (-)	0.033 (-)	0.043 (-)
Car-F6	+	0.239 (+)	0.283 (+)	0.495 (+)
Car-F7	+	0.103 (+)	0.044 (-)	0.871 (+)
Car-F8	+	0.211 (+)	0.206 (+)	0.708 (+)
Car-F10	-	0.987 (+)	0.043 (-)	0.362 (+)
Car-F11	-	0.039 (-)	0.067 (+)	0.035 (-)
Car-F15	-	0.033 (-)	0.032 (-)	0.033 (-)
Car-F17	-	0.039 (-)	0.382 (+)	0.189 (+)
Zar-F2	+	0.060 (+)	0.112 (+)	0.554 (+)
Zar-F5	-	0.123 (+)	0.320 (+)	0.980 (+)
No. of positives	4	6	6	7

^a Plant bait species were *Lycopersicon esculentum* var. Supermarmande, *Nicotiana tabacum* var. Xanthi, and *N. benthamiana*.

^b ELISA absorbance value at 405 nm. Samples with ELISA absorbance values higher than the mean of the negative controls plus three standard deviations were considered positive. Positive (+) or negative (-) samples are indicated for field soil and bait plants.

humidity, as they change with altitude, may be factors influencing the degree of association among regions. Temperature and humidity have been noted to affect other plasmodiophorid vector-virus associations (7).

The data from the soil bioassay showed that transmission of soilborne PMTV occurred readily (Table 5), despite our inability to detect *S. subterranea* by ELISA in four of the eight PMTV-positive samples. The inability to detect *S. subterranea* suggests reduced sensitivity of the ELISA test relative to the bioassay, which would affect survey results. A similar difference in sensitivity between ELISA and bioassay was demonstrated by Walsh et al. (38). Additionally, differential susceptibility to *S. subterranea* infection has been noted between potato roots and tubers (21). Thus, root or stolon infection may be occurring in the field without a corresponding tuber infection. Both circumstances would affect the results of the survey and could explain the low degree of association between *S. subterranea* and PMTV found in this study and other studies (25,30).

The degree of association and the actual infection rates for both diseases were possibly underestimated in the present work due to the limitations of the detection technique employed. ELISA is known to have lower sensitivity than PCR-based methods, such as reverse-transcriptase PCR (1,24,27,30). Detection may be hampered due to the erratic distribution of PMTV in the tissue (11,27,39) as well as the dormancy status and the storage conditions of the tubers (24,27,39). Thus, the timing and details of the sampling methodology impact PMTV detection. In the case of *S. subterranea*, there are PCR-based methods available that are more sensitive than ELISA (26,32). Moreover, the ELISA antibodies used in this work only detect sporosori (38); thus, developing infections in the tubers may not have been detected. Testing soil samples directly by ELISA for the presence of *S. subterranea* may be affected by soil compounds or the distribution and concentration of the sporosori in the soil (37,38). On the other hand, bioassay with soil samples has greater sensitivity than the ELISA technique due to an amplification effect (secondary zoospores). In future studies, it is recommended that PCR-based techniques be used, tuber samples should include tissue from both ends of the tuber, and root system samples should be analyzed as well.

Regarding the visual evaluation, no potato tuber collected during the survey showed PMTV-like spraing symptoms: slightly raised, necrotic, or partly necrotic, concentric rings (4). It is possible that cultivar resistance, environmental conditions, PMTV strain, or an interaction of one or more of these factors suppressed the development of these symptoms. It is in-

teresting to note that, sometimes, a reticulate cracking on the surface of a tuber was observed, which has been strongly correlated with PMTV infection in the Andes (30). With respect to foliar infection, about 82% of the foliar samples testing positive for PMTV were asymptomatic and several foliar samples with symptoms typical of PMTV tested negative with ELISA. The symptoms of PMTV infection in potato can be confused with several other viral infections. For example, aucuba symptoms caused by PMTV in leaf tissue may be confused with symptoms caused by *Alfalfa mosaic virus* or *Potato aucuba mosaic virus* (28,39).

The results obtained in this study indicate clearly that relying on symptoms to select PMTV-free plants and tubers or to make disease management decisions is not reliable (30). In this study, detection of PMTV in tubers by ELISA was greater than in foliar tissue, coinciding with former results (27). Interestingly, the detection of PMTV in tubers was not coincident with its detection in leaf tissue (i.e., few samples tested positive for PMTV in leaves and tubers simultaneously). Such inconsistencies may be due to the ELISA technique limitations discussed previously or to uneven distribution of the virus. The results indicate the need for testing of both tissues when surveying fields to avoid underestimation of virus incidence.

In contrast to PMTV, only 47 of 146 samples that tested positive for *S. subterranea* by ELISA were completely asymptomatic. These results suggest that visual inspection of tubers by farmers, especially in developing countries where other testing techniques may not be available, should be helpful in reducing the number of infected tubers in a seed lot. However, it should be noted that high frequency of latent tuber infection has been reported in other studies, which can be explained by unfavorable conditions for disease development (33).

Overall, it appears that vector-transmission of PMTV occurs in Costa Rica and that its importance in the transmission of the virus is determined by additional factors not revealed in this study. Future research for *S. subterranea* and PMTV should focus on understanding the factors that determine the relative importance of soilborne or seedborne inoculum in the development of the disease and modeling this virus-vector interaction. Determining the effects of climate, soil moisture, and soil characteristics should be the first step to gaining a better understanding of disease development (3) and, thus, disease management strategies. Second, PMTV is not homogeneously distributed in the plant as evidenced from ELISA data and erratic symptom development (30). Thus, additional research should be conducted to resolve the factors involved in field symptom development of PMTV. Lastly, the chemical control of the plas-

modiophorid vectors of viruses is very difficult and unjustified due to economical or environmental restraints (16). The use of resistant cultivars is a control measure that can be used once a field is contaminated, but such cultivars are not yet commercially available. A possible measure to manage the spread of the diseases is to implement strict seed surveillance to avoid the contamination of disease-free fields. Additionally, field soils should be tested to evaluate soil level of infestation and use this information in an integrated management approach (6,20). It is very important, in the case of Costa Rica, to evaluate the susceptibility of new cultivars prior to their commercial release in order to avoid epidemic outbreaks of the diseases as has occurred in Europe and Australia (13,20,35).

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