Identification of Maize Viruses and Mollicutes and Their Potential Insect Vectors in Peru

L. R. Nault, D. T. Gordon, R. E. Gingery, O. E. Bradfute, and J. Castillo Loayza

Professor, Department of Entomology; professor, Department of Plant Pathology; research chemist, Science and Education Administration, U.S. Department of Agriculture; associate professor, Department of Plant Pathology, all at the Ohio Agricultural Research and Development Center (OARDC), Wooster, 44691; and associate professor, Department of Plant Pathology, Universidad Nacional Agraria, La Molina, Lima, Perú.

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ABSTRACT

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Maize viruses and mollicutes and their potential vectors were surveyed in Peru. Locations and departments were the coastal valleys between Lima and Barranca (Lima), the Andean valleys of Urubamba and Calca (Cuzco), a high tropical valley near Tarapoto (San Martin), and the Callejon de Huaylas, a mountain valley between Malpaso and Caraz (Ancash). Leaf samples from 27 diseased plants were assayed. The following pathogens were detected by the methods indicated: corn stunt spiroplasma (CSS) by dark-field light microscopy; maize bushy stunt mycoplasma (MBSM) by *Dalbulus maidis* transmission and diagnostic symptoms in sweetcorn; maize rayado fino virus (MRFV) by enzyme-linked immunosorbent assay (EIA) and immunomicroprecipitin assay (MPA); a rhabdovirus, presumed to be maize mosaic virus (MMV), by electron microscopy of negatively stained sap; maize stripe virus (MStpV) by immunofluorescence (IF) and immune agar-gel double diffusion assay; maize dwarf mosaic virus strain A (MDMV-A) by EIA; and maize chlorotic mottle virus (MCMV) by IF and MPA. Maize rayado fino virus, MMV, and MCMV were detected in maize samples and MDMV-A from Johnson grass from Lima; MBSM, MRFV, MMV, and MCMV from Ancash; and CSS, MRFV, MMV, MStpV, and MDMV-A from San Martin. No maize with virus symptoms was observed in Cuzco. Prior to this survey, only MCMV and MRFV had been identified from Peru. *Dalbulus maidis* (vector of CSS, MBSM, and MRFV) and *Peregrinus maidis* (vector of MMV and MStpV) were collected from Lima, Ancash, and San Martin; these are the first reports of these species from Peru.

RESUMEN

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Se realizó una encuesta con el objeto de identificar los virus y mollicutes que causan enfermedades al maíz en el Perú. Los Departamentos y localidades visitados fueron los valles costeros entre Lima y Barranca (Departamento de Lima), los valles andinos de Urubamba y Calca (Departamento de Cuzco), el valle tropical de selva alta de Tarapoto (Departamento de San Martín), y la parte del Callejón de Huaylas comprendida entre Malpaso y Caraz (Departamento de Ancash). Se obtuvieron y analizaron 27 muestras de hojas de plantas enfermas. Se detectaron los siguientes patógenos por los métodos que se indican para cada caso. El espiroplasma del achaparramiento (CSS) por microscopia de luz en campo oscuro. El micoplasma del achaparramiento (maize bushy stunt mycoplasma—MBSM) por transmisión por *Dalbulus maidis* y síntomas en maíz dulce. El virus del rayado fino (MRFV) por pruebas serológicas del método ELISA y microprecipitación. Por microscopia electrónica de savia teñida negativamente se observó un rhabdo-virus que se presume sea el virus del mosaico del maíz (MMV). El virus mosaico del bandeado del maíz (maize stripe virus) por immunofluorescencia y doble difusión en agar-gel. El mosaico del enanismo del maiz, raza A (maize dwarf mosaic-A) por ELISA. El virus del moteado clorótico (maize chlorotic mottle virus) por immunofluorescencia y microprecipitación. En Lima, el MRFV, MMV, y MCMV se detectaron en muestras de maiz, mientras que el MDMV-A fue detectado en Johnson grass. En Ancash el MBSM, MRFV, MMV, y MCMV se detectaron en muestras de maíz e igualmente el CSS, MRFV, MMV, MStpV, y MDMV-A en muestras procedentes de San Martin. No se observaron síntomas de enfermedades virósicas en campos de maíz del Cuzco. Antes de realizarse esta encuesta sólo se habían identificado el MCMV y MRFV en el Perú. Se colectaron especimenes de *Dalbulus maidis* (vector del CSS, MBSM, y MRFV) y *Peregrinus maidis* (vector del MMV y MStpV) de los lugares visitados en Lima, Ancash, y San Martín.

Although generally it is agreed that ancestral maize, *Zea mays L.*, evolved in Mexico (8,9,19), it has been suggested that Peru also may have been an independent center for its domestication (14). Maize has been cultivated for at least 3,000 yr on the coast and at middle altitudes of the Sierras in Peru. It seems likely that during this period many insect pests and pathogens, including insect-borne viruses and mollicutes, would have become established. However, only maize chlorotic mottle virus (MCMV) (5) and maize rayado fino virus (MRFV) (11) have been identified. The presence of sugarcane mosaic virus (SCMV), maize dwarf mosaic virus (MDMV), and corn stunt spiroplasma (CSS) is suspected in Peru

(5) but this has not been confirmed. This paper reports the results of a survey for insect-borne maize pathogens and their potential vectors in Peru.

MATERIALS AND METHODS

Survey sites and collection of maize samples and insects. The survey for diseased maize samples was made from 21 February through 6 March 1978, to coincide with maize maturation in the four major production regions of Peru (Fig. 1, Table 1). At each locality, preliminary diagnoses were based on symptoms (Table 2) and two or more plants with each symptom type were selected for sampling. Three or four upper leaves (1-2 linear meters on the longitudinal axis) of a diseased plant were collected and placed in a plastic bag which was stored in a styrofoam insulated box.

Fig. 1. Collection sites in a survey of viral pathogens of maize and their potential insect vectors in Peru: Juan Guerra-1, Tarapoto-2, Malpaso-3, Caraz-4, Barranca-5, Acos-6, Huaral-7, Puente Chillon-8, La Molina-9, Urubamba-10, Calca-11.

After the maize samples were collected, a standard 30-cm-diameter insect net was used to sweep maize foliage and surrounding weeds. Leafhoppers, planthoppers and chrysomelid beetles were aspirated from the nets, killed with HCN, and placed in labeled pill boxes. In some cases insects were directly aspirated from corn foliage.

Detection of pathogens. *Corn stunt spiroplasma.* Sap expressed from leaf samples was processed according to techniques described by Davis (7) and examined by dark-field light microscopy for detection of CSS. Leaves from healthy and Rio Grande corn stunt-infected corn plants maintained in greenhouse culture were used for controls. At least 20 fields of each of two preparations were scanned before a leaf sample was recorded as negative for the presence of spiroplasma.

Maize bushy stunt mycoplasma. One hundred *Dalbulus maidis* (DeLong & Wolcott) were placed in a petri dish with 7.5–10.0 linear centimeters of diseased leaf tissue for a 48-hr acquisition access period. After a 19-day incubation period, leafhoppers were placed, five per two sweet corn seedlings (cultivar Aristogold Bantam Evergreen), for a 3- or 4-day inoculation access period. Leafhoppers were transferred serially to four sets of test plants. Test plants were placed in an environmental chamber set for a 16-hr day and an 8-hr night at 30 and 25 C, respectively. Plants were observed for 6 wk for symptom development. An isolate of maize bushy stunt mycoplasma (MBSM) from Texas was used as the control for symptom expression. *Dalbulus maidis* from stock colonies were placed on test plants to check for the presence of pathogens. None transmitted MBSM.

Maize rayado fino virus. Samples were prepared for assays by grinding 1 g of leaf tissue in 4 ml of PBS-Tween (0.15 M NaCl, 0.02 M sodium phosphate, and 0.02% sodium azide, pH 7.4, plus 0.05% polyoxyethylene sorbitan monolaurate [Tween-20]) in a mortar with a pestle. The extract was filtered through two layers of fine-mesh cheesecloth, and the filtrate was centrifuged at 12,000 *g* for 10 min. The supernatant fraction (clarified extract) was recovered and tested in the enzyme-linked immunosorbent (EIA) and microprecipitin (MPA) assays.

Antiserum to MRFV was produced by injecting a rabbit twice intravenously (1.0 ml per injection) and three times intramuscularly (2.0 ml per injection) with purified virus suspended in PBS (0.15M NaCl and 0.01 M potassium phosphate, pH 7.0). Virus for intramuscular injections was emulsified in an equal volume of Freund's complete adjuvant. Injections were made over a period of 9 wk. Virus was purified as described previously (13), except that a second rate-zonal centrifugation on sucrose gradients in 0.05 M

TABLE 1. Locations and characteristics of regions in Peru sampled in a survey for insect-borne maize viruses and mollicutes

Department and description of region	Average daily temperature extremes in February (C)	Elevation (m)	Types of maize planted (%)	Period of cultivation
San Martin—tropical valley, draining to the Amazon basin	34–17	300–360	Flint (100)	Continuous
Ancash—Callejon de Huaylas, Andes valley draining to the Pacific coast	23–8	2,200–2,800	Floury (70) Flint (30)	Summer
Lima—irrigated coastal valleys	28–20	near sea level to 500	Flint (98) Floury (2)	Continuous
Cuzco—high Sierra valleys	31–16	2,800–3,200	Floury (80) Flint (20)	Summer

potassium phosphate, pH 7.0, was substituted for the isopycnic banding in cesium chloride. A total of 0.85 mg of purified virus was injected.

Purification of γ-globulin, conjugation of alkaline phosphatase with γ-globulin, and the assay procedure for EIA was performed according to the methods of Clark and Adams (6), except as follows: One-tenth milliliter of coating γ-globulin, clarified extract, and enzyme-antibody conjugate each were added per well of polystyrene microtiter plates (Dynatech Laboratories Inc., 900 Slaters Lane, Alexandria, VA 22314). Incubation times and temperatures for the three added preparations were 37 C for 3 hr, 6 C overnight, and 37 C for 3 hr, respectively. After each incubation, wells were washed five times with PBS-Tween. The coating γ-globulin concentration was 5.0 μg/ml, and the conjugate dilution was 1:800. Clarified extracts were tested in duplicate wells. The substrate (1.0 mg/ml substrate buffer) was added at 0.15 ml/well and incubated at room temperature. Visual observations for *p*-nitrophenol formation (yellow coloration) were made at 30 min and 2 hr after substrate addition. PBS-Tween control wells were colorless in all tests, and healthy corn extract controls were colorless or very faintly colored. Wells in which a positive reaction occurred had an appreciably brighter yellow coloration.

Extracts for MPA were prepared as described above and diluted twice in twofold dilutions with PBS. The extract and two dilutions (1:2 and 1:4) were tested. The MRFV antiserum was diluted 1:32 with PBS. Antigen controls included extracts from corn plants infected with maize dwarf mosaic virus strain A (MDMV-A), maize chlorotic mottle virus (MCMV), and foxtail mosaic virus (FMV) and from healthy corn plants. These controls were negative in MPA. The MRFV extract reacted positively. The MPA was performed as described previously (16).

Maize stripe virus. Virus extracts for agar-gel double diffusion tests were prepared by grinding 0.5 g of leaf tissue in 0.2 ml of PBS containing 0.5% 2-mercaptoethanol and filtration through cheesecloth. Tests were done in plastic petri dishes (9 cm diameter) containing 13 ml of 0.5% Ionagar No. 2 (Colab Laboratories, Inc., Chicago Heights, IL 60411) in PBS with 0.02% sodium azide. Plant extracts and antiserum were placed in 6-mm diameter wells spaced 9 mm apart. Sealed plates were held at room temperature for 3 days and observed for formation of precipitin lines.

Immunofluorescence tests for maize stripe virus were done by the procedure for maize chlorotic dwarf virus (12).

Maize mosaic virus. Sap expressed directly from leaf samples was negatively stained with 2% phosphotungstic acid (neutralized to pH 7.0 with potassium hydroxide) on Formvar-coated grids, then examined by electron microscopy. Rhabdovirus-like particles that occurred in high frequency on grids prepared from leaves with symptoms characteristic of maize mosaic were presumed to be maize mosaic virus (MMV).

Maize dwarf mosaic virus. Samples for EIA and MPA were pre-

TABLE 2. Results of assays for viruses and mollicutes infecting Peruvian maize (*Zea mays*) and Johnson grass (*Sorghum halepense*)[a]

Sample no.	Collection site (see Fig. 1)	Preliminary Identification[b]	Pathogen									
			CSS[c]	MBSM[d]	MRFV[e]	MStpV[f]	MMV[g]	MDMV-A[h]	MDMV-B[i]	MCMV[j]	FMV[k]	MCDV[l]
1	San Martin-1	MSV			−	++		−	−	−−	−	−
2	San Martin-1	CSS	+		−	−−		−	−	−	−	
3	San Martin-1	CSS	+		−	−−		−	−	−	−	
4	San Martin-1	MSV			−	++		−	−	−−	−	−
5	San Martin-1	MSV			−	++		−	−	−−	−	−
6	San Martin-1	CSS	+		−	−−		−	−	−	−	
7	San Martin-2	MMV			−−	−−	+	+−	−	−−	−	
8	San Martin-2	MMV			−−	−−	+	−	−	−	−	
9	San Martin-2	MMV			−−	−−	+	−	−	−	−	
10	San Martin-2	MSV			−	++		−	−	−−	−	−
11	San Martin-2	MSV			−	++		−	−	−−	−	−
12	San Martin-2	CSS	+		+−	−−		−	−	−	−	
13	San Martin-2	CSS	+		+−	−−		−	−	−	−	
14	Ancash-3	MBSM	−	+	−−	−−		−−	−	−−	−	
15	Ancash-3	MBSM	−	+	++	−−		−	−	−−	−	
16	Ancash-3	Mosaic			−	−−	−	−	−	++	−	−[m]
17	Ancash-3	MRFV			++	−−	−	−	−	−	−	
18	Ancash-3	MRFV			++	−−	−	−	−	−−	−	
19	Ancash-3	Mosaic			−	−−	−	−	−	++	−	−[m]
20	Ancash-3	MBSM	−	+	−	−−		−	−	−−	−	
21	Ancash-4	MMV			−	−−	+	−	−	−−	−	−[m]
22	Ancash-4	MMV			−	−−	+	−	−	−−	−	
23	Lima-5	Mosaic			++	−−		−	−	++	−	
24	Lima-9	MCMV			−−	−−	−	−	−	++	−	
25	Lima-9	MCMV			−	−−		−	−	++	−	
26	Lima-9	MMV			−	−−	+	−	−	++	−	
27	Lima-9	MDMV			−	−−	−	++[n]	−	−	−	

[a] Symbols: +, positive assay; −, negative assay, and no symbol indicates that no assay was performed.
[b] Identification based upon field symptoms.
[c] Corn stunt spiroplasma (CSS) was detected by dark-field light microscopy.
[d] Maize bushy stunt mycoplasma (MBSM) was detected by transmission with *Dalbulus maidis* and by symptoms in test-maize indicator.
[e] Symbols in the first column under maize rayado fino virus (MRFV) represent results for enzyme-linked immunosorbent assays (EIA), the second column for immunomicroprecipitin assays (MPA).
[f] The first column under maize stripe virus (MStpV) gives immunofluorescence assay (IF) results, the second column agar-gel double diffusion assay results.
[g] Maize mosaic virus (MMV) tentatively was identified by the presence of rhabdovirus in electron micrographs of negatively stained sap plus diagnostic symptoms on sample leaves.
[h] The first column under maize dwarf mosaic virus, strain A (MDMV-A), gives results for EIA, the second for MPA.
[i] Maize dwarf mosaic virus, strain B (MDMV-B), was assayed by EIA.
[j] The first column under maize chlorotic mottle virus (MCMV) gives results for IF assays, the second column for MPA.
[k] Foxtail mosaic virus (FMV) was assayed by EIA.
[l] Maize chlorotic dwarf virus (MCDV) was identified by a rate-zonal centrifugation assay.
[m] Samples 16, 19, and 21 contained isometric particles which banded either at or slightly above the depth of MCMV in centrifuged gradients.
[n] Johnson grass leaves were collected from several plants in the vicinity of collection sites for samples 24, 25, and 26.

pared as described above for MRFV assays. Antisera to strains A and B of MDMV were prepared as previously described (16), and the EIA and the MPA were performed as for MRFV. For EIA the coating γ-globulin concentration of both antisera was 5.0 μg/ml and the conjugate dilutions were 1:200 for MDMV-A and 1:400 for MDMV-B. For MPA, only MDMV-A was tested and the antiserum dilution was 1:8. Antigen controls of MRFV, FMV, MCMV, and healthy corn extracts were negative, whereas that of the MDMV-A control was positive.

Maize chlorotic mottle virus. Samples for MPA were prepared as described above for MRFV assays. Antiserum to MCMV was used at a dilution of 1:64. Antigen controls of MRFV, MDMV-A, FMV, and healthy corn extracts were negative, whereas the MCMV control was positive. Immunofluorescence tests for MCMV were done according to the procedure for maize chlorotic dwarf virus (13).

Maize chlorotic dwarf virus. Maize chlorotic dwarf virus (MCDV) was assayed by a centrifugation method described previously (2). The sample volume layered onto gradients was 0.1 ml. Virus controls centrifuged in the same set of gradients as test preparations were MCDV, MCMV, MRFV, and tobacco mosaic virus (80 μg/ml). A healthy corn preparation was centrifuged in one set. Material that absorbed at 260 nm, from about 2.6 to 4.0 ml in centrifuged gradients, was collected and added to another cellulose nitrate tube of the Beckman SW50.1 rotor. Each tube was filled with 0.01 M potassium phosphate, pH 7.0, and centrifuged at 45,000 rpm for 1 hr at 20 C. Pellets were recovered and suspended in a drop of distilled water. A sample of this suspension was placed on Formvar-coated grids, carbon-platinum shadowed, and examined with an electron microscope at 30,000 magnification.

Foxtail mosaic virus. Samples for EIA and MPA were prepared and assays performed as described above for MRFV assays. Antiserum to foxtail mosaic virus (FMV) used in the EIA was prepared by injecting a rabbit twice at a 10-day interval intravenously (1.0 ml per injection) with purified virus suspended in PBS. A total of 1.83 mg of virus protein was injected. Injections were given, and antiserum was collected 1 wk after the second injection. Antiserum was diluted with an equal volume of glycerol and stored at −10 to −20 C. The virus isolate was obtained from a weed grass collected in a Louisiana corn field by one of us (OEB), and increased in corn for purification. Virus was extracted in 0.1 M potassium phosphate, adjusted to pH 5.0 by the addition of citric acid, at 1 g leaf tissue to 1 ml of buffer. The extract was clarified by emulsification with one-half volume of chloroform followed by centrifugation at 12,000 *g* for 10 min. The aqueous phase was recovered and layered onto linear 10% to 40% sucrose gradients which were centrifuged in a Beckman SW27 rotor at 27,000 rpm for 150 min. Centrifuged gradients were fractionated with the ISCO Model 640 fractionator and Model UA-4 UV analyzer. Virus-peak fractions were collected, diluted with the extraction buffer, and centrifuged in a Beckman SW27 rotor at 27,000 rpm for 170 min or in the Beckman Type 35 rotor at 35,000 for 144 min. Material in pellets was suspended in the extraction buffer. These suspensions were layered on sucrose gradients and recovered from fractions under conditions described above. Material recovered from sucrose fractions of the second SW27 centrifugation was suspended in PBS.

For EIA, the coating γ-globulin concentration was 5.0 μg/ml, and the enzyme γ-globulin conjugate dilution was 1:100. For MPA, the antiserum dilution was 1:32. For EIA, the healthy corn extract was negative and for MPA, MRFV, MDMV-A, MCMV, and healthy corn controls were negative. In both assays FMV in extracts gave a positive reaction.

RESULTS AND DISCUSSION

At least one pathogen was detected in all 27 samples from three of the four regions surveyed. Seven of the 10 assayed pathogens were found (Table 2). No maize plants with viruslike symptoms were observed in the department of Cuzco, therefore, no samples were tested from there. In the department of Lima, MCMV, MRFV, and MMV were detected in maize and MDMV-A in Johnson grass. In the department of Ancash, MCMV, MRFV, MMV, and MBSM were detected. Symptoms of MBSM in test plants (samples 14, 15, and 20) appeared similar to those produced by a Texas isolate. Assay leafhoppers also transmitted MRFV from sample 15. MCMV had not been reported from Ancash. In San Martin, MStpV, CSS, MMV, and MDMV-A were found. Strain B of MDMV was detected by EIA in a sugarcane (*Saccharum officinarum* L.) leaf sample collected in San Martin (L. R. Nault et al, unpublished).

In general, field diagnoses agreed with assay results. However, in doubly infected plants the symptoms of one pathogen masked the presence of the second. For example, MMV masked MDMV-A and MCMV (samples 7 and 26, respectively), CSS masked MRFV (samples 12 and 13), and MBSM masked MRFV (sample 15). Samples 16 and 19 were diagnosed only as mosaic; MCMV was detected in both. A third sample (23) diagnosed as mosaic was doubly infected with MCMV and MRFV. Maize dwarf mosaic virus strain A was detected in one maize sample, but no plants were field diagnosed for this disease. Johnson grass collected near Lima city was selected because leaves had typical MDMV symptoms, and that virus was detected in these leaves.

Four viruses (MRFV, MStpV, MDMV-A, and MCMV were assayed by two different serological tests which did not always give similar results. For example, MRFV was detected in samples 12 and 13 by the more sensitive EIA method, but not by MPA. Even by EIA only three of the four tests involving these two samples were positive. Also, MDMV-A was detected in sample 7 by EIA, but not MPA. Dual assays for MStpV agreed for all 27 samples, and dual assays for MCMV agreed for 18 samples.

In all departments except Cuzco, the corn leafhopper, *D. maidis*, and the corn planthopper, *Peregrinus maidis* Ashmead were collected (Fig. 1). *Dalbulus maidis* was collected from Puente Chillon, Huaral, Acos, La Molina, Barranca (Lima), Juan Guerra, Tarapoto (San Martin), and from Malpaso and Caraz (Ancash). Leafhoppers from at least 17 genera also were collected in maize fields and surrounding vegetation. Leafhoppers were deposited in the insect museum, Department of Entomology, The Ohio State University, Columbus. *Peregrinus maidis* was collected from Huaral, La Molina, Barranca, Juan Guerra, and Caraz. Several other Fulgoroidea in the families Delphacidae, Cixidae, and Derbidae were collected. Planthoppers were deposited in the insect museum at Florida Agricultural and Mechanical University, Tallahassee. Neither *D. maidis* nor *P. maidis* (1) previously have been reported from Peru.

Five of the seven pathogens detected in Peru are transmitted by *D. maidis* or *P. maidis*. *Dalbulus maidis* is the vector of CSS (7), MBSM (4), and MRFV (10). *Peregrinus maidis* is the vector for MMV (15) and MStpV (17). *Diabrotica* spp., in particular *Diabrotica speciosa* (Germar), were found in all corn-growing regions of Peru. Chrysomelid beetles, including three *Diabrotica* spp., have been identified as vectors of a Kansas isolate of MCMV (18). Numerous aphid species, including *Rhopalosiphum maidis* (Fitch), were observed in Peruvian corn fields and in surrounding vegetation. This species, as well as several others, are vectors of MDMV (3).

The intent of this survey was to detect and identify the principal insect-borne maize viruses and mollicutes and their vectors in Peru. Although not enough samples were taken to measure quantitatively the relative occurrence of each pathogen it seems useful to speculate on the importance of each pathogen and the potential for it to become established elsewhere in Peru. These speculations are based on field observations as well as on data for pathogen and vector distribution. The absence of *D. maidis* and *P. maidis* in Cuzco would account for the absence of CSS, MBSM, MRFV, MMV, and MStpV. However, the presence of aphids as well as *D. speciosa* suggest that potentially MDMV and MCMV could become established in this important maize growing department. In the department of Lima, plants with MCMV symptoms were more numerous than those with MRFV or MMV symptoms and, despite the presence of appropriate vectors, maize infected with CSS, MBSM, or MStpV symptoms were not seen. In contrast, maize with CSS and MStpV symptoms were the most frequently observed diseases in San Martin. Marked differences were

observed between diseases in the two departments even though temperatures and altitudes as well as continuous corn production are similar. These differences may relate to several mountain ranges which separate Lima and San Martin and which may act as natural barriers excluding interchange of vectors and pathogens. In Ancash, maize with MCMV and MRFV symptoms were observed more frequently than those with MMV or MBSM symptoms. The cool summer temperatures in this region (Table 1) would favor the relative importance of pathogens with short latent periods in the plant (MCMV and MRFV) rather than those with long ones (MBSM and MMV). Populations of *D. maidis* were higher here than at the other sites surveyed in Peru. *Peregrinus maidis* populations were low. The absence of corn and the low temperatures during the winter months would make overwintering of *D. maidis* and *P. maidis* in Ancash unlikely, and thus the pathogens they transmit less important. But MRFV was important, which indicated that infective insects are introduced into this region perhaps by the strong winds which blow from the coast early in the growing season. Subsequent surveys in Peru should be more extensive and include a measure of the frequency of occurrence of each pathogen. The results of this survey suggest that different pathogens will predominate in each region, and thus future approaches to control, including breeding for resistance, will vary in the different major maize-growing regions of Peru.

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