SURVEY OF VIRUSES AFFECTING EAST AFRICAN MAJOR FOOD CROPS

by

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DECLARATION

I hereby declare that this thesis has not been submitted for a degree in any other University.

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SUMMARY

A survey of virus diseases of maize (*Zea mays* L.) and bean (*Phaseolus vulgaris* L.) in East Africa was made to assess the economic significance of the individual pathogens; to identify the viruses critically on the basis of host range, physical properties, particle morphology and serology and to evaluate possible sources of resistance. The results of several extensive field surveys in Kenya, Tanzania and Uganda indicated that, in addition to maize streak virus and maize mottle virus, maize was infected with sugarcane mosaic virus, maize stripe virus, maize line virus, described here for the first time and a new pathogenic condition, maize tassel abortion disease. Beans were widely infected with bean common mosaic virus; a new bean viral pathogen, yellow-spot virus, is also reported and described. Detailed studies on all these viruses except maize streak and maize mottle viruses are reported in this thesis.

Sugarcane mosaic virus (SCMV) was found to be the most widespread and prevalent virus infecting maize; it reduced maize and sorghum yields by 25% and 73% respectively. Strains of East African SCMV were similar to American SCMV strains A and B. No resistance was found in any of the local maize varieties tested.

Maize stripe virus (MSV) and a new maize virus, designated maize line virus (MLV), both transmitted by Peregrinus maidis Ashm., were shown to be isometric; purified preparations of MSV contained 35 and 40 nm, and those of MLV 28 and 34 nm diameter particles. Antisera were prepared against both MSV and MLV; reciprocal serological tests indicated the two viruses to be unrelated; and also unrelated to maize streak virus and maize mottle virus.

A new disease of maize, designated maize tassel abortion disease, was described from Kenya. It caused severe stunting, produced abortive tassels, and was transmitted by Malexodes farinosus Fennah, but not by sap inoculation. The symptomatology and vector type of this disease suggest either a virus or a mycoplasma to be the causal agent.

Field surveys indicated total virus incidence in maize to be 43% during the years of survey; in some localities 63% infection was observed. Distribution of these viruses in East Africa and their relative economic importance is discussed.

A bean virus of wide occurrence in East Africa was identified as bean common mosaic virus (BCMV) on the basis of particle morphology, aphid transmission, physical properties and serology. Resistance tests indicated four American bean varieties to be probably immune to the virus.

A new viral pathogen of bean, designated bean yellow-spot virus (BYSV) was found to be widespread in East Africa; it was transmitted mechanically, by Aphis fabae Scop. and through seed. Infectivity was associated with 738 ± 31 nm filamentous particles. BYSV reduced yield by 20%. The virus was apparently related to seven other viruses of the potato Y group, including East African BCMV and pea mosaic virus, but not to European BCMV and bean yellow mosaic virus (BYMV) nor to five other viruses of the potato Y group. Seven bean varieties were probably immune to the virus.

A high proportion of a representative sample of aphids trapped in bean plots in the Kenya Highlands were vectors of BCMV (61%) and BYMV (39%). However, BYMV was never isolated from beans during the survey. Correlation of virus incidence and aphid population was demonstrated.

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PART - I

INTRODUCTION, MATERIALS AND METHODS

1. GENERAL INTRODUCTION

Virus diseases have been recognised as important plant pathogens affecting yields of agricultural crops in East Africa for many years. Using the then acceptable criteria of symptomatology, host range and insect vector, the presence of a number of viruses was established. These were cassava brown streak (Storey, 1936c; Nichols, 1950), cassava mosaic (Briant & Johns, 1940; Jameson, 1964; Storey, 1936c; Storey & Nichols, 1938a, 1938b), groundnut rosette (Storey, 1935b; Storey & Ryland, 1950, 1955, 1957), maize streak (Storey, 1936a, 1936b), maize stripe (Storey, 1936a), sugarcane mosaic (Hansford, 1935; Storey, 1936a), sweet potato viruses A and B (Sheffield, 1957, 1958) and tobacco leaf-curl (Storey, 1935a). Subsequent to much of Storey's work, virus diseases have been named in several East African plant disease check lists (Hansford, 1938, 1945; McDonald, 1936; Riley, 1960; Robinson, 1960; Wallace, 1937, 1939, 1944, 1947; Wallace & Wallace, 1949). In the absence of any evidence to the contrary, the identification of these virus diseases apparently was made purely on symptomatology: no work was done on critical characterization using the criteria of particle morphology, physical properties, host range, vector relationships and serology. Even with the classic researches of Storey, characterization of the viral pathogens was not undertaken, and the causal agents of such well known and widely distributed diseases as cassava mosaic and maize streak remained unknown.

In 1967, a project proposal for the initiation of an East African survey of virus diseases of main food crops was accepted by the East African Community Research Councils. The project had two main objectives: the critical identification of economically important plant viruses in relation to viruses occurring in other regions of the world and the subsequent search for field resistance, locally and overseas.

More recently, subsequent to the initiation of the E.A.A.F.R.O. programme, pawpaw decline viruses (Kulkarni, 1970), cowpea mosaic virus (Bock, 1971) and courgette leaf distortion virus (Bakker, 1971) have been characterised.

Maize (Zea mays L.) and beans (Phaseolus vulgaris L.), rank as the major food crops of East Africa by virtue of their total acreage and food value. A survey was therefore undertaken to evaluate the economic importance of virus infections of maize and bean.

The work reported in this thesis is a record of part of the author's contribution to the E.A.A.F.R.O. research project. It consists of detailed studies on sugarcane mosaic virus in maize, maize stripe virus, a new maize line virus and a new pathogenic disease of maize, maize tassel abortion disease; bean common mosaic virus and a new virus of bean, bean yellow-spot virus.

For the sake of convenience, the thesis is divided into two parts, each dealing with viruses of a particular crop; the individual viruses being described separately. To avoid repetition, certain experimental procedures which were used in studies of more than one of these viruses are mentioned in the chapter on materials and methods; any techniques or materials specific to individual viruses are referred to in the appropriate text.

2. GENERAL MATERIALS AND METHODS

Mechanical inoculation. Healthy test seedlings at cotyledon- or 1-2 leaf-stage, raised in insect proof glasshouses at 20-25°C, were pre-darkened for 24 h (Bawden & Roberts, 1947), dusted with Carborundum No.600 and inoculated with infective sap extracted in distilled water or an appropriate buffer. Inoculated leaves were rinsed briefly with distilled water. The plants were then observed for periods ranging from 1-3 months before final records were made and the plants discarded. The duration of observation related to the time normally taken by a particular virus to induce symptoms in its natural host under glasshouse conditions, and was always 2-3 wk in excess of this.

Insect inoculations. Aphid or hopper vectors of the viruses under study were collected from field plants infected with the viruses. The aphids were freed of virus by single aphid culture and reared on appropriate healthy plants under an insect cage at room temperatures of 18-22°C.

Hoppers were freed of virus by breeding them on healthy plants in cages at high temperatures (c 33°C) for about 1 month (Kunkel, 1937), after which individuals were tested for the presence of virus by feeding them on test seedlings susceptible to the virus being studied. Those that were found to be uninfected were used for raising a colony at the temperatures suitable to the particular hopper.

In transmission tests, insects were fed on monocotyledonous plants at the coleoptile stage, and dicotyledonous plants at the cotyledon stage.

All acquisition and test feeds were made at ambient room temperature (18-22°C) following which aphids were killed by spraying with 0.0005% nicotine sulphate; hoppers were killed by moving the test seedlings to glasshouses containing Vapona (2, 2-dichlorovinyl dimethyl phosphate) insecticidal strips. Control plants received uninfected insects and were subjected to the same procedures as test seedlings.

Virus assay. Presence of a manually transmissible virus was checked by inoculating sap from test seedlings to species that produced local lesions or systemic symptoms. Where a virus was not sap transmitted, its insect vector was fed on the test seedlings and then transferred to a susceptible host.

Physical properties determination. Crude sap extracted from virus infected leaves without addition of distilled water or buffer, was centrifuged at 4000 r.p.m. for 5 min and used for studying physical properties as under. Infectivity was checked by inoculating treated sap to appropriate susceptible hosts producing local lesions or systemic symptoms.

Dilution end point. Serial dilutions of 1:10-1:100,000 were made by diluting 1 ml of the sap in distilled water and inoculating separately to virus-assay hosts.

Thermal inactivation point. Normally 0.5 ml aliquots of the sap were put into thin walled glass tubes and heated at temperatures ranging from 40°C to 80°C for 10 min in a water bath. The treated sap was cooled immediately by holding the tube under running water and then inoculated to virus assay hosts.

Longevity. Crude sap was kept at room temperature of 18-22°C and assayed at 24 h intervals upto a week or more depending on the viability of the virus.

Purification. Routinely, 100 g leaves together with the appropriate buffer (containing reducing agents or enzyme inhibitors, 1:2 w/v) were homogenized in a Waring blendor for 1-2 min and the resultant extract passed through muslin. After addition of organic solvents and stirring on magnetic stirrers, the extracts were subjected to differential centrifugation (20,000 g for 20 min followed by 100,000 g for 90 min or 150,000 g for 60 min for filamentous particles; 100,000 g for 120 min or 150,000 g for 90 min for spherical particles) in a Beckman Model L-2 Ultracentrifuge operated at 4°C. The supernatant following the first low speed centrifugation was filtered once or twice through Whatman filter paper No.4. The pellets were resuspended in the original

extracting buffer without reducing agents or enzyme inhibitors and left overnight at c 5°C. The virus suspension was then centrifuged at 12,500 g for 5 min and the pellet discarded; such preparations are referred to as partially purified virus.

Density gradient centrifugation. Sucrose density gradients were made by layering 4, 7, 7 and 7 ml of 10, 20, 30 and 40% sucrose solutions, normally made in the virus extracting buffer without addition of any agents, in 3 x 1 in cellulose nitrate centrifuge tubes (Brakke, 1960) and placing these at c 5°C for 16-20 h. Routinely, 0.5 ml partially purified virus preparation was layered onto the gradients, and these were centrifuged at 24,000 r.p.m. for 120 min in a Beckman SW 25 rotor. Preparations made with uninfected leaves were included for comparison. Methods that did not produce distinct light scattering zones were normally abandoned: since the visible threshold of a light scattering zone is generally assumed to be approximately 1 mg/ml virus (M. Hollings, private communication), lower concentrations of virus were considered inadequate for immunological work.

Infectivity test of partially purified virus preparation. Infectivity of partially purified preparations of viruses not transmitted mechanically was checked by feeding hopper vectors on solutions extracted from the light scattering zones formed by the viruses in sucrose density gradients.

The zones were extracted separately by inserting a hypodermic needle through the wall of the density gradient tube. Each of the sucrose solutions was then put in a 1 cm wide glass tube, one end of which was covered with a Parafilm "M" membrane (American Can Co., Marathon Products, Wisconsin) and inserted in a 4 cm wide glass tube through a rubber cork. Uninfected hoppers were released in the wider tube and its free end closed with cotton wool. The free end of the tube containing the sucrose solution was directed towards a source of light so that the insects were attracted towards the membrane and were likely to feed through it. The insects so fed were then transferred to healthy test seedlings.

Electron microscopy. Partially purified virus preparations, negatively stained with 2% potassium phosphotungstate (K-PTA) or uranyl acetate, were mounted on carbon coated grids and examined in an electron microscope (Carl Zeiss Model 9 A). The particle size of filamentous viruses was determined by calculating mean length of 200-300 particles using the standard deviation method.

Antiserum production. Partially purified virus preparations in 0.85% saline were used to immunise rabbits. One intravenous injection of 1 ml was followed after 7 days by an intramuscular injection of 1 ml virus preparation with 1 ml Freund's incomplete adjuvant (Difco Laboratories).

Fourteen days later a second, similar, intramuscular injection was given and serum was obtained 28 days after the last injection. Serum was cleared by centrifugation at 10,000 r.p.m. for 15 min in a Spinco SW 25 rotor, preserved by the addition of an equal volume of glycerol and stored at 2°C.

Normal serum was routinely obtained from rabbits used for immunisation by bleeding an ear vein prior to the course of injections.

Serological techniques. Serological tests were performed by using the standard tube precipitin test for filamentous or rod-shaped viruses (Matthews, 1957) and the agar gel double diffusion test for isometric viruses (Crowle, 1961).

Tube precipitin test. One ml each of a partially purified antigen at dilutions ranging from 1/50 to 1/150 in 0.85% saline was mixed with 1 ml antiserum dilutions starting from 1/4 and incubated in 7 x 125 mm thin walled bacteriological glass tubes in a water bath at 37°C. Observations were made 15, 30 and 60 min and thereafter at 1 h intervals up to 5 h from the start of the incubation. As a control, healthy antigen/antiserum reaction was also studied.

Agar gel diffusion test. 0.5 g Ionagar No.2 was dissolved in 100 ml of 0.85% saline containing 0.02%

sodium azide and was autoclaved at 15 lbs p.s.i. for 15 min. Twenty ml of this agar was then poured into 9 cm glass petri dishes; wells, 6 mm in diameter and 4 mm apart from the central well, were made when the agar had set.

Crude clarified sap, obtained by grinding 10-20 g infected leaves in 0.85% saline (1:1/2 w/v) and centrifuging at 20,000 g for 20 min, was put in the peripheral wells and each centre well filled with one of a range of antiserum dilutions. The plates were left at room temperature (18-22^{o}C) and were observed for 5 days before discarding.

PART - II

MAIZE VIRUSES

3. MAIZE MOTTLE INCITED BY SUGARCANE MOSAIC VIRUS

SUMMARY

A virus inducing a faint mottle in maize and sugarcane leaves was identified on the basis of particle morphology, physical properties and serology as sugarcane mosaic virus (SCMV). Field surveys showed SCMV to be the most widespread and predominant virus of maize in East Africa, causing infection of up to 30% of the plants in maize fields. Using serology and symptoms induced in SCMV sugarcane strain differentials as criteria, the East African strains were found to be closely related to the American strains A and B but not to strains D, H, or I. Two of the maize isolates of the virus reduced yields of maize by 25% and of sorghum by 73%. Traditional East African maize varieties, early synthetics and more recently developed composite maize selections were all susceptible.

INTRODUCTION

In 1969, maize (*Zea mays* L.) at the National Agricultural Research Station, Kitale (Kenya) was reported to have a high incidence of a mottling symptom. In subsequent surveys, the condition was found to be widespread in maize in Kenya, Tanzania and Uganda (Table 6) and was usually associated with the maize aphid, *Rhopalosiphum maidis* Fitch.

In sap inoculation tests, the first symptoms of infection in maize leaves were minute chlorotic spots at the base of the inoculated, or the next youngest leaf. The spots increased in number and coalesced in subsequently produced leaves to form chlorotic stripes of uneven length, which covered approximately 2/3 of the leaf area. Later formed leaves showed symptoms in the form of a general faint streak mottle. Circular to elongated areas with dark margins appeared on leaf sheaths, nodes and internodes. These leaf symptoms usually disappeared with age without leaving any signs of infection. Cobs were occasionally reduced and poorly filled. The virus was easily transmitted mechanically and by R. maidis.

Since Brandes (1920) first recorded SCMV in maize, the virus has been observed in the crop in several parts of the world. Storey (1924) recorded it in maize, sugarcane (Saccharum officinarum L.) and some grasses in South Africa. It was observed in sugarcane in East Africa (Hansford, 1935; McDonald, 1936; Wallace, 1937) but not recorded in maize in Uganda (Hansford, 1938), Tanzania (Wallace, 1947) or Kenya (Robinson, 1960). The only recent but unconfirmed reference to SCMV being present in maize in East Africa (Tanzania) is that of Riley (1960); infection of maize with SCMV appears in general not to have been noticed.

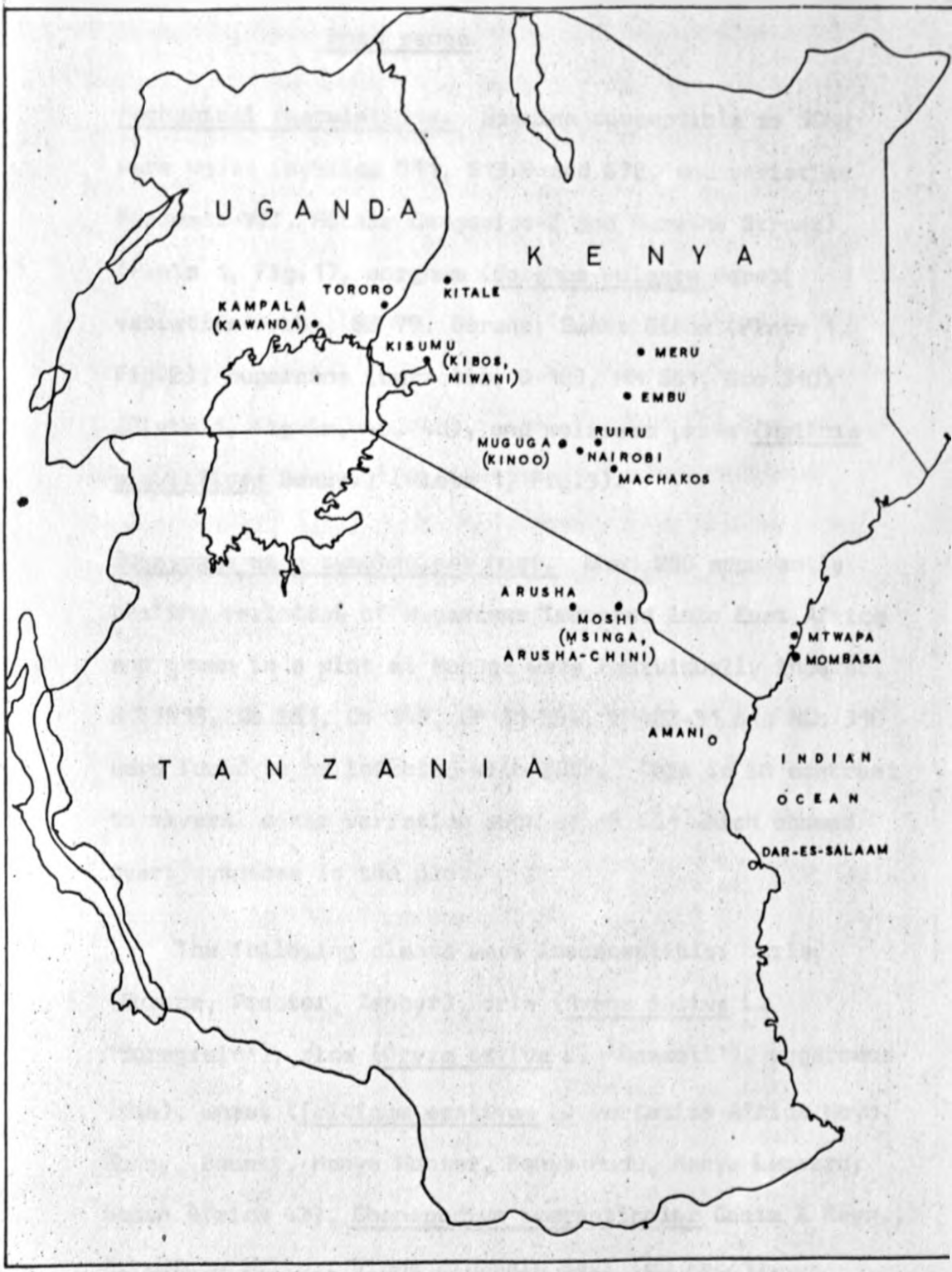
Though the virus was assumed to be present in sugarcane in East Africa, it was never critically identified on the basis of vector, particle morphology, physical properties and serology. For this reason, and because of a manifest effect on yield, detailed studies of the virus were made.

MATERIALS AND METHODS

Forty one isolates of the virus from maize and twelve from sugarcane were collected from widely separated areas in East Africa. Maize isolates were obtained from Kitale and Muguga (Kenya), Arusha-Moshi (Tanzania) and Kawanda (Uganda); Sugarcane isolates were obtained from Kibos, Miwani, Mombasa, Muguga and Nairobi (Kenya), Arusha-Chini (Tanzania) and Kawanda (Uganda) (Map 1). Isolates from both hosts were maintained in maize. Serial passages of the isolates were made by manual inoculations of infected sap extracted in 0.01 M phosphate buffer, pH 7.5. The maize aphid, R. maidis, was collected from maize fields at Muguga; virus-free colonies were reared on healthy barley (Hordeum vulgare L. 'Europa'), an insusceptible species, at temperatures of 18-22 °C.

Healthy maize (Hybrid 511) seedlings were used as a standard test plant for virus assay and for all back-tests in host range studies.

Map 1. Map of East Africa showing the main localities surveyed for maize viruses



● Sites surveyed,

o Not surveyed.

RESULTS

Host range

Mechanical inoculations. Species susceptible to SCMV were maize (Hybrids 511, 613 B and 632, and varieties Katumani-VII, Kitale Composite-E and Muratha Streak) (Plate 1, Fig.1), sorghum (Sorghum vulgare Pers., varieties Dobbs, S8 79, Serena, Sweet Sioux (Plate 1, Fig.2), sugarcane (varieties D 109, HM 661, NCo 310) (Plate 1, Fig.4b, 4c, 4d), and molasses grass (Melinis minutiflora Beauv.) (Plate 1, Fig.3).

Sugarcane as a symptomless host. When 280 apparently healthy varieties of sugarcane imported into East Africa and grown in a plot at Muguga were individually indexed, B 51415, Co 281, Co 349, CP 31-294, M 423-51 and NCo 310 were found to be infected with SCMV. This is in contrast to several other varieties such as HM 661 which showed overt symptoms in the plot.

The following plants were insusceptible: barley (Europa, Proctor, Zephyr), oats (Avena sativa L. 'Suregrain'), rice (Oryza sativa L. 'Basmati'), sugarcane (Uba), wheat (Triticum aestivum L. varieties Africa Mayo, Bonny, Bounty, Kenya Hunter, Kenya Kudu, Kenya Leopard, South Africa 43), Chenopodium amaranticolor Coste & Reyn., C. quinoa Willd., Vigna sinensis Savi (cv Mak/1), Phaseolus vulgaris L. (Prince), Glycine max Merr. (cv HLS 241) and Petunia hybrida Vil.

Insect transmission. Virus free R. maidis was given acquisition feeds of 5 to 30 min on infected maize, followed by test feeds of 3 days. Maize, sorghum and sugarcane became infected, whereas nine, ten and twenty four plants of barley, oats and wheat respectively remained symptomless, and no virus could be recovered from them in subsequent mechanical inoculations to maize.

The aphid could also transmit the virus from mottled sugarcane to maize. Acquisition feeds of 1 min on infected sugarcane leaves, followed by test feeds of 24 h on healthy maize or sugarcane using five insects per plant as an inoculum unit, resulted in no transmission; however, the virus was easily transmitted by increasing acquisition feeds to 5 min.

Seed transmission. To test the possibility of seed transmission, 100 seeds derived from infected maize were germinated: no symptoms were observed on any of the resultant plants and no virus was recovered from them in inoculations to maize seedlings.

Physical properties

Freshly expressed infective maize sap was used to determine the physical properties of the virus. Dilution end point varied between 10^{-3} (Muguga isolates) and 15×10^{-3} (Kitale isolates); all infectivity was lost at dilutions of 10^{-4}. When infective sap was heated for 10 min,

Muguga isolates were inactivated at 40°C and Kitale isolates at 50°C. Both Muguga and Kitale isolates lost infectivity in less than 48 h at 18-22°C.

Purification

Maize leaves were harvested 20-28 days after inoculation and homogenised in 0.01 M phosphate buffer (1:3 w/v) containing 0.1% Thioglycollic acid, pH 7.6. The extract was expressed through muslin and stood overnight at 5°C; 8.5% n-butanol was then added, the mixture stirred for 45 min, and clarified by centrifugation. The supernatant was centrifuged at 100,000 g and the resultant pellets were resuspended in 0.01 M phosphate buffer, pH 7.6. Such partially purified preparations were highly infectious and produced a single specific, intense light scattering zone at a depth of 14-16 mm below the meniscus, when centrifuged in sucrose density gradients. This zone contained characteristic SCMV filamentous particles; was highly infectious, and induced typical symptoms of SCMV in maize.

The virus could not be purified by extraction in 0.5 M tri-sodium citrate buffer + 1% 2-mercaptoethanol (pH 8.0), and 7 ml n-butanol/100 ml extract. Preparations so obtained did not form any light scattering zones in sucrose density gradients.

Electron microscopy

Partially purified preparations contained high concentrations of stiff viral rods (Plate 1, Fig. 5). Kitale and Muguga maize isolates and a Miwani sugarcane isolate measured 699 ± 17, 646 ± 56 and 742 ± 37 nm respectively; their modal lengths being 756 (27 particles), 765 (101 particles) and 756 nm (26 particles) respectively.

Serology

Antiserum was prepared against partially purified preparations of a Muguga isolate which, in tube precipitin tests, had a precipitin dilution end point against the homologous virus of 1/8192. The serum did not react with purified preparation of healthy maize.

SCMV strain determination

When the Muguga maize isolate of the virus was tested against antisera prepared against American strains A, B, D, H and I of SCMV (obtained from the United States Sugarcane Field Station, Houma, Louisiana) in tube precipitin tests, it reacted strongly with its homologous and with strain A and B antisera; weakly with antiserum to strain D; and not with antisera to strains H and I (Table 1). None of these antisera reacted with purified preparations of healthy maize. Similar reactions were obtained with SCMV maize isolates from Arusha-Moshi and Kawanda and SCMV sugarcane isolates from Arusha-Chini, Kawanda, Kibos, Miwani, Mombasa and Nairobi.

Table 1. Serological relationships between an East African strain of SCMV (Muguga isolate) and 5 American SCMV strains (A, B, D, H and I)

Antiserum dilution	Antisera prepared against: Muguga maize isolate	Strain A	Strain B	Strain D	Strain H	Strain I
1/4	+	+	+	+	-	-
1/8	+	+	+	+	-	-
1/16	+	+	+	+	-	-
1/32	+	+	+	+	-	-
1/64	+	+	+	+	-	-
1/128	+	+	+	+	-	-
1/256	+	+	+	+	-	-
1/512	+	+	+	-	-	-
1/1024	+	+	+	-	-	-
1/2048	+	-	+	-	-	-
1/4096	+	-	+	-	-	-
1/8192	+	-	-	-	-	-

Few of the sugarcane differentials used for determining SCMV strains were available locally. Maize isolates from Arusha-Moshi, Kitale and Muguga and sugarcane isolates from Arusha-Chini, Miwani, Muguga and Nairobi were each mechanically inoculated to glasshouse - grown young plants of sugarcane varieties Co 281, CP 29-291, CP 31-294 and HM 661 (Plate 1, Fig. 4b, 4c, 4d). Symptoms incited are summarised in Table 2.

Table 2. Reactions of SCMV strain differential cane varieties to infection by East African maize and sugarcane isolates of SCMV

Isolates	Differential varieties			
	Co 281	CP 29-291	CP 31-294	HM 661
Maize				
Kitale	o	o	c	d
Muguga	o	o	c	d
Sugarcane				
Kibos	a	o	c	a
Miwani	b	a	c	a
Muguga	o	a	c	a
Nairobi	o	a	c	a

a: Mottle, with dark-green elongated areas on lighter background.

b: As for (a), but with elongated white necrotic lesions upto 1 cm.

c: As for (a), but with numerous minute white specks.

d: Mottle, with few elongated white specks.

e: As for (d), white specks extensive, giving a bleached appearance.

o: No infection.

Symptoms caused by East African SCMV isolates were compared with characteristic symptoms induced in the same sugarcane varieties by strains A to H (Abbott & Tippett, 1966) and I (Tippett and Abbott, 1968). In CP 31-294, none of the East African isolates caused the severe stunting and excess tillering which is typical of strain H, or the severe stunting and necrosis typical of strain I (Table 2). None, except sugarcane isolates from Kibos and Miwani infected Co 281, and these did not induce the severe chlorosis and necrosis characteristic of strain C. Strain F induces a fine mosaic with large green islands; D a severe necrosis, stunting and tillering; and E chlorotic stripes with red margins in variety CP 31-294. None of these severe reactions was induced by infection with East African isolates.

Yield trial

Yields of maize Hybrid 511, Katumani-VII, and Kitale Composite - E infected with SCMV maize isolates from Kitale and Muguga were determined under field conditions at Muguga (altitude 2,096 m). Healthy maize seedlings were either raised and inoculated in a glasshouse prior to transplanting in the field, or were directly grown and inoculated in the field; any inoculated plant that did not show symptoms was replaced by an infected one. Healthy,

uninoculated controls from the same batch of seed were grown under the same conditions as the inoculated plants. Four pairs of plots were planted with healthy and infected maize alternating; each plot contained six rows of five plants, the rows being spaced 1 m apart and the individual plants in the row 30 cm apart. Because SCMV is highly infectious and readily infects maize in the field by accidental handling during cultural practices such as watering and weeding, four guard rows of healthy maize surrounded each plot. This reduced accidental spread of the virus from the infected to the control plots.

As a precaution against maize stalk-borer (Busseola fusca Fuller), plots were treated with 'Didimac-5' (5% DDT) two weeks after planting. Subsequently, 'Rogor-40' was sprayed at fortnightly intervals to control the maize aphid population.

Dry grain was harvested and yields of infected and control plots assessed statistically. The Kitale isolate reduced yield by 24% (significant at P = 0.05 level) and 16% (significant at P = 0.05) in Katumani-VII and Kitale Composite-E varieties respectively; the Muguga isolate reduced yield by 25% (significant at P = 0.01) in Hybrid 511.

Sorghum yields were also severely affected. Ten plants each of the commonly grown variety Dobbs were inoculated with the Kitale and Muguga isolates, an equal number of healthy plants being used as controls. Yield

was reduced by 37% and 73% respectively by the two isolates.

Screening for resistance

A preliminary search was made for resistance in local maize varieties. In addition, recently introduced and widely grown varieties were compared with varieties grown before the advent of improved varieties in an attempt to find out whether the present high incidence was attributable to higher susceptibility of improved lines. Five plants each of the following twenty two maize selections were inoculated both mechanically and by *R. maidis* using the maize isolate of SCMV.

(1) Present-day widely grown maize varieties: Ecuador 573 (R 12) C3; Ilonga Composite and Ilonga Composites A and B; Kawanda Composite A; Kitale Composite A, B, C, E and F; K II (R 11) C_3; K III and Ukiriguru Composites A and B.

(2) Early maize hybrids (widely grown c 1963): Hybrids 611 B, 611 (R) CO, 613 (R) CO and SR 52.

(3) Pre-hybrid maize varieties (prior to 1963): Hickory King, Kitale II (un-selected), Kitale Station maize and traditionally grown local maize.

Every plant developed SCMV symptoms: no variation in degree of susceptibility among the three groups was detected.

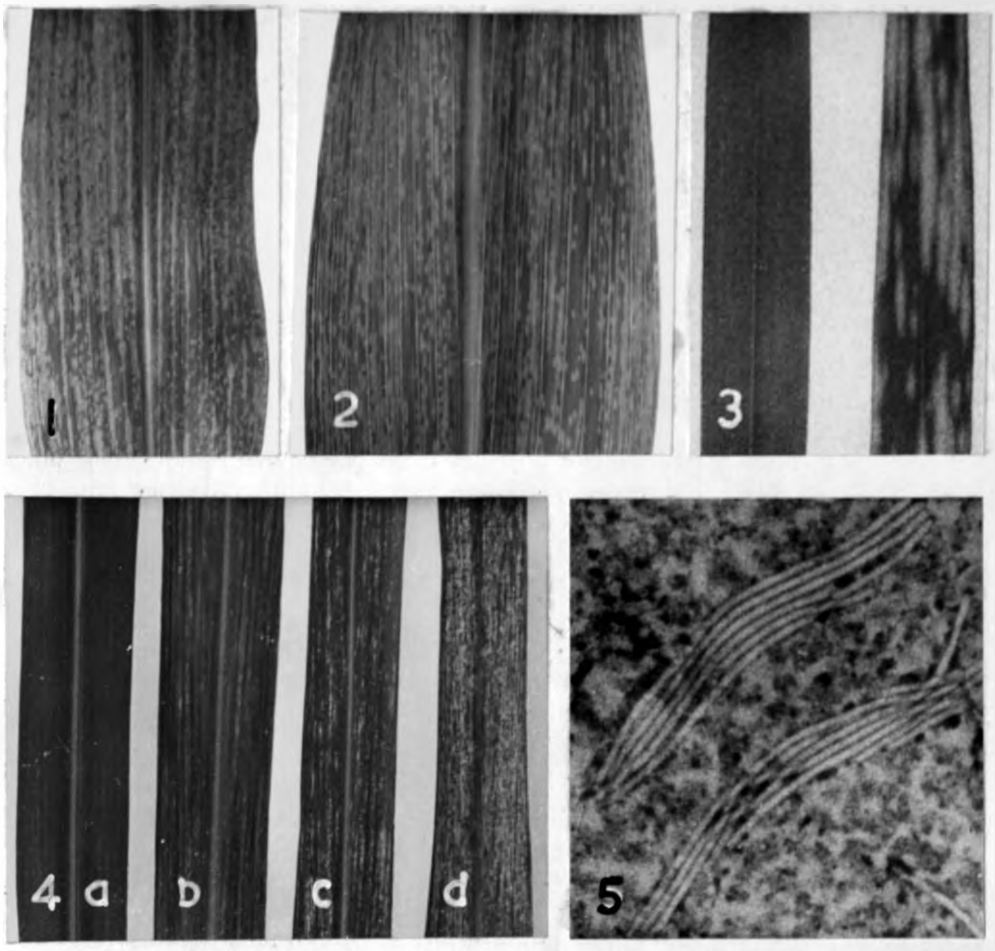
DISCUSSION

Partially purified preparations of East African isolates of the virus contained filamentous particles 646-742 nm long; all were readily mechanically transmissible, none infected sugarcane variety Uba and all were transmitted by R. maidis in the non-persistent manner. These characteristics closely resemble those of sugarcane mosaic virus, which has 630 nm to 770 nm filamentous particles, (Gold & Martin, 1955; Brandes, 1964; Gardner, 1969; Shepherd, 1965; Herold & Weibel, 1963; Pirone & Anzalone, 1966), which is also transmitted mechanically and by different species of aphids including R. maidis (Smith, 1957; Abbott, 1961; Shepherd, 1965) and which will not infect Uba (Storey, 1924). In vitro properties are also similar: type SCMV has a thermal inactivation point of 53° - 65° C (Smith, 1957; Abbott, 1961), a dilution end point of 1 in 1000 (Smith, 1957) and a longevity in crude sap of 24 h (Abbott, 1961). Slight variation in the properties of the East African virus lies within the limits of variation defined by Abbott, 1961. Serological studies indicate the East African virus to be similar to strains A and B of SCMV from the U.S.A; serological evidence being confirmed by a predictable and similar reaction of the sugarcane strain differentials to infection (Abbott & Tippett, 1966; Tippett & Abbott, 1968). On this evidence, the East African virus in maize and sugarcane was

therefore identified as sugarcane mosaic virus.

The East African SCMV seems not to be related to other described strains which occur in North America and India. Maize dwarf mosaic virus (Williams & Alexander, 1965), which has c 750 nm particles (Frazier, Freitag & Gold, 1965; Shepherd, 1965; Kraas & Ford, 1969) is a strain of sugarcane mosaic virus (Frazier, Freitag & Gold, 1965; Shepherd, 1965), causes severe stunting in maize and remains infective for 6–8 days in crude sap (Williams & Alexander, 1965). Maize mosaic virus (Paliwal, Raychaudhuri & Renfro, 1968) although related to SCMV, is not similar to East African SCMV: this virus has a very low dilution end point, remains infective in crude sap for several days and is unable to infect sugarcane.

PLATE - 1



EXPLANATION OF PLATE - 1

Fig. 1. Leaf of maize (hybrid 511) infected with SCMV.

Fig. 2. Leaf of sorghum (var Dobbs) infected with SCMV.

Fig. 3. Healthy (left) and SCMV-infected leaves of molasses grass.

Fig. 4. Sugarcane leaves (HM 661):

(a) Healthy, (b) and (c) infected with SCMV maize isolates from Kitale and Muguga respectively showing mottle with few elongated white specks and (d) infected with SCMV sugarcane isolate from Miwani showing extensive white specks.

Fig. 5. Electron micrograph of SCMV particles negatively stained with 2% K-PTA (79,000).

4. MAIZE STRIPE AND MAIZE LINE VIRUSES

SUMMARY

Two similar viruses isolated from maize in East Africa induced two distinct symptom types in maize. One, designated maize stripe virus (MSV), showed broad yellow stripes or a yellowing of the entire leaf, acute bending of the shoot apex and severe stunting. The second, maize line virus (MLV) induced continuous, narrow yellow lines along the leaf veins and did not cause apical bending or stunting. MSV and MLV were both transmitted by Peregrinus maidis, but not by Cicadulina mbila or by sap inoculation. Both viruses were purified by extracting systemically infected leaves in 0.5 M sodium citrate buffer and clarifying with 7 ml n-butanol/100 ml extract, followed by differential, and finally sucrose density gradient, centrifugation. Partially purified preparations of both viruses contained isometric virus-like particles of two sizes; MSV particles were 35 and 40 nm in diameter with sedimentation coefficients (S^{o}_{20w}) of 109 and 166 respectively; MLV particles were 28 and 34 nm in diameter. Antisera prepared against MSV and MLV had dilution end points of 1/128 and 1/64 respectively in agar-gel diffusion tests.

MSV did not react with MLV antiserum and MLV did not react with MSV antiserum. In the presence of antiserum containing antibodies to both MSV and MLV, the two viruses formed precipitin bands which crossed in the pattern of

non-identity. Maize streak virus and maize mottle virus showed no serological relationship with MSV or MLV.

On the basis of particle size, serology and cross protection tests, MSV and MLV are shown to be two distinct, unrelated viruses and MLV to be a new maize virus for which the name Maize Line Virus is proposed.

MSV and MLV apparently are dis-similar from any characterized viruses of the Gramineae, although they may be in the same group as rice hoja blanca virus on the basis of particle morphology and transmission by a hopper vector.

INTRODUCTION

Storey (1936a) reported a stripe virus of maize (Zea mays L.), transmitted by Peregrinus maidis Ashm., from Amani, Tanzania (Map 1), and concluded that it was possibly the same as the maize stripe virus occurring in Trinidad (Briton-Jones, 1933), Hawaii (Kunkel, 1927) and Cuba (Stahl, 1927). No detailed work was done on the East African virus; this precluded critical comparison with other hopper-borne viruses of maize.

Storey noted two types of symptoms in virus-infected maize: narrow, closely spaced yellow stripes which coalesced to give a completely yellow leaf; and broad, yellow stripes separated by wide green areas. During the present investigations similar symptoms were distinguished, but were invariably found

in different plants. In addition, the former symptom type was always associated with severe bending of the shoot apex; the latter was not. This suggested the two symptoms were possibly induced by two different viruses or virus strains. The isolate inducing broad yellow stripes and apical bending was designated maize stripe virus (MSV); and the isolate causing widely spaced yellow lines maize line virus (MLV).

MSV was locally common in different areas in Kenya and Tanzania, but MLV was only recorded in isolated areas in Kenya. Because MLV symptoms are masked in the presence of maize streak virus, which occurs throughout East Africa, it is possible that MLV occurs more frequently than is suggested.

SYMPTOMATOLOGY

Maize infected experimentally with MSV and MLV under glasshouse conditions developed symptoms similar to those observed in the field.

Maize infected with MSV. When plants were infected at the coleoptile stage, chlorotic specks or streaks developed on the fourth or fifth leaf in 12-24 days. The next leaves showed whitish to bright yellow bands, 1 cm or more in width, usually on one half of the leaf (Plate 2, Fig. 3b). These bands originated from the base of the leaf and extended half to two thirds along its length. Later formed

leaves were more or less completely chlorotic (Plate 2, Fig. 3c, 3d). At this stage, when infected plants were c 0.5 - 1 m in height, acute bending of the apical portion of the shoot occurred (Plate 2, Fig. 1); apical leaves were much reduced and plants were severely stunted. Tasselling was apparently normal, but cob formation was often stimulated in each leaf axil; these were always reduced and poorly filled. Symptoms, in particular apical bending, were always more severe with early infection.

Maize infected with MLV. Initial symptoms in plants infected at the coleoptile stage developed 12-16 days after inoculation; symptom expression was delayed to 28-33 days when plants were inoculated at the 5-6 leaf stage. First symptoms appeared as minute chlorotic spots at the leaf base, followed by a mottle and chlorosis of many of the veins. In the fully developed infected leaf, the yellow veins were 5-7 mm apart, and the interveinal tissue was mottled (Plate 3, Fig. 4b,4c). Lamina tissue about 1 mm on either side of the chlorotic veins was bright to whitish yellow: this chlorosis often extended the entire length of the vein. The lower surfaces of yellowed veins became prominent giving a thickened, ridged texture to the undersurface of the leaf. Plants infected with MLV were only slightly stunted and in contrast to MSV, never developed apical shoot bending (Plate 3, Fig. 1). Tassel and cob development were apparently normal.

MATERIALS AND METHODS

Isolates of the MSV were collected from Muguga, Ruiru, Embu and Meru in Kenya, and from Arusha in Tanzania (Map 1). MLV was observed in the field only at Muguga and Machakos in Kenya, and was consequently collected from these two sites only. Maize streak virus was obtained from Storey's (1967b) culture. Maize plants infected with maize mottle virus (Storey, 1937) were collected from fields at Mtwapa, Kenya (Map 1) and only the material found to be free from maize streak or sap transmissible viruses, as described below under virus isolation, was used for experimental work.

Maize Hybrid 511 was used in all experimental work. In attempted manual inoculation experiments, infective maize sap was extracted in 0.01 M phosphate buffer, pH 7.7.

P. maidis, collected from field-infected maize at Muguga, was freed of virus by subjecting the insects to high temperatures (c 33°C) for 1 month; cultures of virus free individuals were reared on barley (Hordeum vulgare L. 'Europa') at 20-25°C.

Uninfected Cicadulina mbila (Naude) China used for transmission studies were obtained from a virus free culture maintained on healthy maize at c 33°C (Storey, 1967b).

RESULTS

Virus isolation from field infected maize

Both MSV and MLV usually occurred as single virus infections in nature and only rarely were they contaminated with sugarcane mosaic virus or maize streak virus. P. maidis collected from field plants showing only MSV or MLV symptoms were separately transferred to caged healthy maize seedlings in a glasshouse. Plants that developed typical MSV and MLV symptoms were subsequently indexed for maize streak virus by test-feeding virus-free C. mbila on them for 4 days and transferring the insects to healthy maize seedlings. Concurrently the plants were tested for the presence of sap-transmissible viruses, including sugarcane mosaic virus, by sap inoculation assay to healthy maize seedlings. Plants that were free of these viruses were used for bulking MSV and MLV. Infected source plants and young healthy maize were interspersed in an insect proof glasshouse in which uninfected P. maidis were released. When seedlings developed initial symptoms, they were moved to a glasshouse containing Vapona.

Transmission

Sap inoculations. Attempts to transmit MSV and MLV mechanically to the following species were not successful: maize varieties Ecuador 573 (R 12) C_3, Hybrids 511 and 613 B,

Kawanda Composite A, Ukiriguru Composite A, barley, sorghum (Sorghum vulgare Pers. 'Serena'), Chenopodium amaranticolor Coste & Reyn., C. quinoa Willd., Glycine max Merr. (cv HLS 241), molasses grass (Melinis minutiflora Beauv.), Petunia hybrida Vil. and Phaseolus vulgaris L. (Prince, Canadian Wonder).

Transmission tests using P. maidis.

Transmission of MSV from maize to maize. Table 3 indicates that a minimum acquisition feed of 5 days, followed by a test feed of 6 days, was apparently sufficient for P. maidis to transmit the virus (Table 3, Experiments 2, 3, 5, 7). In general, however, percent transmission was unpredictable even with longer acquisition and test feeds (Table 3, Experiments 6, 9). There was some evidence, that nymphs were possibly more efficient than adults as vectors (Table 3, Experiment 7).

Transmission of MLV from maize to maize. P. maidis seems not to be an efficient vector of MLV. When one to thirty infected insects obtained from a field source of MLV were fed on healthy maize seedlings for 5-7 days, only two out of twelve plants, one receiving five and another thirty insects, developed typical MLV symptoms (Table 4, Experiment 1). In a subsequent experiment, again using insects from a field source of MLV, ten plants which were removed after 9 days failed to become infected (Table 4, Experiment 2), while five of the remaining six plants which

were exposed for 5 wk became infected (Table 4, Experiment 4). Unlike MSV, long incubation periods were necessary to effect transmission of MLV (Table 4, Experiments 3, 4, 6).

Transmission tests using C. mbila.

C. mbila, the vector of maize streak virus (Storey, 1925), did not transmit MSV to any of twenty seven maize seedlings when units of five insects were given acquisition feeds of 3-10 days, followed by test feeds of 2-15 days. Similarly, C. mbila did not transmit MLV to any of thirty seedlings after acquisition feeds of 2-7 days followed by test feeds of 1-18 days using one to fifteen insects per plant.

Table 3. Transmission of MSV from maize to maize by P. maidis

Expt. No.	Acquisition feed (days)	Test feed (days)	Insect per plant	No. infected/ No. inoculated
1	unknown ex field source	3-4	5-13	0/10
2	"	6	5	4/5
3	"	7	5	3/5
4	3	5	8	0/5
5	5	6	20	2/5
6	7	6	10-20	0/3
7	7	6	5 (nymphs)	5/5
8	8	3	3	0/4
9	11	8	3	0/3
			Total	14/45

Table 4. Transmission of MLV from maize to maize by P. maidis

Expt. No.	Acquisition feed (days)	Test feed (days)	Insect per plant	No. infected/ No. inoculated
1	unknown ex field source	5-7	1-30	2/12
2	"	9	c 20	0/10
3	"	20	c 20	2/4
4	"	5 wk	c 20	5/6
5	3	1-11	1	0/12
6	8	13	10	2/5
			Total	11/49

Host range symptomatology

P. maidis, from cultures bred on MSV or MLV infected maize at 20-25°C transmitted the viruses to the following species or varieties. Five to ten insects were fed on each test seedling for 5 days.

Species susceptible to MSV:

Maize. Hybrid 511, hybrid 613 B, Katumani-VII and Wisconsin 641 AA were all susceptible; symptoms induced by the virus in these varieties were identical.

Barley (Europa). One out of eight plants developed symptoms: newly formed leaves showed elongated yellow specks 15 days after inoculation. Subsequent leaves developed more or less continuous yellow stripes (Plate 2, Fig. 4b).

There was excessive tillering, stunting, and reduction in the number of floral heads (Plate 2, Fig. 2).

Sorghum (Serena). The virus induced a distinct mottle on young leaves of one of five inoculated plants, followed by yellow stripes similar to those induced in maize leaves but rather brighter; the infected plant was slightly stunted and there was an increase in tillering. Sorghum infected with MSV was observed in the field at Kagari/ Gichera, Embu, Kenya.

Seedlings of oats (Avena sativa L. 'Suregrain'), rice (Oryza sativa L. 'Basmati'), sugarcane (Saccharum officinarum L. 'CP 29-291, CP 31-294'), wheat (Triticum aestivum L. 'Trophy') and molasses grass remained symptomless when similarly inoculated with MSV.

Species susceptible to MLV: P. maidis transmitted MLV to maize hybrid 511, hybrid 613 B, Katumani-VII and Wisconsin 641 AA; reaction to infection was similar in all the maize varieties. Inoculation of other species was not attempted.

Virus purification and density gradient centrifugation

Only those areas of infected maize leaves showing chlorosis of the lamina or veins (Storey, 1928) were used for purification of MSV and MLV. Excised material was

homogenised in a range of buffers under test, the extracts clarified with various agents and subjected to differential centrifugation and the partially purified preparations to density gradient centrifugation.

Purification of MSV. The methods and procedures used and the results of density gradient centrifugation of the MSV preparations are summarised in Table 5.

Table 5. Effect of extractant buffer and clarification agent on concentration of MSV in purified preparations

Buffer	pH	Clarification agents per 100 ml extract	Zones produced in sucrose density gradients
0.5 M NaC + 1% Me	8.0	6.5% n-butanol	2 distinct zones, 12-13 mm, 20-21 mm
0.01 M PO_4 + 1% TGA	7.6	7.5% n-butanol	3 distinct zones, 5-7 mm, @ 11-13 mm
0.01 M PO_4 + 1% TGA	7.5	chloroform (1:1)	2-4 faint zones
0.01 M PO_4 + 1% TGA	7.5	5% n-butanol	0
0.01 M PO_4 + 1% TGA	7.5	chloroform (1:2)	0
0.01 M PO_4	7.5	33 ml chloroform & 20 g ammonium sulphate	0
0.05 M PO_4 + 1% TGA	7.6	7 ml n-butanol, 25 ml chloroform & 25 g ammonium sulphate	0
0.1 M PO_4	5.5	(a) chloroform (2:1) (b) 25 g ammonium sulphate (c) 40 g ammonium sulphate	0

NaC = Tri sodium citrate,
Me = 2-mercaptoethanol,
PO_4 = Sodium/potassium phosphate,
TGA = Thioglycollic acid,
@ = Extracting buffer without Me or TGA used for resuspending virus and dissolving sucrose for density gradients except in #,
0 = No zone formed.

Of the three methods that gave specific light scattering zones in density gradient centrifugation, sodium citrate/butanol gave the cleanest preparations and this was adopted as standard purification procedure. Systemically infected leaf tissue was homogenised in 0.5 M tri-sodium citrate + 1% 2-mercaptoethenol and 7 ml n-butanol added to every 100 ml expressed sap. The extract was stirred for 15 min; stood overnight at 5°C; clarified by centrifuging at 20,000 g for 20 min and subsequently at 100,000 g for 120 min. The pellets were resuspended in 0.01 M sodium tetraborate, pH 8.5 and 0.5 ml of the partially purified preparation was layered on sucrose density gradients made in 0.01 M borate buffer, pH 8.5. These were centrifuged at 24,000 r.p.m. for 120 min. The resultant specific light scattering zones, at c 12-13 mm and c 20-21 mm below the meniscus, were withdrawn separately by inserting a hypodermic needle through the wall of the tube; the virus was centrifuged out of sucrose, resuspended in 0.01 M phosphate buffer, pH 7.5 and these together with the partially purified preparations were examined in the electron microscope.

Purification of MLV. Sodium citrate/butanol preparations of MLV, as described for MSV (Table 5), produced two distinct, but very faint, light scattering zones in density gradients at c 11-12 mm and c 20-21 mm below the meniscus; in contrast to MSV, however, preparations with phosphate/butanol did not result in the formation of light scattering zones; nor did phosphate/chloroform/ammonium sulphate preparations.

Electron microscopy

Partially purified preparations of MSV and MLV stained with 2% K-PTA or uranyl acetate contained isometric, virus-like particles when examined under an electron microscope. MSV preparations contained 'empty' and complete particles 35 nm and 40 nm in diameter respectively (Plate 2, Fig.5). Seventy two percent of the particles measured were 40 nm in diameter. 'Empty' particles, that is, where stain had penetrated, appeared hexagonal with 3 capsomere-like structures on each side of the hexagon (Plate 2, Fig.7).

Particles associated with MLV preparations were smaller, and measured 28 nm and 34 nm in diameter (Plate 3, Fig. 2). Seventy eight percent of the particles were 34 nm in diameter.

Infectivity tests of purified preparations

Attempts to check whether the virus-like particles found in partially purified preparations were the causative agents of MSV and MLV type symptoms were not successful. Adult *P. maidis* were fed through a membrane on each of the two light scattering zones formed by MSV and MLV in the sucrose density gradients and then transferred to healthy maize seedlings. The insects were allowed acquisition feed of 3-5 days on zones formed by MSV followed by 4-12 days test feed; 4 days acquisition feed on MLV zones followed by 3-25 days test feed; each seedling receiving

1-5 insects. None of the seedlings so treated developed any symptoms. Attempts at needle inoculation of the insects (Storey, 1933) with partially purified virus preparations, followed by their transference to healthy maize seedlings also failed.

Analytical ultracentrifugation

When an unfractionated preparation of MSV was examined in a Beckman Model E analytical ultracentrifuge, two peaks with $S^{o}_{20}w$ values of 166 and 109 were observed (Plate 2, Fig. 6); it was assumed that these related to whole and 'empty' particles respectively.

Serology

Antisera to MSV and MLV were prepared and the titres determined in agar-gel diffusion tests: homologous titres of MSV and MLV antisera were 1/128 and 1/64 respectively. Titre of antiserum to MSV was the same when serum was obtained 15 or 28 days after completion of the immunization procedure. However, antisera to MLV obtained after similar periods had titres of 1/16 and 1/64 respectively.

When crude clarified sap of MSV, MLV and the hopper transmitted maize streak virus and maize mottle virus were tested against MSV and MLV antisera, MSV and MLV produced specific precipitin bands only against their respective antiserum (Plate 2, Fig. 8; Plate 3, Fig. 3) and maize streak and

maize mottle viruses did not react at all. Healthy antigen reacted against both the antisera with a precipitin end point of 1/16. A mixture of MSV and MLV antisera (1:1) reacted specifically against MSV and MLV antigens (Plate 2, Fig. 9) but not against maize streak virus and the precipitin bands produced by MSV and MLV crossed in the pattern of intersection (Crowle, 1961). These results clearly indicated MSV and MLV to be two distinct, serologically unrelated viruses bearing no relationship with either maize streak virus or maize mottle virus.

Antisera to cucumber mosaic virus, dahlia and iris strains (supplied by M. Hollings) or Nottonia strain (prepared by K.R. Bock) failed to react with MSV, MLV or maize streak virus.

None of the above antigens reacted with sera normal to MSV or MLV.

Cross protection tests

Groups of 7 healthy maize seedlings were each infected with MSV and MLV and maize streak virus; MSV and MLV were inoculated using a P. maidis culture bred on maize infected with the respective viruses and maize streak virus using infective C. mbila which had fed on infected maize for 4 days (Storey & Howland, 1967b). As soon as initial symptoms of infection developed, five plants from each group were challenge-inoculated with one of the two other

viruses, the remaining two plants being left as controls. The challenging virus was also inoculated to healthy maize seedlings which served as an additional set of controls. Results of these challenge-inoculations were:

MSV followed by maize streak virus. When C. mbila infective for streak virus was fed (five insects per plant, 5 days test feed) on MSV infected maize, subsequent growth of test plants was completely arrested, followed by drying of the apical leaves, and subsequent death in 1½-2 months, but without the appearance of streak symptoms. The two sets of control plants developed typical stripe (MSV) and streak symptoms respectively and their growth was not severely affected.

MLV followed by maize streak virus. In contrast to the lethal effect of dual infection of MSV and streak virus, introduction of streak virus (five C. mbila per plant, 5 days test feed) into MLV infected plants did not restrict growth; 1 month after the introduction of maize streak virus newly formed leaves of three of the test plants developed severe streak, masking the line (MLV) symptoms. The two sets of controls showed typical line and streak symptoms.

Maize streak virus followed by MSV. Inoculation of MSV (twenty P. maidis per plant, 11 days test feed) into maize already infected with maize streak virus resulted in cessation of apical growth, whitening and then drying of

leaves, and death of the plants in 2-2½ months. No leaves developed stripe (MSV) symptoms.

Simultaneous inoculation of MSV and maize streak viruses. P. maidis infective for MSV and C. mbila infective for maize streak virus were fed (five insects of each genus per plant, 5 days test feed) simultaneously on ten maize seedlings. After 2 months, four plants had developed symptoms typical of MSV, and six plants typical of maize streak virus.

DISCUSSION

Attempts to prove that the spherical particles contained in purified preparations of maize infected with MSV and MLV were the agents responsible for inducing diseases were unsuccessful. However, the exclusive presence of these particles in MSV and MLV infected maize and the absence of similar particles in both maize and P. maidis infected with maize mosaic virus-1 (Smith) (Herold, Bergold & Weibel, 1960; Herold & Munz, 1965), or in healthy P. maidis (Herold & Munz, 1967), indicate that the 35 and 40 nm and the 28 and 34 nm particles are in fact the causative agents of stripe and line diseases. They were identified as virus particles in view of their close structural similarity to known spherical viruses.

The two viruses have certain features in common such as their natural host, their spherical shape and similar, though not identical, sizes and transmission by P. maidis.

These features might suggest that the two viruses are strains. However, failure of MSV and MLV to react with antiserum to the heterologous antigen, crossing of their precipitin bands in the presence of antiserum containing antibodies to both MSV and MLV and results of cross protection tests, all suggest MSV and MLV to be unrelated.

Adult P. maidis apparently is not an efficient vector and it possibly needs longer incubation periods than were used in these experiments to effect transmission. With maize mosaic virus-1, for example, incubation periods as long as 58 days were required (Herold & Munz, 1965) before P. maidis could transmit the virus. There are other possibilities in this connection: Jennings & Alicia (1971) showed that rice hoja blanca virus had a deleterious effect on its vector Sogata orizicola Muir and that only 5-15% of the hoppers transmitted the virus; and active (transmitting) and inactive (non-transmitting) races of the insect as noted for C. mbila (Storey, 1932) may operate in P. maidis.

Of the characterized maize viruses, only maize rough dwarf virus (MRDV) is isometric, measuring 55-60 nm in diameter; it is transmitted by Laodelphax striatellus Fallen (Wetter et al., 1969). The maize selection "Wisconsin 641 AA" is highly susceptible to MRDV: it failed to produce MRDV symptoms when infected with MSV or MLV.

Maize mosaic virus-1 is also transmitted by P. maidis (Smith, 1957; Herold & Munz, 1965; Kunkel, 1927) but its particles are rod-shaped, 242 nm long (Herold, Bergold & Weibel, 1960). Thus, on the basis of particle morphology or particle size, neither of these resembles MSV or MLV.

On the basis of symptomatology, MSV and MLV resemble maize mosaic virus-1, maize stunt, maize stripe mosaic and maize streak viruses, all of which induce yellow striping in maize. Maize mosaic virus-1 differs widely in particle morphology; maize stunt is caused by a mycoplasma (Maramorosch, Shikata & Granados, 1968; Shikata, Maramorosch & Ling, 1969); maize stripe mosaic virus is related to sugarcane mosaic (Szirmai, 1968) and maize streak (Storey, 1925, 1936b) and maize mottle (Storey, 1937) viruses, which have not yet been characterized morphologically are serologically unrelated to MSV and MLV and have a different vector.

Several rice and sugarcane viruses have spherical particles, but with one exception are different from MSV and MLV in particle size and vector. Rice dwarf virus has 70 nm spherical particles (Fukushi, Shikata & Kimura, 1962); rice black-streaked dwarf virus, which has 60 nm isodiametric particles (Kitagawa & Shikata, 1969), is serologically related to maize rough dwarf virus (M. Conti, personal communication). Rice yellow mottle virus has 32 nm polyhedral particles (Bakker, 1970), but it is sap-and beetle-transmitted. Sugarcane grassy shoot virus and sugarcane dwarf disease exhibit stripe symptoms on leaves, but the former is sap-and aphid-transmitted; transmission

of the latter is still unknown (Steindl, 1964). Fiji disease of sugarcane has 65-70 nm spherical particles (Teakle & Steindl, 1969). Barley stripe mosaic virus is rod-shaped and is mechanically transmitted (Gold et al., 1954; Gibbs et al., 1963).

Of the 30-40 nm spherical viruses, cucumber mosaic virus (CMV) is known to infect maize, producing stripes and stunting (Slykhuis, 1967). However, this is aphid borne; serological tests with antiserum to dahlia, iris and Nottonia strains of CMV failed to indicate relationship with MSV or MLV.

Rice hoja blanca virus (RHBV) induces white stripes in leaves (Atkins & Adair, 1957) and is isometric, 42 nm in diameter (Herold, Trujillo & Munz, 1968). It may hence be related to MSV and MLV and would appear to form a group of hopper transmitted viruses, circulative in their vectors and of c 40 nm diameter, which infect Gramineae. The possibility of serological affinities between the maize viruses reported here and RHBV was not investigated.

Martyn (1968) lists maize stripe virus Storey, 1936 as a synonym of maize (corn) mosaic virus Kunkel, 1921, (maize mosaic virus-1 Smith). The results reported here show conclusively that the two viruses are different and it is suggested that Storey's name for the disease be retained. The name Maize Line Virus is proposed for MLV which is shown in the present studies to be a new virus of maize unrelated to Storey's maize stripe virus.

EXPLANATION OF PLATE - 2

Fig. 1. MSV-infected maize plant (hybrid 511) showing typical apical banding and broad yellow striping or complete chlorosis of the leaves.

Fig. 2. Healthy (left) and MSV-infected barley plants (var Europa).

Fig. 3. Healthy (a) and MSV-infected maize leaves showing characteristic broad yellow stripes (b) and partial (c) or complete (d) chlorosis.

Fig. 4. Healthy (left) and MSV-infected barley leaf showing yellow stripes.

Fig. 5. Electron micrograph of MSV particles negatively stained with 2% K-PTA (x 98,750). Many particles appear to be 'empty', due to penetration of stain.

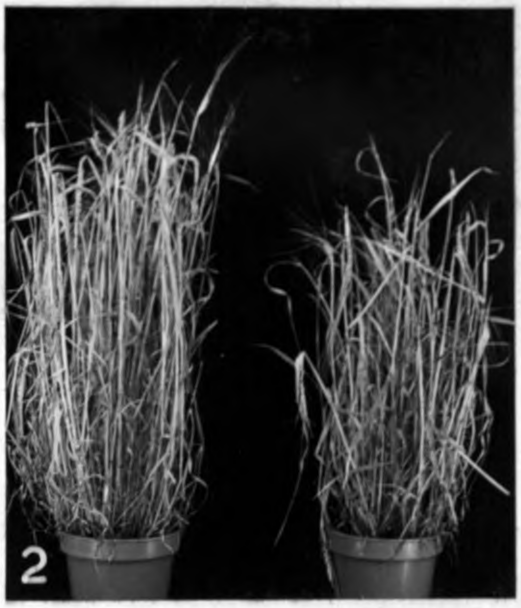
Fig. 6. Schlieren pattern of partially purified preparation of MSV.

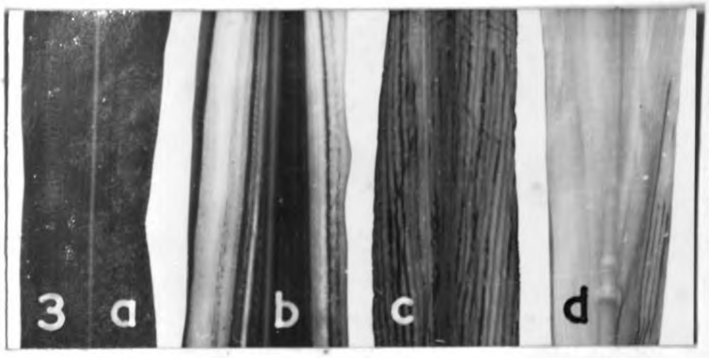
Fig. 7. Highly magnified (x 207,375) 'empty' particles of MSV showing hexagonal shape and three capsomere-like structures on each side.

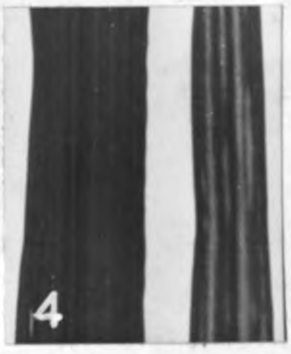
Fig. 8. Agar gel diffusion test of crude saps of healthy maize (H) and maize infected with MSV (S), MLV (L), maize streak virus (St) and maize mottle virus (M) against antiserum to MSV (aS) at 1/16 dilution. Pronounced precipitin bands produced by MSV antigens only; fainter bands are formed with healthy antigens.

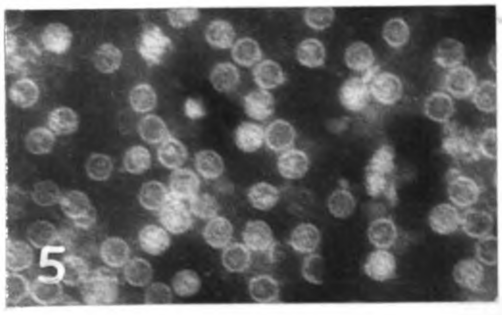
Fig. 9. Agar gel diffusion test of a 1:1 mixture of antisera to MSV and MLV (SL) at 1/8 dilution against healthy (H) and against MSV (S), MLV (L) and maize streak virus (St) antigens in crude maize saps. MSV and MLV form distinct precipitin bands which intersect in a pattern of non-identity; bands against St and H are formed with healthy antigens.

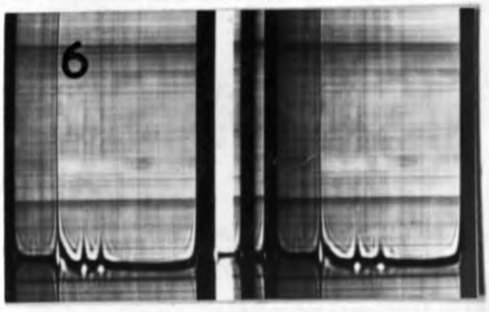
PLATE - 2

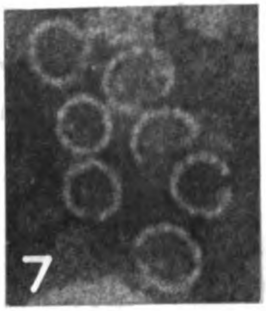


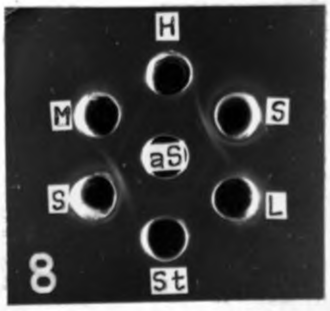












EXPLANATION OF PLATE - 3

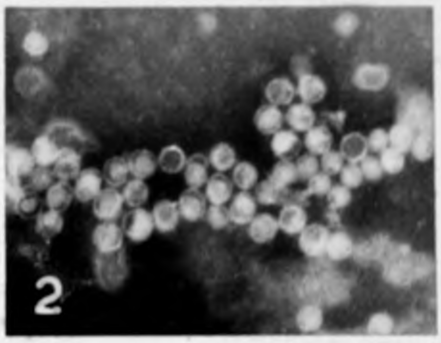
Fig. 1. Maize plant (hybrid 511) infected with MLV. Note distinct, typical line pattern on the leaves.

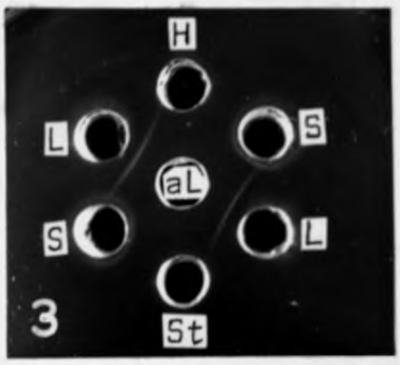
Fig. 2. Electron micrograph of MLV particles negatively stained with 2% K-PTA (x 98,750). Particles exhibit varying degrees of penetration of the stain and occur in two sizes (28 and 34 nm diameter).

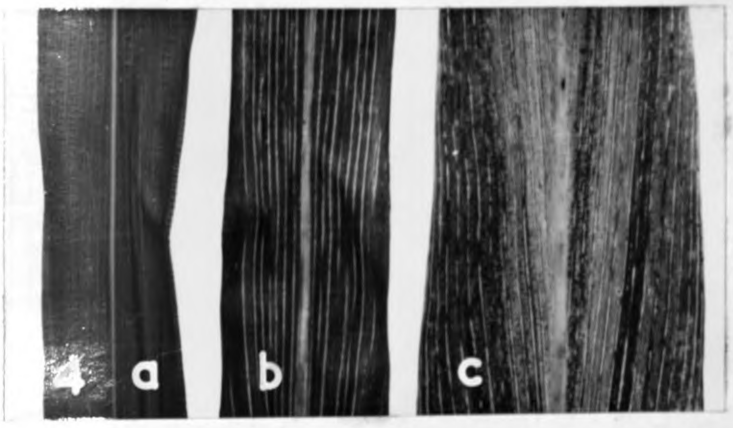
Fig. 3. Agar gel diffusion test using MLV antiserum (aL) at 1/32 dilution against healthy (H), and against MSV (S), MLV (L) and maize streak virus (St) infected crude maize saps. Distinct precipitin bands are formed by MLV antigens only.

Fig. 4. Healthy (a) and MLV-infected maize leaves showing yellow lines 5-7 mm apart (b) and closer (c).

PLATE - 3







5. MAIZE TASSEL ABORTION DISEASE

SUMMARY

An apparently new disease of maize, designated maize tassel abortion disease, is described from Kenya. Typical field symptoms of severe stunting, poorly formed cobs and characteristic abortive tassels were reproduced under glasshouse conditions by inoculation of healthy maize seedlings using infective Malaxodes farinosus. Acquisition feeds of 24 h followed by test feeds of 6 days, resulted in transmission; test feeds of 3 days did not. Although the disease symptoms do not resemble those induced by any known maize virus or mycoplasma, it is considered that one of these types of pathogens is involved.

INTRODUCTION AND SYMPTOMATOLOGY

Maize (Zea mays L.) plants showing severe stunting with other associated symptoms were observed during the course of maize virus surveys in the Kikuyu and Machakos Districts in Kenya. Subsequent counts of affected plants indicated incidence of 14 to 35%. In the field, leaves of affected plants, which were scattered among normal plants of the same age and variety, were chlorotic, much reduced in size and borne horizontally on the stem (Plate 4, Fig.1). The male inflorescences of such plants were often trapped by the apical leaves and were without spikelets (Plate 4, Fig. 2); cobs were either absent or deformed and poorly filled.

Because similar symptoms were recorded when maize was infected experimentally with the pathogen of molasses dwarf disease (Kulkarni, 1969), an attempt was made to investigate the possibility of natural field infection of maize with the pathogen of molasses dwarf disease.

Results.

Uninfected Malaxodes farinosus Fennah, the vector of molasses dwarf disease, were obtained from a 3 year old culture maintained on healthy molasses grass (Melinis minutiflora Beauv.) at c 33°C (Kulkarni, 1969). The insects were given an acquisition feed on the stunted maize from a field source and transferred to healthy maize seedlings (Hybrid 511) at the coleoptile stage. Groups of ten to fifteen insects were fed on each test seedling; acquisition and test feeds were at ambient temperatures of 18-22°C. Controls received uninfected insects and were treated the same way as the test seedlings.

With acquisition feeds of 24 h and test feeds of 3 days no transmission was achieved. However, when the same insects were transferred serially to a second batch of healthy seedlings for 3 days, every seedling developed typical symptoms.

When sap from leaves of the abnormal maize, expressed in 0.01 M phosphate buffer, pH 7.5, was inoculated to healthy maize seedlings, none developed any visible symptoms.

DISCUSSION

The fact that the stunted maize was scattered in the field and was present among apparently healthy maize of the same age and variety suggested the condition to be of pathogenic origin. Results of the transmission experiments confirm that a pathogen is involved.

M. farinosus was not found on affected maize in the field; experience with cultures has shown that the insect will not colonize maize. Furthermore, molasses grass, the natural host, does not occur in the Highlands East of the Rift Valley: this suggests the presence of an alternative vector.

A species of Delphacodes is widespread on maize in East Africa (Le Pelley, 1959). As the insects of this genus are known to transmit viruses (maize rough dwarf virus, Conti, 1966; European wheat striate mosaic, Slykhuis & Watson, 1958; Serjeant, 1967) and mycoplasma (aster yellows disease, Blattny & Prochazkova, 1965), it could well be the natural vector. Cicadulina mbila and Peregrinus maidis are other possibilities.

Maize tassel abortion disease does not resemble any of the known maize virus or mycoplasma diseases. It does not induce enations characteristic of maize rough dwarf (Vidano, Lovisolo & Conti, 1966) or shoot proliferation and stripes characteristic of maize stunt (Shikata, Maramorosch & Ling, 1969), nor can it be confused with

maize streak virus (Storey, 1925, 1936b) and maize stripe or maize line viruses on the basis of symptomatology. Maize mosaic virus (Paliwal, Raychaudhuri & Renfro, 1968) and maize dwarf mosaic virus (Williams & Alexander, 1965), both cause stunting, but these viruses are aphid borne and sap transmissible.

Transmission by hopper but not by sap inoculation and chlorosis and severe stunting of affected maize resulting in interference with flowering and seed setting suggest a virus or a mycoplasma to be the cause of the condition.

PLATE - 4

EXPLANATION OF PLATE - 4

Fig. 1. Maize plant (hybrid 511) infected with maize tassel abortion disease.

Fig. 2. Abortion of tassel spikelets of a maize plant infected with MTAD.

6. FIELD INCIDENCE AND ECONOMIC IMPORTANCE

In order to evaluate the economic importance of the various maize viruses in East Africa, estimates of incidence in the field were made. Areas surveyed included Embu-Meru, Machakos, Muguga and Kinoo (Kikuyu) in Kenya, and Arusha and Moshi in Tanzania (Map 1). Fields were selected at random and every maize plant in blocks of 100-200 plants was scored for visible symptoms (Table 6).

The survey indicated that 43% of plants examined were affected by virus diseases. At Arusha and Muguga, virus incidence was high, 63% and 55% being recorded respectively. Sugarcane mosaic virus (19% of the plants) and maize streak virus (17% of the plants) were the most common viruses; the former was recorded in every field inspected. Incidence of maize tassel abortion disease was high at Machakos (14-35%) and Kinoo (14%) but more localised in distribution.

On the basis of estimates of acreage and yield, Kenya grows approximately twenty million bags of maize a year. Experiments have indicated that the East African SCMV isolates reduce maize yields by about 25%, and that nearly 20% of the crop surveyed was infected with this virus. Thus, at a conservative estimate, SCMV alone accounted for a 5% loss, equivalent to approximately one million bags during 1970-71.

Table 6. Field incidence of maize viruses, 1970-71

Area surveyed	Date	Healthy	# MStV	SCMV	MSV	MLV	MTAD	MStV + SCMV	SCMV + MSLV	Total checked	Total infected	Infection (%)
* Embu-Meru	July'71	223	12	3	3	-	-	-	-	241	18	7.4
Machakos	July'71	245	-	82	-	1	57	-	-	385	140	36.3
Muguga	April'70	458	260	133	15	21	-	8	-	895	437	48.8
"	July'71	68	6	50	20	6	-	2	1	153	85	55.5
Kinoo	April'70	240	7	26	-	-	45	-	-	318	78	24.5
Arusha	June'71	430	350	345	1	-	-	33	-	1159	729	62.8
Moshi	May'71	454	7	69	1	-	-	1	-	532	78	14.6
Individual virus infection			17.4%	19.2%	1%	< 1%	2.7%	1.1%	<1%			
									Total	3683	1566	42.5

MStV = Maize streak virus, SCMV = Sugarcane mosaic virus, MSV = Maize stripe virus, MLV = Maize line virus, MTAD = Maize tassel abortion disease.

* The plants were rather old to notice virus symptoms, especially those of SCMV.

SCMV is of great economic importance as, besides maize, it is known to infect other food and cash crops such as rice, sorghum and sugarcane, in addition to a number of grass species. It is considered to be the most destructive disease of sugarcane, responsible for upto 33.4% loss in yield (Abbott, 1961) in susceptible varieties.

Since its first and only previous record in maize in East Africa (Riley, 1960), the virus apparently has become widespread and of common occurrence over a 10 year period. However, because of lack of severe symptoms, it is equally likely that, in spite of extremely wide occurrence in East Africa, the virus has escaped attention. The situation is rendered more serious because (1) the virus is present throughout East Africa over a wide altitude range and (2) no resistance was found in these investigations, where at least one Composite variety of maize tested contains an extremely wide range of recently introduced maize germplasm.

Although the incidence of maize streak virus varies from season to season and from place to place, it causes substantial losses in yield (Storey & Howland, 1967a). It is known to infect only sugarcane and relatively few grass species in addition to maize (Storey, 1936b), but apparently is no threat to sugarcane production (Storey & Thomson, 1961).

Maize stripe virus could become equally serious, particularly because infection inevitably results in poorly filled cobs. However, the low incidence in maize of both MSV and MLV appears to be an ecologically stable phenomenon. This is deduced from the fact that 35 years since they were first described in East Africa (Storey, 1936a), they are still contained in restricted localities.

Tassel abortion disease also appears to be of limited occurrence: it is not possible to deduce whether this disease, like MSV and MLV, is in an ecologically stable state. It could prove to be a disease of recent occurrence in maize.

In any event, the recent advent and widespread use of new maize germplasm in East Africa may alter the balance of several of the viruses. It is essential that their field incidence be kept under constant review and that, when possible, a search for resistance be made.

PART - III

BEAN VIRUSES

7. BEAN COMMON MOSAIC VIRUS AND VIRUS INCIDENCE IN RELATION TO BEAN APHID POPULATIONS

SUMMARY

A c 750 nm filamentous virus causing dark green vein banding, crinkling and blistering in leaves of bean (*Phaseolus vulgaris*) and transmitted mechanically and by *Aphis fabae* was identified by particle morphology, physical properties and serology, as bean common mosaic virus (BCMV). Four of ten American bean varieties were resistant to the virus.

Sixtyone percent and 39% of a representative sample of aphids trapped in bean plots in the Kenya Highlands, were known vectors of BCMV and bean yellow mosaic virus (BYMV) respectively; BYMV was however not found in beans in East Africa. Virus incidence was found to be related to the increase in aphid populations.

INTRODUCTION

In East Africa, French beans (*Phaseolus vulgaris* L.) are usually interplanted among maize (*Zea mays* L.) and sweet potato (*Ipomoea batatas* Lam.) by smallholders for consumption or are grown on a large scale, especially in the Kenya Highlands and Northern Tanzania, for export.

During the present investigations, virus disease incidence was high in certain seasons and considerable overall losses in yield were being experienced. Although bean common mosaic virus (BCMV) has been recorded in Kenya (McDonald, 1936; Robinson, 1960) and Tanzania (Wallace, 1939, 1944) and bean yellow mosaic virus (BYMV) in Tanzania (Wallace, 1944), identification was based solely on field symptoms and an economic assessment of the effects of these viruses was not made.

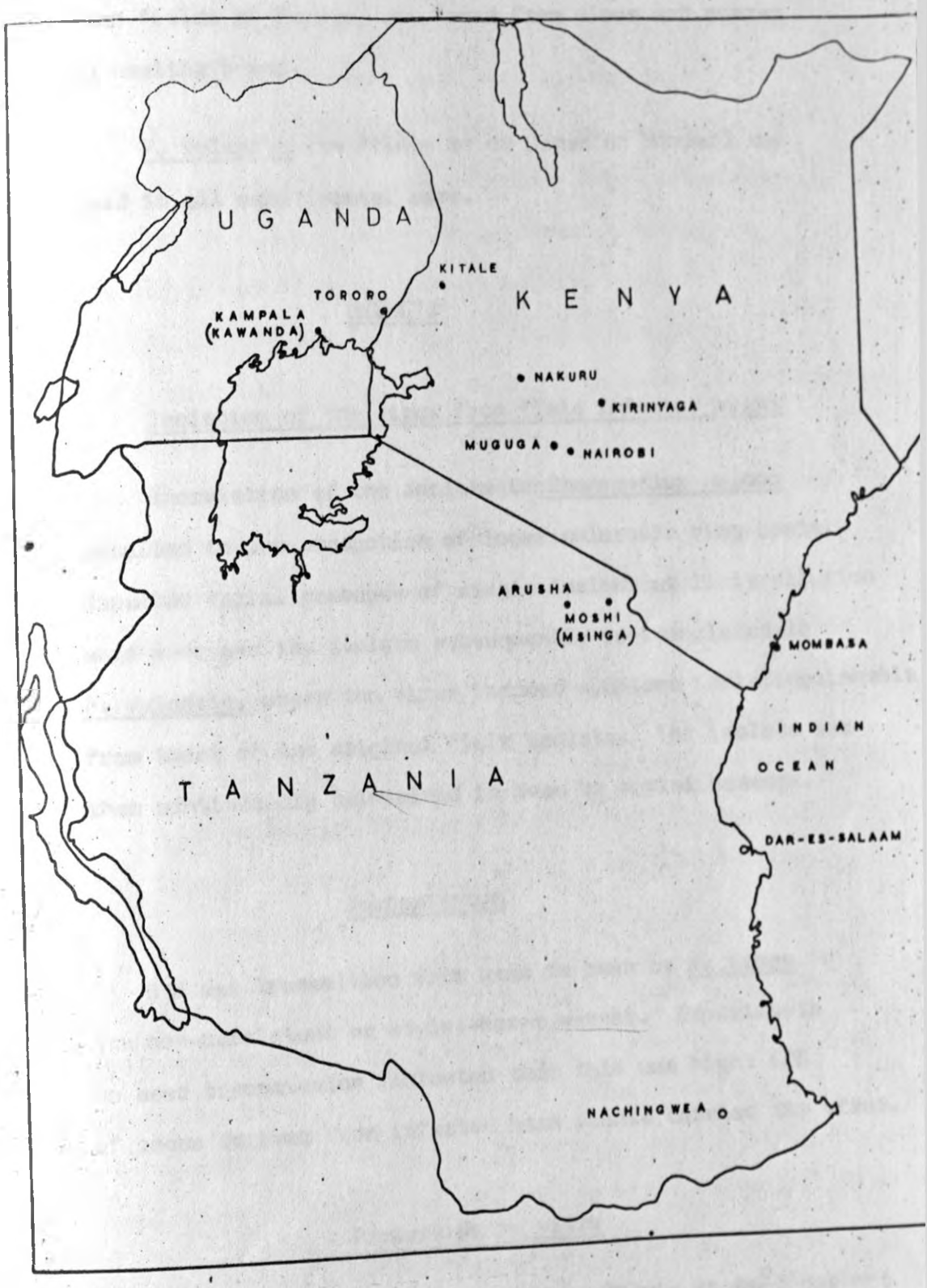
Symptoms of many of the bean virus infections observed in the field in isolates collected from different parts of East Africa resembled those of BCMV (Smith, 1957). Such plants were stunted and their leaves showed broad, dark green, undulated vein banding (Plate 5, Fig. 1). Their younger leaves were often reduced in size, twisted, chlorotic and blistered. Small enations were occasionally seen arising from the undersurface of veins; pods were apparently normal.

Studies on the critical identification of this virus are reported here.

MATERIALS AND METHODS

Virus isolates collected from many areas of East Africa were cultured in the glasshouse in P. vulgaris. An isolate, B18, collected from Kirinyaga, Kenya (Map 2) was selected for detailed study and subsequent comparison

Map 2. Map of East Africa showing the main localities surveyed for bean viruses



● Sites surveyed,

o Not surveyed.

with other isolates. Aphis fabae Scop., collected from bean fields at Muguga, was freed from virus and reared on healthy beans.

P. vulgaris (cv Prince or cv Canadian Wonder) was used in all experimental work.

RESULTS

Isolation of the virus from field infected beans

Inoculation of the isolate to Chenopodium quinoa resulted in the production of local chlorotic ring-spots. Repeated serial passages of single lesions at limit dilution were made and the isolate subsequently re-inoculated to P. vulgaris, where the virus induced symptoms indistinguishable from those of the original field isolate. The isolate was then continuously maintained in bean by serial passage.

Transmission

818 was transmitted from bean to bean by A. fabae in the non-persistant or stylet-borne manner. Experiments on seed transmission indicated that this was high: 60% of seeds derived from infected bean plants carried the virus.

Properties in vitro

Sap from bean was infective after 10 min at 55°C but not at 60 C; after dilution to 10^{-3} but not 10^{-4} and after 48 h but not 72 h at 18-22°C.

Purification

The virus was purified using the method described by Ross (1967) for purification of soybean mosaic virus. Systemically infected bean leaves harvested 21-35 days after inoculation were homogenized in 0.5 M tri-sodium citrate, pH 8.0 containing 1% 2-mercaptoethanol; the homogenate was filtered through muslin, n-butanol added (7.5 ml/100 ml) and the extract stood overnight at c 5°C. Following alternate low and high speed centrifugation, the sedimented virus was resuspended in 0.01 M sodium tetraborate, pH 8.3 for 16 h and the suspension clarified. When such partially purified preparations were layered on sucrose density gradients made in borate buffer and centrifuged, a single sharply defined, bright light-scattering zone was seen 18-20 mm below the meniscus.

The partially purified preparation and the extracted zone were highly infective; when assayed to *P. vulgaris* they induced formation of 115 and 43 local lesions per ½ leaf respectively.

The isolate could not be purified by either of the following methods: (1) by extracting bean sap in 0.1 M phosphate buffer, pH 8.5, with the addition of 25 g ammonium sulphate, 25 ml chloroform and 7 ml n-butanol to every 100 ml extract, although some infectivity was retained; (2) by homogenizing 100 g bean leaves sprinkled with 0.5 g sodium diethyldithio-carbamate (Stace-Smith & Tremaine, 1970)

followed by clarification with 20 ml of 95% alcohol per 100 ml sap, when all infectivity was lost. No light scattering zones were produced with these two methods.

Electron microscopy

Partially purified preparations, stained with 2% K-PTA, showed numerous 722 ± 25 nm long (modal length of 147 particles was 738 nm) filamentous virus particles when examined in an electron microscope (Plate 5, Fig. 2).

Serology

Immunisation of a rabbit, using partially purified preparations, resulted in an antiserum with a homologous titre of 1/1024 in tube precipitin tests. The virus reacted specifically with antiserum to a European isolate of BCMV to a dilution end point of 1/16,384, but not with antiserum to a European isolate of BYMV. Partially purified preparations of healthy bean material did not react to any of the three antisera.

Isolates from widely separated areas in East Africa were tested against B18 (BCMV) antiserum (Table 7). Viruses collected from Arusha, Tanzania (2 isolates), from Muguga (1) and Kitale (2), Kenya and from Tororo (1) and Kawanda (2), Uganda (Map 2), all reacted strongly with the antiserum, with no indication of serological variation between geographically distinct isolates.

Table 7. Serological reactions of East African bean isolates to antisera to bean viruses

Isolate	Collected from	Antiserum	Antiserum titre
B18	Kirinyaga	B18	1/1024
		BCMV (European)	1/16, 384
		BYMV (European)	0
B10	Arusha	B18	1/1024
B20	Muguga	B18	1/512
B23	Kitale	B18	1/1024
B31	Tororo	B18	1/1024
B44	Kawanda	B18	1/2048

Screening for resistance

Ten American bean varieties were checked for resistance to East African BCMV (B18) by manual and aphid inoculation tests. Table 8 indicates that the varieties Great Northern 1140, Tendercrop, Topcrop and Selection 184 may have useful resistance to the East African strain of BCMV.

Aphid populations and their relationship to BCMV

Eastop (1957) studied seasonal variation of aphid populations in East Africa but his more detailed observations were made in a pyrethrum plot at Muguga in Kenya and in

Table 8. Resistance of American bean varieties to an East African isolate of BCMV

Bean varieties tested	Mechanical inoculation	Aphis fabae inoculation
Gallatin 50	1/5 *	-
Great Northern 1140	0/5	0/5
Pinto 5	5/5	-
Pinto 14	5/5	-
Pinto 111	5/5	-
Red Mexican No.36	2/5	1/10
Selection 184	2/5	0/5
Selection 780	3/5	1/5
Tendercrop	0/5	-
Topcrop	0/5	-

* No. infected/No. inoculated,

\- Not tested.

mixed vegetation at Nachingwea in Southern Tanzania (Map 2). Because little was known of aphid populations in relation to bean crops, a preliminary study was made of aphid species found in beans in the Kenya Highlands and an attempt was made to relate population density and species with virus incidence. In addition, general observations were made on aphid populations and virus incidence in beans in Northern Tanzania in July, 1969 and June, 1971.

One hundred plants each of three bean varieties (Masterpiece, Mexico 142 and Prince) were grown in an open plot at Muguga (2096 m altitude), Kenya during the rainy season April-June, 1969. Aphids were trapped daily in Moericke trays (shallow dishes painted yellow with Robbialac 'Maize' colour paint and partially filled with water) which were placed at random within the plots at an average height of the bean plants. In April and May, aphid infestation was low; this built up during June and dense populations were present throughout July. Table 9 summarises the results of trapping and indicates the relative frequencies of the aphid species.

In July, 1969, the bean crop in the Arusha-Moshi area of Northern Tanzania was surveyed for virus incidence and observations were also made on aphid populations. Field infection was high (upto 100% in some areas) and aphid infestation heavy. The same areas were surveyed again in June, 1971, when light incidence of virus infection and low aphid populations were encountered.

Table 9. Aphid species and their relative frequency of occurrence in bean plots at Muguga during April-June, 1969 *

Species	Per cent total aphids
Aphis craccivora Koch.	2.8
A. fabae Scop.	36.2
A. gossypii Glover	21.2
A. nerii Boy.	0.6
A. sp.	0.6
Hyperomyzus lactucae L.	4.0
Lipaphis erysimi Kalt.	11.5
Myzus ornatus Laing	0.6
M. persicae Sulz.	4.0
Rhopalosiphum maidis Fitch	3.4
Paoliella (Unipterus) commiphorae Doncaster	0.6
Smynthurodes betae Westw.	0.6
Tetraneura nigriabdominalis (=hirsuta) Baker	13.2
Toxoptera citricidus Kirk.	0.6
Total	100

*

Month	Rainfall mm	Temperature Minimum °C	Temperature Maximum °C	Wind speed Kph
April, 1969	60.1	12.0	22.7	15.3
May, 1969	159.4	11.1	20.6	10.0
June, 1969	9.6	10.1	19.8	10.4

DISCUSSION

On the basis of symptomatology in bean, high rate of seed transmission (60%), aphid (A. fabae) transmission in the non-persistant manner, particle morphology (c 750 nm flexuous rods) and an extremely close serological relationship, the virus studied in East Africa is confirmed as a strain of BCMV. Evidence also suggests that East African BCMV is similar to European BCMV but differs markedly from some American strains of the virus.

The Idaho (Dean & Wilson, 1959) and Mexican (Silbernagel, 1969) strains of BCMV are able to infect the Great Northern bean varieties, whereas at least one of these varieties is resistant to the East African isolates. The Florida strain (Zaumeyer & Goth, 1964) has a dilution end point of less than 1 in 4000 and induces local solid brown lesions and a pronounced dark green pod mottle: no East African isolate was similar in any of these respects.

Neither can the East African virus be confused with bean (Western) mosaic virus (Skotland & Burke, 1961), which has a low seed transmission rate (2-3%) and which induces veinal necrosis in Great Northern bean and Pinto 111; nor with the severe bean mosaic virus (Yerkes & Patino, 1960) which is a strain of the beetle-transmitted bean (Southern) mosaic virus (Grogan & Kimble, 1964).

Serologically, it is unrelated to BYMV.

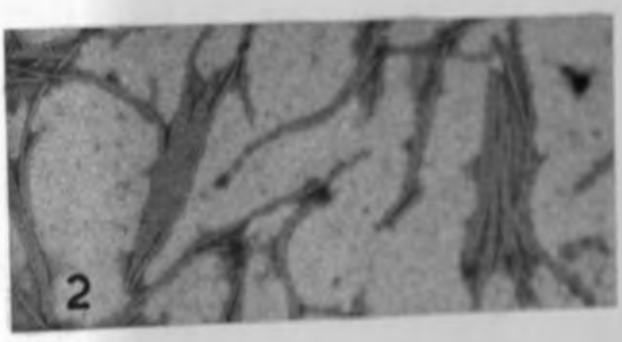
Of significance is the wide distribution of BCMV in East Africa; the virus was found to occur in all the major bean-producing areas throughout Kenya, Tanzania and Uganda and it probably occurs wherever beans are grown. The wide distribution is, however, countered by the apparent lack of dissimilar strains, all isolates studied being remarkably uniform and by the existence of several varieties which are resistant to the virus.

Observations indicate a fairly consistent pattern of aphid population densities in the Kenya Highlands and Northern Tanzania; infestation is low in April and May, there is a marked increase in June, and in July high densities are encountered within the bean crop. These results confirm for beans the more general observations of Eastop (1957) who showed that the aphid population in East Africa decreases from February to April and that half the number of aphids trapped in a year are trapped in June and a quarter in July. Virus incidence is obviously related to the initial population increase in early June and their migrations.

Of the thirteen species of aphids trapped in the bean plots at Muguga, Aphis fabae (Zettler, 1967), A. gossypii (Smith, 1957) and Myzus persicae (Smith, 1957; Zettler, 1967) are known vectors of BCMV; it is significant that they collectively represented 61% of the total population.

That at least two vectors of BYMV (A. fabae, Smith, 1957 and A. craccivora, Evans & Zettler, 1968) are also present is of interest. There is no evidence that BYMV occurs in East Africa, although pea mosaic virus is relatively common in pea (Pisum sativum L.), broadbean (Vicia faba L.) (K.R. Bock, personal communication) and Trifolium semipilosum Fres. var. glabrescens Gillett, the common clover of the Kenya Highlands. During the extensive surveys for bean viruses in East Africa, BYMV was not isolated, nor were symptoms similar to those induced by BYMV recorded. In contrast A. fabae transmits bean yellow-spot virus and this virus is in fact fairly common.

PLATE - 5



EXPLANATION OF PLATE - 5

Fig. 1. Healthy (left) and BCMV-infected bean plants (*P. vulgaris* cv Prince).

Fig. 2. Electron micrograph of BCMV particles negatively stained with 2% K-PTA (x 44,655).

8. BEAN YELLOW-SPOT VIRUS

SUMMARY

Mottle, yellow spotting and apical vein fusion symptoms of bean (*Phaseolus vulgaris*) leaves were associated with a 738 ± 31 nm filamentous virus which was transmitted mechanically, by *Aphis fabae*, and through bean seed and which reduced yield by 20%. The virus was purified by extracting sap from systemically infected leaves in 0.1 M phosphate buffer, pH 8.5 and clarifying the extract with ammonium sulphate, chloroform and n-butanol; such preparations were infectious and were used to produce an antiserum with a homologous titre of 1/4096. The virus was serologically related to seven of twelve viruses of the potato virus Y group, was apparently closely related to East African cowpea aphid-borne mosaic virus, and, distantly, to East African bean common mosaic virus and pea mosaic virus. It showed no relationship with bean yellow mosaic virus. On the basis of symptomatology, host range, physical properties and serology, the virus is considered to be a new virus of bean and is designated bean yellow-spot virus (BYSV). Tests for resistance indicated 7 of 19 New World bean varieties to be immune.

INTRODUCTION

In the course of surveys for viruses affecting bean (Phaseolus vulgaris L.) in East Africa, virus isolates were collected from many different localities in Kenya, Tanzania and Uganda. Several of these were identified as bean common mosaic virus (BCMV); however, other isolates which induced a distinct systemic mottle and yellow spotting, together with apical vein fusion, did not resemble those of BCMV or bean yellow mosaic virus (BYMV, Smith, 1957). Nor were symptoms similar to those induced by the following viruses: dark green mottled pods as found in plants infected with bean (Southern) mosaic virus (Zaumeyer & Harter, 1943) and bean pod mottle virus (Zaumeyer & Thomas, 1948); reddish nodes and concentric rings on pods caused by bean red-node virus (Thomas & Zaumeyer, 1950); pin-head size yellow spots on leaves and reddish discolouration of stems and petioles caused by bean yellow-dot virus (Thomas, 1951); black streaking of stems and petioles induced by bean stipple streak (Smith 1957); veinal necrosis resulting from Western bean mosaic virus (Skotland & Burke, 1961); and severe stunting associated with bean stunt virus (Echandi & Hebert, 1971). Symptoms were however, similar to those produced by bean yellow stipple virus (Zaumeyer & Thomas, 1950).

In view of this general dis-similarity of symptoms detailed studies of the virus were made; results of the characterization are reported here.

SYMPTOMATOLOGY

Beans infected manually with the virus produced local chlorotic or necrotic rings which became diffuse with ageing of the leaf. The next few leaves showed a network of dark green veins against a chlorotic background; later formed leaves developed the characteristic mild mottle (Plate 6, Figs. 1, 2), accompanied by interveinal yellow spotting (Plate 6, Fig. 3), as seen in the field. Frequently, vein thickening and vein fusion occurred at the tips of the leaves (Plate 6, Fig. 4). Occasionally, systemically infected leaves remained small, developed dark green vein banding similar to that induced by BCMV, and showed dark green blisters (Plate 6, Fig. 5); infrequently, small enations developed on the under surface of the veins. Pods were normal, and infection, which always resulted in slight stunting (Plate 6, Fig. 1), was generally milder than that produced by the East African isolate of BCMV.

MATERIALS AND METHODS

An isolate of the coded virus 81 collected from Nairobi, Kenya (Map 2) and maintained in P. vulgaris (Prince or Canadian Wonder) by serial inoculations was used for detailed studies. Similar isolates were collected from

Kitale (3 isolates), Kenya, Arusha (1) and Maings (1), Tanzania and Kawanda (1), Uganda.

A partially purified virus preparation obtained by using chloroform as the clarifying agent may produce a spontaneous precipitate in tube precipitin tests (M. Hollings, private communication). To avoid this, an equal volume of 0.3 M saline was added to a partially purified preparation of B1 virus; the preparation was incubated at room temperature for 16 h, centrifuged at 12,500 g for 5 min and the supernatant used in serology tests.

RESULTS

Virus isolation from field infected beans

Unlike bean common mosaic virus, B1 induced local chlorotic lesions in *Chenopodium quinoa* Willd. Single local lesions were excised; after three or four passages at limit dilution in *C. quinoa*, the virus was returned to *P. vulgaris*.

Transmission and host range

The virus was transmitted from bean to bean by sap inoculation, *Aphis fabae* Scop. in the non-persistant manner and through 3% of bean seed.

In addition to C. quinoa and P. vulgaris, the following plants were susceptible to B1: Calapogonium mucunoides Desv., Cassia occidentalis L., Centrosema pubescens Benth., Chenopodium amaranticolor Coste & Reyn., Clitorea ternatea L., Crotalaria intermedia Kotschy, C. juncea L., C. pauline Schrank, Glycine max Merr. (HLS 241), Lathyrus odoratus L., Medicago sativa L., Nicotiana clevelandii Gray, Pisum sativum L. cv Greenfeast, Tephrosia candida DC., and Vigna sinensis Savi (Mak/1).

The plants that could not be infected were: Antirrhinum majus L. (Mammoth Mixed), Arachis hypogaea L. (Natal Common), Capsicum annuum L., Cucumis sativus L. (National Pickling), Desmodium discolor Vog., D. intortum Fawc. & Rendle, D. ovalifolium Guill & Perr, D. sandvicense E. Mey., D. uncinatum (Jacq.) DC., Lycopersicon esculentum Mill (Money Maker), Melilotus alba Desr., Nicotiana glutinosa L., N. tabacum L. (White Burley), Petunia hybrida Vil., Stylosanthes gracilis Kunth, Tetragonia expansa Murr., Trifolium repens L., T. pratense L. (red clover) clone Ky C 71-8 which is highly susceptible to some strains of bean yellow mosaic virus and Vicia faba L.

Properties in vitro

Dilution end point: Sap usually lost infectivity when diluted more than 10^{-3} with distilled water.

Thermal inactivation point: Using freshly extracted sap, infectivity was much decreased after 10 min at 55°C and abolished after 10 min at 60°C.

Longevity in vitro: Infectivity of sap was reduced after 48 h and lost at 72 h, at 18-22°C.

Purification

Table 10 summarises the attempts at purification of isolate 81; only 0.1 M phosphate buffer with butanol/ ammonium sulphate/chloroform clarification resulted in clear, infective preparations with good virus concentration. Systemically infected bean leaves 21-35 days after inoculation were homogenized in 0.1 M phosphate buffer, pH 8.5 (1:2 w/v), the extract strained through muslin, and 25 g ammonium sulphate, 25 ml chloroform and 7 ml n-butanol, were added to every 100 ml. The mixture was stirred for 20 min, clarified by centrifugation at 20,000 g and the clear supernatant centrifuged for 90 min at 100,000 g. Pellets were resuspended in 0.1 M phosphate buffer, pH 8.5. Density gradient centrifugation of such preparations resulted in single, specific well defined light scattering zones at c 16-18 mm below the meniscus. Rarely, a second fainter zone was observed at c 19-20 mm.

Partially purified preparations and the upper and lower light scattering zones were inoculated to P. vulgaris where they induced 203, 164 and 86 lesions per ½ leaf respectively.

Table 10. Effect of extractant buffer and clarification agent on concentration of 81 in purified preparations

Buffer	pH	Clarification agents per 100 ml extract	Zones produced in sucrose density gradients
* 0.1 M PO_4	8.5	7 ml n-butanol, 25 g ammonium sulphate & 25 ml chloroform	Distinct zone, 16-18 mm
0.1 M PO_4	5.0	(a) chloroform (2:1) (b) 25 g ammonium sulphate (c) 40 g ammonium sulphate	0
0.1 M PO_4	5.0	(a) 25 g ammonium sulphate (b) 40 g ammonium sulphate	0
0.1 M PO_4	8.5	7.5 ml n-butanol & 25 ml chloroform	0
0.05 M PO_4 + 1% TGA	7.6	8.5 ml n-butanol	0
0.5 M NaC + 1% Me	8.0	8.5 ml n-butanol	0

* PO_4 = Sodium/potassium phosphate,

TGA = Thioglycollic acid,

NaC = Tri sodium citrate,

Me = 2-merceptoethanol,

0 = No zone formed.

Electron microscopy

Electron microscopy of partially purified preparations, stained with 2% K-PTA, showed numerous filamentous particles; mean length of 390 particles was 738 ± 31 nm and modal length of 337 particles was 734 nm (Plate 6, Fig. 6).

Serology

Antiserum prepared against B1 virus had a homologous titre of 1/4096. Purified preparations of the virus reacted with antisera to the following East African viruses: bean common mosaic virus (BCMV, precipitin end point 1/1024 against B1 virus), cowpea aphid-borne mosaic virus (CAMV, 1/4096), pea mosaic virus (PMV, clover isolate, 1/256) and sugarcane mosaic virus (SCMV strains A and B, 1/128); to American SCMV (strain A, 1/128) and soybean mosaic virus (SMV, 1/128) and to European PMV (1/256) and lettuce mosaic virus (1/32). With the exception of CAMV, reactions were weak.

The virus shared no antigens with European BCMV, bean yellow mosaic virus (BYMV), celery mosaic virus, clover yellow vein virus, iris mosaic virus, potato virus Y and tobacco severe etch virus.

Isolates similar to B1 from Kenya, Tanzania and Uganda were tested serologically; all reacted specifically with B1 antiserum, apparently without any serological differences.

Yield trial

One hundred seedlings of bean (Prince) were inoculated with 81 virus and then grown to maturity in a glasshouse; an equal number of uninoculated seedlings were grown in the same house as controls. When the dry bean seeds of each treatment were harvested and weighed, those derived from diseased plants showed a loss of 19.3% in yield.

Screening for resistance

Locally grown bean varieties. Varieties of P. vulgaris locally available in East Africa, including several that are widely grown, were screened for resistance to 81 virus by sap inoculation to ten seedlings of each variety. Four to 6 wk later the plants were assayed for presence of the virus: equal sized leaf discs were ground in standard volumes of buffer and the inoculum assayed to C. quinoa; virus concentration was determined by relative numbers of lesions produced. Apparently complete resistance was found in Burpee's Greenpod Stringless, Contender Stringless and Kentucky Wonder; all other varieties were highly susceptible to the virus (Table 11).

Table 11. Susceptibility to B1 virus of bean varieties grown in East Africa

Bean variety	Symptoms	Pods	Lesions per ½ leaf of C. quinoa
Burpee's Greenpod Stringless (Lima bean)	r	+	0
Canadian Wonder	1	-	69
Contender Stringless	r	+	0
Kentucky Wonder	r	+	0
Masterpiece (Victory)	2	-	79
Mexico 142	2	+	49
Primeur	1	+	74
Prince (Long Tom)	1	+	68
Tendergreen	3	-	96

r = Resistant; no symptoms induced, no virus recovered in back test,

Reaction to infection:
1 = Mild; 2 = severe and 3 = very severe symptoms,

\+ = Pods present,

\- = No pods produced.

Imported North American bean varieties. Ten bean varieties imported from the U.S.A. were checked for resistance to B1 by sap or aphid inoculation (Table 12). Gallatin 50, Tendercrop and Topcrop were immune when inoculated mechanically and Red Mexican No.36 could not be infected by A. fabae.

Table 12. Susceptibility of American bean varieties to B1 virus

Bean variety	Mechanical inoculation	A. fabae inoculation
Gallatin 50	0/5*	-
Great Northern 1140	3/5	2/5
Pinto 5	4/5	-
Pinto 14	5/5	-
Pinto 111	5/5	-
Red Mexican No.36	5/5	0/10
Selection 184	1/5	1/5
Selection 780	4/4	1/5
Tendercrop	0/5	-
Topcrop	0/5	-

* No. infected/No. inoculated,

- Not tested,

DISCUSSION

B1 virus was antigenically related to seven viruses of the potato virus Y group, closest affinity being with CAMV. Both B1 and CAMV give weak serological reactions against antiserum to East African BCMV, but the precipitin end point of CAMV is 1/256 (Bock, 1972) in contrast to that of B1, which was 1/1024. Furthermore, CAMV is not related to SCMV and does not infect Lathyrus odoratus and Medicago sativa.

B1 virus, although distantly related to East African BCMV, did not react serologically with antisera to European BCMV or BYMV. It failed to infect red clover clone Ky C 71-8, which is highly susceptible to certain strains of BYMV (S. Diachun, personal communication). Its dilution end point and longevity *in vitro* differ from those of BCMV, BYMV and pea mosaic virus (Yerkes & Patino, 1960). In addition, failure to purify the virus using the method successfully employed for the purification of BCMV also suggests the two viruses to be unrelated. *Pisum sativum*, *Trifolium* spp. and *Vicia faba* are key differential hosts of BYMV (Zaumeyer & Goth, 1964), but none of these could be infected with B1 virus. Bean yellow-dot virus also seems unrelated to B1 because of wide differences in symptoms and dilution end points and the ability of bean yellow-dot to infect Kentuky Wonder and Topcrop beans, *Cucumis sativus*, *Melilotus alba*, *Nicotiana tabacum*, *Trifolium pratense* and *Vicia faba* (Thomas, 1951) which, under conditions of these tests were immune to B1. One recorded virus bears some resemblance to B1 virus. Bean yellow stipple virus (Zaumeyer & Thomas, 1950) is transmitted manually, produces mildly mottled and yellow stippled leaves and has many hosts common with the virus under study. However, yellow stipple virus can infect Kentucky Wonder, Topcrop, *Phaseolus lunatus* L., *C. sativus*, *Lycopersicon esculentum*, *M. alba*, *N. tabacum*, *T. pratense*, *T. repens* and *V. faba*, whereas these

are immune to the East African virus; in addition, yellow stipple virus is not seed borne and can withstand high dilutions in crude sap.

On this evidence 81 virus is considered to be a new virus; because of the characteristic systemic yellow spot symptoms it induces in beans, it is designated as bean yellow-spot virus (BYSV).

EXPLANATION OF PLATE - 6

Fig. 1. Bean plant (*P. vulgaris*, cv Prince) infected with BYSV.

Fig. 2. BYSV-infected bean leaf showing mottling.

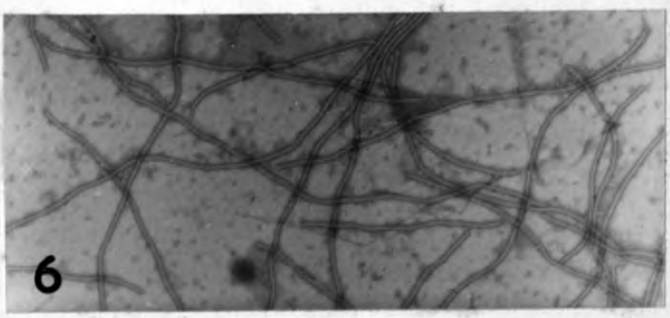
Fig. 3. BYSV-infected bean leaf showing characteristic interveinal yellow spots.

Fig. 4. BYSV-infected bean leaf showing deformation, vein fusion and blistering.

Fig. 5. BYSV-infected bean leaf showing vein banding, blistering and extreme reduction in size.

Fig. 6. Electron micrograph of BYSV particles negatively stained with 2% K-PTA (x 34,562).

PLATE - 6



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