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NOTES ON EAST AFRICAN PLANT VIRUS DISEASES
II. ALFALFA MOSAIC VIRUS[1]

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Alfalfa mosaic virus (AMV) is worldwide in distribution affecting 305 species of dicotyledonous plants in 47 families (1, 2, 4). There have been three reports of AMV affecting crops in the African continent. These were from Morocco (5), Sudan (8), and South Africa (6). In Morocco, AMV was isolated from peppers (*Capsicum annuum* L.), while in Sudan, AMV was recovered from naturally-infected hyacinth bean (*Lablab niger* L.), broadbean (*Vicia faba* L.), lucerne (alfalfa) (*Medicago sativa* L.), and *Sonchus cornutus* Hochst. ex Oliv. and Hiern. In South Africa, the virus affected bean (*Phaseolus vulgaris* L.), lucerne, and other unspecified legumes. The authors isolated AMV from naturally-infected lucerne in Tanzania and potato (*Solanum tuberosum* L.) in Kenya. This is the first report of AMV from East Africa.

Several strains of AMV have been reported which vary in pathogenicity and host range (1, 2, 4). To assess the potential importance of AMV to East African agriculture, studies were initiated to investigate various properties of the virus, including host range and methods of transmission.

The Virus Isolates

Isolate from Lucerne.—The virus was isolated from diseased lucerne plants growing in a 2-3 ha planting on the estate of the East African Kenaf Industries Limited near Moshi, Tanzania in 1974. Diseased plants from which the virus was isolated exhibited mosaic and leaf deformation symptoms.

Isolate from Potato.—The virus was isolated from two plants in a crop of Kenya Akiba growing in the Timau area of Meru District, Kenya. The plants, exhibiting bright yellow calico symptoms, were collected in 1972.

Materials and Methods

Plants were grown in steam-sterilized soil in glasshouses at 15-25°C. Insects were controlled by use of 20 per cent Vapona resin strips (2, 2-dichlorovinyl dimethyl phosphate) and/or periodic sprays with wide spectrum insecticides.

The lucerne isolate was maintained in *Nicotiana clevelandii* Gray, and the potato isolate in *Nicotiana tabacum* L. "White Burley". Sap from systemically-infected leaves was used for host range, physical property, purification, and serological studies.

Host range studies were made by manual inoculation with sap extracted from AMV-infected leaf tissue in either 1 per cent K_2HPO_4 (lucerne isolate) or distilled water (potato isolate). The leaves of the test plants, previously dusted with carborundum, were rinsed with water immediately after inoculation. Plants that did not develop any symptoms were tested for latent infection by back-inoculation to bean cv. Long Tom.

Physical properties were determined with crude infective sap of *N. clevelandii* and clarified sap of *N. tabacum* "White Burley" for the lucerne and potato isolates, respectively. Determination of the dilution-end points was made using serial dilutions in distilled water. The isolates were assayed by inoculation to the fully expanded primary leaves of Long Tom bean.

Vector transmission studies were conducted using *Aphis craccivora* Koch, *Macrosiphum euphorbiae* (Thos.) and *Myzus persicae* Sulz. Aphids were starved for 1-2 h, given acquisition feeds of 1-30 min and test feeding periods of 18-24 h (5 to 10 aphids/test plant). Plants were sprayed with an aphicide immediately after test feeds.

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For virus purification, leaves of *N. clevelandii* systematically infected with the lucerne isolate were homogenized in 0.1M phosphate buffer (2 my/g) containing 1 per cent mercapto ethanol, pH 7.5. The homogenate was stirred with equal volumes of a 1:1 mixture of n-butanol and chloroform for 10 min and clarified by low speed centrifugation for 10 min. The clarified extract was left at 4°C for 16 h, centrifuged at 13,000 g for 20 min and then at 78,000 g for 120 min. The pellets were resuspended in 0.01M phosphate buffer for 60 min, and briefly centrifuged for 10 min, followed by a second similar cycle of differential centrifugation.

Sucrose density gradients were prepared by layering 4, 7, 7, and 7 ml of 10, 20, 30, and 40 per cent (w/v) sucrose in 0.01M phosphate buffer, pH 7.7, respectively, in 3 × 1 in centrifuge tubes. Partially purified virus preparations (usually 0.5 ml) of the lucerne isolate were layered onto the density gradients which were centrifuged for 120 min at 24,000 g. Gradients were sampled by inserting a hypodermic syringe through the side of the tube.

An antiserum was prepared by injecting rabbits intravenously with 1 ml of a partially purified preparation of the lucerne isolate, followed seven days later by an intramuscular injection of 1 ml virus emulsified with 1 ml Freund's incomplete adjuvant, and again 14 days later by a second, similar injection. Rabbits were bled 28 days after the final injection.

The serological reactions of East African AMV isolates were studied in Ouchterlony immunodiffusion tests (3) using AMV antisera produced in Kenya, the Netherlands, United Kingdom, and United States.

Results

Host Range and Symptoms

In host range studies, the lucerne isolate affected plants in 29 genera from 7 families, while the potato isolate infected 27 genera in 7 families (Table I).

Medicago sativa L.—Both isolates of AMV produced mosaic and leaf deformation symptoms (Fig. 1) in Hairy Peruvian lucerne similar to that observed in naturally-infected lucerne from Tanzania.

Solanum tuberosum L.—The first leaves emerging from a tuber of Kenya Akiba infected with the potato isolate developed bright yellow interveinal blotches, usually towards the leaf margins (Fig. 2). The leaves which developed later showed a diffuse light green mottle and eventually growth became symptomless. The symptoms in Kenya Baraka, Roslin Eburu and Anett were more intense with ofter large areas of leaflets exhibiting bright yellow to golden calico symptoms. The lucerne isolate induced calico symptoms on Kenya Baraka potato.

Glycine max (L.) Merr.—Mosaic symptoms (Fig. 3) developed on 14 varieties inoculated with the lucerne isolate; in some, mosaic symptoms were accompanied by leaf deformation and stunting of the plant.

Phaseolus vulgaris L.—Necrotic local lesions within four days appeared on inoculated leaves of all bean varieties tested with both isolates (Fig. 4). No systemic symptoms were observed.

Chenopodium quinoa Willd.—Both isolates produced chlorotic local lesions on inoculated leaves (Fig. 5) followed by a systemic mosaic.

Physalis floridana Rydb.—The potato isolate induced a bright-yellow vein banding of the midrib and main veins extending from the base of the newly expanding leaves which developed into a bright yellow mosaic (Fig. 6). The lucerne isolate produced mosaic symptoms in inoculated plants.

Physical Properties

The properties *in vitro* of the two isolates are presented in Table 2.

Electron Microscopy

Leaf squash preparations from *C. quinoa* infected with the potato isolate, made in either 0.5M ammonium acetate solution or distilled water and stained with 1 per cent aqueous uranyl acetate, showed bacilliform particles with typical bottom, middle and top components (Fig. 7). Similar bacilliform particles were observed in negatively stained leaf dip preparations of *Gomphrena globosa* L. infected with the lucerne isolate.

Serology

In Ouchterlony agar double-diffusion tests, partially purified virus preparations of the lucerne isolate reacted with its homologous

TABLE I—HOST RANGE OF EAST AFRICAN ISOLATES OF ALFALFA MOSAIC VIRUS

Hosts [a]	*Isolate from*	
	Alfalfa	*Potato*
Amaranthaceae		
Amaranthus hybridus L. ssp. *incurvatus* (Gren. & Godr.) Brenan..	NS	M, LD [b]
Gomphrena globosa L.	NLL, M	CLL, M
Chenopodiaceae		
Beta vulgaris L.	NS	NS
Chenopodium amaranticolor Coste & Reyn.	CLL, M	CLL, M
C. quinoa Willd.	CLL, M	CLL, M
Compositae		
Chrysanthemum cinerariifolium (Trev.) Vis.	NS	—
Lactuca sativa L. "Boston"	M	M
Zinnia elegans Jacq.	M	M
Cruciferae		
Brassica napus L.	—	NS
B. oleracea var. *gemmifera* DC	NS	—
B. rapa L.	—	NS
Curcubitaceae		
Cucumis sativus L. "Cool and Crisp"	—	CLL, M
C. sativus L. "National Pickling"	CLL, M	CLL, M
Cucurbita pepo L. "Buttercup"	CLL	—
Graminae		
Zea mays L.	NS	—
Labiatae		
Ocimum basilicum L.	YM	YM
Leguminosae		
Arachis hypogaea L. "Natal Common"	NS	—
Leguminosae		
Cajanus cajan (L.) Millsp. "T-21"	NLL	NLL, M, LD
Cicer arietinum L.	M, W	M, W
Crotalaria juncea L.	NLL	—
Glycine max (L.) Merr. "HLS 541"	M	M
Lablab purpureus (L.) Sweet	NS	—
Lens esculenta Moench	M	M
Medicago sativa L. "Hairy Peruvian"	M, LD	M, LD
Melilotus alba Desr. "Spanish Sweet Clover"	NS	NS
Phaseolus lunatus L. "Jackson Wonder"	M, LD	NS
P. vulgaris L. "Canadian Wonder"	NLL	NLL
P. vulgaris "Long Tom"	NLL	NLL
Pisum sativum L. "Onward"	—	SSI
P. sativum L. "Wisconsin Perfection"	M, LD	SSI
Trigonella foenum-graecum L.	M	SSI
Vicia faba L. "Aguadulce"	NLL, M	NLL, M
Vigna radiata (L.) Wilczek	NLL, M	NLL, M
Vigna unguiculata (L.) Walp. "California No. 5"	NLL	NLL
Voandzeia subterranea (L.) Thou.	M	—
Malvaceae		
Hibiscus cannabinus L.	NS	—

TABLE I—HOST RANGE OF EAST AFRICAN ISOLATES OF ALFALFA MOSAIC VIRUS—(*Contd.*)

Hosts [a]	Isolate from: Alfalfa	Isolate from: Potato
Solanaceae		
Capsicum annuum L. "Puerto Rico Bell"	M, TN	M, LD
C. annuum "Yolo Wonder"	—	YM
Datura metel L.	NS	M
D. stramonium L.	M	M
Lycopersicon esculentum Millsp. "Money Maker"	TN, S	NLL, TN, S
L. esculentum "Rutgers"	NLL, TN	NLL, TN, S
Nicotiana clevelandii Gray	M, LD	YM
N. glutinosa L.	M	M
N. tabacum L. "White Burley"	M	M
Nicranda physalodes (L.) Gaertn.	M	M, VN
Petunia hybrida Vilm.	M	M
Physalis floridana Rydb.	M	YM
Solanum demissum Lind. × *Solanum tuberosum* L. "A-6"	C	M
S. melongena L.	M	NLL
S. nigrum L.	M	CLL, M, LD
S. tuberosum "Kenya Baraka"	C, M	C, M
Umbelliferae		
Daucus carota L.	NS	SSI

[a] Hosts are listed alphabetically by family and genus.

[b] NS, not susceptible; C, calico; CLL, Chlorotic local lesions; NLL, necrotic local lesions; LD, leaf deformation; M, mosaic; S, stunting; SSI, symptomless systemic infection; TN, top necrosis; VN, Vein necrosis; W, wilting; YM, yellow mosaic; and —, not tested.

TABLE II—PHYSICAL PROPERTIES OF THE LUCERNE AND POTATO ISOLATES OF ALFALFA MOSAIC VIRUS FROM EAST AFRICA

Physical Property Test	AMV Isolate: Lucerne, Infectivity at	Lucerne, No Infectivity at	Potato, Infectivity at	Potato, No Infectivity at
Thermal Inactivation (after 10 min. heating)	60°C	65°C	60°C	65°C
Dilution End Point	10^{-4}	10^{-4}	10^{-4}	10^{-5}
Longevity in Vitro (Days at room temperature)	4	5	1	2

Plate 1—Chlorotic local lessions on a leaf of *Chenopodium quinoa.*

Plate 2—Necrotic local lesions on a leaf of *Phaseolus vulgari* **"Top Crop".**

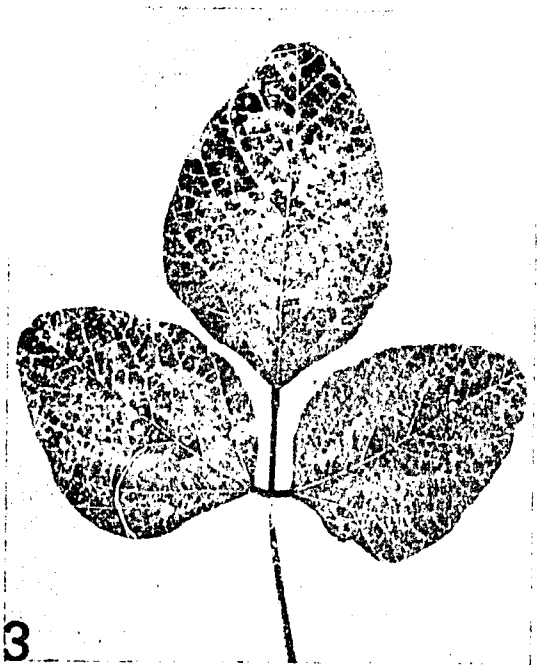

Plate 3—Mosaic symptoms on *Glycine max* **"Jupiter".**

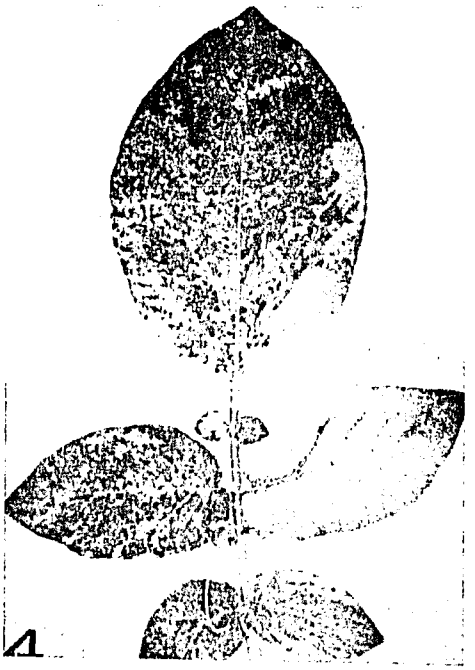

Plate 4—Calico symptoms on *Solanum demissum* x *S. tuberosum* **"A-6".**

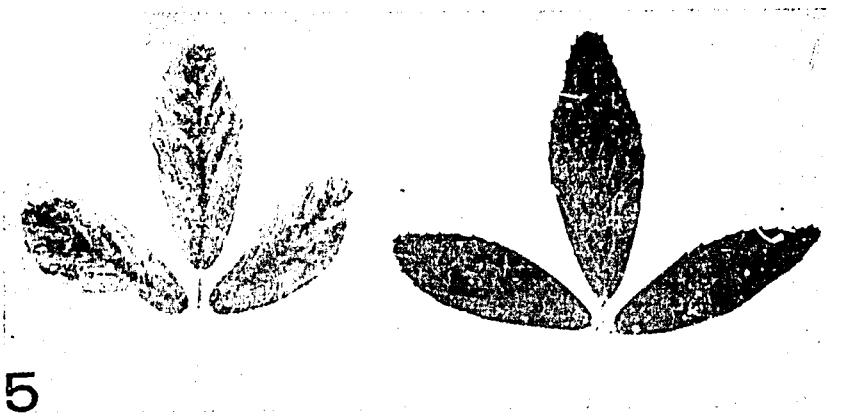

Plate 5—*Medicago sativa* "Hairy Peruvian" infected with L-AMV (left); healthy trifoliate leaf, right.

Plate 6—Yellow mosaic symptoms on leaf of *Physalis floridana* infected with P-AMV.

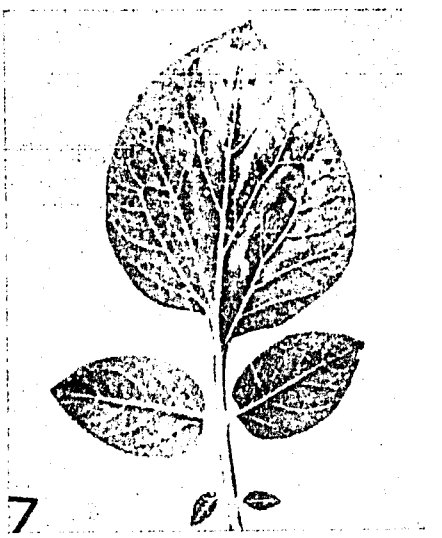

Plate 7—Interveinal chlorosis on young leaf of *Solanum tuberosum* "Kenya Akiba" resulting from tuber infection with P-AMV.

antiserum at dilutions up to 1/32. Crude extracts of *N. clevelandii* infected with the lucerne isolate also reacted specifically to AMV antisera from the Netherlands, United Kingdom and United States. The potato isolate was shown to be related serologically to an AMV antiserum from the United Kingdom.

Insect Transmission

The lucerne isolate was transmitted from AMV-infected *N. clevelandii* and *P. floridana* by *Myzus persicae* to healthy *N. clevelandii* and *P. floridana*. The lucerne isolate was not transmitted by *Aphis craccivora*. The potato isolate was transmitted by *M. persicae* from AMV-infected *N. clevelandii* to *P. floridana*. No transmission occurred with *Macrosiphum euphoribae*.

Seed Transmission

The lucerne isolate was seed-borne in *N. clevelandii* (9/219), but not in *M. sativa*

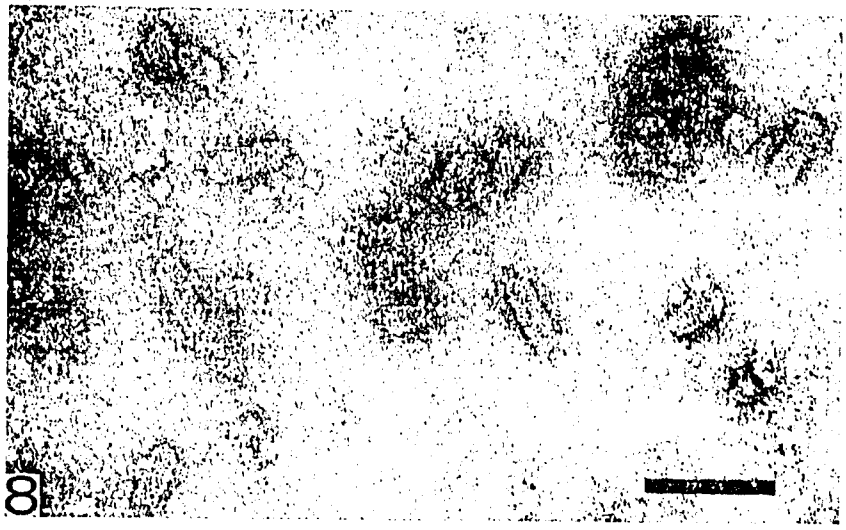

Plate 8—Bacilliform particles in a leaf squash preparation of *Chenopodium quinoa* systemically infected with P-AMV (x172,000). Bar represents 100 nm.

(0/156, *G. max* (0/126), *P. floridana* (0/408), or *Solanum nigrum* L. (0/420). The potato isolate was seed-transmitted in *Nicrandra physalodes* (L.) Gaerth. (107/403), *S. nigrum* (7/1341), but not in *P. Floridana* (0/428). Both isolates were transmitted in potato tubers collected from AMV-infected plants.

Discussion

Although AMV was first reported to occur on the African continent in 1955 (6), the virus was not recorded from East Africa until 1972 when it was found infecting potato. Lucerne was shown to be a natural host of AMV in South Africa (6) and Sudan (8). However, this is the first report of AMV affecting potato in Africa.

The experimental host range of both East African isolates of AMV is extensive which emphasizes the potential importance of the virus to East African agriculture. The virus did not systemically infect bean or maize which are two of the most important food crops in East Africa, but it was transmitted to other food crops, and to widely distributed weeds, including *Amaranthus hybridus* L. ssp. *incurvatus* (Gren. and Godr.) Brenan, *Datura stramonium* L., *Nicrandra physalodes*, *Physalis floridana*, and *Solanum nigrum*.

Numerous strains of AMV have been described (1, 2, 4). AMV strains are differentiated on the basis of pathogenicity, host range and/or symptomatology. The East African isolates of AMV from lucerne and potato differed markedly in host range and symptom expression, especially within the Solanaceae. These differences indicate that there are at least two different strains of the virus in East Africa.

The lucerne planting at Moshi, Tanzania from which AMV was isolated was established about four years ago. No other lucerne is grown in the vicinity. The lucerne seed (variety Hairy Peruvian) had been imported from Israel where AMV affects lucerne naturally (7). As AMV is reported to be seed-borne in lucerne (1, 2, 4), it is likely that the virus was introduced into the Moshi area on imported lucerne seed. The potato variety Kenya Akiba must have become naturally-infected with AMV in Kenya since it was selected in 1968 from imported true seed.

Seed transmission of AMV in lucerne and pepper (*Capsicum annuum*) has been demonstrated by several investigators (1, 2, 4). In our studies, the lucerne isolate was seed-borne in tobacco (*N. clevelandii*), but not in several other hosts, including lucerne, soybean, limabean, black nightshade (*S. nigrum*) and *P. floridana*. The potato isolate was transmitted

in seeds of *S. nigrum* and *N. physalodes* which are common weeds and which could serve as reservoir hosts of AMV in East Africa.

Summary

A potentially important virus was isolated from naturally-infected lucerne (alfalfa) (*Medicago sativa* L.) in Tanzania and potato (*Solanum tuberosum* L.) in Kenya. Symptomatology, host range, serology, and particle morphology were utilized to identify the virus as alfalfa mosaic (AMV). This is the first record of AMV from East Africa. The experimental host range of both virus isolates was extensive. The lucerne isolate infected plants in 29 genera from 7 families, while the potato isolate affected plants in 27 genera from 7 families. AMV was seed-borne in several solanaceous plants, some of which could serve as alternate reservoir hosts of the virus in nature. *Myzus persicae* was a vector of the lucerne and potato isolates. Field surveys in Kenya and Tanzania indicate that AMV is at present probably restricted in its distribution in both countries.

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