STUDIES ON VIRUSES ISOLATED FROM LEAF VEGETABLES IN LAGOS, WITH REFERENCE TO *CELOSIA ARGENTEA* AND *CUCURBITA MOSCHATA*.

BY

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DEDICATION

Dedicated to my loving and caring wife, Iyabo and my children, Labake, Fayoke, Bamidele and Ayodeji jr. For their patience and understanding.

SCHOOL OF POSTGRADUATE STUDIES

UNIVERSITY OF LAGOS

CERTIFICATION

THIS IS TO CERTIFY THAT THE THESIS:-

'STUDIES ON VIRUSES ISOLATED FROM LEAFY VEGETABLES IN LAGOS, WITH REFERENCE TO *CELOSIA ARGENTEA* AND *CUCURBITA MOSCHATA*'.

SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES UNIVERSITY OF LAGOS FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY (MICROBIOLOGY) IS A RECORD OF ORIGINAL RESEARCH CARRIED OUT BY OWOLABI, AYODEJI TIMOTHY IN THE DEPARTMENT OF BIOLOGICAL SCIENCES.

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SCHOOL OF POSTGRADUATE STUDIES
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ABSTRACT

Monthly surveys for the occurrence and distribution of viruses of leafy vegetables were carried out in four commercial vegetable farms at Amuwo odofin, Abule Ado, Tejuoso and Oko Oba in Lagos in order to isolate and identify previously unreported viruses in Nigeria. Viruses were isolated from *Amaranthus hybridus, Celosia argentea, Cucurbita moschata, Telfairia occidentalis* and *Brassica oleracea.* The identities of Amaranthus mosaic virus (AMV) and Telfairia mosaic virus (TeMV) previously reported in Nigeria were confirmed. AMV and the Celosia virus isolate were the most prevalent. Beside *B. oleracea*, no virus infection was recorded on the other exotic vegetables examined.

The properties of two previously uncharacterized viruses, one each from *C. argentea* and *C. moschata* are reported in this work. The Celosia virus isolate, for which the name Celosia leaf curl virus (CLCV), has been suggested and the Cucurbita virus designated as Cucurbita mosaic virus (CuMV), had narrow host ranges, were transmitted non-persistently by *Aphid spiraecola* and *Toxoptera citricidus* and reacted positively with universal potyvirus monoclonal antibody in Indirect enzyme-linked immunosorbent assay. No serological relationship was detected when both viruses were tested against 22 antisera prepared against 17 putative potyviruses in immunodiffusion tests. The Celosia

virus has a molecular weight of 30.2 Kda. It reacted positively with polyclonal antibodies to asparagus virus-1 (AV-1) turnip mosaic (TuMV), maize dwarf mosaic (MDMV), watermelon mosaic (WMV-2), plum pox (PPV), Soybean mosaic (SOyMV), lettuce mosaic (LMV), bean common mosaic (BCMV) and beet mosaic (BMV) potyviruses in at least one of the serological methods used which included plate-trapped antigen (PTA), double antibody sandwich (DAS) enzyme-linked immunosorbent assays, electroblotting immunosorbent assay (EBIA/Western blot) and immunosorbent electron microscopy plus decoration (ISEM-D) test. The Cucurbita mosaic virus (CuMV) induced striated lamellar and `pinwheel' inclusion bodies in infected host tissues. Electron microscopy of both viruses revealed flexuous rod-shaped particles.

Comparatively, CuMV was transmitted more frequently by *Aphid spiraecola* to *L. siceraria* than to *C. moschata* using varying number of aphids, acquisition/inoculation access feeding and postacquisition starvation periods. The presence of a dense covering of hairs on both the adaxial and abaxial leaf surfaces of *C. moschata* and the lack of this on *L. siceraria* probably accounted for the differential transmission rate of CuMV to both plants.

Studies on the mechanical inoculation of *C. moschata* var. `TLV 8' with CLCV at weekly interval beginning from when the plants were three weeks through to when they were seven weeks old showed that early virus infection induced more severe foliar symptoms, caused significant reduction in leaf size and number, plant height, top fresh and dry

weights as well as those of fresh and dried leaves than did late inoculations. Losses due to virus infection at advanced plant ages were not significant when compared to the controls.

Foliar symptoms were more severe and were expressed faster in plants of field-grown *C. moschata* inoculated with CuMV at the first true leaf stage than did inoculations performed when the vine had started to run and at first perfect flower stage. Virus inoculation had little or no effect on the number of staminate and perfect flowers produced irrespective of time of inoculation. Generally, inoculated plants produced smaller and fewer fruits, albeit insignificantly different when compared to the control. However, mean fruit from such plants differed significantly from those of buffer inoculated control regardless of the age at time of inoculation.

Plants of both *C. argentea* and *C. moschata* inoculated with CLCV and CuMV respectively and examined at 3, 4, 5 and 6 weeks after inoculation generally contained higher amount of potassium, sodium, phosphorus, nitrogen and crude protein than in healthy plants. Conversely, healthy plants had higher ether extract (fat) and crude fibre than infected plants.

Although a few leafy vegetable viruses have been reported in Nigeria, the properties of the causal agents of the leaf curl disease of *C. argentea*, designated as CLCV and the mosaic disease of *C. moschata* referred to as CuMV, are

described for the first time. Both viruses which are members of the potyvirus group are of economic importance.

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CHAPTER ONE

DISTRIBUTION, PREVALENCE AND PRELIMINARY IDENTIFICATION OF VIRUSES OF LEAFY VEGETABLES IN LAGOS.

ABSTRACT

Monthly surveys for the occurrence and distribution of viruses on local and exotic leafy vegetables in four commercial vegetable farms at Amuwo Odofin, Abule Ado, Tejuoso and Oko-Oba in Lagos were conducted between October 1989 - September 1990. Viruses were isolated from *Amaranthus hybridus, Celosia argentea, Cucurbita moschata, Telfairia occidentalis* and *Brassica oleracea*.

Two previously uncharacterized viruses, one each from *C. argentea* and *C. moschata* were identified and partially characterized. The identities of other viruses such as Telfairia mosaic virus (TeMV) and Amaranthus mosaic virus (AMV) previously reported in Nigeria were confirmed on the basis of host range, symptomatology and insect transmission.

Amaranthus mosaic virus (AMV) and the Celosia virus were isolated from all the farms. Both viruses were more prevalent at Amuwo Odofin relative to the other farms. The highest incidence of AMV was 19.7% at Amuwo Odofin farm compared to 0.7 and 0.6% at Tejuoso and Abule Ado farms respectively. For Celosia virus the highest incidence of 27.05% was recorded at Amuwo Odofin while those for Tejuoso

and Abule Ado farms were 0.6 and 1.37% respectively Telfairia mosaic virus (TeMV), Cucurbita virus and a virus from Brassica were only observed at Tejuoso farm.

The two previously undescribed viruses isolated from *Celosia argentea* and *Cucurbita moschata* formed the subjects of investigation in subsequent studies.

1.1 INTRODUCTION

Vegetables have been described as edible plant parts such as leaves, petioles, stems, roots, rhizomes, bulbs, tubers, inflorescence, seeds and fruits which may be consumed raw or in cooked form (Duckworth, 1966; Okigbo, 1975).

Hundreds of crops are regarded as vegetables depending on the locality. For instance, the leaves of innumerable species of crops considered as pot-herbs in Africa, Asia and Latin-America are in some places regarded as weeds (Martin and Ruberte, 1975; Obmen and Grubben, 1978).

Vegetables as used in the context of this research refer to those plants which are recognized and consumed as vegetables in Nigeria and other African countries. These include *Abelmuschus esculentus* (L.) Moench. (Okro), *Capsicum* spp. (peppers), *Citrullus lanatus* (Thunb) Mansf. (Watermelon), *C. vulgaris* Schrad., *Cucumis melo* L. (melon), *C. sativus* L. (cucumber), *Cucumeropsis edulis* (Hook F.) Cogn. (melon), *Solanum melongena* L. (egg-plant), and *S. macrocarpon* L. which are cultivated for their fruits and seeds. Others such as *Amaranthus* spp., *Celosia* spp., *Corchorus olitorius* L., *Cucurbita* spp., (pumpkins) and *Telfairia occidentalis* Hook (fluted pumpkin) are mainly cultivated for their leaves. Exotic vegetables such as *Apium graveolens* L. (celery), *Brassica oleracea* L. (cabbage), *Cichorium intybus* L. (chicory), *Lactuca sativa* L. (lettuce), *Petroselinum cripsum* (mill) Nym. ex. Hill

(parsley), and *Raphanus sativus* L. (radish) have also been introduced into the Nigerian horticultural industry (Personal observation).

The importance of vegetables lies in their nutritive values as sources of vitamins, minerals, proteins and energy (Oyenuga and Fetuga, 1975; Omueti, 1980; Atiri and Osemobor, 1991). If fully exploited, by consuming sufficiently large amounts, vegetables can play a significant role by providing the much needed minerals and vitamins to supplement the diet of the tropical populace (Oke, 1968).

Besides producing vegetables for local consumption, certain African countries such as Morocco, grow vegetables principally for export thus contributing to their foreign exchange earnings (Ladipo, 1988a). Medicinally, some vegetables are known to have laxative and therapeutic properties (Okigbo, 1978).

Virus diseases have been recognised to constitute one of the major factors limiting vegetable crop production world wide (Grogan, 1980). In Africa, they sometimes result in significant yield losses (Ladipo, 1988a).

There are several reports of viral diseases of vegetable crops. For instance, celery mosaic virus (CeMV) (Albert *et al.*, 1989), celery yellow mosaic virus (CeYMV) (Edwardson, 1974) and celery latent virus (CLV) (Luisoni, 1966) incite diseases in celery. Lettuce mosaic virus (LMV) is considered a serious threat to lettuce production

in California where it causes frequent failures of lettuce fields (Grogan, 1980). Other important viral diseases of lettuce include lettuce speckles mottle, beet western yellows (Falk *et al.*, 1986) and lettuce infectious yellows (Duffus *et al.*, 1986).

In continental Africa, several viruses mostly from fruit vegetables have been isolated and characterized. These include pepper veinal mottle virus (PVMV) (Brunt and Kenten, 1971; De Wijs, 1973; Lana *et al.*, 1974; Ladipo and Roberts, 1977; Igwegbe and Waterworth, 1982), watermelon mosaic virus 1 (WMV-1) (Hafidi, 1983), a strain of WMV-2 (Igwegbe, 1983a), tobacco mosaic virus (TMV) (Igwegbe, 1983b), Okra mosaic virus (OMV) (Atiri, 1984; Igwegbe, 1983c), okra leaf curl virus (OLCV) (Lana, 1976; Fauquet and Thouvenel, 1987), tomato mosaic virus (Fauquet and Thouvenel, 1987), tomato bunchy top virus (Ladipo, 1973; Fischer and Lockhart, 1977), tomato leaf curl virus (TLCV) (Kisha, 1981; Cherif and Russo, 1983), tomato vein yellowing virus (TVYV) (El-Maalaoui *et al.*, 1985), eggplant green mosaic virus (EGMV) (Ladipo *et al.*, 1988a), and eggplant severe mottle virus (ESMV) (Ladipo *et al.*, 1988b), and potato virus Y (PVY) (Fischer and Lockhart, 1974).

Leafy vegetable viruses have received very little attention in Nigeria and indeed Africa. In Nigeria, the first report of a leafy vegetable virus was by Nwauzo and Brown (1975) who described a mosaic disease of *T. occidentalis*. The agent, provisionally designated as

Telfairia mosaic virus (TeMV), has been established to be a potyvirus (Shoyinka *et al.*, 1987). Cucumber mosaic virus (CMV) had earlier been reported on the crop (Atiri, 1985). Taiwo *et al.*, (1988) reported another potyvirus inciting a mosaic disease in *Amaranthus hybridus L.*

In a comprehensive review of viruses of vegetable crops in Africa, Ladipo (1988a) remarked that a few of the reported viruses were yet to be fully characterized and some were yet to be isolated.

Apparently, there is a dearth of information on viruses of leafy vegetables. This study was therefore initiated to investigate the distribution and prevalence of leafy vegetable viruses in Lagos with a view to isolating and identifying previously unreported viruses of leafy vegetables in Nigeria.

2.0 MATERIALS AND METHODS

2.1 STUDY SITES

Four sites within Lagos and environs were chosen for this study. They were

1) the Tejuoso vegetable farm located in the swamp between the National Sports Commission and its staff quarters,
2) Amuwo Odofin vegetable farm situated behind Amuwo Odofin housing estate,
3) Abule Ado vegetable farm situated a few meters away from the International Trade Fair Complex and
4) a vegetable farm owned by the Lagos State Ministry of Agriculture. It is located at Oko-Oba in the Agege area of Lagos (Fig.1.1).

The sites were chosen on the basis of **(a)** the size of the farms which were several hectares each. **(b)** the variety of vegetables grown which included a reasonable number of local as well as exotic varieties, **(c)** water availability at the farms throughout the year and **(d)** the method of cultivation which was crop rotation.

In the farms, seeds were usually sown in nurseries. Seedlings were transplanted at close spacing in well defined rows and columns thus making sampling and assessment of extent of infection easy. At Oko-Oba farm however, seeds were sown by broadcasting and cultivation was rain-dependent.

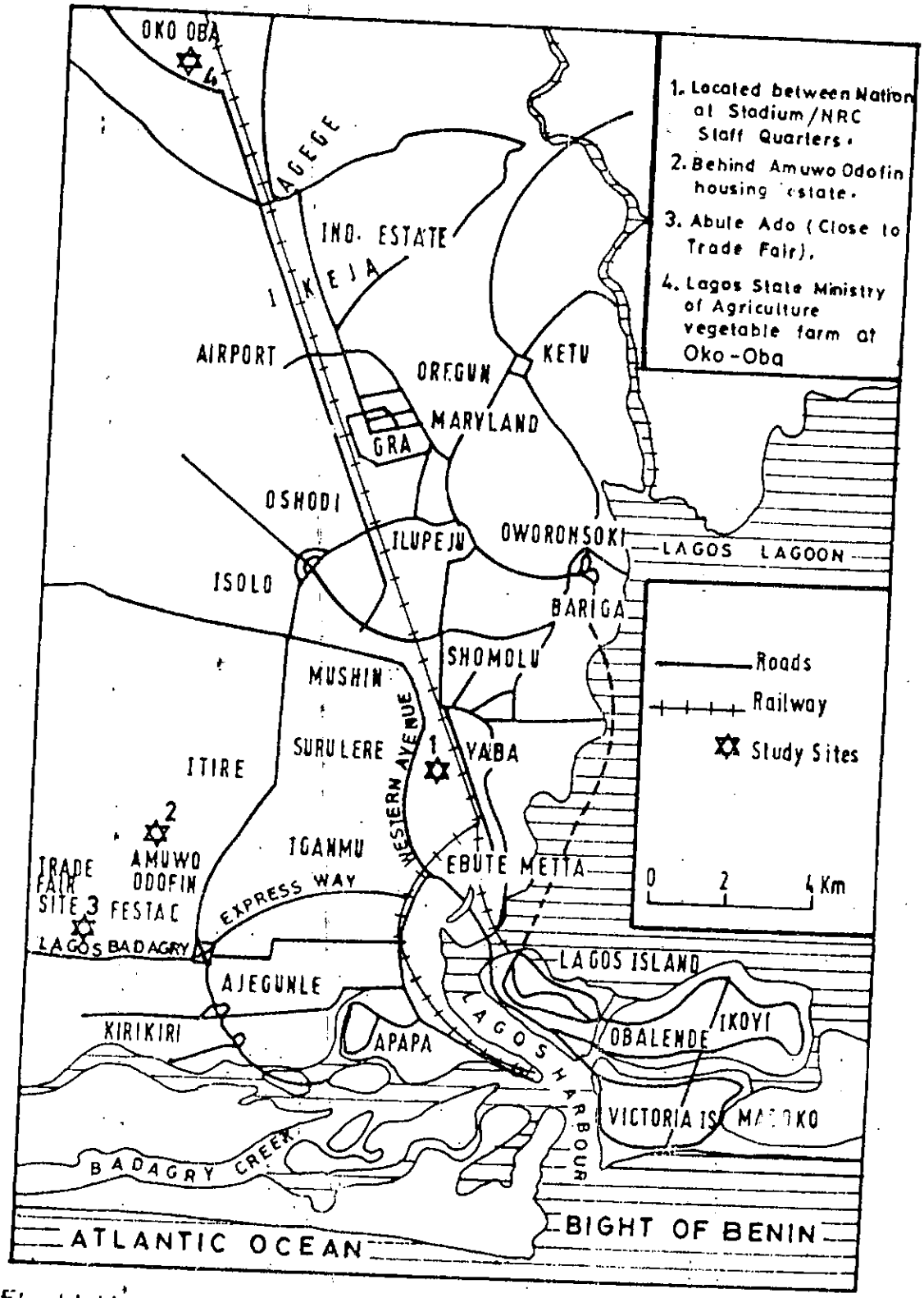


Fig.1.1 Map of Lagos area showing the study sites.

1.2.2 SAMPLING TECHNIQUE

Sampling was by physical examination of the various vegetable types. Plants which showed discernible viral-like symptoms were adjudged infected. The number of such plants was noted. Also the total number of plants examined per bed was noted. Beds were chosen at random from all sections of each farm during any particular sampling exercise. Surveys were conducted usually during the last week of each month beginning from October 1989 through to September 1990 spanning both the dry and rainy seasons.

The type of vegetables available for sampling and the method of cultivation determined the information gathered at the Oko Oba farm.

1.2.3 VIRUS ISOLATION AND PRELIMINARY IDENTIFICATION

Leaf samples from infected vegetables were collected and brought to the greenhouse in clean polythelene bags. Where practicable, infected plants were also transplanted into pots. Inocula were prepared from infected leaves of *C. argentea, A. hybridus, C. moschata, T. occidentalis, B. oleracea* and *S. macrocarpon* by triturating the leaf tissues in 0.03M sodium phosphate buffer (Na_2HPO_4/NaH_2PO_4) pH 8.0 or 0.5M potassium phosphate buffer (K_2HPO_4/KH_2PO_4) pH 7.5. The inocula were used to mechanically inoculate a number of test plants in the greenhouse which had temperatures ranging from 28-33°C.

Identification of the virus isolates obtained from *A. hybridus* and *T. occidentalis* was made by comparing their host range, symptomatology and mode of insect transmission with those of previously reported viruses from these plants. The virus isolates from *C. argentea*, *C. moschata*, *B. oleracea* and *S. macrocarpon* that had not been previously characterized in Nigeria, were temporarily named Celosia virus, Cucurbita virus, Brassica virus and Solanum `virus' respectively.

1.3 RESULTS

1.3.1 DISEASE SYMPTOMS ON INFECTED PLANTS IN THE FIELD

Virus - like symptoms were observed on *A. hybridus, B. oleracea, C. argentea, C. moschata, S. macrocarpon* and *T. occidentalis* during the period of the survey.

The Amaranthus virus caused green vein-banding, mottle and mosaic of the infected leaves (Plate 1.1). Infected plants were generally stunted and prostrate. Mosaic was the major symptom induced in *Brassica* by the Brassica virus (Plate 1.2). The disease in *C. argentea* was characterised by severe leaf curl, mosaic, leaf puckering, moderate to severe stunting of infected plants and apparent reduction in leaf size (Plate 1.3). Symptoms of virus infection in *C. moschata* included green vein-banding, mosaic and blistering on the leaves (Plate 1.4). On *S. macrocarpon* the observed symptoms included mosaic, blistering and leaf puckering (Plate 1.5) while mosaic and leaf malformation were the most common symptoms observed on *T. occidentalis* (Plate 1.6).

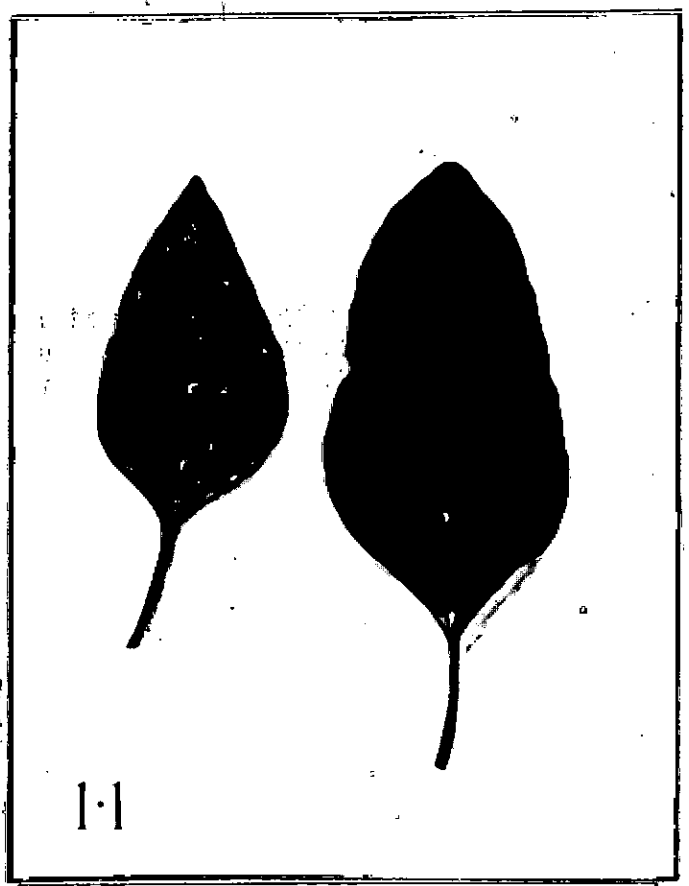
1.3.2 VIRUS DISTRIBUTION AND PREVALENCE

Amaranthus and Celosia viruses were the most prevalent and widespread of all the viruses and were recorded in all the farms. Virus infections of *C. moschata, T. occidentalis* and *B. oleracea* were observed only at Tejuoso farm. Virus-induced symptoms were observed on *S. macrocarpon* only at Amuwo Odofin. No discernible virus induced symptoms were recorded on `Cusbara', *C. olitorus,*

A. graveolens, C. intybus, L. sativa, R. sativus and *P. crispum* in any of the farms throughout the period of the survey (Table 1.1).

Plate 1.1. *Amaranthus hybridus* leaf naturally infected by Amaranthus mosaic virus (AMV) Leaf from healthy plant is shown on the right

Plate 1.2. A naturally infected *Brassica oleracea* plant showing mosaic symptom.



1·1

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Plate 1.3. Leaf curl, mosaic and leaf malformation in *Celosia argentea* naturally infected by Celosia virus.

Plate 1.4. Blistering, vein-banding and mosaic symptoms induced in the leaf of *Cucurbita moschata* naturally infected by Cucurbita virus. Healthy leaf is shown on the right.

1·3

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Plate 1.5. Leaf of *Solanum macrocarpon* showing virus - induced symptoms. Healthy leaf is shown on the right.

Plate 1.6. Mosaic and leaf malformation induced in naturally infected leaf of *Telfairia occidentalis*. Healthy leaf is shown on the right.

1·5

1·6

TABLE 11 Incidence of Viruses on leafy vegetables in four farms in Lagos

Leafy vegetable	FARM			
	Amuwo Odofin	Tejuoso	Abule Ado	Oko Oba
Amaranthus hybridus	+[a]	+	+	+
Celosia argentea	+	+	+	+
Cucurbia moschota	-[b]	+	-	-
Telfairia occidentalis	-	+	-	-
Brassica oleracea	-	+	-	*[c]
Solanum macrocarpon	+	-	-	-
Corchorus olitorus	-	-	-	-
'Cusbara' (Malvaceae)	-	-	-	*
Apium graveolens	-	-	-	*
Lactuca sativa	-	-	-	*
Petroselinium cripsum	-	-	-	*
Raphanus sativus	-	-	-	*

+[a] = Virus infection was observed.
-[B] = No virus infection was observed.
* = Vegetable not cultivated.

The results of the virus incidence study are summarized in Fig. 1.2. The rainfall data during the period of survey showed two prominent peaks. The wet seasons began about March through to July with a brief dry spell in August - September followed by an upsurge in the amount of precipitation in September - October (Fig. 1.3).

Generally, the results showed that the incidence of viral and viral-like diseases was highest during the wet months of the period of survey except for Amaranthus mosaic virus (AMV) at Amuwo Odofin and Tejuoso farms. At Amuwo Odofin farm, AMV had the highest incidence of 19.17% in August 1990 and the lowest incidence of 3.18% in April of the same year (Appendix 1). The highest incidence of the virus at Tejuoso farm was 0.68% recorded in January 1990 and the lowest in March 1990 with a percentage incidence of 0.07% (Appendix 2) while Abule Ado farm had a maximum incidence of 1.25% of AMV in October 1989 and zero percent in March and May 1990 when no infected plants were observed (Appendix 3).

The Celosia virus had a percentage incidence ranging from 1.66 - 27.05 in Amuwo Odofin with the highest in June 1990 and the lowest in February of the same year. The values recorded for Tejuoso and Abule Ado farms were comparatively lower than that of Amuwo Odofin. At Tejuoso, the incidence of the virus ranged from 0 - 1.43% with the highest in July and the lowest in January and June

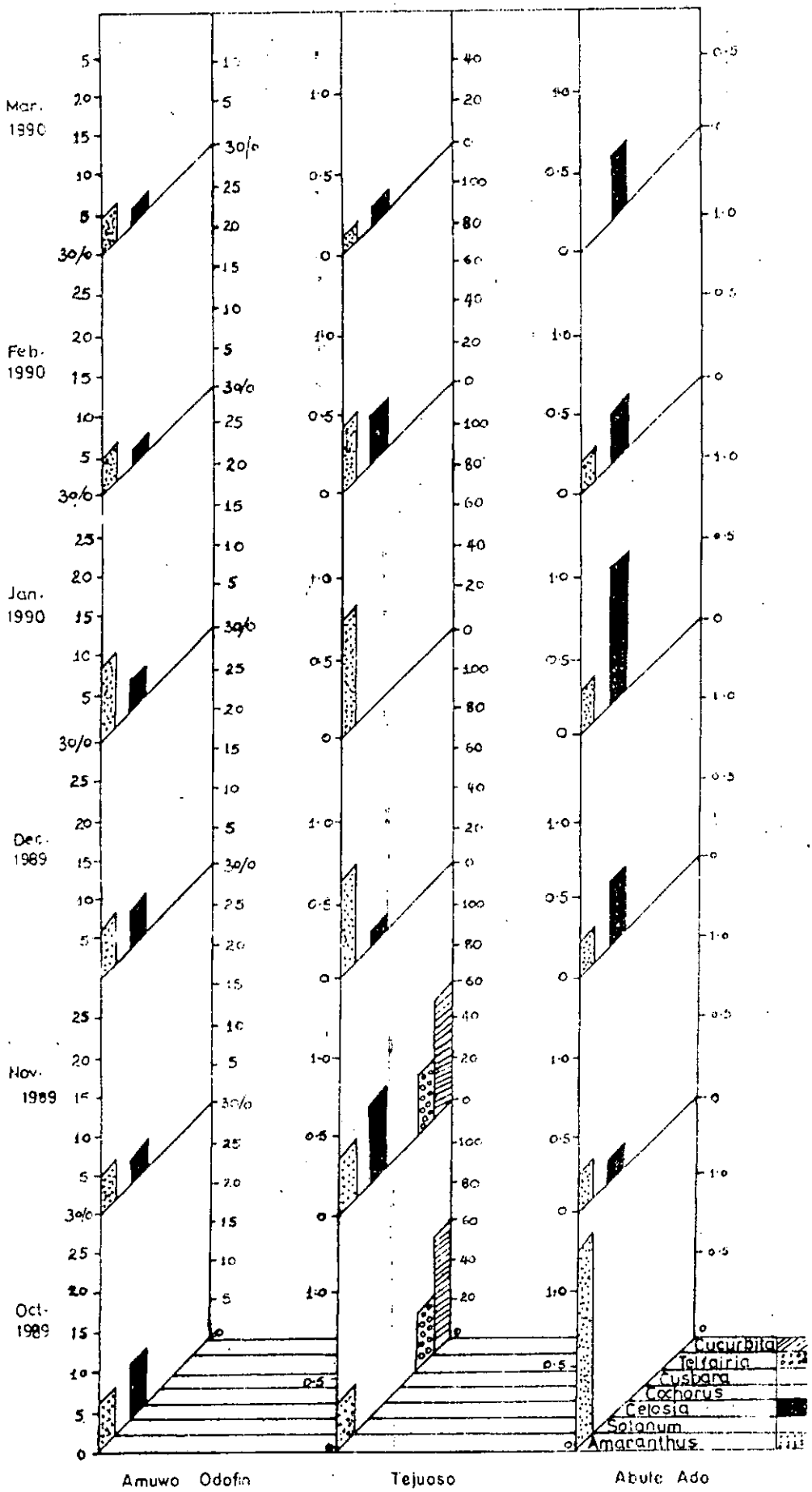

Fig 1.2 Percentage incidence of leafy vegetable viruses in three farms in Lagos.

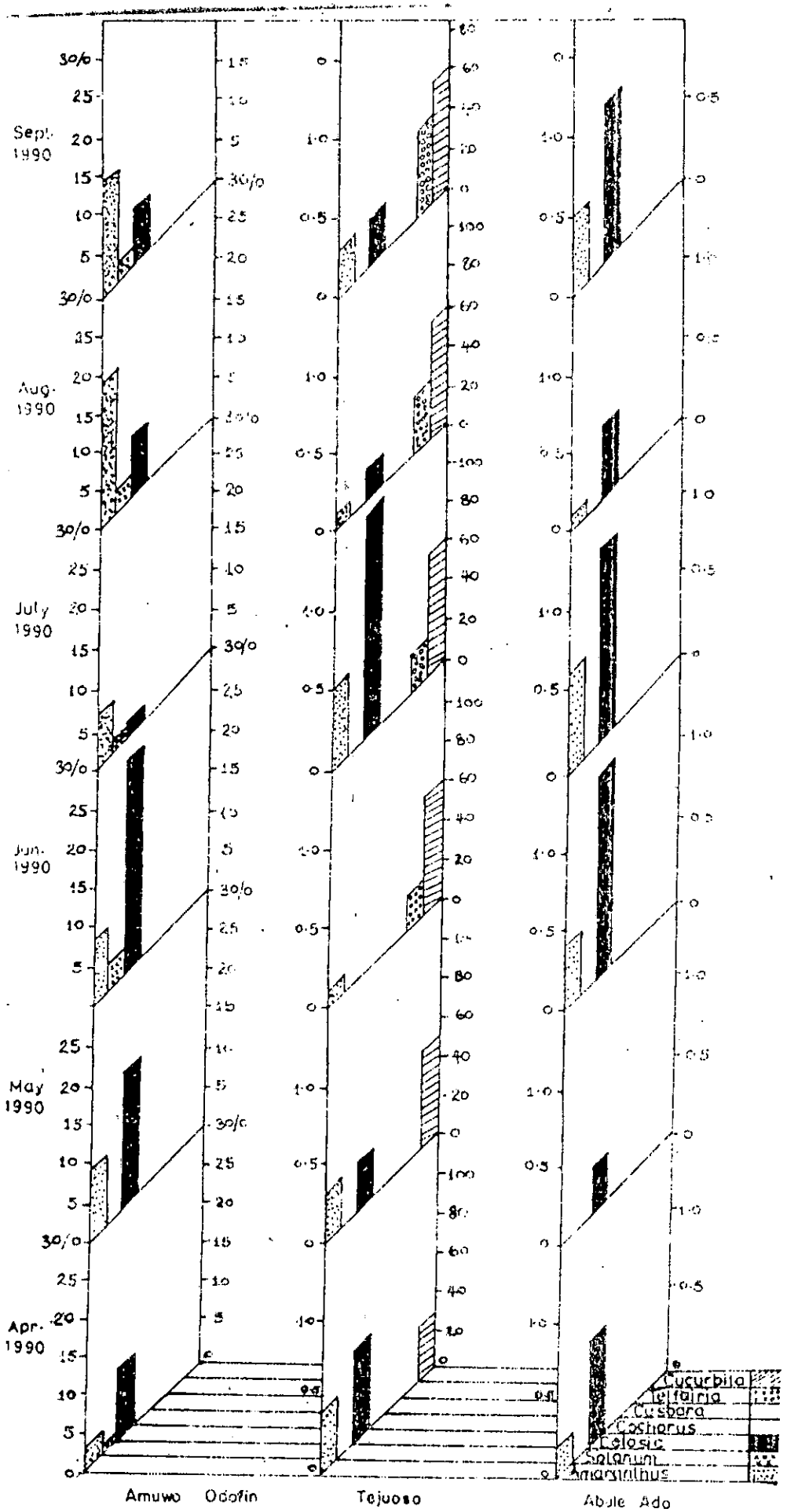

Fig. I.2 Percentage incidence of leafy vegetable viruses in three farms in Lagos.

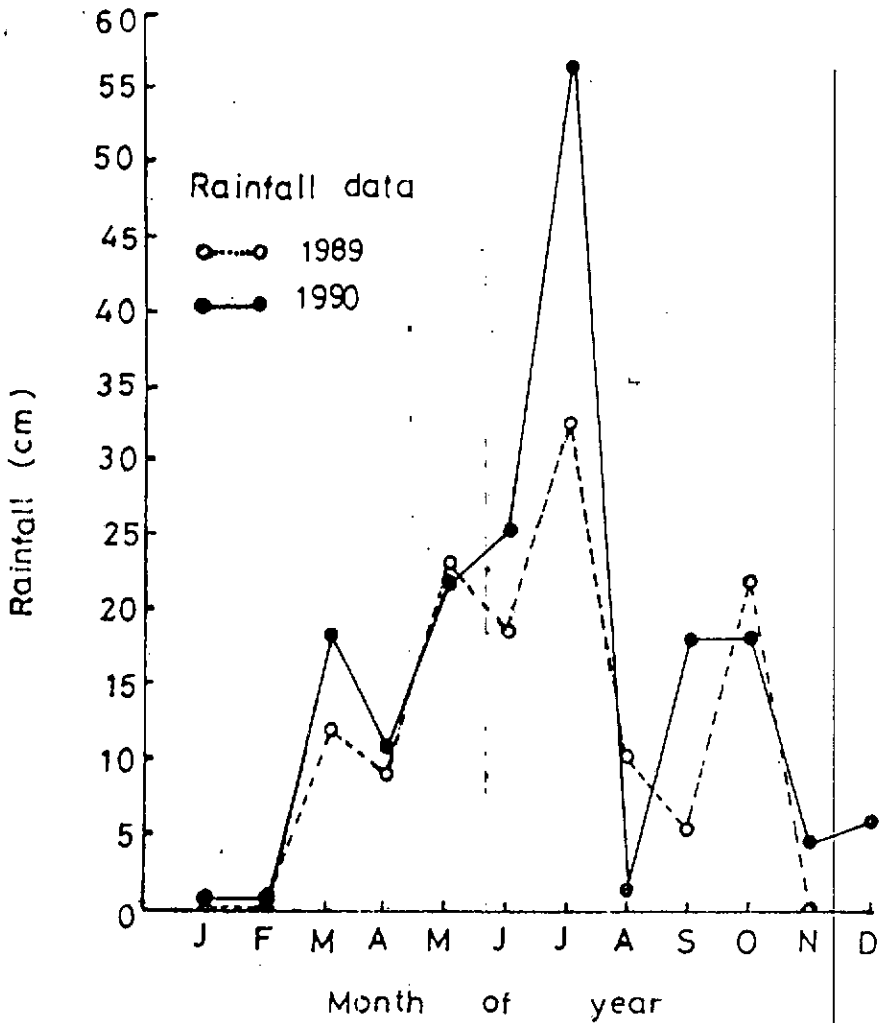

Fig. 1.3 Monthly rainfall distribution pattern in Lagos in 1989 and 1990 (Source: Meteorological Services Department, Oshodi)

1990. For Abule Ado farm, the range was between 0 - 1.34% the lowest in October 1989 while the highest was recorded in June 1990.

The incidence of the Cucurbita virus in Tejuoso farm was 60% in October 1989 as 3 out of every 5 plants were infected. In September 1990 when another survey for Cucurbita virus infection was conducted 11 out of 18 plants were infected, representing an incidence of 66.11%. For the Telfairia mosaic virus (TeMV) the data showed a 29% incidence in October 1989 as 9 out of 31 plants of *T. occidentalis* were infected (Appendix 2) while 12 out of 27 plants (44.4%) showed symptoms of infection in September 1990.

Symptoms of 'virus' infection were observed on *S. macrocarpon* only at Amuwo Odofin farm. The incidence ranged from 0 - 2.86% with the lowest in November 1989 to March and including May and August 1990 when no infected plants were recorded and the highest in June 1990 (Appendix 1).

Virus infection of *B. oleracea* was recorded at Tejuoso farm in October 1989 and April 1990. Out of the 500 plants examined in October 1989, 459 were infected, representing an incidence of 91.6%. The survey conducted in April gave an incidence of 0.24% as only 2 out of 848 plants were infected (Appendix 2).

1.3.3 VIRUS ISOLATION AND IDENTIFICATION

The agents causing the observed disease symptoms in *A. hybridus, B. oleracea, C. moschata, Celosia argentea* and *T. occidentalis* were mechanically transmissible. Attempts to isolate and transmit the agent that induced the virus-like symptoms in *S. macrocarpon* were not successful.

The Amaranthus virus isolate induced mottling, mosaic, green vein-banding, reduced leaf size and stunting in *A. hybridus, A. crentus, A. caudatus and A. viridis.* The following test plants: *Chenopodium amaranticolor, Gomphrena globosa, Nicotiana glutinosa, N. rustica, N. tabacum* cvs. 'White Burley' and 'Samsum', *Datura, stramonium* and *C. argentea* were non hosts. The virus was transmitted by *Aphis craccivora* in a non-persistent manner.

The Celosia virus was successfully mechanically transmitted to *Celosia* spp. in which it caused leaf curl, leaf tip necrosis, leaf malformation and mosaic.

Enation, severe leaf malformation and mosaic were elicited when the Cucurbita virus was inoculated onto *Cucumeropsis edulis.*

The mosaic disease observed on cabbage in the field was successfully reproduced on cabbage in the greenhouse. No further information could be obtained on the virus as it could not be reactivated from infected leaf tissues dried over calcium chloride ($CaCl_2$).

The Telfairia virus isolate produced necrotic local lesions in *C. argentea*. On *T. occidentalis* mosaic, leaf malformation and reduction in leaf size were observed. *Datura metel, D. stramonium, N. glutinosa* and *N. Rustica* were not susceptible to the virus. The virus was transmitted by *A. spiraecola* from *Telfairia* to *Telfairia* but not by *A. craccivora*.

1.4 DISCUSSION

The incidence of viruses in the farms where the study was conducted was generally higher during the wet than during the dry months. This is expected, because favourable growing conditions such as nutrition and water among others predispose plants to virus infection as well as favour vector multiplication and behaviour (Bos, 1981).

Amaranthus mosaic virus (AMV) and Celosia virus were the most prevalent and widespread of the viruses isolated in the course of this investigation. The two viruses were observed in all the four farms. They were more prevalent in Amuwo Odofin farm than in Tejuoso and Abule Ado farms. Field observations at Amuwo Odofin farm showed that remnants of previous season's crops, some of which were infected, were commonly left unharvested in the farm. These remnants or volunteer plants could serve as sources of inoculum which could later be spread by vectors to newly transplanted healthy seedlings. Furthermore, volunteer plants could harbour the vector and thus ensure the spread of the viruses throughout the year. This probably explains the occurrence of these viruses in the farm all year round.

The role of volunteer crops in the ecology and epidemiology of plant viruses such as wheat streak mosaic virus (WSMV), several diseases of rice, peanut mottle and groundnut rosette viruses has been documented (Demski, 1975, Sojawa, 1976; Thresh, 1982; Bos, 1981).

The relatively high incidence of AMV and Celosia viruses in Amuwo Odofin farm particularly during the wet months could also be attributed to an increase in the number or activity of aphid vectors. High population of aphids was noticed on the farm during the peak of the rainy season. The occurrence of potato virus Y (PVY) in pepper in Morocco (Lockhart and Fischer, 1974) and the widespread occurrence of maize streak mosaic virus (MSMV) in Nigeria (Fajemisin *et al.*, 1976) were reported to coincide with heavy infestation by vectors.

In all the farms with the exception of Oko Oba, the land was fragmented into small holdings. Within each holding, the farmers repeatedly cultivated the vegetables in rotation. However, sometimes, the same vegetable was grown repeatedly on the same piece of land or in close proximity to a piece of land from which the same crop had just been harvested. This practice probably contributed to the high incidence of AMV and the Celosia virus at Amuwo Odofin farm. Lockhart and Fischer (1974) remarked that the cultivation of the same crop or related crops in succession increased inoculum build up and disease severity. The outbreak of WSMV in wheat in the United States and beet yellow vein virus (BYVV) in beet in Yugoslavia was attributed to repeated use of the same site (Sultic and Milvanovic, 1980). Other factors that might have contributed to the incidence of the viruses at Amuwo Odofin farm are the degree of genetic uniformity of the vegetables

and the dense uniform stands occasioned by close spacing. These two factors have been recognised as important in plant disease epidemics (Day, 1974). Hill *et al.* (1980) have shown that close spacing of soybean facilitated plant-to-plant spread of soybean mosaic virus (SMV) by its aphid vectors.

Although, the same situation prevailed at Tejuoso and Abule Ado farms where the same piece of land was repeatedly grown with genetically uniform plants, at close spacing, resulting in dense stands as in Amuwo Odofin, the relatively low incidence of viruses in both farms was probably due to what could be described as `clean harvesting' since it was uncommon to find remnants or volunteer plants growing on previously harvested plots.

Throughout the period of this study no virus infection was observed on *C. olitorus*, `*Cusbara*' (Malvaceae) and the exotic vegetables like *A. graveolens*, *L. sativa*, *P. cripsum* and *R. sativus*. That virus infection was not observed on these exotic vegetables might be due to strict adherence to quarantine regulations that govern the movement of plant materials across international boundaries by the companies that produced them for export. The seed packs of some of the vegetables often carried the inscription `virus tested seeds'. Lettuce mosaic virus (LMV), for example, is frequently seed transmitted (Tomlinson, 1970) and seeds have been considered the most important source of inoculum

(Grogan, 1980). It is also possible that these exotic vegetables are not susceptible to the vegetable viruses already reported in Nigeria or that the viruses associated with them are not present here.

As for the identification of some of the viruses isolated in the course of this study, based on host range, symptomatology and insect transmission, the AMV isolate was essentially similar to that described by Taiwo *et al.* (1988) while the virus isolated from *T. occidentalis* was similar to that described by Shoyinka *et al.* (1987).

Three previously undescribed viruses in Nigeria, one each from *C. argentea, C. moschata* and *B. oleracea* were isolated. The virus isolates from *C. argentea* and *C. moschata* form the subject of separate studies in subsequent investigations while the Brassica virus which could not be reactivated from infected tissues chopped, dried and stored over calcium chloride ($CaCl_2$) was lost.

The results of this study confirm the opinion expressed by Ladipo (1988) that some viruses of vegetable crops are yet to be isolated and characterized. The viruses inciting the symptoms observed in *C. argentea, C. moschata and B. oleracea* are probably some of these viruses.

CHAPTER TWO

CHARACTERIZATION AND IDENTIFICATION OF A VIRUS CAUSING LEAF CURL DISEASE OF *CELOSIA ARGENTEA L.*

ABSTRACT

The properties of a virus causing the leaf curl disease of *Celosia argentea* were studied. The virus, which was sap transmissible induced leaf curl, leaf malformation, mosaic and stunting in infected plants. It had a rather narrow host range infecting a few plants in the Amaranthaceae, Chenopodiaceae and Solanaceae families.

The virus had a dilution end point between 10^{-2} - 10^{-3}, thermal inactivation between 35 - 40°C and longevity `in vitro' of 15 - 20 hr. The virus was transmitted in a non-persistent manner by *Aphis spiraecola* and *Toxoptera citricidus* but not by *Aphis craccivora, A. fabae, A. nasturtii, Acyrosiphon pisum, Aulacorthum solani, Rhopalosiphon maidis* and *Myzus persicae*. No evidence of seed transmission was obtained.

Electron microscopy of purified preparations revealed flexuous rod-shaped particles measuring about 750 nm.

The virus reacted positively with the universal potyvirus group monoclonal antibody (PVY-1) and thus belongs to the potyvirus group. It also reacted with monoclonal antibody P-3-3H8 raised against pea stripe mosaic virus and with

polyclonal antibodies to asparagus virus-1 (AV-1), turnip mosaic (TuMV), maize dwarf mosaic (MDMV), watermelon mosaic (WMV-2), plum pox (PPV) soybean mosaic (SoyMV), lettuce mosaic (LMV), bean common mosaic (BCMV) and beet mosaic (BMV) viruses in at least one of the serological assays used including plate-trapped antigen (PTA), double antibody sandwich (DAS) enzyme-linked immunosorbent assays, electroblotting immunoassay (EBIA/Western blot) and immunosorbent electron microscopy plus decoration (ISEM-D).

In reciprocal tests using Celosia virus antiserum raised in mice, cross reactivity was observed with AV-1, TuMV, WMV-2, MDMV, PPV, CeMV and LMV in PTA-ELISA while in Western blotting the virus reacted exclusively with its homologous antiserum. The antiserum also reacted strongly with BMV but gave weak reactions with PsbMV, LMV and SoyMV in ISEM plus decoration test. The virus appears more serologically related to AV-1 than to any of the other viruses because of the consistency of reaction of its antiserum with the virus. However, differences in host range and symptoms induced in diagnostic plants susceptible to both viruses, and lack of cross reactivity of the antiserum raised against the Celosia virus indicate that AV-1 and the virus are distinct viruses. This virus, for which the name Celosia leaf curl virus (CLCV) has been suggested is apparently a new member of the *Potyviridae* family and its exact taxonomic relationship will become

clearer when sequence data of the coat protein become elucidated.

2.1 INTRODUCTION

The family Amaranthaceae to which *Celosia argentea L.* belongs occurs mainly in the tropics and subtropics often as weeds or escapes. There are fourteen (14) genera of which *Celosia* and *Amaranthus* are of commercial importance (Hutchinson and Dalziel, 1954).

There is no unequivocal evidence of the origin of *C. argentea*. It is widely distributed in tropical Asia, Africa and America where it is cultivated (Hooker, 1954). It is an erect, branched annual herb reaching a height of 60 - 120cm. The flowers which are borne in simple, continuous and very dense silvery white spikes, are characterized by perianth segments of 6 - 10 cm long and short bifid style (Hutchinson and Dalziel, 1954).

C. argentea, locally referred to in Yoruba as `Sokoyokoto', is widely used as edible vegetable in Nigeria (Okigbo, 1975). Its leaves when boiled as spinash or pot-herbs are a good source of vitamins such as vitamin C (ascorbic acid) and vitamin B complex (Oke, 1966).

Several members of the family Amaranthaceae have been widely used as experimental hosts for a number of plant viruses (Edwardson, 1974), only a few viruses have so far been reported to infect these plants naturally (Lovisolo and Lisa, 1976).

Phatak (1965) reported a virus disease of *Amaranthus blitum and A. viridis* in India whose causative agent was

designated as Amaranthus mosaic virus. Also in India, Ramakrishnan *et al.* (1971) reported a virus disease on *A. gangeticus* that was transmitted to non-Amaranthaceae host plants. Singh *et al.* (1972) isolated pigweed mosaic virus from *A. viridis*. The virus which was aphid-transmitted had particle length of 750 nm.

A virus of *A. deflexus* with a 70% incidence and characterised by mottling, blistering and growth reduction was described in Italy and Spain by Lovisolo and Lisa (1976) and in Morocco (Lovisolo and Lisa, 1979). The virus, called Amaranthus leaf mottle virus (ALMV) with experimental host range spanning 6 families, was serologically related to bean yellow mosaic virus (BYMV). It was transmitted non-persistently by *Macrosiphon euphobiae* Thos. and *Myzus persicae* Sulz. A closely related strain designated as ALMV-C was isolated fron *Cirsium arvense* Scop. It showed serological relationship with BYMV (Casseta *et al.*, 1986).

Taiwo *et al.* (1988) described a sap transmissible virus isolated from *A. hybridus* in Nigeria inciting mosaic or mottling, vein-banding and leaf malformation in the host plant. The virus serologically unrelated to ALVM and BYMV had flexuous rod-shaped particles of 750 - 760 x 12 nm dimensions.

Reports of natural infection of *C. argentea* by viruses are rather scanty. Provvidenti (1975) reported the natural

infection of *C. argentea* by cucumber mosaic virus (CMV) in a commercial greenhouse in Western New York State. Leaf symptoms consisted of ringspot, chlorotic mottle, necrosis and prominent malformation. Atiri and Osemobor (1991) have also reported an uncharacterized virus of *C. argentea* which was experimentally transmitted to *Arachis hypogaea*, *Capsicum annuum*, *Lycopersicum esculentum*, *Nicotiana glutinosa*, and *Chenopodium amaranticolor* besides its natural host.

In October, 1989 a severe leaf curl disease of *C. argentea* was observed in a commercial vegetable farm at Amuwo Odofin, a Lagos suburb. Infected plants also showed mosaic, leaf malformation and stunting. Similar symptoms were observed on the crop in other vegetable farms within Lagos during surveys for leafy vegetable viruses (Chap. 1). Virus infected *C. argentea* were also noticed in some farms at the Institute of Agricultural Research and Training (IAR&T) and the National Horticultural Research Institute (NIHORT) both at Ibadan.

Although, virus diseases have been observed on some Amaranthus species, this is the first attempt at characterizing and identifying a virus causing the leaf curl disease of *C. argentea* in Nigeria.

2.2 MATERIALS AND METHODS

2.2.1 VIRUS ISOLATION AND TRANSMISSION

In order to isolate the virus infecting the *Celosia* plants, one of the naturally infected plants was transplanted into a porcelain pot in the greenhouse. Young symptomatic leaves were removed from the infected plant and ground in sterilized mortar with pestle, in 0.03M phosphate buffer pH 8.0. The inoculum was used to inoculate carborundum (500 mesh) dusted leaves of young seedlings of *C. argentea*. The inoculated plants were then rinsed with water, kept in the greenhouse with a temperature range of 28-33°C and observed for symptom development.

2.2.2 VIRUS PROPAGATION

The virus isolate was inoculated mechanically onto *Chenopodium quinoa* on which it induced chlorotic local lesions. Four successive single lesion transfers were carried out and the virus was subsequently maintained in *Nicotiana clevelandii, N. benthamiana* or *Celosia argentea.* by periodic transfer of the virus to young seedlings transplanted into sterilized soil in polyethylene bags or paper pots.

2.2.3 PREPARATION OF BUFFER

The buffer used throughout the study was 0.03M sodium phosphate buffer. pH 8.0 prepared by dissolving 4.26g of

Na_2HPO_4 in one litre of distilled water. The resultant solution which had an initial pH of 8.6 was adjusted by carefully adding 0.5M NaH_2PO_4 until the desired pH of 8.0 was obtained. The buffer was chilled by refrigeration. A solution of 0.1% sodium sulphite (Na_2SO_3) was also added to the inoculum (in the ratio of 1:10) during each inoculation exercise when it was observed that within minutes of trituration the inoculum turned brownish suggesting the presence of some inhibitory substances that could result in loss of inoculum infectivity (Walkey, 1985).

2.2.4 HOST RANGE AND SYMPTOMATOLOGY

The seeds of the test plants for this study except those of legumes and cucurbits were sown in wooden seed trays in sterilized manure-supplemented garden soil. The seedlings were later transplanted into 16 cm diameter polyethylene bags containing sterilized soil. They were inoculated at the 5 - 6 leaf stage. For the legumes and cucurbits, seeds were sown directly in bags containing sterilized soil and were inoculated at the 2-leaf stage.

Inoculation was performed as described in section 2.2.1 above. At least five plants of each of 76 plant species or varieties belonging to eleven families were inoculated. The inoculated plants were kept in the greenhouse for at least four weeks. Extracts from plants which did not show observable symptoms were back inoculated

onto *C. quinoa* or *Celosia argentea* var.`TLV 8' to detect latent infection. A set of 5 plants of each plant species or variety was inoculated with buffer only, to serve as control.

2.2.5 PROPERTIES IN SAP

Thermal inactivation point (TIP), longevity `*in vitro*' (LIV) and dilution end point (DEP) determination for the virus were carried out as described by Green (1971).

In order to determine the TIP, 2ml of virus containing sap was carefully dispensed into each of 9 screw-capped tubes. The content of each test tube was heated for 10 min in a water-bath preheated to the required temperatures (30,35,40,45,60,65,70°C). The heated homogenates were cooled under running tap water. Each solution was assayed by mechanical inoculation onto 5 seedlings of *C. argentea* var.`TLV 8'. Inoculation of the same number of the assay plant with unheated sap constituted the control.

For the determination of LIV for the Celosia virus, 2 - 5 ml of expressed sap from symptomatic propagation host plant was pipetted into each of 9 screw-capped bottles. The content of each tube was used to inoculate 5 seedlings of the test plant in succeeding days. The experiment was repeated by inoculating the test plant at 5 hr interval when it was observed that infectivity of the inoculum was lost when kept beyond twenty four hours.

DEP was carried out by grinding virus-infected tissues in buffer (1g leaf to 1.5ml buffer). The homogenate was

filtered through gauze. The filtrate was serially diluted ten fold (1ml sap: 9ml distilled water) up to 10^{-7}. Each dilution was assayed on 5 seedlings of the test plant. All inoculated plants were kept in the greenhouse and observed for symptom development for at least 4 weeks. Each of the three tests was conducted thrice.

2.2.6 SEED TRANSMISSION

Mature inflorescence harvested from at least 15 mechanically infected plants of each of *C. argentea* var.`TLV 8', `purple leaved' and `narrow leaved' varieties were dried in the sun. The seeds were collected by rubbing the inflorescence between the palms. The seeds were then sown in sterilized manure-supplemented soil in seed trays measuring 120 x 30 cm. The resulting seedlings in the trays were kept in insect screen cages in the greenhouse for 8 weeks and observed for symptom development.

2.2.7 INSECT TRANSMISSION TEST

Apterous nymphs and adults of *Aphis craccivora* Koch., *A. spiraecola* Pach. and *Toxoptera citricidus* Kirk. collected from *Glirisidia maculata, Chromolaena odorata* (= *Eupatorium odoratum*) and *Citrus sinensis*, their natural hosts respectively, *Aphis craccivora, A. fabae, A. nasturtii, Acyrosiphon pisum, Aulacorthum solani, Rhopalosiphon maidis* and *Myzus persicae* were used for

insect transmission studies. Seedlings of *C. argentea* var. 'TLV 8' were used as virus source and test plants for all the tests.

The aphids were starved for 2 hr and allowed 10 min acquisition feeding on detached infected leaves floated on water in Petri dishes. Ten to fifteen insects were transferred onto each of 8 - 10 plants. They were allowed 10 min inoculation access feeding before they were killed by spraying with Actellic 50 EC (10 ml/l), a broad spectrum insecticide or Pirimor. The plants were kept in the greenhouse and observed for symptom development for at least 4 weeks. Each of the aphid species was tested twice for its ability to transmit the Celosia virus.

Control test consisted of each aphid species feeding on healthy leaves before they were transferred to seedlings of the test plants.

2.2.8 PURIFICATION

Initial attempts to purify the Celosia virus were unsuccessful. Several purification methods using leaf materials from *C. quinoa*, *N. clevelandii* or *N. occidentalis* and different grinding buffers such as potassium phosphate buffer, pH 8.0 containing 5% Na_2SO_3, 0.078 ml phenylmethyl sulphonyl fluoride (PMSF) in 0.5 ml dioxan, ammonium citrate buffer, pH. 6.5 containing 0.5% Na_2SO_3 or glycine buffer, pH 8.5 containing 0.4% 2-mercaptoethanol were tried. Similarly, different purification procedures

including differential centrifugation at various low and high speeds, addition of 2 to 5% Triton X-100, followed by precipitation with polyethylene glycol (PEG 600) and 0.1 M NaCl were also tried. The virus was successfully purified from frozen *N. benthamiana* leaf tissues harvested 2 - 3 weeks after inoculation. The grinding buffer consisted of 0.5 M sodium citrate, pH 7.4 containing 0.005 M ethylenediaminetetracetic acid (EDTA) and 0.015 M diethydithiocarbamate (DIECA). The homogenate was squeezed through two layers of cheese cloth and subjected to 3 differential cycles of low and high speed centrifugation. Details of the purification procedure are illustrated in Fig.2.1. The yield and the extinction coefficient ($E_{2206/208}$) of virus preparations were determined spectrophotometrically.

2.2.9 ELECTRON MICROSCOPY

Drops of undiluted purified preparation of the virus were placed on clean glass slides. Electron microscope grids pre-coated with carbon were placed on the drops for 5 min. Each grid was later floated on one drop of distilled water. Excess water was drained off the grids which were then stained with a drop of 2% phosphotungstic acid (PTA) pH 6.0. Excess stain was drained off the grids and the grids were allowed to dry thoroughly before they were examined under a Philips TEM-500 electron microscope. The electron micrographs were prepared at the Scottish Crop

1. Frozen Virus infected leaf tissues

↓

2. Homogenized in 0.5M Sodium borate buffer, pH 7.4 containing 0.005 M EDTA and 0.015 M DIECA (1gm : 2ml buffer)

↓

3 Centrifuged at 5,000 rpm/15 min, supernatant plus 5% Triton - 100 (5% of homogenate)

↓

4. Centrifuged at 30,000 rpm/2h. Sediment in 0.01M Sodium citrate buffer, pH 7.4 containing 1M Urea plus 0.1% 2-Mercaptoethanol (to a vol. of $^{1}/_{10}$ of original homogenate)

↓

5. Centrifuged at 6,000 rpm/10 min

↓

6 Supernatant laid over 20% sucrose followed by centrifugation at 40,000 rpm/2h sediment in 0.05M borate buffer, pH 8.0 (to a vol.of $^{1}/_{60}$ of original homogenate)

↓

7. Centrifuged at 6,000 rpm/10 min

↓

8. Supernatant mixed with equal vol. of Caesium chloride (CsCl) followed by centrifugation at 33,000 rpm/17$^{3}/_{4}$ h

↓

9 Virus band collected in 10 fold by vol. of borate buffer, pH 8.0, followed by centrifugation at 40 000 rpm/1$^{1}/_{2}$h

↓

10. Virus pellet resuspended in borate buffer (stored in cold room).

↓

Purified virus

Fig 21. Flow chart of the procedure for the purification of Celosia virus

Research Institute, (SCRI) Invergowrie, Dundee, United Kingdom.

2.2.10 DETERMINATION OF MOLECULAR WEIGHT OF COAT PROTEIN

The molecular weight (*Mr*) of the Celosia virus coat protein (CP) was determined by sodium dodecyl sulphate polyacrylamide gel eletrophoresis (SDS - PAGE) using Laemmli's method (1970). Celosia virus infected leaves were triturated in distilled water (1:3 w/v) and centrifuged at 12,000 rpm for 3 min. One hundred microlitres (100 µl) of the supernatant were mixed with equal volume of SDS - dissociation buffer (2% SDS, 0.625M Tris-HCl buffer pH. 6.8, 5% 2-mercaptoethanol, 5% glycerol and 0.001% bromophenol blue), boiled for 5 min and stored at -20°C until required for use. Fifteen microlitres (15µl) of the samples were analysed on 10% polyacrylamide gels (prepared from a stock solution of 30% acrylamide and 0.8% of N, N'-bis-methylene acrylamide). The final concentration of the separation gel (5.5 x 9cm) consisted of 2ml of acrylamide solution, 1.2ml of 1.88M Tris-HCl pH 8.8, 1.2ml of 0.5% SDS and 1.6ml distilled water. Polymerization of the gel was by the addition of 5µl of tetramethylethylenediamine (TEMED) and 30µl of 10% ammonium persulphate (APS). The stacking gel of 5% acrylamide consisted of the stock solution to which appropriate volumes of 0.625M Tris-HCl buffer, pH 6.8 and 0.5% SDS

(0.33ml stock solution, 0.4ml Tris-HCl buffer pH 6.8, 0.4ml SDS) were added and made up to 2ml by adding 0.87ml of distilled water. Polymerization was by the addition of 2µl TEMED and 10µl APS respectively. Electrophoresis was performed in a vertical Minigel - Twin electrophoresis apparatus (Biometra, Gottingen, Germany). The running buffer consisted of 3g Tris-HCl, 14.4g glycine and 1g SDS in 1 litre of distilled water adjusted to pH 8.3. The separated proteins were transferred to nitrocellulose membrane (NC) (0.45 pore size, Hybond-C Amersham, UK) in fast buffer, pH 8.3 consisting of 3g Tris-HCl, 11.2g glycine and 0.1ml methanol at low temperature (cooled by ice) at 200 Am for 30 min using a Fast Blot B32 (Biometra).

After transfer, the NC was washed, by agitation, for 15 min and was incubated for 1hr with potyvirus group specific monoclonal antibody P-3-3H8 raised against peanut stripe potyvirus (supplied by Dr. H.J.V. Vetten, BBA, Braunschweig) diluted to 1:1000 in Tris buffer containing 5% defatted milk. This was followed by 15 min washing with the same buffer used as diluent for the antibody. Enzyme conjugate was alkaline phosphatase (AP) - conjugated sheep anti-mouse IgG diluted to 1:1000. After two rounds of washing for 15 min, first with tris buffer pH 8.0 and second with 0.1M Tris-HCl buffer, pH 9.0 containing 10mM $MgCl_2$, bound antibody was detected by adding substrate solution (5-bromo-4-chloro-3-indolyphosphate-p toluidine

salt) containing nitrobluezoliumchloride in 0.1M Tris-HCl buffer, pH 9.0. The membrane was then treated with 10% sodium hypochloride (NaOCl). Rainbow marker (*Mr* 14.2 to 97 kDa) was included in the analysis. The *Mr* weight of the virus coat protein was estimated graphically.

2.2.11 ANTISERUM PRODUCTION

A polyclonal antiserum was raised in two mice against the virus by administering three intraperitoneal injections with 1.54 mg of purified virus mixed with equal volume of Freund's adjuvant at weekly interval. The first injection was administered with complete adjuvant while the virus was mixed with incomplete adjuvant for the subsequent injections. After temporary anaesthesis, the animals were bled two weeks after the last injection by insertion of glass tubing under the right eye. The collected blood was overlaid with one or two drops of brine (NaCl) solution, left overnight at room temperature and centrifuged at 10,000 rpm for 5 min to remove cell debris. The antiserum was then used to determine relationship with some potyviruses in plate-trapped (PTA) enzyme-linked immunosorbent assay, electroblotting immunoassay (EBIA/Western blot) and immunosorbent electron microscopy plus decoration (ISEM-D) assays.

2.2.12 SEROLOGICAL STUDIES

Plate trapped antigen (PTA)-, double antibody sandwich (DAS) ELISAs, electroblotting immunoassay (EBIA/Western blot) and Immunosorbent electron microscopy plus decoration (ISEM-D) were used to determine the serological relationship between the Celosia virus and some potyviruses.

Leaves of plants that had been inoculated for 2 - 3 weeks were collected in polyethylene bags. The samples were separately passed into a motorized leaf sap extractor (Eric Pollahle, GMBH, Germany) and the expressed sap was collected in small test tubes. Healthy leaves were also treated similarly. Sap expressed in this way was used in all enzyme-linked immunosorbent assays.

Antisera (IgGs) raised against the following viruses (obtained from the antiserum bank, Institut fur Pathogendiagnostik, Aschersleben, Germany) were tested: asparagus virus-1 (AV-1), bean common mosaic (BCMV), bean yellow mosaic (BYMV), potato virus V (PVV), maize dwarf mosaic (MDMV), soybean mosaic (SoyMV), turnip mosaic (TuMV), plum pox (PPV), watermelon mosaic-2 (WMV-2), beet mosaic (BMV), lettuce mosaic (LMV), pea seed-borne (PsbMV) and celery mosaic (CeMV) viruses. Lyophilized or fresh leaf samples infected by these viruses constituted the positive controls.

2.2.12.1 PLANT-VIRUS GROUP DETERMINATION

The determination of the plant virus group for the Celosia virus isolate was carried out at IAR&T.

Ten fold dilutions of infectious sap obtained from Celosia virus infected plants were tested against 1:1000 dilution of the Universal potyvirus group monoclonal antibody (Agdia, Inc. Elkhart, IN) Kindly supplied by Dr. S.A. Shoyinka, IAR&T, Ibadan) in plate-trapped indirect enzyme-linked immunosorbent assay (I - ELISA) as described by Anonymous (1991).

Virus extract was made in carbonate coating buffer (1:20 w/v) consisting of 1.59 g Na_2CO_3, 2.93 g $NaHCO_3$ and 0.2 g NaN_3 dissolved in 1 litre of distilled water to which 2% polyvinnyl pyrolidone (PVP) was added. The extract was filtered through cheese cloth. One hundred microlitres of the extract was added to each well of the ELISA plate and incubated for 3 hr at 27°C. The plates were washed thrice with phosphate buffered saline - Tween (PBS-Tween) consisting of 8.0 g NaCl, 0.2 g KH_2PO_4, 1.188 g Na_2HPO_4, 0.2 g KCl, 0.2 g N_aN_3 and made up to 1 litre with distilled water to which 0.05% Tween-20 was added. The plates were again filled with 150 µl of 'blocking solution' (PBS + 3% fat free milk in PBS-Tween), incubated for 1 hr at 27°C and emptied by inverting the plates which were then blotted dry. Each well was filled with 100 µl of the potyvirus group monoclonal antibody and the plates were incubated for

3 hr at 27°C. The plates were washed again as previously described. One hundred microlitres of enzyme-labelled (alkaline phosphatase) goat antimouse conjugate diluted 1:1000 in conjugate buffer (PBS-Tween containing 2% PVP was added to the wells to inhibit non-specific binding of the conjugate. The plates were left in the refrigerator overnight. They were washed, filled with 100 µl of freshly prepared p-nitrophenyl phosphatase (PNPP) and incubated at 27°C for 2 hr.

Extract from cowpea aphid-borne mosaic virus (CabMV - a potyvirus) infected plant was included as standard. All ELISA reactions were assessed by reading the absorbance at 405 nm in a Dynatech ELISA reader.

2.2.12.2 **Plate trapped antigen - ELISA**

PTA-ELISA was performed as outlined by Converse and Martin (1990). Virus in crude sap diluted (1:10) in 0.05M carbonate coating buffer, pH 9.0 was added to the wells of microtitre plates and incubated at 4°C overnight. Blocking was carried out by the addition of 1% bovine serum albumin (BSA) or defatted milk in phosphate buffered saline (PBS) followed by incubation for 2hr at 37°C. The plates were washed three times with PBS containing 0.05% Tween-20 (PBS-T), filled with IgG in PBS buffer containing 1% BSA and then incubated for 2hr at 37°C. After another round of washing, the wells were filled with enzyme-antibody

conjugate [goat anti-rabbit IgG coupled to alkaline phosphatase (AP)] diluted 1:1000 in 0.5M Tris-HCl buffer, pH 8.0 containing BSA and then incubated for 1hr at 37°C. The plates were washed again and absorbance at 405nm was recorded using a Titreplus version 3.5 ELISA reader after adding 200µl p-nitrophenyl phosphatase as substrate and incubating for 1hr at room temperature. In the other steps the wells were filled with 100 µl of the reactants. Each sample was tested in three wells and reaction was considered positive if the mean absorbance exceeded twice the mean value of healthy sample (Mumford *et al.*, 1994; Walkey *et al.*, 1994).

2.2.12.3 Double antibody Sandwich - ELISA

The procedure used is as described by Clark and Adams (1977). Briefly, plates were coated with 1µl or 10µl/ml of IgG (depending on antiserum titre) in 0.05M carbonate coating buffer for 3 to 4 hr at 37°C. The plates were rinsed four times with PBS-T, filled with plant extract containing virus in extraction buffer [PBS-T containing 2% polyvinylpyrolidone (PVP)] and were incubated overnight at 4°C. The plates were washed again and this was followed by the addition of AP-IgG appropriately diluted depending on the antiserum titres. Addition of enzyme substrate (1 mg/ml p-nitrophenyphosphate in diethanolamine buffer, pH

8.0), incubation and photometric measurements were as previously described.

2.2.12.4 **Electroblotting Immunoassay (EBIA)/Western Blot**

EBIA was performed using the procedure described by Richter *et al.* (1994). After SDS-PAGE in a vertical Minigel Twin electrophoresis apparatus, proteins transfer to nitrocellulose membranes (NCs), blocking, incubation with antisera, washing and detection of bound antisera were as described for coat protein molecular weight determination.

2.2.12.5 **Immunosorbent Electron Microscopy plus Decoration Test**

`Zaponlack' carbon-coated grids were floated for 15 min on drops of antisera diluted 1:1000, rinsed three times and floated on virus containing clarified plant extract in 0.05 M potassium phosphate buffer. After two rounds of washing with distilled water, the grids were floated for the second time on drops of antisera diluted 1:50 and washed twice with buffer. Finally, the grids were stained with 2% uranyl acetate and examined with a JEM 100 electron microscope.

2.2.12.6 **IMMUNODIFFUSION TESTS**

Immunodiffusion tests were carried out in agar gel containing sodium dodecyl sulphate (SDS). The immunodiffusion medium, consisting of 1 g Noble agar, 0.6% SDS and 1.2 g sodium azide (N_aN_3), was prepared as described

by Purcifull and Batchelor (1975). The agar was dissolved in 75 ml of distilled water and autoclaved for 5 min at 121°C/1.1 kg cm^{-2}. After autoclaving sodium azide was added and dissolved by stirring. To the agar-azide solution was added SDS dissolved in 40 ml of distilled water. The final volume was brought to 125 ml by adding hot water. About 12 ml of the medium was poured into each Petri dish and allowed to gel. The plates were wrapped in polythelene bags and stored in the refrigerator.

Just before use, one central and 6 peripheral wells were punched in each plate using a cork borer. The agar plugs were carefully removed using pipette connected to a suction pump.

Crude extracts from systemically infected *C. argentea* were tested against antisera to the following viruses: bean common mosaic virus (BCMV) isolates from Germany, New York and the Netherlands, pepper veinal mottle virus (PVMV), maize dwarf mosaic virus isolates (MDMV-A, MDMV-IITA), Telfairia mosaic virus (TeMV), peanut mottle virus (PMoV), soybean mosaic virus (SMV), Amaranthus leaf mottle virus (ALMV), pepper mild mottle virus (PMMV) beet mosaic virus (BMV), blackeye cowpea mosaic virus (BlCMV), CabMV from Onne and IITA, bean yellow mosaic virus New York isolate (BYMV-NY). (The antisera were obtained from Dr. G. Thottappilly, IITA, Ibadan). The crude extracts were also tested against antisera to watermelon mosaic virus 1 (WMV-1), WMV-2, zucchini yellow mosaic virus (ZYMV) (Supplied by

Dr. V. Lisa, Instituto Di Fitovitologia Applicata, Torino, Italy), cucumber mosaic virus (CMV), squash mosaic virus (SqMV), papaya ringspot virus (PRV) (Kindly supplied by Dr. D. Gonsalves, N.Y.S. Agric. Exp. Station Geneva, NY), BYMV - Germany and pumpkin virus Niger Republic isolate (obtained from Dr. H.J. Vetten, Biologische Bundesanstalt B11/13 Messeweg Braunscheweig. Germany).

In all the tests, the central wells were filled with the different antisera while the peripheral wells were loaded with crude extracts from virus infected *Celosia* plants. Extracts from healthy leaves of *C. argentea* were included as controls. Plates were incubated at room temperature and observations were made after 24 - 48 hours.

2.3 RESULTS

2.3.1 HOST RANGE AND SYMPTOMATOLOGY

The results of the host range study presented in Table 2.1 showed that the Celosia virus isolate had a rather narrow host range infecting some species in Amaranthaceae, Chenopodiaceae and Solanaceae families. All the varieties of *C.argentea* tested were susceptible to the virus. None of the species or varieties in the *Aizoaceae, Cruciferae, Cucurbitaceae, Fabaceae (= Leguminosae), Lamiaceae (=Labiatae), Malvaceae, Poaceae (=Graminae), and Tiliaceae* families tested became infected. Also, no latent infection was detected in them. In *C. argentea* var. 'TLV 8', infection began as tiny chlorotic spots near the margin of the lower half of the third leaf above the inoculated leaf. Subsequent leaves bunched up and often developed necrotic tips (plate 2.1a). As the leaves became older they turned (almost through 180°) and became characteristically curled. Other leaves showed varying degrees of leaf shape alterations in form of abnormal serration (Plate 2.1b) or truncated leaf tip or leaf apex drawn into long filiform structure (Plate 2.1c) and mosaic (Plate 2.1d).

C. argentea 'purple variety' besides showing leaf malformation, curling and mosaic, lost its purple

pigmentation. The `narrow leaved variety' also showed leaf malformation, mottle and mosaic (Plate 2.2).

Disease reactions were more drastic in *C. trigyna*. Inoculated plants showed severe stunting, `shoe stringing' and `witches broom'-like symptom characterized by abnormal axillary shoot proliferation (Plate 2.3). *Chenopodium amaranticolor*, *C. quinoa*, *C. urbicum*, *C.morale*, *C. rubrum*, and *Gomphrena globosa* were local lesion hosts. Beside local lesion induction, *C. amaranticolor* and *C. quinoa* were systemically infected by the virus. Other susceptible plants were *N. clevelandii*, *N. occidentalis*, *Celosia argentea* var.`Deutsche', *N. benthamiana* and *Chenopodium foetidum* with varying degree of symptom severity ranging from mild mottle to severe leaf malformation.

Table 2.1: REACTION OF TEST PLANTS TO MECHANICAL INOCULATION WITH CELOSIA VIRUS

TEST PLANT	SYMPTOM [a]/ RESULTS OF BACK INDEXING[b]
AIZOACEAE	
Tetragonia expansa	NS/(-)
AMARANTHACEAE	
Amaranthus caudatus L.	NS/(-)
A. cruentus L.	NS/(-)
A. hybridus L.	NS/(-)
A. viridis L.	NS/(-)
Celosia argentea L.	
`commercial var'	CLL, Cu, lDi, Mo, St.
`purple var'	Cu, lC, Pu, lM, Mo.
narrow leaved var'	lC, Mot, Mo, (±)NLL
var `TLV 8'	CLL, Cu, Pu, lM, lC, St, (±)NL.
C. trigyna L.	lDi, SS, ASP, sST.
Gomphrena globosa L.	RNLL
CHENOPODIACEAE	
Chenopodium amaranticolor Coste & Reyn	NLL/SLL
C. quinoa	CLL, SCP, lM.
C. capitatum	NS/(-)
C. foetidum	Mot
C. foliosum	NS/(-)
C. morale	CLL
C. rubrum	CLL
C. urbicum	CLL
CRUCIFERAE	
Brassica pekinensis	NS/(-)
B. oleracea var. capitata	NS/(-)
CUCURBITACEAE	
Citrullus lanatus (Thurb.) Marsum & Nakai	NS/(-)
Colocynthis citrullus L.	NS/(-)
Cucumeropsis edulis L.	NS/(-)
Cucumis sativus L.	
Cv. `Poinsett'	NS/(-)

TEST PLANT	SYMPTOM [a]/ RESULTS OF BACK INDEXING[b]
Cucurbita maxima Duch. ex` Lam.	NS/(-)
C. moschata (Duch.) Poir	NS/(-)
C. pepo L.	
var. `Encore'	NS/(-)
var. `Consul'	NS/(-)
var. `Corona'	NS/(-)
Luffa acutangula Roxb.	NS/(-)
L. cylindrica Roem.	NS/(-)
Telfairia occidentalis Hook.	NS/(-)
***FABACEAE* (- LEGUMINOSAE)**	
Cajanus cajan Mill sp.	NS/(-)
Canavalia ensiformis DC.	NS/(-)
Glycine max (L.). Merr.	NS/(-)
Phaseolus lanatus L.	NS/(-)
P. vulgaris L.	
Cv. `Saxa'	NS/(-)
Vigna mungo L.	NS/(-)
V. unguiculata (L.) Walp	
Cv. `Ife Brown'	NS/(-)
Cv. `K59'	NS/(-)
Cv. `Mascara'	NS/(-)
***LAMIACEAE* (= LAMIATAE)**	
Ocimum basilicum L.	NS/(-)
O. canum L.	NS/(-)
O. gratissimum L.	NS/(-)
MALVACEAE	
Abelmuschus esculentus (L.) Moenh.	NS/(-)
***POACEAE* (= GRAMINAE)**	
Zea mays Gaertn Fruct.	NS/(-)
SOLANACEAE	
Capsicum annuum L.	
var. `Cerasiform'	NS/(-)
var. `Longum'	NS/(-)
C. frutescens L.	NS/(-)
Datura metel L.	NS/(-)
D. stramonium L.	NS/(-)
Physalis angulata L.	NS/(-)
P. floridana Rydb.	NS/(-)
Lycopersicum esculentum Mill.	NS/(-)

TEST PLANT	SYMPTOM [a]/ RESULTS OF BACK INDEXING[b]
Nicotiana benthamiana Domin.	lR,Mo, lM
N. clevelandii	Pu, VY
N. glutinosa L.	NS/(-)
N. occidentalis Wheeler	Mo, GVB
N. rustica L.	NS/(-)
N. megalosiphon	NS/(-)
N. tabacum L.	
var. `Samsum'	NS/(-)
var. `White Burley'	NS/(-)
Solanum macrocarpon L.	NS/(-)
S. melongena L.	NS/(-)
TILIACEAE	
Corchorus olitorius L.	NS/(-)

Abbreviations for Symptoms[a]: NS = No symptoms, CLL = chlorotic local lesion; Cu = cupping; lDi = leaf distortion, Mo = mosaic; Mot = mottle; St = stunting; lC = leaf curl; Pu = puckering; lM = leaf malformation; SS = shoe stringing; ASP = axillary shoot proliferation; sST = severe stunting; (±) NLL = necrotic local lesion (inconsistent); RNLL = reddish necrotic local lesion; SLL = Systemic local lesion; GVB = Green vein-banding; VY = Vein yellowing; SCP = Systemic chlorotic patches; lR = Leaf roll; [b](-) = no infectious virus recovered.

Plate 2.1. Disease symptoms induced in *Celosia argentea* var. `TLV 8' mechanically inoculated with Celosia virus. The symptoms included leaf tip necrosis (a), abnormal serration (b), leaf tip drawn into filiform structure (c) and mosaic (d). Healthy leaf is shown at the extreme right (e).

Plate 2.2. Mosaic in *Celosia argentea* `narrow leaved' (left) and `purple leaved' (right) varities caused by Celosia virus. Healthy leaves are shown below.


a
b
c
d
e
2.1


2.2

Plate 2.3. *Celosia trigyna* showing abnormal axillary shoot proliferation and severe stunting caused by Celosia virus. Right is the buffer inoculated control.


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2.3.2 PROPERTIES IN SAP

The Celosia virus had a dilution end point between 10^{-2} - 10^{-3}. Crude sap was infectious when treated at 35°C for 10 min, but not when heated at 40°C. Longevity `*in vitro*' was between 15-20 hr at room temperature.

2.3.3 SEED TRANSMISSION

Out of the 1,275 seedlings derived from seeds of 15 plants of *C. argentea* var. `TLV 8' systematically infected with the Celosia virus none showed any symptoms. Also, no seedling from the 347 and 500 seedlings derived from seeds of 15 plants of each of the `purple' and `narrow' leaved varieties respectively, infected with the virus showed any symptom of infection. Similarly, seeds from healthy plants of the three varieties did not show symptom of infection.

2.3.4 INSECT TRANSMISSION

The virus was transmitted by *A. spiraecola* and *T. citricidus* from *C. argentea* to *C. argentea* in a non-persistent manner. The rate of transmission was, however, low. When *A. spiraecola* was used as vector 5 out of 19 plants (in 2 trials) developed symptoms while for *T. citricidus* 6 out of 20 plants showed symptom of infection from the same number of trials. Attempts to transmit the virus using *A. craccivora*, *A. fabae*, *A. nasturtii*,

Acyrthosiphon pisum, *Aulacorthum solani* and *M. persicae* were not successful.

2.3.5 PURIFICATION

The virus was successfully purified using the procedure outlined in Fig.2.1 with approximate yield of 1.54mg/kg of leaf tissue and $E_{206/208}$ of 1.22. The other purification methods were unsuitable as virus particles were either lost, broken or aggregated together when examined under the EM.

2.3.6 INFECTIVITY ASSAY OF PURIFIED PREPARATIONS

The purified preparations when inoculated onto *C. quinoa*, *N. benthamiana* and *N. clevelandii* induced similar symptoms earlier observed using crude inoculum of the virus.

2.3.7 ELECTRON MICROSCOPY

Purified preparation of the virus when viewed under the electron microscope revealed flexuous rod -shaped particles characteristic of potyviruses (Plate 2.4). The mean particle length was about 750 nm.

2.3.8 MOLECULAR WEIGHT DETERMINATION OF COAT PROTEIN

The *Mr* as determined in SDS-PAGE followed by Western blotting was 30.2kDa.

Plate 2.4. Electron micrograph of the Celosia virus isolate showing flexuous rod-shaped particles (x 11,500).

2·4

2.3.9 PLANT VIRUS GROUP DETERMINATION

The Celosia virus reacted positively with the universal potyvirus group monoclonal antibody (PVY-I) and with potyvirus group specific monoclonal antibody P-3-H8 in ELISA.

2.3.10 SEROLOGICAL RELATIONSHIP WITH OTHER POTYVIRUSES

2.3.10.1 PLATE-TRAPPED (PTA) - ELISA

The Celosia virus reacted with antisera to AV-1, MDMV, TuMV, LMV and BMV in PTA-ELISA while antiserum to PVV gave a weak reaction. No reactivity was observed with antisera to BCMV, BYMV, SoyMV, CeMV and PPV (Fig.2.2). In reciprocal tests, the virus reacted with its homologous antiserum as well as with AV-1, TuMV, WMV-2, MDMV, PPV, CeMV and LMV.

2.3.10.2 DOUBLE ANTIBODY SANDWICH (DAS) - ELISA

The result of DAS-ELISA tests presented in Fig. 2.3 indicated that the Celosia virus reacted positively only with antiserum to AV-1 while other viruses reacted specifically with their homologous antisera. No reciprocal tests were carried out

2.3.10.3 ELECTROBLOTTING IMMUNOSORBENT ASSAY (EBIA)/WESTERN BLOT ASSAY

In Western blotting, antisera to AV-1, TuMV, MDMV, PPV and BCMV detected SDS-dissociated coat protein of the Celosia virus. In contrast, no cross reactivity was observed with antisera to PPV, SoyMV and WMV-2. Antisera to

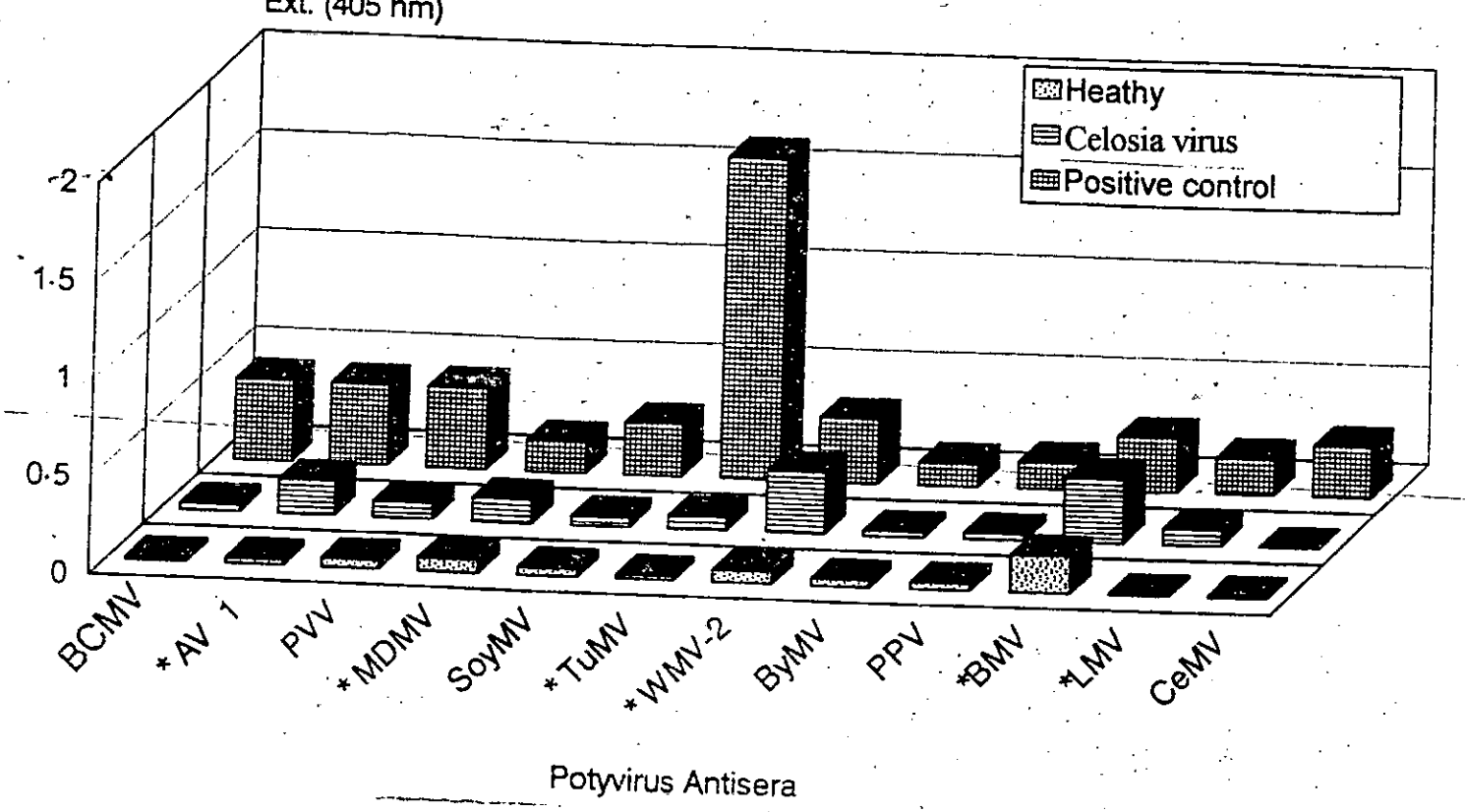


Fig.2.2 Serological reaction of Celosia virus to some potyvirusesin plate - trapped antigen (PTA) enzyme linked immunosorbent assay.

Virus acronyms: BCMV = bean common mosaic virus; AV-1 =aparagus virus 1; PVV = Potato virus V; MDMV = maize dwarf mosaic virus; SoyMV = soybean mosaic virus; turnip mosaic virus; WMV-2 = watermelon mosaic virus-2; ByMV = bean yellow mosaic mosaic virus; PPV = plum pox virus; BMV = beet mosaic virus; LMV = lettuce mosaic virus; CeMV =celery mosaic virus.

* Values > 2x healthy control.

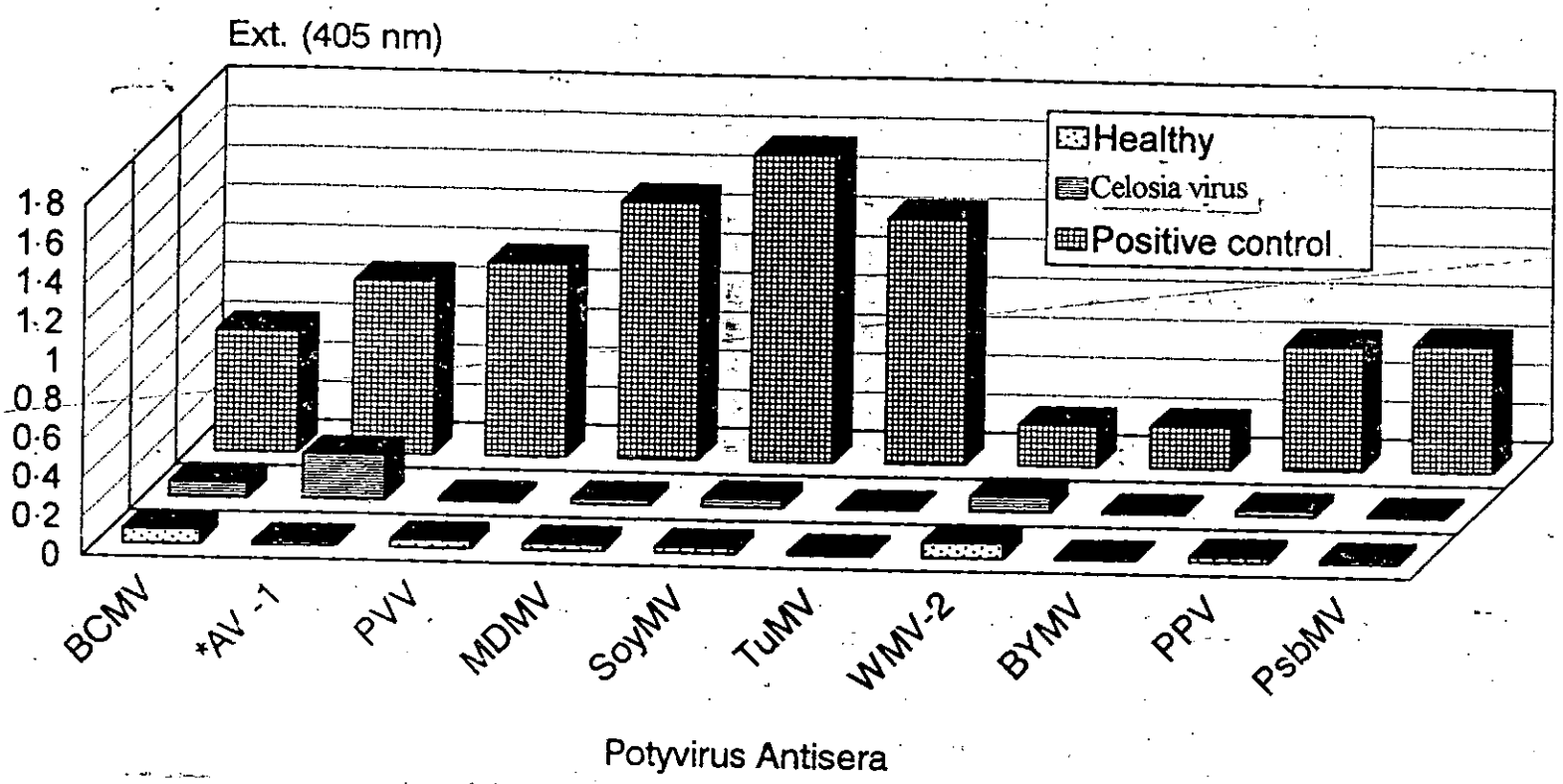


Fig. 2.3. Serological reaction of Celosia virus to some potyviruses in double antobody-sandwich (DAS) enzyme-linked immunosorbent assay.

Virus acronyms: BCMV = bean common mosaic virus; AV-1 =aparagus virus 1; PVV = Potato virus V; MDMV = maize dwarf mosaic virus; SoyMV = soybean mosaic virus; turnip mosaic virus; WMV-2 = watermelon mosaic virus-2; ByMV = bean yellow mosaic mosaic virus; PPV = plum pox virus; PsbMV = pea seed-borne mosaic virus.

* Values > 2x healthy control.

BMV, CeMV and LMV were not tested. Reciprocal tests in Western blotting showed that the virus reacted exclusively with its antiserum. No cross reactivity was observed with AV-1, TuMV, MDMV, WMV-2, PPV, PVV, PVY and SoyMV. Also, the antiserum did not react with BMV, LMV and CeMV.

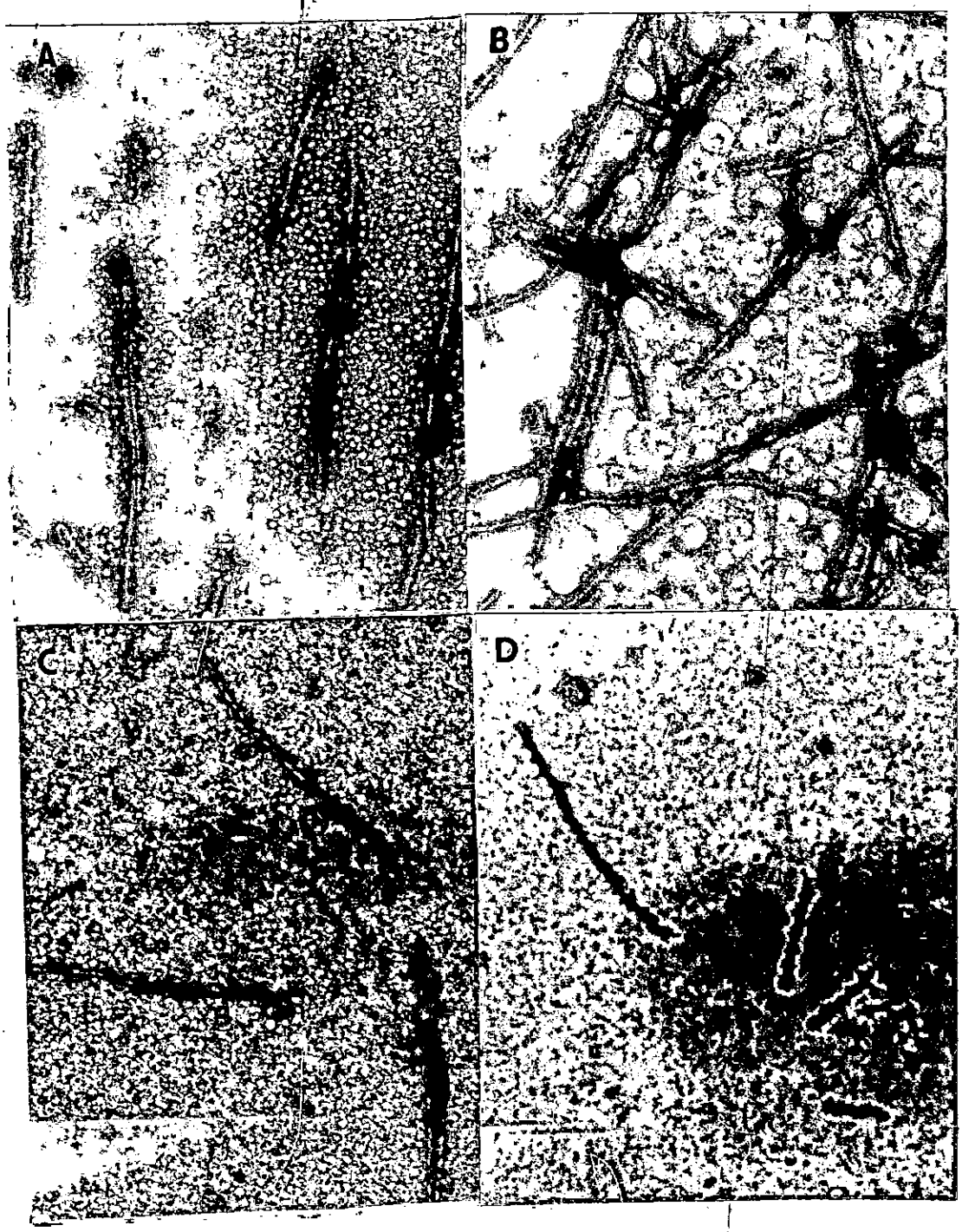
2.3.10.4 IMMUNOSORBENT ELECTRON MICROSCOPY PLUS DECORATION (ISEM-D) TEST

The Celosia virus particles were strongly decorated by antiserum to AV-1 and MDMV while SoyMV antisera produced slight decoration reactions (Plate 2.5). On the other hand, no decoration was observed with antisera to TuMV, WMV-2, PVV, BCMV and PVV. The virus was typically decorated by its homologous antiserum (Fig.2.5a). It also reacted strongly with BMV but gave weak reactions with PsbMV, LMV and SoyMV in ISEM plus decoration test.

2.3.11 IMMUNODIFFUSION TESTS

The virus did not react with any of the 24 antisera to 17 potyviruses tested in immunodiffusion test.

Plate 2.5: Decoration of Celosia virus particles (purified prep.) with its homologous antiserum (A) (x 29,900) and with heterologous antisera to asparagus virus-1 (B) (x 28,900), soybean mosaic virus (C) and maize dwarf mosaic virus (D) (x 48,400).


A
B
C
D

2.4 DISCUSSION

The successful mechanical transmission of the causal agent of the leaf curl disease of *C. argentea* from its natural host to some experimental plants of the same species in the greenhouse suggested a viral etiology.

The result of this study indicates that the Celosia virus had a rather narrow host range infecting some species in the families Amaranthaceae, Chenopodiaceae and Solanaceae. The limited host range sets the Celosia virus aside from other viruses such as ALMV (Lovisolo and Lisa, 1976; 1979), AMV (Taiwo *et al.*, 1988) and PWMV (Singh *et al.*, 1972) reported on *Amaranthus deflexus, A. hybridus* and *A. viridis* respectively and CMV which was reported to naturally infect *C. argentea* (Provvidenti, 1975). Amaranthus leaf mottle virus infected 26 plant species spanning 6 families including Aizoaceae, Amaranthaceae, Chenopodiaceae, Lamiaceae (=Labiatae), Fabaceae (= Leguminosae) and Solanaceae. Cucumber mosaic virus is known to infect several species in diverse plant families (Tanne and Zimmermann - Gries, 1980; Lakshmann *et al.*, 1985). Beside, CMV has icosahedral particles and therefore differ from the Celosia virus. The virus in this study also seems to be different from that reported by Atiri and Osemobor (1991). Their virus isolate infected several plant species which were not susceptible to the Celosia virus.

The Celosia virus also appears to differ from these viruses in its physical properties as it seems less stable under `*in vitro*' conditions. While the Celosia virus had a DEP between 10^{-2} and 10^{-3}, TIP between 35-40°C and LIV of between 15-20 hr, AMV had a DEP of between 10^{-2} - 10^{-3}, TIP of 60-65°C and LIV of between 6 and 7 days. For ALMV the DEP was between 10^{-5} - 10^{-6}, a TIP of between 58-60°C and LIV of at least 30 days at room temperature.

The Celosia virus is also different from the potyviruses that have so far been reported on vegetables in Nigeria. Beside the rather wide host range of PVMV (Lana *et al.*, 1975; Ladipo and Roberts, 1977, 1979; Igwegbe and Waterworth, 1982) the Celosia virus did not react with its antiserum, neither was it transmitted by *A. craccivora* which vectors PVMV (Lana *et al.*, 1975; Ogungbenro and Ladipo, 1987). It also differs from eggplant green mottle virus (EGMV) and eggplant severe mottle virus (ESMV) (Ladipo, 1976; Ladipo *et al.*, 1988a,b) whose natural host, *Solanum melongena* was not susceptible to the Celosia virus.

With respect to the WMV strain described by Igwegbe (1983a), the Celosia virus failed to infect some cucurbits which were susceptible to the former. Similarly, *T. occidentalis*, the natural host of TeMV (Shoyinka *et al.* 1987) was immune to the Celosia virus and it is serologically unrelated to it. Moreover, these potyviruses differ from the Celosia virus in their physical properties.

From the result of this study the Celosia virus was transmitted by *A. spiraecola* and *T. citricidus* in a non-persistent manner but not by *A. craccivora, A. fabae, A. nasturtii, Acyrosiphon pisum, Aulacorthum solani, Rhopalosiphon maidis* and *Myzus persicae.* No evidence of seed transmission in *C. argentea* var.`TLV 8', the `purple' and `narrow leaved' varieties was obtained. The non-persistent transmission of the virus by aphids, a property which the virus shares with several members of the potyvirus group (PVY) (Edwardson, 1974) seems to indicate that the Celosia virus is a member of the potyvirus group. This is further confirmed by the flexuous rod-shaped particles of the purified preparation of the virus, a coat protein with a molecular weight of 30.2kDa, as well as the positive reaction of the virus with the PVY-group and P-3-3H8 monoclonal antibodies and its detection with a broad-reacting potyvirus group specific polyclonal antiserum TUMV 314 (Richter *et al.*, 1994).

The virus failed to react with any of the 22 antisera prepared against 17 potyviruses and antisera to CMV and SqMV in immunodiffusion tests, suggesting that the Celosia virus might be different from these viruses. However, this result should be interpreted with caution since there was no reciprocal testing of antigens.

The virus reacted with heterologous antisera to AV-1, TuMV, MDMV, WMV-2, PPV, SoyMV, LMV, BCMV and BMV in at

least one of the serological assays used in this study. On the other hand, no cross reactivity was observed with antisera raised against PVV, PVY, and BYMV. The cross reactivity of these antisera particularly those raised against TuMV, MDMV WMV-2 and LMV which showed reciprocal serological relationship with the Celosia virus in PTA-ELISA may not necessarily indicate close relationship as antisera directed against the conserved core regions of the coat proteins of potyviruses have been demonstrated to detect potyviruses differing in biological properties (Shukla *et al.*, 1989a, b; Richter *et al.*, 1994). It is also probable that these antisera were obtained after long period of immunization at a time when a mixed population of antibodies lacking in specificity predominate (Regenmortel and Wechmar, 1970; Shepard *et al.*, 1974; Mernaugh *et al.*, 1990). This idea is reinforced by lack of cross reactivity of the Celosia virus antiserum obtained 5 weeks after the first injection with any of these viruses in Western blotting. One outstanding result emerging from the immunological assays is the positive reaction of the virus with AV-1 antiserum in all the serological tests including DAS-ELISA, a serodiagnostic tool generally held to be strain-specific (Koenig and Paul, 1982; Regenmortel, 1982) and capable of distinguishing between virus species (Richter, 1992; Richter *et al.*, 1994). The AV-1 antiserum strongly decorated the Celosia virus in ISEM plus

decoration tests, detected the CP of the virus in Western blotting while the virus also showed the same electrophoretic mobility, with a *Mr* of 30.2kDa (determined in this study) similar to that of the Celosia virus. These results are strong indications that the Celosia virus is more closely related to AV-1 than to any of the viruses tested. Interestingly however, polyclonal antibody raised against the Celosia virus failed to react with AV-1 in ISEM plus decoration test, Western blotting and even in PTA-ELISA suggesting a lack of complete identity of the two viruses. This type of unidirectional serological relationship has been observed between BYMV and LMV (Hollings and Brunt, 1991) and between johnsongrass mosaic virus (JMV) and WMV-2 (Shukla *et al.*, 1988). From the results of the immunological studies it becomes apparent that the serological data become more complex and serological relationship more difficult to interpret with increasing number of viruses included in the tests. The double antibody sanwich-ELISA is most often preferred in diagnostic work. Richter (1992) and with his colleagues (Richter *et al.*, 1994) obtained positive reactions only with corresponding homologous virus-antiserum combinations involving 19 potyviruses but did not fail to point out the possibility of cross reactivity between heterologous virus-antiserum combinations. Graichen and Rabenstein (1995) could not distinguish between isolates of beet western

yellows virus and beet mild yellowing virus serologically using DAS-ELISA, two viruses that are considered distinctive on the basis of RNA sequence data (Guilley *et al.*, 1995). The unidirectional serological relationship between Celosia virus and AV-1 is strong evidence that the Celosia virus is distinct from AV-1 and points to the danger of making hurried deductions from serological data regarding taxonomic relationships between viruses without recourse to other diagnostic methods. Since this property is coat protein dependent (Shukla and Ward, 1991) the exact taxonomic relationship of the Celosia virus with other members of the *Potyviridae* will become clearer when sequence data of the coat protein and the genome become elucidated.

Although, not less than eight potyviruses have been reported to infect *C. argentea* experimentally (Edwardson, 1974; Shoyinka *et al.*, 1987) this is the first report of a partially characterized virus naturally infecting *C. argentea* in Nigeria. The name Celosia leaf curl virus (CLCV) is hereby proposed for the agent.

CHAPTER THREE

EFFECT OF SUCCESSIVE WEEKLY INOCULATIONS WITH CELOSIA LEAF CURL VIRUS ON THE GROWTH AND DEVELOPMENT OF *CELOSIA ARGENTEA*

ABSTRACT

Celosia argentea var `TLV 8' was mechanically inoculated with Celosia leaf curl virus (CLCV) at weekly intervals for five consecutive weeks beginning from when the plants were three weeks through to when they were seven weeks old. Foliar symptoms became less severe as the plant grew older before inoculation was carried out. Early inoculations (at 3 and 4 weeks after planting) with CLCV induced severe disease symptoms which included mosaic, characteristic leaf curl and reduced leaf lamina. In later inoculated plants the disease symptoms were characterized by mosaic, mild leaf curl or no curling of leaves.

The effects of CLCV on the parameters investigated indicated that the reductions were almost proportional to plant age at time of inoculation. Early virus infection (3 and 4 weeks after planting) significantly reduced leaf size and number by as much as 11.9 - 42.4%. Percentage reductions in plant height, top fresh and dry weights as well as those of fresh and dried leaves ranged from 15.1 - 37.0% compared to 0.8 - 13.8% losses due to virus inoculation at advanced plant ages (5 - 7 weeks after

planting). These reductions generally did not differ significantly from the controls.

3.1. INTRODUCTION

Celosia argentea L. belongs to the family Amaranthaceae. It is widely cultivated for its leaves and stems. In Nigeria, this leafy vegetable is boiled and prepared as vegetable sauce and usually served with starchy staples such as rice, cassava, and yam flour (Oke, 1966; Omueti, 1980).

In October, 1989 a severe viral disease of *C. argentea* was observed in a commercial vegetable farm in Lagos. Infected plants showed mosaic, severe leaf curl, apparent reduction in leaf size and moderate to severe stunting. The disease has been established to be incited by a potyvirus for which the name Celosia leaf curl virus (CLCV) has been proposed (Chap. 2). The severity of the disease symptoms in all the varieties available renders them unmarketable thus indicating that the virus may be of economic importance to growers.

The effect of virus infection may be influenced dramatically by the host variety, the virus or viroid strain and age of the crop at time of inoculation (Singh *et al.*, 1971; Lana and Adegbola, 1977; Mikel *et al.*, 1981a,b; Agrios, 1989). This has been demonstrated in several plant-virus combinations.

Ladipo (1973) while comparing the effect of bunchy top virus infection on *Lycopersicum esculentum* var. Ife No.1 and *L. peruvianum* reported that the virus significantly

reduced the stem length and number of fruits in both species. However, the number of primary branches per plant and the number of seeds per fruit were affected differently. Atiri and Verma (1983) showed that the reduction in the yield of okra inoculated with okra mosaic virus was dependent on time of inoculation. Early virus infection significantly reduced fruit yield. Similarly, Arhavbarien (1989) reported greater reduction in plant height, leaf number and shoot weight of *Amaranthus hybridus, A. crentus* and *A. caudatus* when they were inoculated with Amaranthus mosaic virus (AMV) at 3 weeks than at 5 and 7 weeks after seeding. Other research works involving several plant - virus interactions such as beet western yellow virus on sugar beet (Mink, 1972), tobacco mosaic virus (TMV) on tomato (Crill *et al.*, 1970; 1973) watermelon mosaic virus on watermelon (Demski and Chalkey, 1974) indicated that early virus infections reduced crop yield than late inoculations.

There have been reports on inoculation with plant viruses at successive weekly intervals on growth and yield of some vegetable crops. The results of inoculating tomato with TMV at 6,7,8,9 and 10 weeks after seeding showed that the virus effect on yield decreased proportionally as inoculation time was delayed. Early inoculations were observed to cause greater yield reductions than late inoculations (Crill *et al.*, 1970). Similarly, the

successive weekly inoculations of pepper with cucumber mosaic virus (CMV) showed that early inoculations resulted in more severe foliar symptoms, significant reduction in plant height, markedly less top weight, fewer and smaller leaves, fewer total and marketable fruits than late inoculations. Plant growth and fruit yield improved in almost direct proportion to the lateness of inoculation of the plant with CMV (Agrios *et al.*, 1985).

Although quantitative data have been provided on the effect of age on the yield components of *C. argentea* (Omueti, 1980), no information is available on virus effect on yield parameters of the vegetable. The objective of this study is to provide information on the effect of CLCV inoculation at different stages of growth on the foliar symptoms, growth and yield of *C. argentea*.

3.2. MATERIALS AND METHODS

3.2.1 SOURCE OF SEED AND RAISING OF SEEDLINGS.

Seeds of *C. argentea* var.`TLV 8' used for this study were obtained from the National Institute of Horticultural Research (NIHORT) Ibadan, Nigeria.

The seeds were sown in manure-supplemented sterilized soil in wooden seed trays. The trays were watered regularly until the seedlings were ready for transplanting. One seedling each was transplanted into 16cm diameter polyethylene bags which were three quarter filled with sterilized manure-supplemented garden soil.

3.2.2 EXPERIMENTAL DESIGN

The polyethylene bags were arranged in five replications in a randomized complete block design as described by Agrios *et al.* (1985). Each replication contained 60 seedlings arranged in triple rows of 20. Each group of 12 seedlings per triple row (4 in each row) constituted a plot (Plate 3.1). There were 5 of such plots in each replication and the plots were inoculated at different ages. The ages of plants at inoculation constituted the treatments.

Each replication was separated from the adjacent one by a distance of 40cm. Successive group of 12 seedlings (plots) within each replication was also separated by a distance of 40cm while plants were spaced 16cm apart within each plot. (Fig. 3.1)

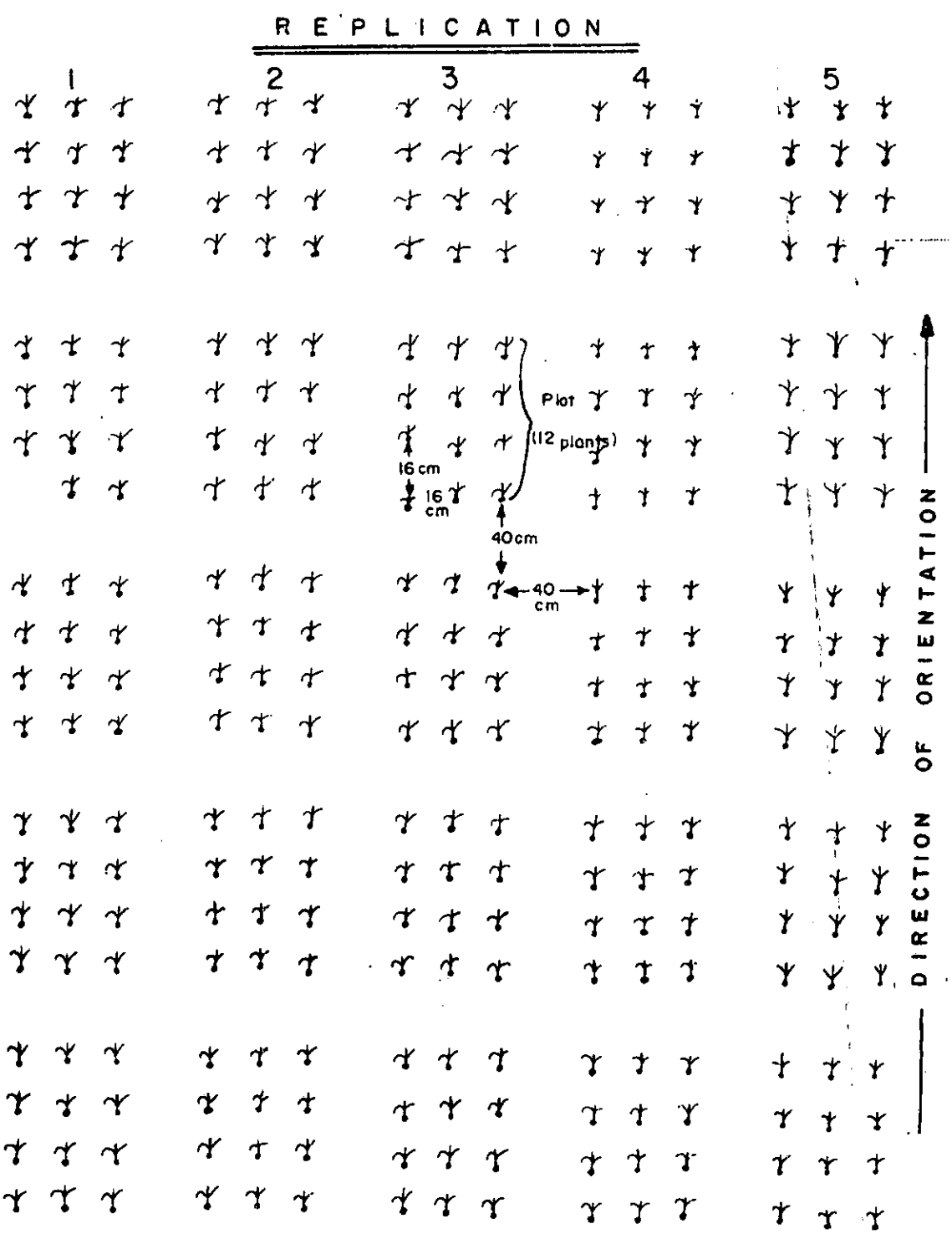


Fig. 3.1 SCHEMATIC REPRESENTATION OF THE EXPERIMENTAL DESIGN

The experiment was conducted in the Biological Garden of the University of Lagos.

3.2.3 RANDOMIZATION PROCEDURE

The treatments were randomized within each replication using the method described by Spiegel (1972). For the first and subsequent inoculation exercises one of 5 pieces of paper discs were marked `INOC' to denote to be inoculated while the rest were marked `No' to denote not to be inoculated. The discs were then rolled and distributed randomly among the plots. The same procedure was adopted for virus application among the 12 plants in each plot (treatment). Six out of 12 paper discs were marked `INOC' (meaning to be inoculated) and the remaining six were marked `BCON' to denote control to be inoculated with buffer only. The plants that had discs marked `INOC' were thus inoculated with CLCV.

3.2.4 INOCULATION PROCEDURE

Inoculations were carried out weekly for five successive weeks beginning on March 15, 1990 (when the plants were 3 weeks old) and ending on April 20, 1990 (when the plants were seven weeks old).

Infectious sap was prepared by grinding symptomatic leaves of *C. argentea* with pestle and mortar in 0.03M Na_2HPO_4 buffer pH 8.0 containing 0.1% (W/V) Na_2SO_3. The inoculum was then applied, by rubbing it onto carborundum-

dusted young leaves of 6 out of the 12 plants in each plot and in all the replications, five replicates for each inoculation age. The inoculated plants were rinsed with water and appropriately labelled. Successful inoculation on individual plants was confirmed by observing symptom development.

3.2.5 EFFECT OF CELOSIA LEAF CURL VIRUS INOCULATION ON LEAF SIZE OF *CELOSIA ARGENTEA*

The effect of the virus on the leaf size was determined by using an Electronic Planimetre (Paton/CSIRO). The samples measured for each treatment (inoculation age) was the tenth leaf to the inoculated leaf on 5 plants which were randomly selected from the five replications. The leaf samples were fed into the transparent conveyor belt of the Planimetre and the values read. Each sample was read five times and the value recorded for each treatment was the mean of the reading for five replicates. Leaves from five healthy plants (one leaf per plant) were also randomly picked from the five replications for each of the inoculation ages to serve as control. The size of each leaf was determined as described above.

3.2.6 EFFECT OF VIRUS INOCULATION ON PLANT HEIGHT

In order to determine the effect of the treatments on plant height the study was terminated on May 17, 1990. Harvesting was done by cutting the plants above the soil line. The effect of the virus on plant height was

established by measuring five inoculated plants from each of the five replications for each treatment. Similar measurements were made for 25 buffer inoculated plants, five plants each for each of the treatments.

3.2.7 EFFECT OF VIRUS INOCULATION ON LEAF NUMBER

Leaves of five harvested inoculated plants for each treatment were counted. Similarly, the leaves on buffer inoculated control plants were counted. Data presented are the means of five replicates.

3.2.8 EFFECT OF VIRUS INOCULATION ON FRESH WEIGHTS OF WHOLE PLANT AND LEAF PER PLANT OF *CELOSIA ARGENTEA*

Plants that received the same treatments were harvested and weighed together. The leaves were later removed and weighed separately. Buffer inoculated plants were treated in similar manner.

3.2.9 EFFECT OF VIRUS INOCULATION ON DRY WEIGHTS OF WHOLE PLANT AND LEAF PER PLANT OF CELOSIA ARGENTEA

After obtaining the fresh weights of both whole plants and leaves, the plant materials were dried in the oven at 70°C for 5 days. The combined weight of the stem and leaf of each plant per treatment was obtained. Data were also obtained for dried leaf only. Data presented are the means of five replicates.

3.2.10 STATISTICAL ANALYSIS

All data were subjected to analysis of variance. Means were compared to detect differences among the treatments by using Duncan's multiple range test at P = 0.05. However, data for the leaf size measurement were analysed by using Student's t-test as described by Clake (1980).

3.3 RESULTS

3.3.1 SYMPTOMS ON *CELOSIA ARGENTEA* INOCULATED WITH CELOSIA LEAF CURL VIRUS

Symptoms of infection on all plants inoculated at 3 and 4 weeks after planting began to show about 5 days following inoculation. Infected leaves developed characteristic leaf curl, mosaic and reduced leaf lamina. Leaves became more upright than normal, giving a bunchy appearance (Plate 3.2).

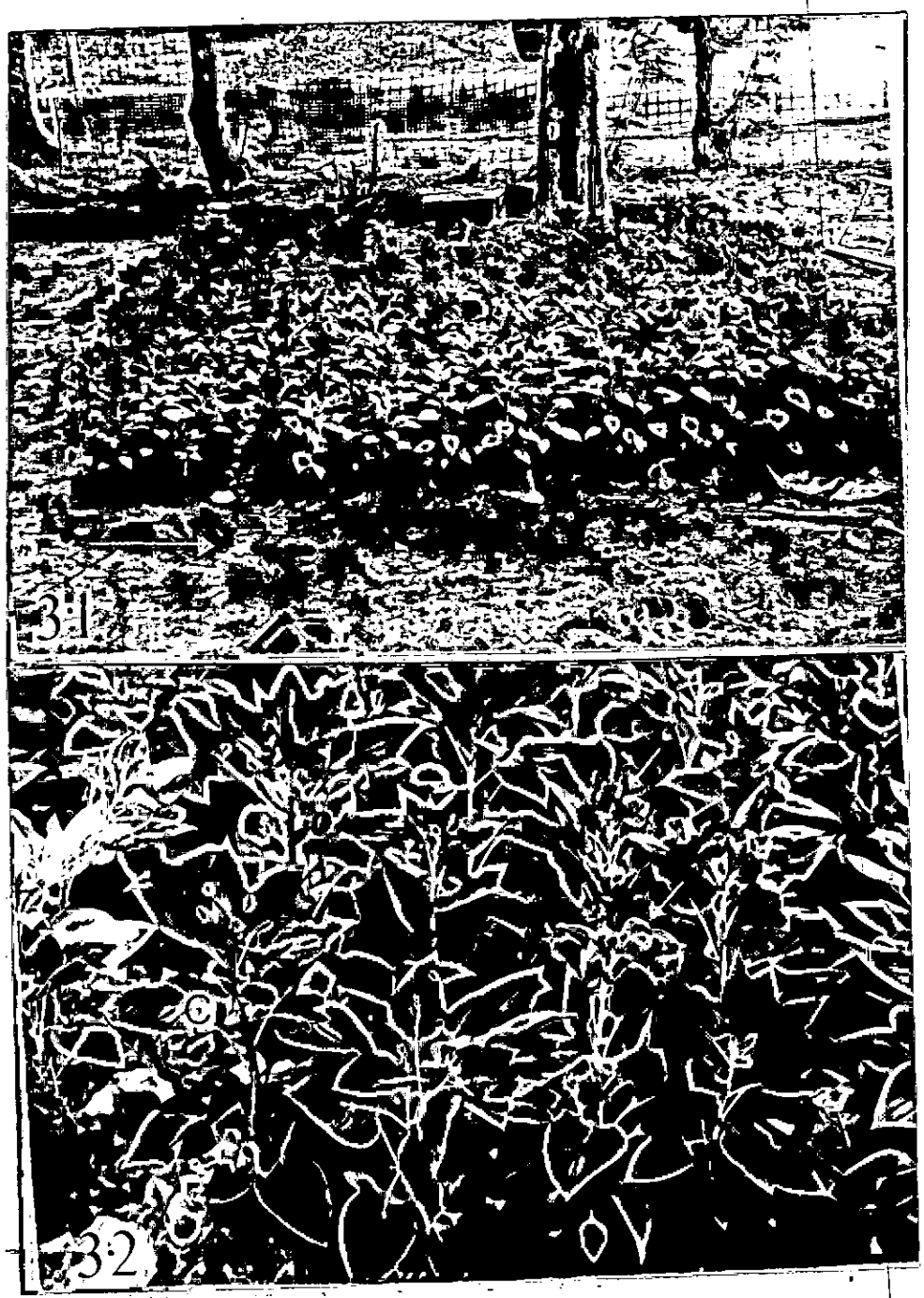
Plants inoculated at 5, 6 and 7 weeks after planting were less affected than plants inoculated at weeks 3 and 4. Symptom appearance was delayed and did not begin to show until about 9 - 12 days after inoculation. Symptomatic leaves showed mosaic, mild leaf curl or no curling at all.

3.3.2 EFFECT OF VIRUS INOCULATION ON LEAF SIZE

The effect of CLCV on the leaf size of *C. argentea* indicated a strong relationship between the age of plant at time of inoculation and leaf size. Significant leaf size reductions of 42.4, 36.8 and 13.8% were observed when plants were inoculated at 3, 4 and 5 weeks respectively (Appendix 4). Thereafter, the differences between the values obtained for the virus inoculated and buffer inoculated control plants were not significantly different (Fig. 3.2). Leaf sizes were reduced by 12.6 and 5.2% when

Plate 3.1. The experimental set-up (Randomized complete block design) showing arrangement of plants in blocks/replications. Arrow shows the orientation of the experimental design.

Plate 3.2. Leaf curl and mosaic symptoms (arrowed) induced on leaves of *Celosia argentea* inoculated with Celosia leaf curl virus. Buffer inolucated control plant is marked C in the foreground.


3·1
3·2

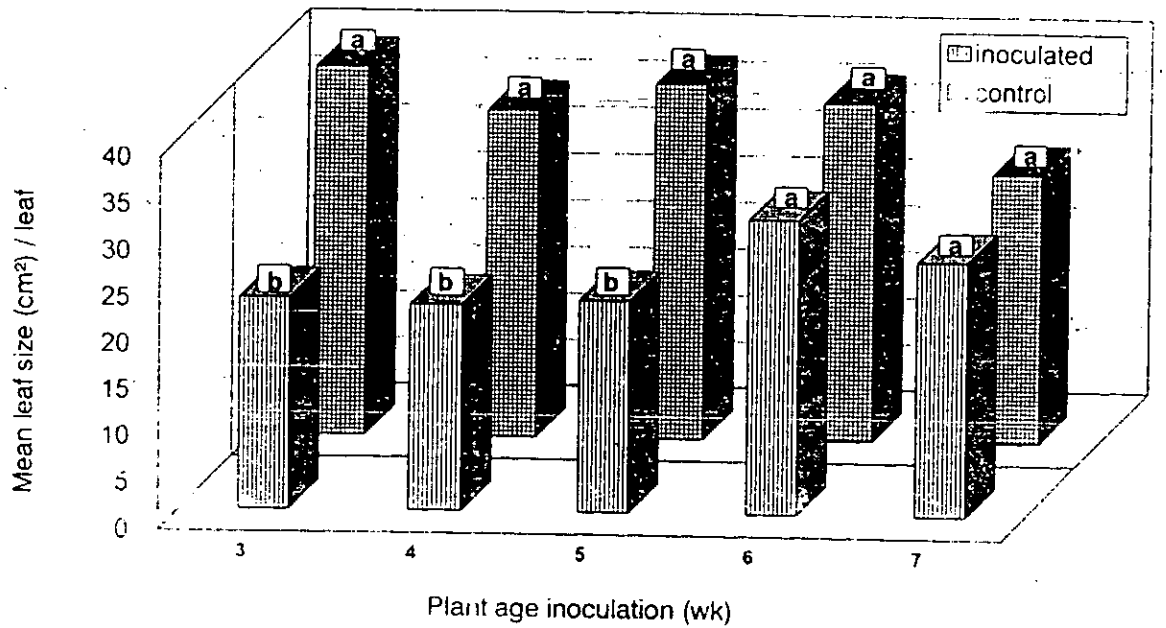


Fig. 3. 2. Mean leaf size of *Celosia argentea* inoculated with Celosia leaf curl virus at different ages. Each pair of bars topped by the same letters are not significantly different using Student's t - test.

plants were inoculated at the age of 6 and 7 weeks respectively.

3.3 EFFECT OF VIRUS INOCULATION ON PLANT HEIGHT

The effect of CLCV inoculation on plant height was influenced by the ages at which inoculations were carried out. Early inoculations (3 and 4 weeks after seeding) resulted in greater reduction in plant height than late inoculations (5-7 weeks after seeding). At the end of the experiment (May 17) the average height of healthy *C. argentea* was 96.7cm, whereas that of inoculated plants ranged from 78.2cm for those inoculated at 3 weeks to 95.5cm for those inoculated at 7 weeks after planting. Percentage reductions in plant height when compared to the control ranged from 1.2 for inoculation at 7 weeks to 19.1 for inoculation at 3 weeks after planting (Appendix 5a).

Data analysis summarized in appendix 5b indicated significant difference in treatment effect (plant age at inoculation). Plant height was significantly reduced when inoculated at 3 and 4 weeks after planting, whereas no significant difference existed between the values for later inoculations when compared to that of the control (Fig.3.3).

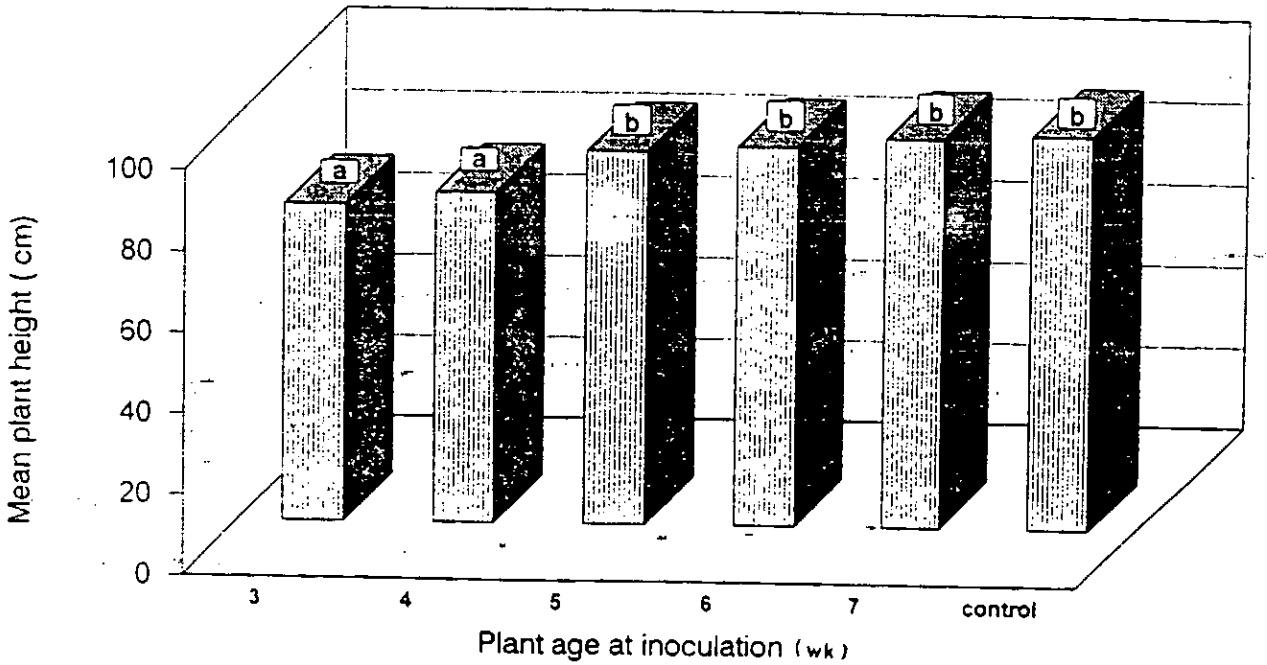


Fig. 3.3 Mean height of *Celosia argentea* inoculated with Celosia leaf curl virus at different ages . Bars topped by the same letter are not significantly different (P = 0.05) according to Duncan's multiple range test.

3.3.4 EFFECT OF VIRUS INOCULATION ON LEAF NUMBER

Plants that were inoculated 3 weeks after planting produced the least number of leaves with an average of 33.6 compared to 39.2 for inoculation at 7 weeks after planting. Average value for the buffer inoculated control was 40.5. The least percentage reduction of 3.2 was obtained for plants inoculated at 7 weeks while the highest reduction of 17.0% was recorded for plants inoculated at the youngest age (Appendix 6a).

The plant age at time of inoculation significantly influenced the number of leaves produced (Appendix 6b). Significant reduction in the number of leaves was observed at all the ages at which plants were inoculated except at 7 weeks when compared to the control. Mean values obtained for plants that were inoculated at 4,5 and 6 weeks were not significantly different from each other but differed significantly from the value for plants inoculated at 3 weeks old. (Fig.3.4).

3.3.5 EFFECT OF VIRUS INOCULATION ON TOP FRESH AND DRY WEIGHTS OF PLANTS

Celosia leaf curl virus infection caused reductions in the top fresh weight of *C. argentea* at all the inoculation ages. The results presented in appendix 7a showed that the mean top fresh weight was 62.2g for inoculation at 3 weeks whereas the values ranged from 64.4 to 93.7g for plants

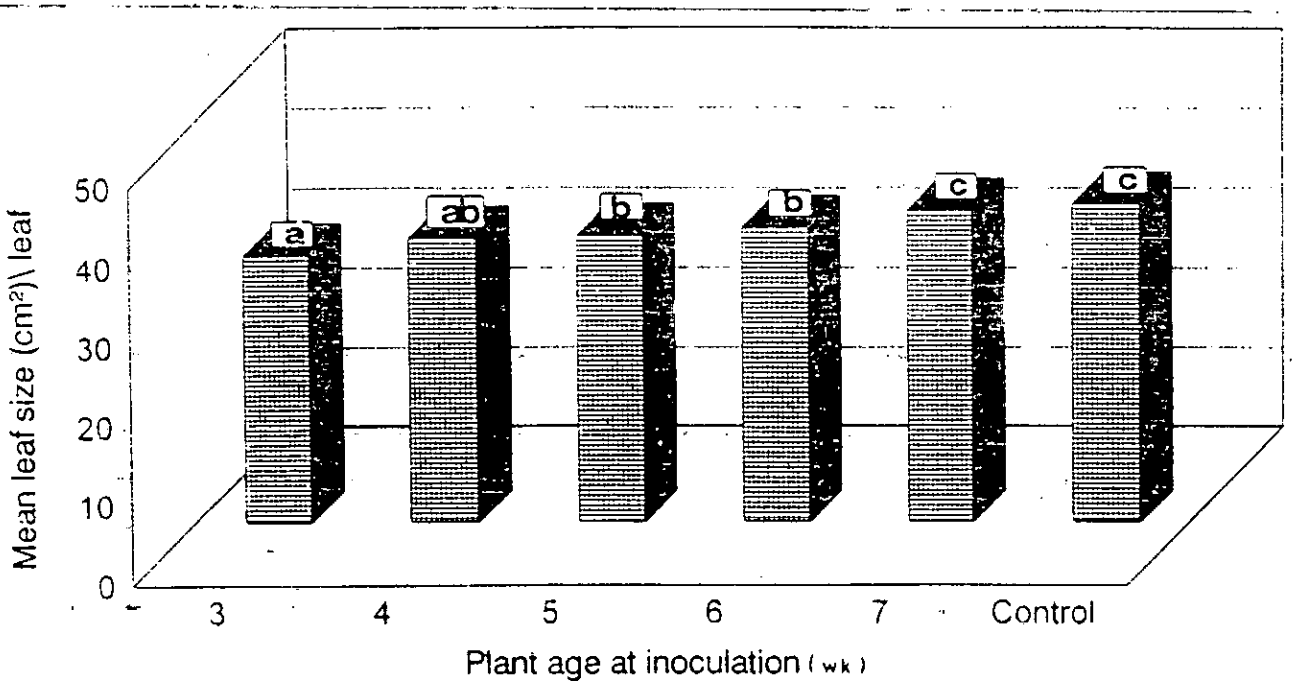


Fig. 3.4. Mean leaf number of *Celosia argentea* inoculated with Celosia leaf curl virus at different ages. Bars topped by the same letter are not significantly different (P = 0.05) according to Duncan's multiple range test.

inoculated in the subsequent 4 weeks. The value for the buffer inoculated control averaged 94.5g. Percentage reductions were between 0.8 - 34.0 for plants inoculated at 7 and 3 weeks old respectively.

Analysis of the data (appendix 7b,) indicated significant difference between treatment effect. Figure 3.5 showed that top fresh weight was significantly reduced at all inoculation ages except at 7 weeks which had comparable value with the control.

Similar to CLCV effect on plant top fresh weight, the least and highest mean values for plant dry weight were recorded for plants inoculated at 3 and 7 weeks respectively. Percentage reductions ranged from 5.0 for plants inoculated at 7 weeks old to 28.3 for plants inoculated at 3 weeks after planting (Appendix 8a).

Analysis of variance showed significant difference in treatment effect (Appendix 8b). Whereas CLCV caused significant reductions in dry weight of plants inoculated at 3 and 4 weeks after planting, values obtained for later inoculation ages did not differ significantly from the control (Fig.3.5).

3.3.6 EFFECT OF VIRUS INOCULATION ON FRESH AND DRY LEAF WEIGHTS

The results of inoculation with CLCV on leaf fresh and dry weights of *C.argentea* presented in appendices 9a and

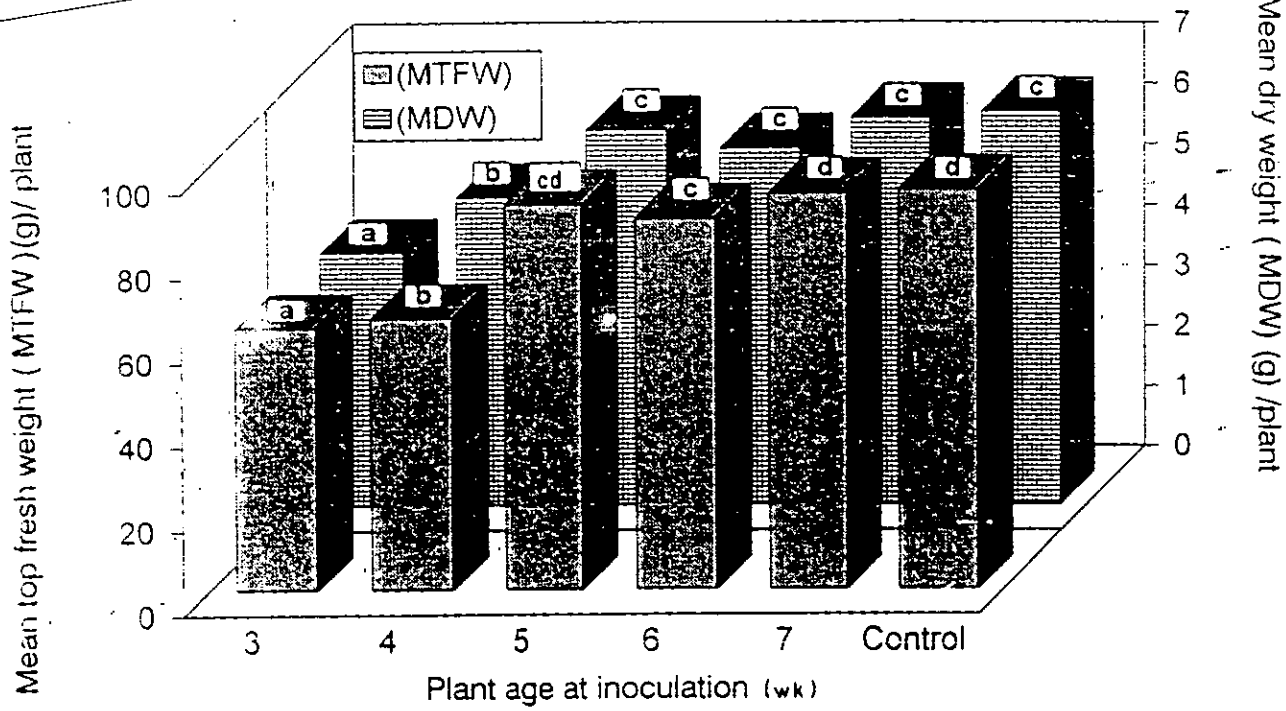


Fig. 3.5. Mean top fresh and dry weight (g) of *Celosia argentea* inoculated with Celosia leaf curl virus at different ages. Bars for each parameter topped by the same letter are not significantly different (P = 0.05) according to Duncan's multiple range test.

10a showed the same trend. Inoculation at 3 and 4 weeks after planting resulted in greater reductions in both parameters (35.4 and 27.1% respectively in fresh leaf and 37 and 29.6% in dry leaf weights) than did late inoculations.

Data analysis for both parameters indicated significant difference in treatment effects (appendices 9b and 10b). No significant difference existed between the values for the two earlier inoculation ages but these values differ significantly from those of later inoculations (at 5,6 and 7 weeks after planting) which had values comparable to the controls. (Fig. 3.6).

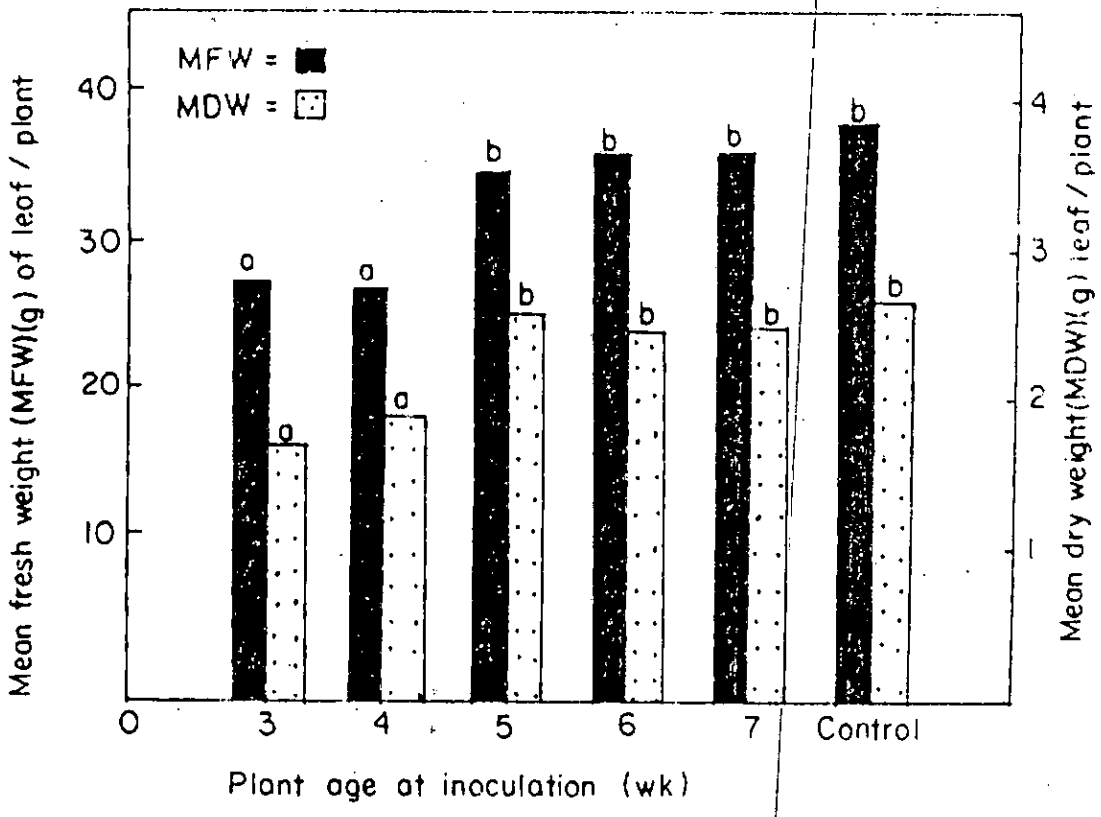


Fig. 3·6 Mean leaf fresh and dry weights (g) of Celosia argentea inoculated with Celosia leaf curl virus at different ages. Bars for each parametre topped by the same letter are not significantly different (P=0.05) according to Duncan's multiple range test.

3.4. DISCUSSION

The severity of disease symptoms in *Celosia* plants inoculated with CLCV was greater in plants that were inoculated at an early age than in plants inoculated at advanced ages. This observation confirms earlier reports of Rosenkranz and Scott (1978), Uyemoto *et al.* (1981) and Owolabi *et al.* (1988) that early infection of plants by viruses resulted in more severe response than infection at advanced stages of growth.

The effects of CLCV inoculation on all the parameters investigated in this study indicate a relationship between age at time of infection and each of the parameters.

C. argentea is a fast growing succulent herb reaching marketable size in about 6 - 7 weeks after seeding. The significant reduction in plant height caused by inoculation of the vegetable with CLCV is due to prevention of stem elongation. Similar results have been presented for tomato infected by bunchy top virus by Ladipo (1976).

Considering the results of the effect of inoculation with CLCV on the number of leaves per plant of *C. argentea* early inoculations (at 3 and 4 weeks after planting) produced significantly fewer leaves than late inoculations at 5-7 weeks of plant age. Agrios *et al.* (1985) obtained similar results when pepper was inoculated with CMV at successive weekly intervals. Ladipo (1976) however, did not detect significant reduction in the number of leaves of

tomato var. Ife No 1 and *L. peruvianum* inoculated with bunchy top virus.

The significantly higher fresh and dry weight values for late inoculations (5 - 7 weeks after seeding) compared to those obtained for early inoculations (3-4 weeks after seeding) was probably due to the attainment of maturity by the plants before inoculation.

The cultivation of *C. argentea* is primarily for the production of the succulent tops which include the leaves and stem. This study has shown that early infection of *C. argentea* by CLVC could result in severe losses. These could be prevented through practices that would prevent early virus infection. At present, none of the four varieties of *C. argentea* tested was resistant to CLCV. However, the use of resistant varieties, when available holds greater promise.

CHAPTER FOUR

CHARACTERIZATION AND IDENTIFICATION OF A VIRUS CAUSING A MOSAIC DISEASE OF CUCURBITA MOSCHATA, DUCH ex. Poir.

ABSTRACT

A mechanically transmissible virus was isolated from naturally infected *Cucurbita moschata*. The virus had a narrow host range confined to the family Cucurbitaceae. Susceptible plants showed green vein-banding, blistering, leaf malformation and mosaic. The virus failed to infect 43 other herbaceous plant species and varieties.

The virus was transmitted by *Aphis spiraecola* and *Toxoptera citricidus* from *C. moschata* to *C. moschata* and *Lagenaria siceraria*' in a non-persistent manner but not by *A. craccivora*. No evidence of seed transmission was obtained.

Electron microscopy of negatively stained leaf extracts and ultrathin sections of *C. moschata* infected with the virus revealed flexuous rod-shaped particles, striated lamellar and `pinwheel' inclusion bodies, typical of potyviruses.

The virus reacted positively with potyvirus group monoclonal antibody. No serological reaction was detected between the virus and 17 putative potyviruses using 22 antisera in immunodiffusion tests. In addition, the virus lost infectivity when diluted in water to 10^{-5}, heated to 55°C for 10min or kept up to 5 days at room temperature.

Based on the mode of insect transmission, electron microscopy and serology the Cucurbita virus is a member of the potyvirus group.

4.1 INTRODUCTION

Cucurbita moschata Duch ex. Poir commonly called pumpkin and referred to in Yoruba as "elegede" is an edible member of the Cucurbitaceae family. It is believed to have originated from Peru or Mexico (Tindall, 1989). Today *C. moschata* has spread to most parts of the world including Southeast Asia, Africa, South and Central America and the Caribbeans (Kochlar, 1981; Tindall, 1989).

C. moschata is an annual running andromonoecious vine. The leaves are large, alternate, simple, long petioled, palmately lobed and slightly harsh to touch. The flowers which are borne singly in the axil of the leaves are large and yellow in colour. The fruit is round to elongated in shape, often streak white (Purseglove, 1976).

It is widely cultivated in the Southern parts of Nigeria, usually in mixed cropping, providing effective cover against soil erosion and smothering weeds. It is often found growing on dunghills around human settlements, obviously from discarded seeds of previous harvest (personal observation).

Pumpkins are used for making pie, for canning and even as cattle feed. The young leaves are eaten as vegetable while the pulp of slightly unripe fruit is eaten raw or occasionally cooked (Kochlar, 1981; Dupriez and De Leener, 1989). In some communities in Nigeria the leaves are

considered more esteemed than the fruits while the reverse is the case in others.

A literature survey revealed a preponderance of information on virus diseases of cucurbits. More than 25 viruses including at least seven potyviruses are known to infect cucurbits naturally (Lovisolo, 1980).

In the Mediterranean region, eleven viruses have been reported to infect cucurbits of which cucumber mosaic virus (CMV), squash mosaic virus (SqMV), watermelon mosaic viruses 1 and 2 (WMV-1 and WMV-2) are known to cause important crop losses (Nameth *et al.*, 1986).

In Europe, zucchini yellow mosaic virus (ZYMV), CMV, SqMV, WMV-1 and 2 and a strain of clover yellow vein virus (ClYW) have been isolated from cucurbits in France, Italy, Hungary and Turkey (Horvath *et al.*, 1975; Lisa and Dellavale, 1981; Lecoq *et al.*, 1983; Davis, 1986).

Viruses that have been reported in cucurbits in the United States include WMV-1 and 2, CMV, SqMV, ZYMV, squash leaf curl viruses (SLCV-1, SLCV-2), lettuce infectious yellows virus (LIYV), tobacco ringspot virus (TRSV), papaya ringspot virus (PRSV), bean yellow mosaic virus (BYMV) and Tomato ringspot virus (TomRSV) (Purcifull *et al.*, 1984; Provvidenti *et al.*, 1984; Nameth *et al.*, 1986; Davis and Mizuki, 1987; Sammons *et al.*, 1989; Stobbs *et al.*, 1990).

In the Asian subcontinent, Hseu *et al.* (1987) reported the occurrence of ZYMV, WMV-1 and 2, cucumber

green mild mottle virus (CGMMV) and CMV in six cucurbits in Taiwan. The Indian record indicated that WMV,CMV, pumpkin enation virus, pumpkin mild virus and CGMMV were among viruses of cucurbits (Mukhopadhyay, 1985) while Yamamoto *et al.* (1984) have documented the incidence of WMV in Japan.

Reports of cucurbit viruses in the Middle East showed the occurrence of ZYMV and WMV-1 in Lebanon (Makkouk and Lesemann, 1980; Lesemann *et al.*, 1983), bottle gourd mosaic virus (BGMV), CMV, SqMV, WMV and squirting cucumber mosaic virus (SCMV) in Isreal (Cohen and Nitzany, 1963) while WMV-1 and 2 and CMV have been reported in Iran (Rahimian and Izadpanah, 1978).

Virus diseases of cucurbits have also been documented in Africa. Watermelon mosaic viruses have been reported in Morocco (Baum *et al.*, 1979; Hafidi, 1983), South Africa (van Regenmortel, 1971) and Niger Republic (Thouvenel *et al.* 1986). Ouf and Scott (1983) reported a strain of WMV in Egypt designated WMV-E which was serologically distinct from WMV-1 and WMV-2. Fauquet and Thouvenel (1987) listed CMV, WMV-1 and Cucurbita mosaic virus (CuMV) as cucurbit viruses in Cote D' Ivoire. CuMV believed to be a potyvirus had as its natural hosts *Cucurbita pepo, Physandra englandulosa and Adenopus guineensis.* It was serologically related to yam mosaic and pepper veinal mottle viruses but distantly related to groundnut eye spot, canavalia mosaic and passiflora ringspot viruses. It was however, not

related to WMV-1 and 2. No evidence of seed transmission was also obtained.

Reports of virus diseases of cucurbits in Nigeria are rather few. Nwauzo and Brown (1975) described a mosaic disease of *Telfairia occidentalis*. Shoyinka *et al.* (1987) convincingly established that the disease was incited by a potyvirus designated as Telfairia mosaic virus (TeMV). The virus, besides infecting non-cucurbitaceous plants was serologically related to but distinct from ZYMV and distantly related to WMV-2 and BYMV. Igwegbe (1983a) reported the occurrence of a virus disease of *Cucumeropsis edulis*. The virus had a thermal inactivation point between 45-50°C, longevity in vitro' between 4-5 days, dilution end point between 10^{-3} - 10^{-4} and flexuous rod-shaped particles. The virus was readily transmitted by *Myzus persicae* and had no serological relationship with muskmelon necrotic spot virus, WMV-1 and 2 or the Moroccan isolate of WMV. It induced mosaic, blisters, leaf malformation and flower abortion in *C. edulis*. It also incited chlorotic lesions in *Chenopodium amaranticolor* and *C. quinoa* but no symptoms in *Luffa acutangula*.

In October 1989, virus induced symptoms were observed on *C. moschata* during a visit to Tejuosho vegetable farm. Naturally infected plants showed mosaic, green vein-banding, blistering and leaf malformation.

So far, no virus disease of *C. moschata* has been reported in Nigeria. This study was therefore undertaken to provide information on the properties of the causal agent of the disease which has been tentatively named Cucurbita virus, with a view to identifying it.

4.2 MATERIALS AND METHODS

4.2.1 VIRUS ISOLATION AND MAINTENANCE

Young symptomatic leaves obtained from naturally infected *C. moschata* plants were ground in cold 0.03M phosphate buffer pH 8.0 in precooled oven-sterilized mortar. The buffer was prepared as previously described (Chapter Two). The homogenate was used to inoculate carborundum (500 mesh) dusted 9 - day old plants of *C. edulis* and *C. moschata* in the greenhouse (temperature range between 28 - 33°C). Inoculated plants were rinsed with water and kept in the greenhouse.

The virus was subsequently maintained by periodic transfer to both plant species.

4.2.2 HOST RANGE AND SYMPTOMATOLOGY

About 60 plant species and varieties belonging to nine plant families were used in this study. Test plants other than those of Cucurbits and legumes were raised in seed trays. The seedlings were later transplanted into polyethyene bags where they were allowed to grow to inoculation age, about 5-6 leaf stage. For the Cucurbitaceous and leguminous test plants, seeds were sown directly into bags and the seedlings were inoculated 8 - 9 days after planting when the cotyledonary leaves were fully expanded. At least five seedlings of each test plant were

inoculated with inocula prepared from infected leaf tissues of the stock plant, in cold buffer.

All inoculated plants were rinsed with water, kept in the greenhouse and examined daily for symptom development for 4 weeks. Back inoculation was performed on *C. edulis*, for the detection of latent infection. At least three plants of each species or variety were inoculated with buffer only, to serve as controls.

4.2.3 SCREENING FOR RESISTANCE

Seeds were obtained from fruits of different varieties of *C. moschata* from Edo, Cross River, Imo, Lagos, Ondo and Oyo States. The varieties differed in fruit shape which varied from spherical to elongated and in the colour and hairiness of the seeds. About 15 - 20 seeds from each seed lot were sown and inoculated with the Cucurbita virus at the cotyledonary stage.

4.2.4 VIRUS RECOVERY FROM FLORAL PARTS, JUVENILE PODS OF INFECTED PLANTS AND SEED TRANSMISSION TEST.

Virus recovery from the floral parts and juvenile pods of natural and mechanically inoculated *C. moschata and Cucumis sativus* var. `Poinsett' plants was carried out as described by Ladipo (1988b). Sepals, petals and anthers were obtained from flower buds and fully opened perfect and staminate flowers randomly chosen from five infected plants. One gram of tissue from each of the reproductive

parts and from five developing pods were ground in 2ml phosphate buffer and each inoculum was assayed on three plants of *C. edulis*. Control tests consisted of inoculating the assay plants with inocula obtained from floral parts and young fruits taken from buffer inoculated plants.

For seed transmission test, the five hundred and thirty seven (537) seeds used in this study were obtained from 9 fruits produced by Cucurbita virus infected *C. moschata*. The seeds were dried in the sun for a few days before they were planted in seed trays containing sterilized soil and kept in screen houses. The resultant seedlings were watered regularly and observed for symptom development. Final observations were made when the fourth true leaf was fully developed.

4.2.5 APHID TRANSMISSION TEST

The nymphs and apterous adults of *A. craccivora, A. spiraecola and T. citricidus* were tested for their ability to transmit the Cucurbita virus. The insects were starved for 6hr and allowed acquisition access feeding of 10min on virus infected leaves of *C. moschata*. Between 10 - 15 insects were transferred to 10 plants in two trials. The insects were allowed inoculation access feeding of 10min before they were killed by spraying with Actellic 50EC (10ml/l). Ten day old seedlings of *C. moschata and Lagenaria siceraria* were used as test plants. Inoculated

plants were kept in screen cages in the greenhouse and observed for symptom development.

4.2.6 PROPERTIES IN SAP

Crude extracts from *C. edulis* inoculated with the Cucurbita virus were used to determine the dilution end point (DEP), thermal inactivation point (TIP) and longevity 'in vitro' (LIV) of the virus. The procedures used for the tests are as previously described (Chapter Two). The tests were conducted using 8 - 9 day old seedlings of *C. edulis* as test host. Inoculated plants were kept in the greenhouse for at least 4 weeks for symptoms to develop. Each test was carried out three times and appropriate controls were included.

4.2.7 SEROLOGY

4.2.7.1 PLANT VIRUS GROUP DETERMINATION

In order to determine the plant virus group to which the Cucurbita virus belongs it was tested in plate-trapped indirect ELISA test against 1:1000 dilution of the potyvirus group monoclonal antibody (Agdia Inc. Elkhart, IN). The procedure used is as previously described (Chapter Two). The plates were later read using a Dynatech reader at A_{405}.

4.2.7.2 IMMUNODIFFUSION TESTS

The serological relationship between the Cucurbita virus and some previously reported potyviruses was determined by testing crude extract from Cucurbita virus infected plants against 22 antisera to 16 putative members of the potyvirus group in immunodiffusion tests. These were bean common mosaic virus (BCMV) isolates from Germany, New York and The Netherlands, pepper veinal mottle virus (PVMV), maize dwarf mosaic virus isolates (MDMV-A, MDMV-IITA), Telfairia mosaic virus (TeMV), peanut mottle virus (PMoV), soybean mosaic virus (SMV), Amaranthus leaf mottle virus (ALMV), blackeye cowpea mosaic virus (BlCMV), cowpea aphid-borne mosaic virus (CabMV-Onne, - IITA), bean yellow mosaic virus (BYMV) New York isolate, watermelon mosaic viruses 1 and 2 (WMV-1, WMV-2), zucchini yellow mosaic virus (ZYMV), papaya ringspot virus, BYMV - German isolate and pumpkin virus isolate from Niger Republic.

The Cucurbita virus isolate was also tested against antisera to cucumber mosaic virus (CMV) and squash mosaic virus (SqMV), which though are not potyviruses have been reported on cucurbits.

Immunodiffusion tests were conducted as previously described (Chapter Two).

4.2.8 ELECTRON MICROSCOPY AND CYTOPATHOLOGY

For the detection of virus particles, crude extracts from infected leaves of *C. moschata* were negatively stained with 2% uranyl acetate and examined under the Philips TEM 300 electron microscope.

Small pieces of virus infected leaves of *C. moschata* were fixed in 2% glutaraldehyde for 1 hr and rinsed in three changes of phosphate buffer (0.05M K_2HPO_4, pH 7.0). The pieces were dehydrated in acetone and infiltrated with acetone - Epon mixtures and with Epon at 40°C in a rotator. Ultrathin sections were cut with a diamond knife on an ultramicrotome. Sections were stained with citrate and examined under the electron microscope.

Electron microscopy of the crude leaf extract for virus particles and cytoplasmic inclusions induced in the host tissues were performed by Mr. George Duncan (Scottish Crop Research Institute (SCRI), Dundee, United Kingdom).

RESULTS

4.3.1 HOST RANGE AND SYMPTOMATOLOGY

The results of the host range study summarized in Table 4.1 indicated that the Cucurbita virus had a rather narrow host range limited to the family Cucurbitaceae. Out of 60 plant species belonging to 9 families, the virus readily infected 17. *Citrullus vulgaris, Luffa cylindrica, Mormodica charanta and Telfairia occidentalis* which are also cucurbits were however not susceptible to the virus. No virus was recovered from them as well as other symptomless plants after back indexing to *C. edulis*.

Most susceptible cucurbits exhibited a common disease syndrome as typified by the reaction of *Cucumis sativus* when plants were inoculated with virus at the cotyledonary leaf stage, 8 - 9 days after planting. The first symptom of infection was usually a green vein-banding on the second true leaf while the first set of leaves remained symptomless. Subsequent leaves showed leaf malformation, chlorosis, blistering, reduction in leaf size and mosaic (Plate 4.1). The severity of infection was however, dependent on the plant species or variety. In *Cucurbita pepo* var. `Consul' (Plate 4.2), for example, symptoms of infection ranged from severe leaf malformation, reduced leaf size to complete defoliation while in *C. edulis* the virus elicited enation beside severe leaf malformation and

TABLE 4.1: RESPONSE OF DIFFERENT PLANT SPECIES TO MECHANICAL INOCULATION WITH CUCURBITA VIRUS

TEST PLANT	SYMPTOMS[a]/ RESULT OF BACK INDEXING[b]
AMARANTHACEAE	
Amaranthus caudatus	NS/(-)[b]
A. hybridus	NS/(-)
A. viridis	NS/(-)
Celosia argentea Var. `TLV 8'	NS/(-)
C. trigyna	NS/(-)
Gomphrena globosa	NS/(-)
CHENOPODIACEAE	
Chenopodium amaranticolor	NS/(-)
CUCURBITACEAE	
Adenopus breviflorus	Mo
Citrullus lanatus	GVB, Bl, lM, Mo
Colocynthis citrillus	GVB, Mo
Citrillus vulgaris	NS/(-)
Cucumeropsis edulis	GVB, lM, En, De, St
Cucumis sativus cv. `Poinsett'	GVB, lM, Cl, Bl, Mo, St
Cucurbita maxima	GVB, lM, Bl, Mo
C. moschata	GVB, lM, VC, Bl, Mo
C. pepo	
var. `Encore'	VC, lM, Mo, Ss
var. `Consul'	VC, Mo, De, Ss
var `Corona'	VC, De, Mo, Ss
Luffa cylindrica	NS/(-)
L. acutangula	GVB, Mo

TABLE 4.1: (CONT'D) **RESPONSE OF DIFFERENT PLANT SPECIES TO MECHANICAL INOCULATION WITH CUCURBITA VIRUS**

TEST PLANT	SYMPTOMS[a]/RESULT OF BACK INDEXING[b]
Lagenaria siceraria	
Calabash `Small var.'	GVB, Mo
`Large var.'	GVB, Mo
`Bitter gourd' `Small var.,	GVB, Mo
`Large var.,	GVB, Mo
`Trumpet gourd'	GVB, Mo
Momordica charánta	NS/(-)[b]
Telfairia occidentalis	NS/(-)
Trichosanthes cucumerina	GVB, Mo
FABACEAE (= LEGUMINOSAE)'	
Arachis hypogaea	NS/(-)
Cajanus cajan	NS/(-)
Canavalia ensiformis	NS/(-)
Glycine max	NS/(-)
Phaseolus vulgaris	
var. `Saxa'	NS/(-)
P. lanatus	NS/(-)
Sesbania sesban	NS/(-)
Vigna mungo	NS/(-)
V. unguiculata	
var. `Ife Brown'	NS/(-)
var. `Mascara'	NS/(-)
var. `K59'	NS/(-)
LAMIACEAE (= LABIATAE)	
Ocimun basilicum	NS/(-)
O. canum	NS/(-)
O. gratissimum	NS/(-)
MALVACEAE	
Abelmuschus esculentus	NS/(-)

TABLE 4.1: (CONT'D) RESPONSE OF DIFFERENT PLANT SPECIES TO MECHANICAL INOCULATION WITH CUCURBITA VIRUS

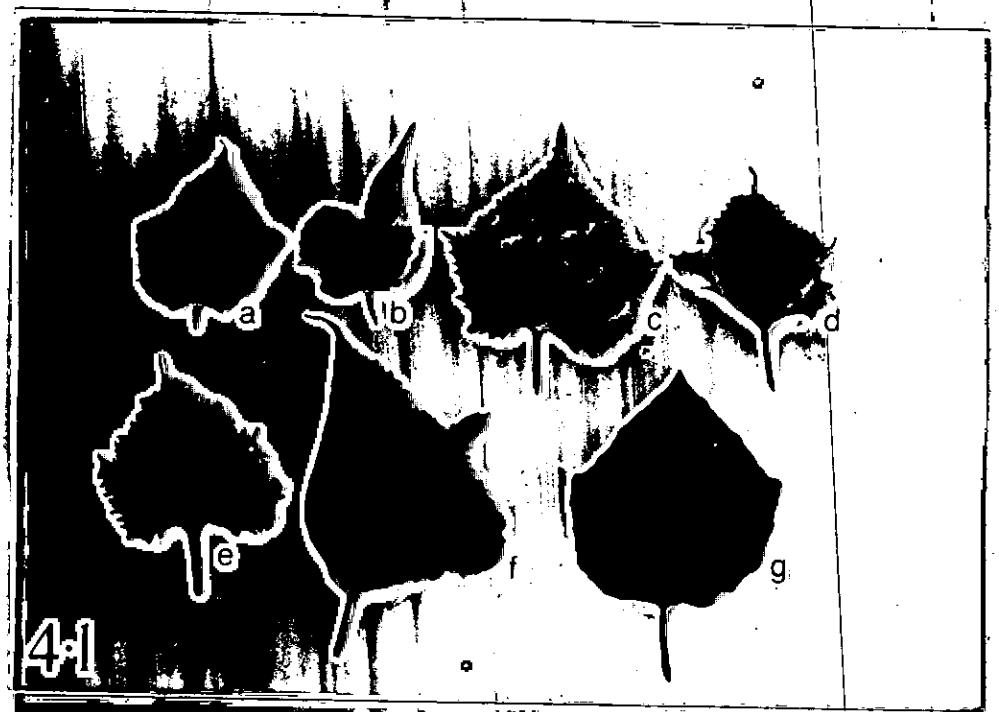
TEST PLANT	SYMPTOMS[a]/RESULT OF BACK INDEXING[b]
POACEAE (= GRAMINAE)	
Zea may	NS/(-)[b]
SOLANACEAE	
Datura metel	NS/(-)
D. stramonium	NS/(-)
Lycopersicum esculentum	NS/(-)
Nicotiana benthamiana	NS/(-)
N. glutinosa	NS/(-)
N. occidentalis	NS/(-)
N. rustica	NS/(-)
N. tabacum	
var. 'White Burley'	NS/(-)
var. 'Samsum'	NS/(-)
Physalis angulata	NS/(-)
P. floridana	NS/(-)
Solanum macrocarpon	NS/(-)
S. melongena	NS/(-)
Capsicum frustescens	NS/(-)
C. annuum	NS/(-)
TILIACEAE	
Corchorus olitorus	NS/(-)

Abbreviations for symptoms[a]: NS = No symptom; GVB = green vein-banding, De = defoliation; VC = vein clearing; St = stunting; lM = leaf malformation; Cl = chlorosis; Mo = mosaic; En = enation; Bl = blistering; Ss = shoestring.

(-)[b] = no infectious virus recovered.

PLATE 4.1: Various reactions of *Cucumis sativus* var. `Poinsett' to Cucurbita virus infection (a -f) typifying progress of disease development in most susceptible cucurbits. Healthy control is shown on the extreme right (g).

PLATE 4.2: Complete defoliation (a) and various forms of leaf malformation (b - e) induced in *Cucurbita pepo* var. `Consul' mechanically inoculated with the Cucurbita virus. Healthy control is labelled f.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b
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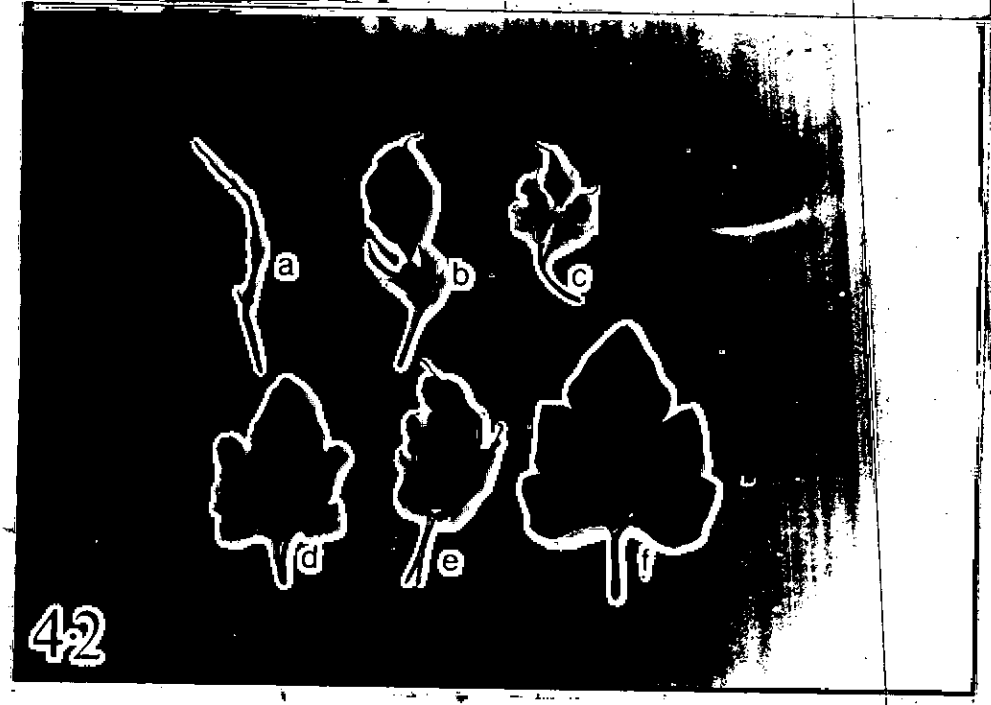
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reduction. In varieties of *Lagenaria siceraria and Citrullus lanatus* symptoms of infection were limited to green vein-banding, mosaic and reduced leaf lamina (Plate 4.3). In *C. moschata* the virus caused leaf malformation, vein clearing and mosaic while in *L. acutangula* symptoms induced were green vein-banding and mosaic. *C. pepo* var. `Encore' showed severe leaf malformation in addition to mosaic and reduced leaf size (Plate 4.4). Generally, infected plants of all susceptible species and varieties were stunted.

4.3.2 SCREENING FOR RESISTANCE

All the seedlings derived from representative samples of all the seed lots of *C. moschata* inoculated with the Cucurbita virus developed symptoms of infection.

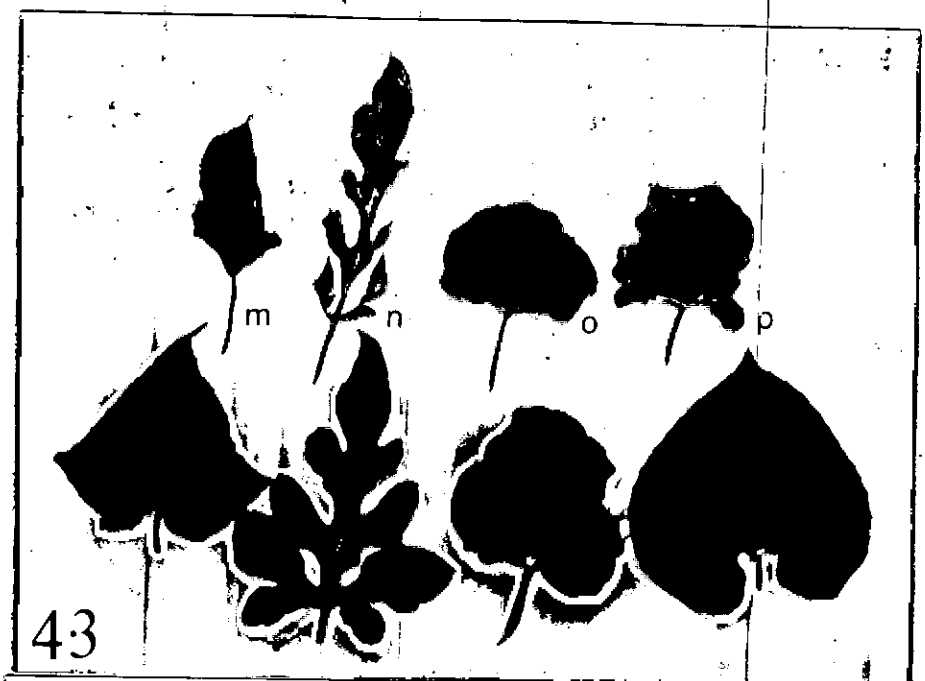
4.3.3 VIRUS RECOVERY FROM FLORAL PARTS, JUVENILE PODS AND SEED TRANSMISSION TEST

In both *C. moschata* and *C. sativus*, infectious virus was recovered from the sepals, petals and anthers of flower buds and fully opened staminate and perfect flowers. No virus was recovered from the juvenile pods of both plants. (Table 4.2).

None of the 537 seeds obtained from infected *C. moschata* plants and screened for seed transmission showed any symptom of infection.

PLATE 4.3: Symptoms induced in some cucurbits mechanically inoculated with the Cucurbita virus: m = *Cucumis sativus*, n = *Citrullus lanatus*, o = *Lagenaria siceraria* (bitter gourd), p = *Cucumeropsis edulis*. Bottom row shows the corresponding leaves from buffer inoculated control plants.

PLATE 4.4: Symptoms induced in some cucurbits mechanically inoculated with the Cucurbita virus: q = *Cucurbita moschata*, r = *Luffa acutangula*, s = *Lagenaria siceraria* (`trumpet gourd'), t = *Cucurbita pepo* var. `Encore'. Bottom row shows the corresponding leaves from buffer inoculated control plants.



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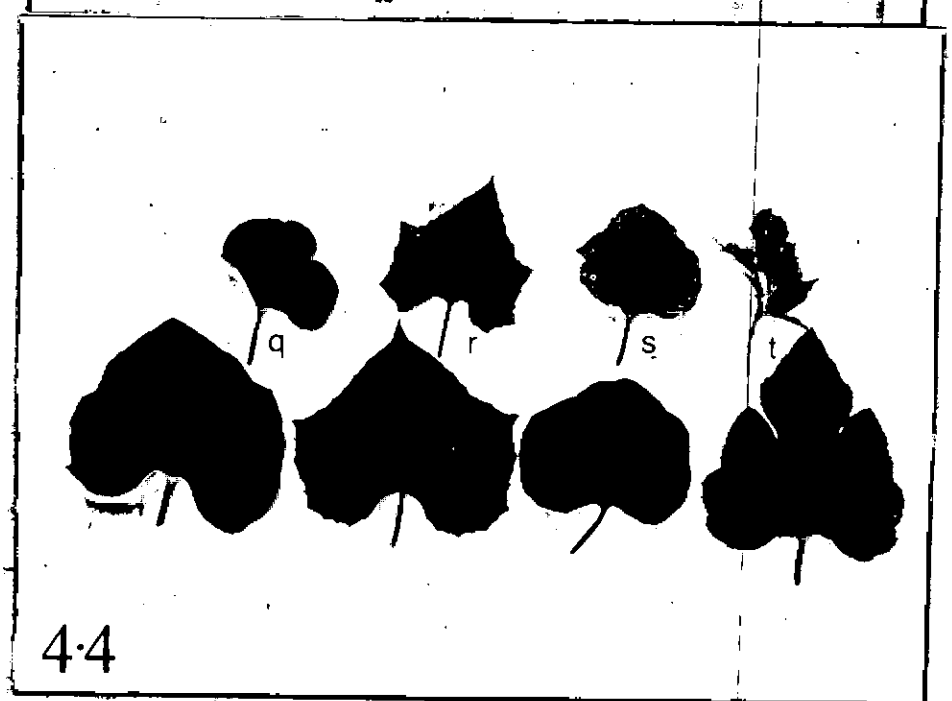

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4.3.4 APHID TRANSMISSION

The Cucurbita virus was transmitted by *A. spiraecola* and *T. citricidus* from *C. moschata* to *C. moschata* and *L. siceraria* in a non-persistent manner. The results of the tests showed that the virus was transmitted by *A. spiraecola* to *L. siceraria* more frequently than to *C. moschata*. In two trials, 15 out of 20 plants of *L. siceraria* were infected compared to 9 of *C. moschata*.

4.3.5 PROPERTIES IN SAP

Infectious sap diluted up to 10^{-4} but not 10^{-5} remained infectious. The virus had a thermal inactivation point between 50-55°C while infectivity was lost when the extract was kept beyond 5 days at room temperature.

4.3.6 SEROLOGY

The Cucurbita virus reacted positively with the potyvirus group monoclonal antibody in I-ELISA. Absorbance values (A_{405}nm) for the virus fell within the range of values recorded for CabMV used as positive control. The absorbance value for the Cucurbita virus range from 0.328 - 0.425 with a mean of 0.365. Cowpea aphid-borne mosaic virus (CabMV) had A_{405}nm value ranging from 0.322 - 0.438 with a mean value of 0.380 while that for healthy plant extract ranged from 0.000 - 0.027.

TABLE 4.2: RECOVERY OF CUCURBITA VIRUS FROM REPRODUCTIVE TISSUES OF *CUCURBITA MOSCHATA* AND *CUCUMIS SATIVUS* VAR. `POINSETT'.

TISSUES TESTED	PLANT SPECIES	
	C. MOSCHATA	*C. SATIVUS*
Perfect flower (flower bud/fully opened)		
Sepals	+[a]	+
Petals	+	+
Anther	+	+
Staminate flower (flower bud/fully opened)		
Sepals	+	+
Petals	+	+
Juvenile pods.	-[b]	-

Key to sign used: +[a] = Virus recovered; -[b] = Virus not recovered.

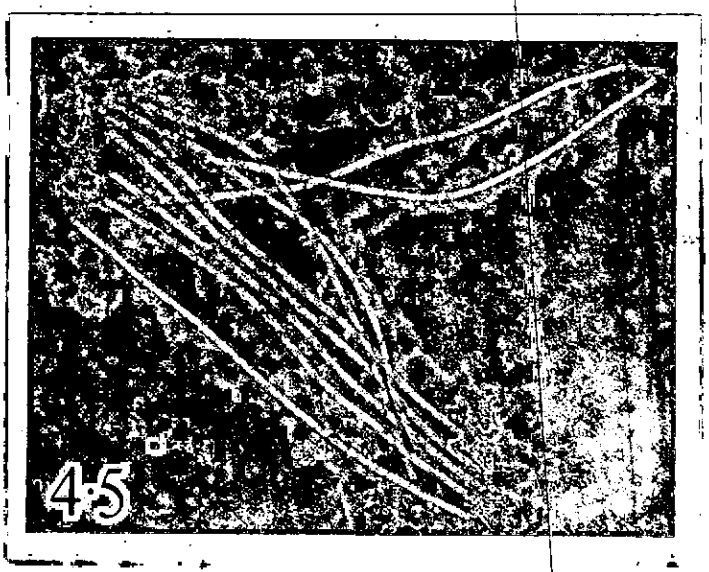
The results of immunodiffusion tests showed that the virus failed to react with any of the antisera to the 22 potyviruses tested. It also did not react with antisera to CMV and SqMV.

4.3.7 ELECTRON MICROSCOPY AND CYTOPATHOLOGY

Crude leaf extracts from infected plants of *C. moschata* using the `leaf dip technique', revealed flexuous rod-shaped particles when viewed under the electron microscope (Plate 4.5). Striate, lamellar inclusions were also detected in crude plant extracts (Plate 4.6). Leaf-dip sections prepared from infected plants showed `pinwheel' inclusion bodies.

PLATE 4.5: Electron micrograph of the Cucurbita virus isolate showing flexuous rod-shaped particles (x 28,500).

PLATE 4.6: Electron migrograph of Cucurbita virus - induced striate lamellar inclusions in crude extracts of infected leaves of *Cucurbita moschata*.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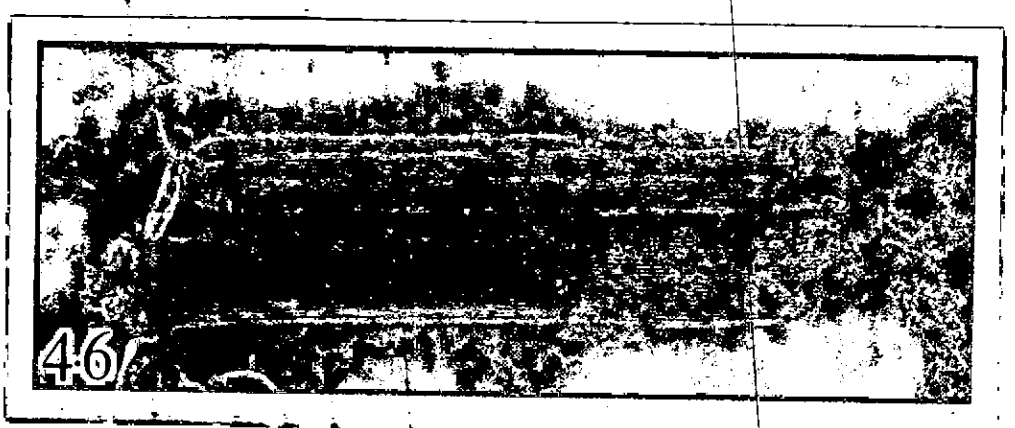
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4.4 DISCUSSION

The most important criteria for assigning viruses into the potyvirus group (PVY-group) include particle length, serological relationship, mode of insect transmission, host range and types of inclusion bodies induced (Edwardson *et al*, 1972; Purcifull and Hiebert, 1979; Shoyinka *et al.*, 1987; Ladipo *et al.*, 1988b).

The results for the host range, insect transmission, electron microscopy, type of inclusion bodies and reaction with the universal potyvirus group monoclonal antibody obtained in this study indicate that the Cucurbita virus isolate is a potyvirus.

The virus had a rather narrow host range restricted to the family Cucurbitaceae. Members of the potyvirus group are known to infect plants in diverse families (Edwardson, 1974) while individual members may have restricted host range (Charudattan *et al.*, 1980; Kaiser *et al.*, 1988).

The Cucurbita virus was transmitted by *A. spiraecola* and *T. citricidus* in a non-persistent manner characteristic of the potyviruses. Edwardson (1974) listed 60 positively assigned, 23 tentatively assigned and 15 strains of the PVY-group out of which 72 were reported to be aphid transmitted in a non-persistent manner. No information was provided concerning insect transmission on quite a number of the remaining 26 viruses. Since 1974, the number of aphid transmitted viruses tentatively assigned to the PVY-

group has increased (Charudattan *et al.*, 1980; Shoyinka *et al.*, 1987; Screenivasulu and Demski, 1988; Bosque-Perez and Buddenhagen, 1990).

No evidence of virus transmission through the seeds of infected *C. moschata* was obtained. This is not unexpected since virus particles were recovered from the floral parts but not from juvenile pods. The failure of the virus to be seed-borne agrees with the reports of other workers on seed transmission of potyviruses (Nameth *et al.*, 1985; Bays and Demski, 1986; Warwick and Demski, 1986; Fauquet and Thouvenel, 1987; Gleason and Provvident, 1990).

The Cucurbita virus reacted positively with the universal potyvirus monoclonal antibody in I-ELISA. This result unequivocally places the virus in the potyvirus group. This is further supported by the flexuous rod-shaped particles of the virus observed under the electron microscope and the induction of 'pinwheel' and striated lamellar inclusion bodies characteristic of potyviruses (Edwardson, 1974; Purcifull *et al.*, 1975; Shoyinka *et al.*, 1987; Ladipo *et al.*, 1988b). The lack of positive serological reaction between the Cucurbita virus and 22 other potyvirus might either be due to lack of serological relationship between them or lack of reactivity of the antisera.

To date, an isolate of WMV considered to be a strain of WMV-2 but serologically distinct from it (Igwegbe,

1983a) and TeMV (Nwanzo and Brown, 1975; Shoyinka *et al.*, 1987) are the two viruses that have been reported on cucurbits in Nigeria. The Cucurbita virus differs from the WMV strain described by Igwegbe (1983a) on the basis of host range. The WMV isolate infected several plants in both Chenopodiaceae and Cucurbitaceae families whereas the Cucurbita virus infected only Cucurbitaceous plants. In addition, *Colocynthis citrullus*, *Cucurbita pepo* and *L. acutangula* which were immune to the WMV isolate were susceptible to the Cucurbita virus. *L. acutangula* has been considered a good differential host plant for distinguishing between WMV-1 and WMV-2 isolates. Watermelon mosaic virus 1 isolates infect the plant whereas WMV-2 isolates do not (Webb, 1965; Webb and Scott, 1965; Purcifull and Hiebert, 1979). On the basis of this, the Cucurbita virus is distinct from WMV-2 but similar to WMV-1. The virus is also different from TeMV. Whereas TeMV infected 29 of 34 plant species from six families, the Cucurbita virus beside its narrow host range, did not infect *T. occidentalis*, the natural host of TeMV. In addition, the Cucurbita virus failed to react with antiserum to TeMV. Similarly, the cucurbita virus is quite distinct from other potyviruses such as AMV (Taiwo *et al.*, 1988) eggplant severe mottle virus (ESMV) (Ladipo *et al.* 1988b and pepper veinal mottle virus (PVMV) (Igwegbe and Waterworth, 1982) previously reported from Nigeria. Unlike

the Cucurbita virus, ESMV infects several solanaceous plants such as *D. stramomium*, *N. benthamiana*, *P. floridana* and *Solanum melongena*. Both AMV and PVMV infected test plants which were immune to the Cucurbita virus. Besides the virus did not react with antiserum to PVMV.

Two cucurbit viruses, Cucurbita mosaic virus and WMV-1 reported from Cote D' Ivoire (Ivory Coast) (Fauquet and Thouvenel, 1987) share some properties with the Cucurbita virus. Both viruses infect only cucurbits, they are transmitted non-persistently by aphids and they are not seed-borne. The Cote D' Ivoire Cucurbita virus, like the Nigerian Cucurbita virus, showed no serological relationship with WMV-1 and WMV-2. The failure of the Nigerian Cucurbita virus to react with antiserum to WMV-1 indicates that they are probably not the same virus. Unfortunately, the antiserum to the Cote D' Ivoire Cucurbita virus was not available for serological testing.

So far, this is the first report of a virus naturally infecting *C. moschata* in Nigeria. Until the results of further test prove otherwise, the name Cucurbita mosaic virus (CuMV) is proposed for the virus because of the similarity between it and the virus reported by Fauquet and Thouvenel (1987) in Cote D' Ivoire.

CHAPTER FIVE

COMPARATIVE STUDY ON THE TRANSMISSION OF CUCURBITA MOSAIC VIRUS BY *APHIS SPIRAECOLA* PACH. TO TWO OF ITS NATURAL HOSTS.

ABSTRACT

A comparative study on the transmission of Cucurbita mosaic virus (CuMV) by *Aphis spiraecola* from *Cucurbita moschata* to *C. moschata* and *Lagenaria siceraria* was carried out. The rate of transmission of the virus to both cucurbit plant species was evaluated using varying number of aphids, acquisition and inoculation access feeding periods and post-acquisition starvation periods.

The rate of transmission of the virus was consistently higher when *L. siceraria* was used as the test plant compared to *C. moschata*.

Epidermal strip preparations of both *C. moschata* and *L. siceraria* showed that the leaf of the latter was glabrous while the former had a dense covering of hairs or trichomes on both the adaxial and abaxial surfaces.

The difference in the leaf surfaces, the presence or absence of hairs, was probably responsible for the observed differential rate of transmission to both plant species since the virus source plant, age of test plant at time of inoculation, the environmental condition and aphid species were kept constant.

5.1 INTRODUCTION

Transmission of potyviruses from plant to plant in nature is accomplished by specific vectors which are aphids for all but a few putative potyviruses (Atrega *et al.*, 1990). Aphids constitute the largest group of plant vectors (Watson, 1972) and have been implicated in the transmission of over 160 different viruses (Green, 1971).

Non-persistent transmission by aphids is one characteristic that is common to all potyviruses. In this mode of transmission, the virus is acquired and transmitted within a few seconds or minutes without any latent period. Transmission is enhanced if the insects are allowed preacquisition starvation while longer acquisition access feeding reduces transmission efficiency (Pirone and Harris, 1977).

A number of factors affect the efficiency with which a particular virus is transmitted by its vector. These factors include the virus strains or isolates (Ogungbenro and Ladipo, 1987; Yokomi *et al.*, 1989; Banik and Zitter, 1990), the species, varieties and conditions of virus source plant, the test and host plants on which the aphids were reared (Mackinnon, 1961), aphid seasonal forms, growth stages and clones (Orlob and Arny, 1960; Rochow and Eastop, 1966; Pirone and Harris, 1977), aphid biotypes (Berger and Toler, 1983), differences in environmental conditions prior

to or during transmission experiments (Gabriel, 1965; Simons, 1966), and the presence of helper components (HC) (Lung and Pirone, 1975; Pirone and Thornbury, 1984; Hunt *et al.*, 1988).

The presence of hairs on the leaf surfaces of some plants has been implicated for their resistance to aphid infestation. In several species of *Nicotiana*, alkaloid exudates from glandular hairs were found to be toxic to aphids (Thurston *et al.*, 1966). Resistance of tomato to *Myzus persicae* (Mckinney, 1938) and *Aphis craccivora* (Johnson, 1956), *Solanum pennelli* to the potato aphid (Gentile and Stoner, 1968), *S. polyadenium*, *S. tarijense* and *S. berthautii* to *M. persicae* and *Macrosiphon euphobiae* (Gibson, 1971) have been attributed to the mechanical gumming effect of an exudate produced by the glandular hairs on the leaf surfaces. The exudates completely immobilized the insects and death ensued as a result of starvation. The inability of *M. persicae* and some species of thrips to infest stringbean was attributed to the presence of strong and curved spines on the leaf surfaces which pierced through the insect upon making contact with them (Mckinney, 1938). Tanne and Zimmerman-Gries (1980) found that the transmission rate of cucumber mosaic virus (CMV) by *Aphis gossypii* and *M. persicae* to eggplant (*S. melongena*) was comparatively low in comparison to pepper

and tomato because of the hairiness of the leaves of *S. melongena*.

Aphis spiraecola is ubiquitous and it is found wherever the Siam weed, *Chromolaena odorata* grows. The aphid has been reported to transmit a number of viruses including pepper veinal mottle virus (PVMV), groundnut chlorotic rosette virus (GCRV), groundnut chlorotic spoting virus (GCSV), passiflora ring-spot virus (PRSV) and Voandzeia distortion mosaic virus (VDMV) (Fauquet and Thouvenel, 1987).

In preliminary aphid transmission tests (Chapter 4) the Cucurbita mosaic virus (CuMV) was transmitted to both *C. moschata* and *L. siceraria* by *A. spiraecola* in a non-persistent manner. Higher transmission rates were observed when *L. siceraria* was used as the test plant compared to when *C. moschata* was used. A visual examination of the leaves of both plant species showed that *C. moschata* had a dense covering of hairs on both the abaxial and adaxial surfaces while *L. siceraria* had glabrous leaf surfaces.

The focus of this study was to evaluate the probable role of hairs (trichomes) for the observed difference in the transmission of CuMV by *A. spiraecola* to *C. moschata* and *L. siceraria*.

5.2 MATERIALS AND METHODS

5.2.1 RAISING AND REARING OF APHID CULTURE

The aphid species used for this study, *A. spiraecola*, was obtained from *Chromolaena odorata* (=*Eupatorium odoratum*). A few apterous adults and nymphs were dislodged from the host plant by breathing heavily on them (Watson, 1972). They were then picked and transferred onto young healthy seedings of *L. siceraria* using a moistened brush.

Rearing was accomplished by periodic transfer of the insects to *L. siceraria* in insect screen cages in the greenhouse with temperature ranging between 28 - 33°C.

5.2.2 MAINTENANCE OF VIRUS

The virus was maintained in *C. moschata*. For each test, non-viruliferous aphids were fed on leaves from the stock plants that had been infected for 2-3 weeks.

5.2.3 PREPARATION OF EPIDERMAL STRIPS OF LEAVES OF *CUCURBITA MOSCHATA* AND *LAGENARIA SICERARIA*.

An area of about one centimetre square was cut from a standard central position of the leaf samples and decolourized by soaking in 70% sodium hypochlorite (NaOCl) for 6 hr. The epidermal strips were carefully teased out and washed in water to which three drops of acetic acid were added to neutralize the action of the bleach. The strips were thoroughly washed again and transferred to 50% ethanol for 2 min to harden the cells. They were then

stained in 1% safranin (in 50% ethanol) for 5 mins and later dehydrated by passing through 50%, 60%, 70% 80%, 90% and absolute ethanol series for 5 min each. The strips were cleared in xylene for 2 min, mounted in glycerine on glass slide, covered with cover slips before they were sealed up with nail vanish. The specimens were viewed and photographed under the Reichert Microstar IV light microscope.

5.2.4 TRANSMISSION STUDIES

The insects were starved for 3 hr in sample bottles covered by fine mesh gauze held tightly over the bottles by their plastic lids whose central parts had been neatly removed. After starvation the insects were transferred with a moistened brush unto excised symptomatic leaves of *C. moschata*, floated on water in Petri dishes. Thereafter, the viruliferous insects were transferred to the test plants for inoculation feeding for a specific length of time depending on the test. Nine-day old seedlings of both test plants were used for all the experiments. Inoculation feeding was terminated by spraying the test plants with Actellic 50 EC (10ml/l). The plants were kept in the greenhouse with temperature between 28-33°C and observed for symptom development for 4 weeks.

Each test was conducted three times and 5 plants were used during each test. *C. moschata* and *L. siceraria* were

used as test plants for each test. The insect culture was tested periodically to ensure they were virus-free.

5.2.5 EFFECT OF APHID NUMBER ON TRANSMISSION OF CUCURBITA MOSAIC VIRUS

After 10 min of acquisition feeding, groups of 1,3,5,7 and 9 insects were transferred to 5 plants of both test plants. They were allowed 10 min inoculation feeding after which they were killed.

5.2.6 EFFECT OF VARYING ACQUISITION/INOCULATION ACCESS FEEDING PERIODS ON THE TRANSMISSION OF CUCURBITA MOSAIC VIRUS.

For these tests, the insects were allowed acquisition/inoculation feeding of 1,5,15 and 180 min. Nine insects were employed on each test plant. When aphid feeding was to be timed for one minute, each insect was observed individually. For longer periods (5,15 and 180mins) a small group was placed on the source plant leaf and timing was commenced when majority had started to feed (indicated by when they remained motionless). Non-feeding insects were carefully removed using moistened brush.

5.2.7 EFFECT OF POSTACQUISITION STARVATION ON TRANSMISSION OF CUCURBITA MOSAIC VIRUS

For each of these series of tests between 50-60 insects were transferred into 5 Petri dishes for post acquisition times of 5,10,15,20 and 25 min after 10 min

acquisition feeding. Batches of 9 insects were then transferred onto 5 test plants for a 10 min inoculation feeding.

5.3 RESULTS

5.3.1 EPIDERMAL STRIPS OF *CUCURBITA MOSCHATA* AND *LAGENARIA SICERARIA*

Epidermal preparations of the leaf of *C. moschata* viewed under the Reichert Microstar IV light microscope (Plate 5.1 a & b) showed numerous long and pointed multiseriate hairs on both the abaxial and adaxial surfaces. Those of *L. siceraria* (Plate 5.2 a & b) had very few and scattered hairs.

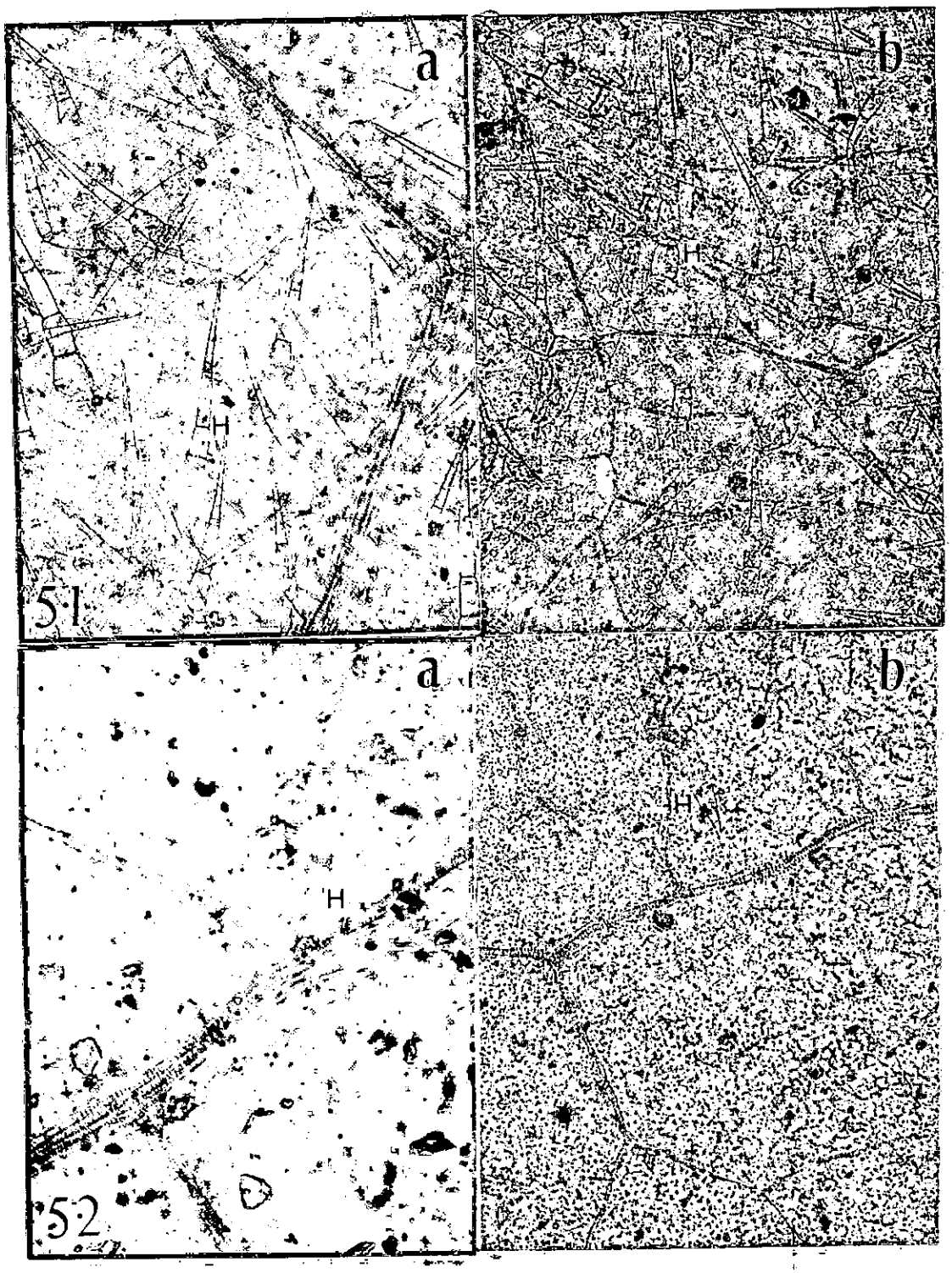
5.3.2 EFFECT OF APHID NUMBER ON TRANSMISSION OF CUCURBITA MOSAIC VIRUS

The percentage transmission of CuMV to both *C. moschata* and *L. siceraria* increased with increasing number of aphids used for virus transmission (Table 5.1). The results also showed that a single aphid was sufficient to effect the transmission of CuMV to both plant species. Generally, the rate of transmission to *L. siceraria* was higher than when *C. moschata* was used as the test plant.

When a single aphid was used per plant 2 out of 15 plants of *C. moschata* were infected representing 13.3% transmission compared to 3 out of 15 for *L. siceraria* which corresponds to 20% transmission. Again, employing 5 and 9 insects per plant, the transmission rates to *C. moschata* were 46.7 and 60% respectively while those of *L. siceraria* were 53.3 and 86.7%.

Plate 5.1: Epidermal strips from the upper (adaxial) (a) and lower (abaxial) (a) leaf surfaces of *Cucurbita moschata* showing numerous hairs (marked H) (x 100).

Plate 5.2: Epidermal strips for the upper (a) and lower (b) leaf surfaces of *Lageneria siceraria* showing few hairs (marked H) (x 100).


a
b
H
5·1
a
b
H
H
5·2

Analysis of the data using Student's t-test showed no significant difference (P=0.05) between percentage transmission when 1-5 insects were used per plant. A significant difference was however found between percentage transmission using 7 and 9 insects per plant for both plant species (Table 5.1)

The transmission values recorded when smaller numbers of aphids (3-5) were used to inoculate *C. moschata* were much the same as the theoretical (expected) values calculated on the basis of transmission by a single aphid. As the number of aphids per plant was increased (7-9) the difference between the observed and the theoretical values widened, though statistically insignificant (Fig. 5.1). Conversely, when *L. siceraria* was used as the test plant and employing 3 and 5 insects per plant observed values differed significantly from the theoretical values. As the number of aphid per plant was increased the observed value gradually approached the theoretical value (Fig. 5.2).

5.3.3 EFFECT OF VARYING ACQUISITION/INOCULATION ACCESS FEEDING ON TRANSMISSION OF CUCURBITA MOSAIC VIRUS

The results of the transmission of CuMV using varying acquisition/inoculation access feeding periods are presented in table 5.2. The results showed that as little as 1 min was required by *A. spiraecola* for the acquisition and transmission of CuMV to both test plants. It was also

TABLE 5.1: EFFECT OF APHID NUMBER ON THE TRANSMISSION OF CUCURBITA MOSAIC VIRUS TO *CUCURBITA MOSCHATA* AND *LAGENARIA SICERARIA* BY *APHIS SPIRAECOLA*.

	C. moschata		*L. siceraria*	
Aphid(s)/ plant	Number infected[a]	Percentage infection	Number infected	Percentage infection
1	2	13.3a	3	20.0a
3	5	33.3a	5	33.3a
5	7	46.7a	8	53.3a
7	8	53.3a	11	73.3b
9	9	60.0a	13	86.7b

[a] Number infected out of a total of 15 inoculated test plants. Pair of values followed by the same letter along the same row are not significantly different (P=0.05) using Student's t-test.

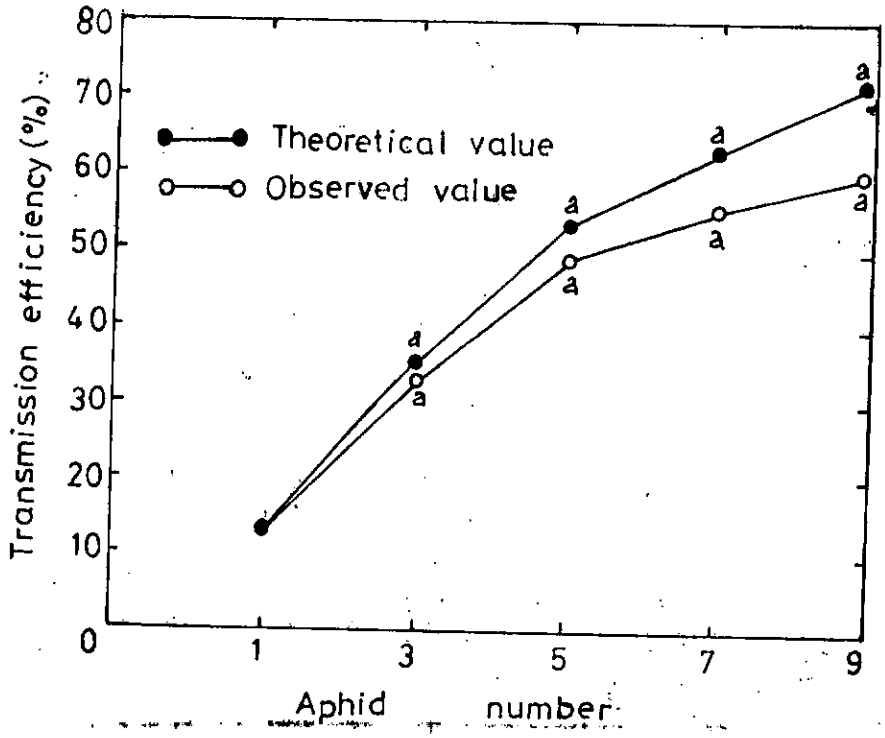


Fig. 5.1 Effect of aphid number on the transmission of Cucurbita mosaic virus to Cucurbita moschata by Aphis spiraecola. Solid dots represent theoretical values determined by the formula $N = 1-(1-Y)^n$, where : N = percentage transmission, Y = percentage transmission for 1 aphid/plant (13.3%), n = number of aphids (Jensen, 1985). Points followed by the same letter are not significantly different (P = 0.05) using Student's t - test.

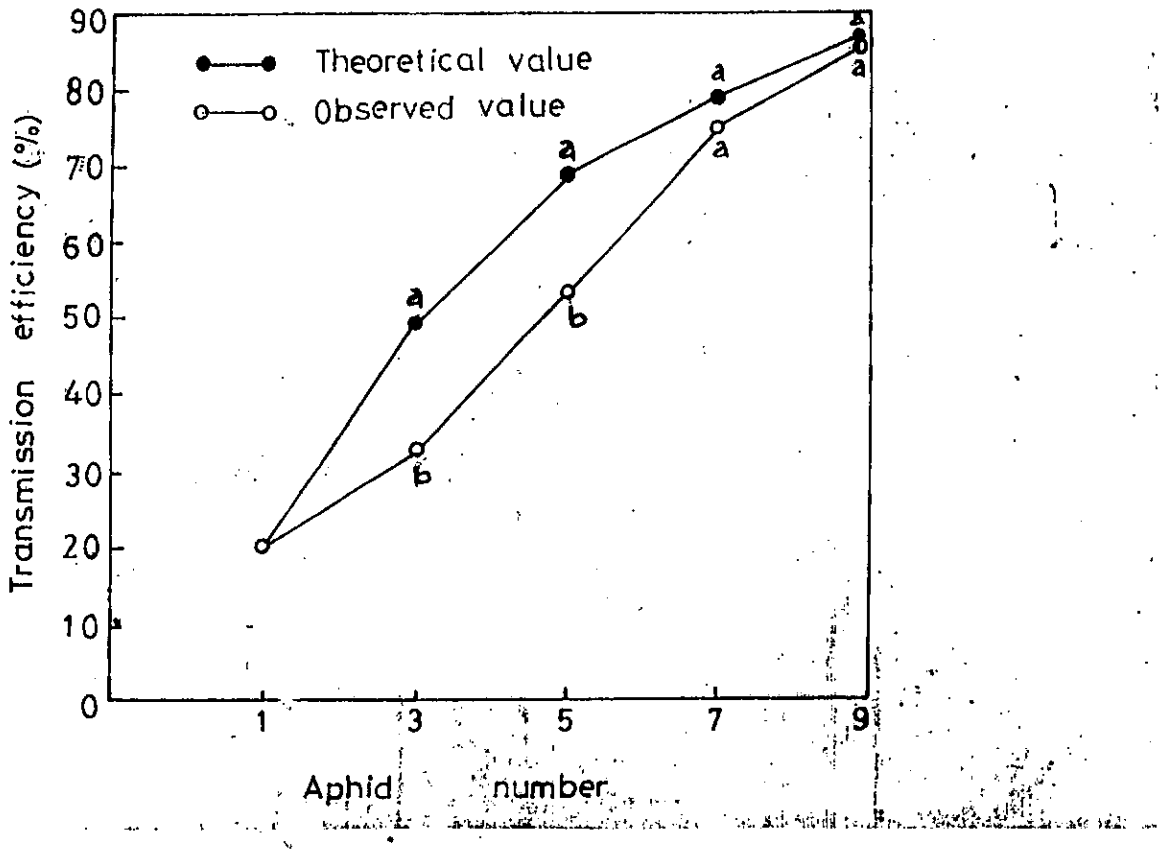


Fig. 5.2 Effect of aphid number on the transmission of Cucurbita mosaic virus to Cucurbita moschata by Aphis spiraecola. Solid dots represent theoretical values determined by the formula $N = 1-(1-Y)^n$ where : N = percentage transmission, Y = percentage transmission for 1 aphid / plant (20.0 %) n = number of aphids (Jensen, 1985) Points followed by the same letter are not significantly different (P = 0.05) using Student's t - test.

observed that transmission rates were higher when *L. siceraria* was used as test plant than when *C. moschata* was employed for all acquisition/inoculation access feeding periods tested.

The highest transmission rates of 66.7 and 86.7% were recorded at 1 min acquisition/inoculation access feeding time for *C. moschata* and *L. siceraria* respectively. Transmission efficiency decreased as acquisition/ inoculation access feeding period was increased. The least percentage transmission rates of 13.3 and 20.0 were recorded for 180 min acquisition/inoculation access feeding period using *C. moschata* and *L. siceraria* as test plants respectively (Table 5.2).

The percentage transmission of CuMV by *A. spiraecola* to *L. siceraria* differed significantly from the values obtained when *C. moschata* was used as the test plant at 1/1, 5/5 and 15/15 min acquisition/inoculation feeding periods. Values obtained for both test plants at 180/180 min were not significantly different.

TABLE 5.2: EFFECT OF VARYING ACQUISITION/INOCULATION ACCESS PERIODS ON THE TRANSMISSION OF CUCURBITA MOSAIC VIRUS TO *CUCURBITA MOSCHATA* AND *LAGENARIA SICERARIA* BY *APHIS SPIRAECOLA*

Acquisition/ inoculation time (min)	*C. moschata*		*L. siceraria*	
	Number infected[a]	Percentage infection	Number infected	Percentage infection
1/1	10	66.7a	13	86.7b
5/5	9	60.0a	12	80.0b
15/15	7	53.3a	11	73.3b
180/180	2	13.3a	3	20.0a

[a] Number infected out of a total of 15 inoculated test plants.
Pair of values followed by the same letter along the same row are not significantly different (P=0.05) using Student's t-test.

5.3.4 EFFECT OF POSTACQUISITION STARVATION ON TRANSMISSION OF CUCURBITA MOSAIC VIRUS

The results presented in table 5.3 showed that the transmission of CuMV by *A. spiraecola* was highest when postacquisition starvation time was shortest. The highest transmission of 60 and 73.3% were recorded for *C. moschata* and *L. siceraria* respectively after 5 min postacquisition starvation. Transmission rates decreased progressively as postacquisition starvation period was prolonged. As low as 6.7 and 13.3% were observed for *C. moschata* and *L. siceraria* respectively after 25 min postacquisition starvation.

The differences in the rate of transmission were however not significant except at 15 min postacquisition starvation (Table 5.3).

TABLE 5.3: EFFECT OF POST ACQUISITION STARVATION ON THE TRANSMISSION OF CUCURBITA MOSAIC VIRUS BY *APHIS SPIRAECOLA* TO *CUCURBITA MOSCHATA* AND *LAGENARIA SICERARIA*

Post-acquisition Starvation (min)	*C. moschata*		*L. siceraria*	
	Number infected[a]	Percentage infection	Number infected	Percentage infection
5	9	60.0a	11	73.3a
10	8	53.3a	10	66.7a
15	4	26.7a	5	33.3b
20	2	13.3a	3	20.0a
25	1	6.7a	2	13.3a

[a] Number infected out of a total of 15 inoculated test plants. Pair of values followed by the same letter along the same row are not significantly different (P=0.05) using Student's t-test.

5.4 DISCUSSION

The transmission of potyviruses by their aphid vectors is accomplished after a short probe and without a latent period (Green, 1971). The results of this study indicate that the relationship between CuMV and *A. spiraecola* is similar to those of other potyviruses and their vectors.

The results indicate that only one insect was required to effect the transmission of CuMV to *C. moschata* and *L. siceraria*. There was a correlation between aphid numbers and frequency of successful transmission of the virus. Higher number of insects per plant resulted in higher percentage transmission. Earlier investigation involving different virus-vector relationships produced similar results. Ogungbenro and Ladipo (1987) reported higher rates of transmission of both tomato streak and tomato mosaic strains of pepper veinal mottle virus (PVMV-S and PVMV-M) by *Myzus persicae* and *Aphis craccivora* when larger numbers of the insects were used than when few insects were used. Similarly, Nault *et al.* (1971) observed higher transmission rates of maize dwarf mosaic virus (MDMV) by *Shizaphis graminum* when many insects were used compared to using few insects. This result is due to the higher probability of virus uptake and transmission by several insects than a single insect.

In this study, the transmission rates of CuMV to *C. moschata* by *A. spiraecola* were found to be below the

theoretical values. On the other hand, with *L. siceraria* the actual percentage transmission values gradually approached the theoretical values with increasing number of insects used per plant. Nault *et al.* (1971) and Jensen (1985) maintained that when there is no interaction between insects on a plant, the number of plants that become infected should be a function of percentage transmission by one insect and the number of insect per plant. According to this proposition the increasing disparity between the actual and the theoretical values when *C. moschata* was used as test plant could be attributed to interaction between the insects. This explanation will only be plausible when there is a higher population build up of the insect on the plant as is often the case under field conditions rather than under greenhouse condition. The reported differential between the observed and theoretical values must be due to some factors other than insect interaction on the plant.

The result of varying the acquisition/inoculation access period is comparable with the non-persistent mode of transmission. As little as 1 min was adequate for acquisition and transmission of CuMV to both plant species.

Longer acquisition periods drastically reduced the transmission efficiency of the virus by the aphid vector. The result also showed that there was a higher rate of transmission of CuMV by *A. spiraecola* to *L. siceraria* than when *C. moschata* was used as the test plant for every

acquisition/inoculation access period tested. The report of several studies have shown that stylet-borne viruses are acquired optimally in brief probes of 10-60 seconds and that there is a decrease in the proportion of aphids acquiring virus with longer acquisition periods (Swenson, 1968; Ogungbenro and Ladipo, 1987).

In this study, increasing the time between aphid acquisition of CuMV and their transfer to either of the test plants resulted in reduced transmission rates. Higher rate of transmission were however recorded when *L. siceraria* was used as the test plant in comparison with *C. moschata*. This result confirms the brevity of virus retention by aphids that transmit stylet-borne viruses (Pirone and Harris, 1977; Berger *et al.*, 1987; Zeyen and Berger, 1990).

Plant trichomes have been reported to be important morphological defenses against pests. Insects have been reported to be more restless on pubescent leaves than glabrous ones (Roberts *et al.*, 1979). Normal movement and probing behaviour were altered (Roberts and Foster, 1983), and feeding by instars were precluded (Dixon, 1985). Exudates from glandular hairs on some Solanaceous plant species have been reported to prevent their infestation by aphids because of their toxicity (Thurston *et al.*, 1966) or because they immobilize them (Gentile and Stoner, 1968; Gibson, 1971). The sharp hairs on stringbean killed off

insects landing on the leaf surfaces by piercing through their body tissues (Mckinney, 1938). The comparatively low transmission rate of cucumber mosaic virus (CMV) by *M. persicae* and *Aphis gossypii* to eggplant, *S. melongena* compared to pepper and tomato was attributed to the hairiness of the leaves of *S. melongena* (Tanne and Zimmerman-Gries, 1980). However, Carter *et al.* (1988) could not implicate trichomes as being responsible for the difference in the resistance of `San Pedro' and `Pls' varieties of alfalfa to spotted alfalfa aphid, *Therioaphis maculata* because both varieties had equal number of trichomes on their leaf surfaces.

C. moschata does not possess glandular hairs nor are the hairs prickly. The consistently lower transmission of CuMV by *A. spiraecola* to *C. moschata* compared to *L. siceraria* may be due to the presence of hair on *C. moschata* which probably prevented the aphids from gripping the leaf surfaces and sucking efficiently within a very short time since other factors such as the virus source plant, age of test plant at time of inoculation, environmental condition and the aphid species which might bring about variation in transmission rate were kept constant.

The development of varieties of *C. moschata* with very dense covering of hairs may offer protection against infection by CuMV by preventing infestation of the crop by its aphid vector.

CHAPTER SIX

EFFECT OF INOCULATION WITH CUCURBITA MOSAIC VIRUS ON THE GROWTH AND YIELD OF FIELD-GROWN *CUCURBITA MOSCHATA*

ABSTRACT

The effect of Cucurbita mosaic virus (CuMV) on *Cucurbita moschata* at three growth stages was investigated using randomized complete block design. Foliar symptoms were more severe and were expressed faster in plants inoculated at the first true leaf stage than in those inoculated at the vegetative and first perfect flower stages. Virus inoculation had little or no effect on the number of both staminate and perfect flowers produced, irrespective of time of inoculation.

Generally, fruits from virus inoculated plants were smaller and fewer but were not significantly different from those of the control. The mean fruit weights from such plants were however significantly different from those of the control regardless of the stage of plant at time of inoculation. Inoculations performed at the first true leaf and vegetative stages resulted in the production of unmarketable fruits, while a 74.4% loss was recorded when inoculated at the first perfect flower stage.

6.1 INTRODUCTION

The pumpkins (*Cucurbita* spp.) are widely grown in mixed cultivation in Southern Nigeria for their leaves and fruits (Okigbo, 1978). The leaves are used as vegetables while the immature unripe fruits are eaten raw or occasionally cooked (Dupriez and De Leener, 1989). The most commonly grown species of *Cucurbita* in the tropics is *C. moschata* (Tindall, 1989) while other species of economic importance include *C. maxima* and *C. pepo*.

Viruses constitute a major constraint to cucurbit production world wide (Makkout and Lesemann, 1980). At least 25 viruses have been isolated from cucurbits; some of which are known to cause significant economic losses (Fischer and Lockhart, 1974; Purcifull *et al.*, 1984; Nameth *et al.*, 1986). Agrios 1989 attributed a loss of 8.5% in cucurbit production in the United States to viruses while unfavourable weather conditions, nematodes and fungi accounted for 21.2%.

Cucurbita mosaic virus (CuMV) has been implicated as the causal agent of a mosaic disease of *C. moschata* cultivated on a commercial vegetable farm in Lagos (Chap.4). Beside mosaic, infected leaves showed green vein-banding and malformation. Under greenhouse conditions infected plants showed a delay in stem `running' and no sources of resistance were identified in seed lots of *C.*

moschata collected from different locations within the Southern belt of Nigeria.

Some reports concerning quantitative estimation of virus induced losses in cucurbits have been documented. Thomas (1971) reported yield losses of 63 and 53% in `Butter Cup' and `Golden' Hubband' squash (*C. maxima*) respectively when both were inoculated with watermelon mosaic virus (WMV) at early stages of growth. Fruits from such plants were distorted and unmarketable. No yield reduction was observed with mid-session inoculation. Demski and Chalkey (1972) recorded yield losses of 43, 28 and 9% from early, mid-term and late inoculations respectively in summer squash (*C. pepo*) inoculated with WMV. Early and mid-term inoculations caused nearly 100% loss in marketability compared to 7% for late inoculated plants. Demski and Chalkey (1974) also recorded yield losses in three varieties of watermelon (`Charleson Gray',`Garrisonian' and Florida Giant') inoculated with WMV at first true leaf stage (early inoculation), when the vine had started to run and when the first fruits were visible (late infection). Yield losses varied from 73% for early inoculation to 19% for late infection. Thirty nine percent (39%) of the infected `Charleson Gray' and 43% of infected `Garrisonian' fruits were malformed as compared to 2 and 11% of fruits from healthy plants of the two varieties respectively. Similarly, Blua and Perring (1989) recorded 96 and 76% reductions in marketable fruits in Cantaloupe

(*Cucumis melo*) inoculated with zucchini yellow mosaic virus (ZYMV) during the vegetative and early flower stages respectively. Plants inoculated after fruit set and healthy control plants produced comparable number of marketable fruits.

A pilot study conducted in 1990 (data not shown) revealed that infected plants of *C. moschata* produced relatively smaller fruits compared to those from healthy plants. Since no resistant varieties were identified among the seed lots screened for resistance (Chapter 4) a knowledge of when the crop can be protected against infection by CuMV to minimize yield loss may be useful.

This study was therefore designed to evaluate the effect of CuMV inoculation on the growth and yield of *C. moschata* at different developmental stages.

6.2 MATERIALS AND METHODS

6.2.1 SOURCE OF SEEDS

Seeds of *C. moschata* used for this study were obtained from `Oyingbo' market in Lagos. After drying in the sun for two days, about 15 seeds were sown in plastic pots in the greenhouse and the resultant seedlings were inoculated to ascertain susceptibility to the virus (CuMV).

6.2.2 PRE-PLANTING AND PLANTING OPERATIONS

A piece of land measuring 15.2m^2 located beside the Biological Garden of the University of Lagos was used for the study. The land was cleared, tilled and properly marked out. Organic manure was added to the marked spots (planting sites for seeds) which were watered daily for about a week prior to planting.

On the 16th of March 1991, the seeds were planted at the rate of three seeds per stand. The seedlings, which were later thinned to one, two weeks after planting, were watered regularly until the onset of the rainy season.

6.2.3 EXPERIMENTAL DESIGN

The randomized complete block design was used. There were three replications (blocks) and each consisted of three plots. Each plot contained 6 plants arranged in three rows of two. The distance between two successive plots and between two replications was 2.5m while the spacing between plants within the plot was 1.6 by 0.8m.

The growth stages at which inoculations were performed constituted the treatments. Inoculations were performed at the first true leaf stage, 9 days after planting (treatment 1), at the vegetative stage, about 5 weeks after planting (treatment 2) and at the first perfect flower stage, about 8 weeks after planting (treatment 3). Buffer inoculated plants served as treatment 4.

The treatments were randomized within each replication and among the plants within each plot as previously described (Chap. 3). As plants began to 'run', their tips were guided to prevent the entanglement of inoculated plants with buffer inoculated controls.

6.2.4 INOCULATION PROCEDURE

Inoculum prepared by grinding CuMV infected leaf tissues of *Cucumis sativus* (maintained in the greenhouse) in 0.03M phosphate buffer pH 8.0 was rubbed onto carborundum dusted cotyledonary leaves in treatment 1 and the two youngest leaves in treatments 2 and 3. The inoculated leaves were rinsed with water and the plants were properly labelled using pegs. Successful inoculation of individual plants was confirmed by observing symptom development.

6.2.5 OTHER CULTURAL OPERATIONS

Plants were sprayed with Ridomil MZ 78 WP (Ciba Geigy) at 2.5 g/l three times at 2 weeks interval to control an attack by a powdery mildew fungus. Actellic 50 EC (ICI) at

the rate of 10ml/l was also sprayed thrice at an interval of 2 weeks to prevent insect infestation. Manual weeding was performed regularly until the onset of fruit set when it was no longer desirable.

6.2.6 EFFECT OF VIRUS INOCULATION ON FLOWER PRODUCTION

Staminate and perfect flowers were counted weekly. Counting was carried out for 3 out of the 4 treatments. Data collection was stopped at the beginning of fruit set so as not to disturb newly set fruits. This consideration did not permit the determination of the effect of CuMV infection on flower production for treatment 3 (first perfect flower stage).

6.2.7 EFFECT OF VIRUS INOCULATION ON YIELD PARAMETRES

Mature fruits were harvested on the 25th of July, 1991. The remaining fruits after the first harvest were harvested on the 4th of August irrespective of their state of maturity because of inclement weather conditions. The effect of virus inoculation on fruit number, fruit weight and fruit size distribution (marketability) were then determined.

The number of fruits per plant was determined by counting all the fruits harvested from 3 replicates (plants) for each treatment. Mean fruit number per plant was obtained by dividing the number of fruits by the number of replicates.

In order to determine the effect of the virus on fruit weight, fruits harvested from each of the 3 plants for each treatment were weighed individually. The combined weight was divided by the number of replicates to obtain mean fruit weight per plant. Fruits from buffer inoculated plants were also weighed to serve as control. The fruits were assigned to classes on the basis of their weights. Percentage values were obtained to determine the relative proportion of fruits in each size class for each treatment.

6.2.8 ANALYSIS OF DATA

All the data were analysed by analysis of variance followed by Duncan's multiple range test, to detect differences between means.

6.3 RESULTS

6.3.1 EFFECT OF VIRUS ON FOLIAR SYMPTOMS AND GROWTH HABIT

Symptoms of infection began to appear about 5-6 days on plants inoculated at the first true leaf stage and about 14 days on those inoculated at the vegetative and the perfect flower stages. The symptoms consisted of vein-clearing, mosaic, and leaf malformation (Plate 6.1).

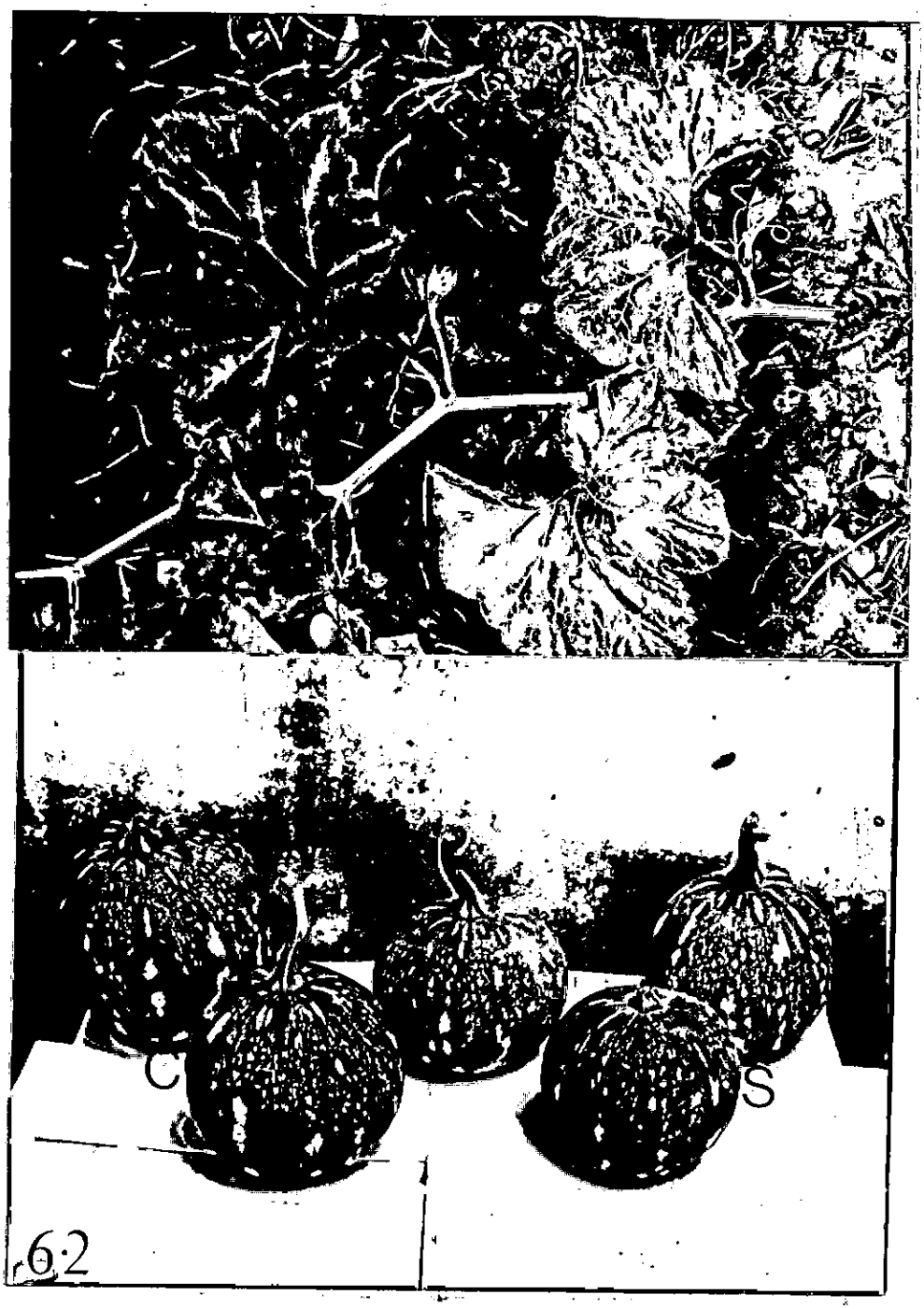
Plant inoculated at the first true leaf stage experienced infection `shock' which resulted in a short delay in stem `running'. Once this was overcome the rate of plant growth was comparable to those of later inoculations. Also, infection at the first true leaf stage caused the induction of more shoots than were observed for the other treatments. No symptoms of infection were observed on fruits produced by infected plants and the fruits were also not deformed (Plate 6.2).

6.3.2 EFFECT OF VIRUS INOCULATION ON FLOWER PRODUCTION

Weekly production of staminate flowers averaged 3.8 per plant for plants inoculated at the first true leaf stage compared to 4.5 and 4.3 for vegetative stage inoculation and control respectively. The results of CuMV inoculation on staminate flower production summarized in Table 6.1 (from appendices 11a - 14a) showed increases from mean values of 5.5 and 6.3 at the first sampling date (6 weeks after planting) to 17.0 and 19.9 at the last sampling date (9 weeks after planting) for treatments 1 and 2

Plate 6.1: A `running' vine of *Cucurbita moschata* with leaves showing symptoms of infection after mechanical inoculation with Cucurbita mosaic virus.

Plate 6.2: Representative samples of *Cucurbita moschata* fruits harvested from buffer inoculated plants, C, (2 fruits) and from Cucurbita mosaic virus inoculated plants, S, (3 fruits) which showed no evidence of deformation.



6.2

TABLE 6.1: MEAN VALUES[y] OF WEEKLY COUNT OF FLOWERS PRODUCED BY *CUCURBITA MOSCHATA* INOCULATED WITH CUCURBITA MOSAIC VIRUS AT THREE GROWTH STAGES.

GROWTH STAGE AT INOCULATION	STAMINATE FLOWER				PERFECT FLOWER	
	SAMPLING AGE AFTER PLANTING (WK)					
	6	7	8	9	8	9
First true leaf (treatment 1)	5.5a[z]	10.0a	14.8a	17.0a	0.9a	1.1a
Vegetative (treatment 2)	6.3a	11.3a	15.7a	19.9a	0.7a	1.0a
Control (treatment 4)	6.3a	12.5a	15.7a	19.2a	1.4a	1.6a

[y] Values presented are treatment means (from appendices 11a -14a). No data were collected for the first perfect flower stage (treatment 3) so as not disturb newly set fruits.

[z] In each column, values followed by the same letter are not significantly different (P=0.05) according to Duncan's multiple range test.

respectively at which times the control had 6.3 and 19.2. Data analysis did not indicate differences between treatment effect (age at time of inoculation) (Appendices 11b - 14b) and no significant differences were observed between values for all the treatments compared to the control (Table 6.1).

The number of perfect flowers produced in all the treatments were comparable with those of the control. While the average weekly counts for treatment 1 were 0.9 and 1.1 for the first and last sampling dates respectively those for treatment 2 were 0.7 and 1.0 compared to the average values of 1.4 and 1.6 for the control at the same time (Table 6.1). No significant differences were observed between treatment effects (age at time of inoculation) (Appendices 15a-16a) and the values for all the treatments when compared to the control did not differ significantly (Table 6.1)

6.3.3 EFFECT OF VIRUS ON FRUIT NUMBER

The number of fruits produced per plant in all the treatments were comparable with those of the buffer inoculated control (Appendix 17a). For treatment 1 the mean fruit number was 1.33 representing a 24.9% reduction when compared to the control with a mean of 1.77. Inoculation with CuMV at the first perfect flower stage (treatment 3) caused a reduction of 12.4%.

The age of plants at the time of inoculation did not seem to affect the number of fruits produced (Appendix 17b) as the values for all the treatments were not significantly different from those of the control (Table 6.2).

6.3.4 EFFECT OF VIRUS ON FRUIT WEIGHT

Inoculating with CuMV resulted in a reduction in the weight of fruits in all the treatments (Appendix 18a). The greatest reduction of 44.8% (mean fruit weight 0.41kg) was recorded for treatment 1 while the least percentage reduction of 25.4% was recorded for treatment 3 (mean fruit weight 0.5kg) (Table 6.2).

Data analysis indicated significant differences between treatment effects (age of plant at inoculation) (Appendix 18b). Mean fruit weights for treatments 1 and 2 were significantly different from the mean value for treatment 3 which in turn differed significantly from the control (Table 6.2).

6.3.5 EFFECT OF VIRUS INOCULATION OF FRUIT SIZE CLASS

Generally, the age of the plants at the time of inoculation affected fruit size. There was a tendency for fruits to be bigger as inoculation was delayed. The plants in treatments 1 and 2 produced greater number of small to medium sized fruits (< 200 to ≤ 600g) when compared to those for treatment 3 and the healthy control. Conversely, all the fruits harvested from plants in treatments 3 and 4

TABLE 6.2: EFFECT OF CUCURBITA MOSAIC VIRUS ON FRUIT NUMBER AND FRUIT WEIGHT OF *CUCURBITA MOSCHATA* INOCULATED AT THREE GROWTH STAGES

GROWTH STAGE AT INOCULATION	YIELD COMPONENTS			
	FRUIT NUMBER		FRUIT WEIGHT (KG)	
	A[W]	B[X]	A	B
First true leaf (treatment 1)	1.33a	24.9	0.37a	44.8
Vegetative (treatment 2)	1.67a	5.6	0.41a	38.8
First perfect flower (treatment 3)	1.55a	12.4	0.50b	25.4
Control (treatment 4)	1.77a	-	0.67c	-

A[W] = treatment means. (from appendices 17a-18a). In each column values followed by the same letter are not significantly different (P=0.05) according to Duncan's multiple range test.

B[X] = percentage reduction calculated by expressing the difference between the control and the treatment as a percentage of values for the control.

weighed above 200g (Fig. 6.1).

Analysis of the data showed significant differences in fruit size class distribution among the treatments (Appendix 19). The percentage of total number of fruits in all the classes of fruits for treatments 1 and 2 differed significantly from those for treatments 3 and 4 (Table 6.3).

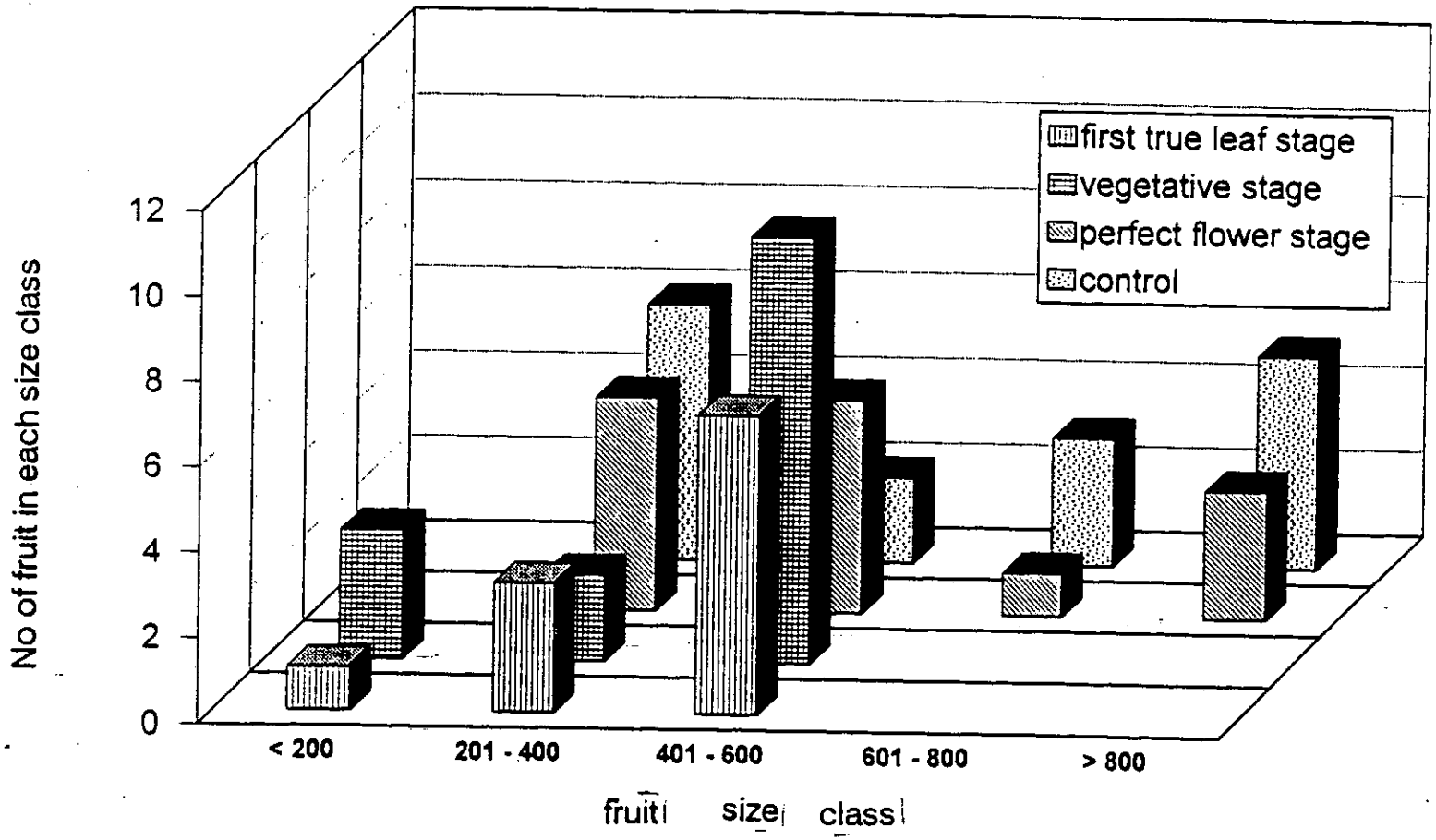


Fig. 6 .1 Class distribution of fruit produced by field-grown *Cucurbita moschata* inoculated with Cucurbita mosaic virus at three growth stages

TABLE 6.3: PERCENTAGE OF THE TOTAL NUMBER OF FRUITS IN DIFFERENT SIZE CLASS PRODUCED BY *CUCURBITA MOSCHATA* INOCULATED WITH CUCURBITA MOSAIC VIRUS AT THREE GROWTH STAGES.

TREATMENT	GROWTH STAGE AT INOCULATION.	PERCENTAGE[a] OF TOTAL NO. FRUIT PER PLANT IN EACH SIZE CLASS (g)				
		<200	200-400	401-600	601-800	>800
1	First true leaf.	9.1a	27.3bc	63.6a	0a	0a
2	Vegetative	20.0a	13.3c	66.7a	0a	0a
3	First perfect flower	0b	35.7b	35.7b	21.4b	7.1b
4	Control	0b	37.5a	12.5c	31.3b	18.7b

[a] Values were obtained by expressing the number of fruits in each size class for each treatment as percentage of the total number of fruits per treatment.

Number in each column followed by the same letter are not significantly different (P=0.05) according to Duncan's multiple range test.

6.4 DISCUSSION

The results of this study have shown the need to delay or completely protect *C. moschata* from infection by CuMV in order to obtain good marketable yield. Inoculation of *C. moschata* with CuMV did not significantly reduce the number of both staminate and perfect flowers irrespective of the time of inoculation. Studies involving other cucurbit - virus combinations did not indicate similar results. Bhargava (1977) reported that early inoculation of zucchini (*C. pepo*) with WMV resulted in a lowered production of female flowers. Blua and Perring (1989) also recorded significantly lower number of staminate and perfect flowers in ZYMV-infected *Cucumis melo*. The observed significant difference was attributed to the generation of a cluster of female flowers in ZYMV-infected plants in contrast to healthy plants on which perfect flowers were borne singly.

The production of staminate flowers by *C. moschata* once commenced, goes on almost indeterminately with one flower at each successive node until interrupted by the development of a perfect flower and yet by another one four or five nodes away from the preceding perfect flower. Inoculation of *C. moschata* with CuMV at all the inoculation ages did not alter this growth habit. This probably accounted for the linear relationship between the number of both staminate and perfect flowers and the weekly counts in contrast to the results of Blua and Perring (1989).

The result of the effect of CuMV infection on the number of fruits produced by *C. moschata* also does not conform with reports involving other cucurbit-virus combinations. Studies by Thomas (1971), Demski and Chalkey (1972,1974) and Blua and Perring (1989) showed that early virus infection caused more significant reductions in the number of fruits produced than did late inoculations. In contrast, the result of this study showed that inoculation of *C. moschata* with CuMV did not result in significant yield losses in terms of the total number of fruits produced regardless of plant age at time of inoculation. This is to be expected, because virus inoculation did not result in statistical difference in the number of perfect flowers between virus inoculated plants and buffer inoculated controls for all the treatments.

The greatest detrimental effect of CuMV on the yield of *C. moschata* is on fruit size (weight) and invariably on fruit marketability. Fruits produced by plants inoculated at the first true leaf and vegetative stages were generally smaller in comparison with those harvested from plants inoculated at the first perfect flower stage and the control. Inoculation at the first true leaf and vegetative leaf stages led to 100% loss while inoculation at the first perfect flower stage caused 74.4% loss in fruit marketability if fruits weighing less than 600g were considered unmarketable. In Nigeria, pumpkin fruits are sold on the basis of size. The larger the fruit the

greater the appeal to prospective buyers and concommitantly, higher economic returns for the farmer. Fruits that weighed below 600g are hardly brought to the market for sale.

The significant reduction in the size of fruits of *C. moschata* caused by CuMV infection observed in this study confirms earlier reports involving other cucurbits-virus systems where significant losses were also recorded in the marketability of fruits from early inoculated plants than those inoculated at advanced plant age (Fletcher *et al.*, 1969; Demski and Chalkey, 1972; Blua and Perring, 1989).

The results of this study have shown the need to delay infection of *C. moschata* by CuMV till, if possible, after fruit set in order to obtain reasonable marketable yield. Several control strategies aimed at protecting crops from virus infection have been tested. Such strategies which include intercropping (Toba *et al.*, 1977), use of reflected mulch (Perring, 1986) and canopy cover (Blua and Perring, 1989) have achieved some degree of effectiveness in delaying the onset of virus infection of some cucurbits. The adoption of one or a combination of these strategies may ensure profitable commercial pumpkin (*C. moschata*) cultivation in the absence of resistant varieties.

CHAPTER SEVEN

CHANGES IN THE NUTRITIVE CONTENTS OF *CELOSIA ARGENTEA* AND *CUCURBITA MOSCHATA* INOCULATED WITH CELOSIA LEAF CURL AND CUCURBITA MOSAIC VIRUSES RESPECTIVELY

ABSTRACT

Changes in the nutritive contents of *Celosia argentea* and *Cucurbita moschata* inoculated with Celosia leaf curl and Cucurbita mosaic viruses respectively at 3, 4, 5 and 6 weeks after inoculation were investigated using flame photometre and micro-Kjeldahl methods.

The potassium, sodium, phosphorus and nitrogen contents were generally higher in infected than in healthy plants of both vegetables. Also, the percentage crude protein content was higher in virus infected than in healthy plants of the two vegetables. However, healthy plants had more ether extract (fat) and crude fibre than infected plants. Significant increases in potassium, phosphorus, nitrogen and crude protein contents were recorded usually after 4 weeks of inoculation. This period seems to coincide with the appearance of severe disease symptoms.

It appears that virus infected plants of both *C. argentea* and *C. moschata* would be more nutritious weight for weight relative to the healthy plants.

The disease symptoms induced in the vegetables by the viruses may however make them less attractive to buyers.

7.1 INTRODUCTION

Vegetables have been recognised as alternative sources of proteins, vitamins and minerals in the dietary requirements of the low income earners who could hardly afford the high cost of animal proteins and other essential nutrients (Omueti, 1980; Reiz and Leitzmann, 1985).

Plants vary in their reactions to viruses. These reactions are often interpreted as a reflection of interference with and altered metabolic activities of infected plants.

The literature is replete with reports of investigations into changes in the metabolic activities of virus infected plants. Such studies have shown a general increase in the content of free amino acids and related compounds (Bozarth and Diener, 1963; Welkie *et al*, 1967; Ford and Tu, 1969), increased transpiration but reduced photosynthetic rates (Tu *et al*, 1968), higher level of activities of some specific enzymes (Solymosy *et al*., 1967; Novacky and Hampton, 1968; White and Blakke, 1982) and decreased hormonal activities in infected plants in comparison with healthy ones (Nibett *et al*., 1974, Whenham, 1989).

Studies have also been conducted to investigate changes in the composition of mineral elements, carbohydrates and lipid contents of virus infected plants.

Moline and Ford (1974a,b, & c) recorded significant increases in the total nitrogen and carbohydrate levels in

the shoots of seedlings of *Zea mays* and *Sorghum halepense* infected with sugarcane mosaic virus (SCMV), *Z. mays*, *Triticum aestivus*, *Avena sativa* and *Hordeum vulgare* inoculated with brome mosaic virus (BMV) and *Pisum sativum* infected by clover yellow mosaic virus. In Chinese cabbage leaves infected with turnip yellow mosaic virus (TYMV) sampled 12-20 days after inoculation, a rise in virus phosphorus was accompanied by a corresponding reduction in the non-virus insoluble phosphorus (Matthews *et al*, 1963).

Bergmann and Boyle (1962) reported non-significant increases in the potassium and phosphorus contents of tomato leaves inoculated with tobacco mosaic virus (TMV). In contrast, the manganese, iron, aluminum, copper and zinc contents were significantly reduced, the last two elements by as much as 50%. Similarly, investigations at various times after inoculation of *Phaseolus vulgaris* with bean common mosaic virus (BCMV) revealed increases in the phosphorus, potassium and crude protein contents but a marked reduction in the ascorbic acid content of infected plants (Suresh *et al*., 1990). Best (1968) reported significant increase in the lipid content of *Nicotiana glutinosa* leaves inoculated with tomato spotted wilt virus (TSWV).

Celosia argentea and *Cucurbita moschata* are two of the popular leafy vegetables cultivated for preparing sauces in the Southern parts of Nigeria. The severe reactions of *C.*

argentea to Celosia leaf curl virus (CLCV) and *C. moschata* to Cucurbita mosaic virus (CuMV) observed in some studies in this work seem to suggest disturbances in the physiology of the host plants.

Since previous investigations have dealt with changes in the nutritive value of some healthy leafy vegetables, including *C. argentea* at various times after seeding (Oke, 1968; Omueti, 1980) the focus of this study is to report changes in the nutritive contents of *C. argentea* and *C. moschata* inoculated with CLCV and CuMV respectively.

7.2. MATERIALS AND METHODS

7.2.1 EXPERIMENTAL DESIGN AND INOCULATION PROCEDURE

Three week old seedlings of *C. argentea* var. 'TLV 8' were transplanted into 16 cm diameter polyethylene bags (one per bag) containing sterilized soil. The bags were arranged in three blocks. Each block contained four groups (plots) of 10 plants each, arranged in two rows of 5 plants such that each block contained 40 plants.

Sap prepared by grinding CLCV-infected *C. argentea* in phosphate buffer was used to inoculate 5 out of the 10 plants in each plot when the plants were four weeks old. The remaining plants were inoculated with buffer only.

The same experimental design was adopted for *C. moschata*. The plants were however inoculated at the primary leaf stage (8 days after planting).

7.2.2 PREPARATION OF SAMPLES FOR ANALYSIS

The succulent tops, about 30cm in length, of 9 virus infected plants (3 plants from any one plot from the 3 blocks) of each of the vegetables were harvested at weekly interval for 4 weeks beginning from 3 weeks after inoculation. The harvested plants were dried in the oven at 70°C for 5 days and milled into powder by grinding in a mortar using a pestle. Three samples weighing 10 g each were packaged for each harvest and taken for analysis.

Nine healthy plants were also harvested, dried and milled into powder for analysis.

Analysis of the samples for changes in the potassium (K), sodium (Na), phosphorus (p), nitrogen (N), crude protein, ether extract (fat) and crude fibre contents of both vegetables were carried out at the Institute of Agricultural Research and Training (IAR&T), Ibadan.

7.2.3 DETERMINATION OF THE POTASSIUM, SODIUM, PHOSPHORUS AND NITROGEN CONTENTS

The potassium, sodium and phosphorus contents of the samples were determined by the flame photometric method while the nitrogen content was determined by the micro-Kejldahl method as described by Juo (1978).

A 0.5 g finely ground sample was put in a porcelain crucible and ignited in a muffle furnace for 6 hr at 500°C (dry ashing). The sample was cooled and 10 ml 1N HCl was added. The sample was evaporated to dryness over a hot plate at low heat. It was returned to the furnace and heated at 400°C for 15 min until a white or greyish white ash was obtained. The sample was cooled, dissolved in 10 ml 0.1N HCl and filtered into a 50 ml volumetric flask.

The crucible and the filter paper were washed with additional 10 ml 0.1N HCl thrice and made up to 50 ml with 0.1N HCL solution. The potassium, sodium, and phosphorus contents were then determined using this sample solution.

For potassium, a 10^{-1} dilution of the sample solution was made and this was read in a flame photometer. The

sodium content was determined by taking readings from 10 ml of the solution in the flame photometer. For phosphorus 5 ml of the sample solution was made up to 50 ml by adding 45 ml distilled water and then 10 ml of vanado-molybdate (ammonium- molybdate + ammonium - metavanadate) reagent was added. The solution was allowed to stand for 10 - 15 min and transmittance was determined using a spectrophotometre at 470nm.

In order to determine the nitrogen content of the vegetables, 0.5 g of the finely ground sample was put in a 50 ml Kjeldhal flask. A tablet of selenium (catalyst) and 4 ml of conc. H_2SO_4 were added. The flask was heated on a digestion rack. After digestion it was allowed to cool. The residue was washed with 10 ml distilled water into a flask. The nitrogen content was determined colorimetrically using a technocon autoanalyser.

Three separate samples were analysed for each element. The concentration of each was estimated from its standard curve.

7.2.4 DETERMINATION OF THE CRUDE PROTEIN CONTENT

The crude protein content of the samples was determined by the Kjeldhal method as described by the Association of Official Analytical Chemists (AOAC, 1975).

A 0.5g of the milled samples was weighed into a 300ml Kjeldhal flask to which about 5-10g of anhydrous sodium sulphate (Na_2SO_4), 1g of copper sulphate ($CuSO_4$) and a trace

of selenium powder were added. After thorough mixing 25ml conc. H_2SO_4 and a few glass beads were added. The mixture was heated over an electric heater until the content of the flask became greenish blue. The digestion was continued for another 15 min after which it was allowed to cool, diluted with cold distilled water and transferred into a 250 ml graduated flask. It was made up to 250 ml and shaken vigorously to mix. Five millilitre (5ml) of diluted sample was transferred into the inner chamber of the Markam micro Kjeldhal apparatus and 2 ml of 60% NaoH solution was added. The distillation process was started and allowed to proceed until the condensate began to drop at the rate of one (1) per minute. A 50 ml conical flask containing 5 ml of 0.5% boric acid was placed under the condenser so that the condenser tip was immersed in the liquid. The distillation was continued for another 1 - 1½ min while allowing a few drops of the condensate to drip into the boric acid solution. The flask was then removed and the ammonium borate formed was titrated against N/100 HCl. The procedure was repeated to obtain replicate titres.

The percentage crude protein content of the sample was calculated as follows:

Calculation

1ml N/100 HCl = 0.00014g Nitrogen (1.4×10^{-4}gN)

Percentage crude protein

= 1.4×10^{-4}g x 100 x 250/5 x 6.25 x titre

$$= \frac{4.735 \times \text{titre}}{\text{wt (sample)}}$$

7.2.5 DETERMINATION OF THE FAT (ETHER EXTRACT) CONTENT

The fat content of the samples was determined as described in AOAC (1975).

Half a gram (0.5g) of the milled samples of *C. argentea* and *C. moschata* was weighed individually into the fat extraction thimble and transferred into a 100 ml Soxhlet extractor. The extractor was fitted into the mouth of a pre-weighed Soxhlet flask, three-quarters filled with petroleum ether (BP 40 - 60°C) and put in a waterbath maintained at 70°C. A double condenser was in turn fitted into the extractor. At regular interval, the ether passed over the sample. After about 7hr the Soxhlet extractor with the thimble was disconnected and the petroleum ether in it was allowed to drain into a stock bottle. The thimble was removed and dried in the air.

The apparatus was reset (without the thimble) in the waterbath to free the fat at the bottom of the flask of the ether. The flask was then removed, wiped dry and placed in an air oven maintained at a constant temperature of 105°C for 12hr to dry off the last traces of ether or water. The flask was then cooled in a dessicator and weighed.

The amount in the sample was obtained using the relationship below.

$$\text{Percentage ether extract (fat)} = \frac{(x - y) \times 100}{0.5}$$

where:

x = weight (wt) of the flask and fat.

y = weight of flask alone

x-y = weight of fat alone

0.5 = sample weight (g) analysed.

7.2.6 DETERMINATION OF THE CRUDE FIBRE CONTENT

The acid-base hydrolysis method as described in AOAC (1975) was used to determine the crude fibre content of the samples. The content of the dry thimble (from fat extraction (section 7.2.5) was transferred into a litre conical flask to which 200 ml of freshly prepared 1.25% H_2SO_4 that had been brought to boil was added. The flask was heated for 30 min using `cooling finger' to keep volume constant and prevent vigorous boiling. The content of the flask was filtered and the residue was washed with four (4) rounds of hot water. The residue was washed back into the flask to which 200 ml of freshly prepared 1.25% NaOH already brought to boil was added. The flask with its content was further boiled for another 30 min under `cooling finger', filtered through No 4 Whatman filter paper, washed as before with hot water and then once with 1% HCl before it was allowed to drain. The washing of the residue was repeated with hot water until it was free of acid, allowed to drain, followed by two (2) rounds of washing with industrial methylated spirit, thrice with petroleum ether and left to drain overnight.

The residue was then transferred into a silica dish and heated in the oven at 105°C for 12 hr. The dish with the content was charred in a muffle furnace to complete ash at 600°C for 30 min. The dish was cooled and weighed.

The percentage amount of crude fibre in the sample was calculated using the following relationship.

$$\text{Percentage crude fibre} = \frac{(x - y) \times 100}{0.5}$$

where:

x = wt of dish + fibre, etc

y = wt of dish + ash + silica

x-y = wt of fibre alone

0.5 = sample weight (g) analysed

7.2.7 STATISTICAL ANALYSIS

All values obtained were expressed as percentages of the samples analysed. Data presented are means of 3 replicates and were subjected to Student's t-test for test of significance.

7.3 RESULTS

7.3.1 EFFECT OF CLCV ON THE POTASSIUM, SODIUM, PHOSPHORUS AND NITROGEN CONTENTS OF *CELOSIA ARGENTEA*

The results of inoculation of *C. argentea* with CLCV on the K, Na, P and N contents of plants, presented in appendix 20 showed a general increase in the level of these mineral elements in infected plants than in healthy ones.

From the marginal difference of 0.4 (mean percentage values of 8.00 and 7.97 for the control and infected plants) the K content was significantly higher at 4 and 5 weeks after virus infection (Fig. 7.1a) by as much as 23.6 and 38.2% when the average values were 6.26 and 6.54 for infected plants and 4.78 and 4.04 for the control.

The Na content in infected plants was higher from 4 to 6 weeks than in control plants. It averaged 0.39, 0.41 and 0.42 at 4, 5 and 6 weeks respectively after virus inoculation whereas 0.31, 0.39 and 0.36 were the corresponding values for the control. The percentage differences were however not significant (Fig. 7.1b).

The P content was not significantly affected by the length of time of virus infection (Fig. 7.1c), although average values for infected plants were slightly higher than for the control.

For the inoculated plants the N content ranged from 1.27 at 3 weeks to 2.87. at 6 weeks after inoculation.

Those for the control ranged from 0.40 to 1.20 with the lowest at 3 weeks after inoculation. Irrespective of when plants were harvested infected plants contained significantly more N than the control (Fig. 7.1d) with percentage differences ranging from 50.2 to 71.4 (appendix 20).

7.3.2 EFFECT OF CLCV ON THE CRUDE PROTEIN, ETHER EXTRACT (FAT) AND CRUDE FIBRE CONTENTS OF *CELOSIA ARGENTEA*

The results which are presented in appendix 21 showed greater amount of crude protein in infected than in healthy plants. Conversely, ether extract and crude fibre contents were higher in healthy than in infected plants.

The crude protein content was higher, regardless of time of harvest after virus infection, in infected than in healthy plants, albeit insignificantly (Fig. 7.2a). For instance, while the crude protein in infected plants averaged 16.92 and 17.5 at 3 and 6 weeks respectively the corresponding values for healthy plants were 14.0 and 16.23.

There was a general reduction in the fat content of virus infected plants relative to the controls. While it fluctuated with age in infected plants (mean values of 2.11, 1.13, 1.58 and 1.36 at 3, 4, 5 and 6 weeks respectively) there was a rise in the fat content in control plants from 3 to 5 weeks (mean values of 1.60 to 1.78) followed by a decline at 6 weeks (average value of 1.54). The fat content differed significantly between

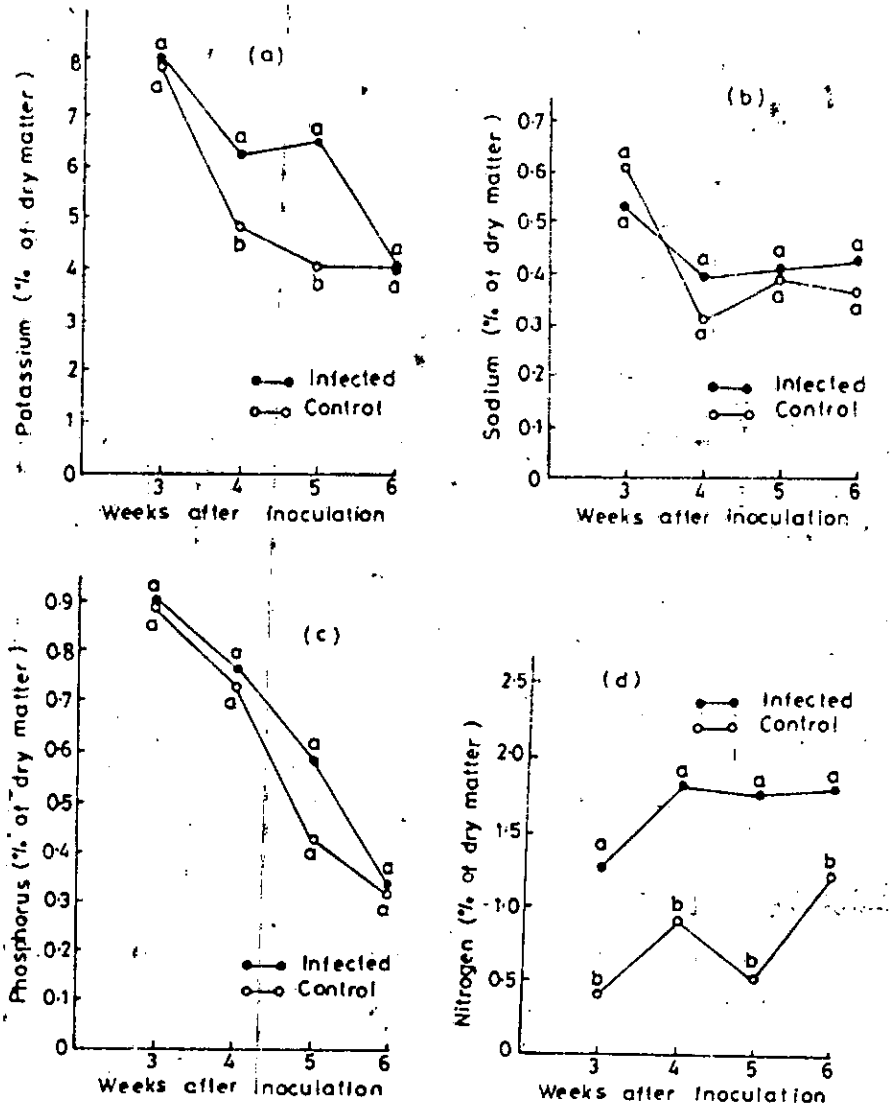


Fig. 7.1 Changes in the potassium (a), sodium (b), phosphorus (c), and nitrogen (d) content of Celosia argentea inoculated with Celosia leaf curl virus at various times after inoculation. Points followed by the same letter are not significantly different (P = 0.05) using Student's t.-test.

infected and the controls at 3 and 4 but not at 5 and 6 weeks after inoculation (Fig. 7.2b).

The crude fibre content of healthy plant was comparatively higher than was observed for infected plants. For the healthy plants there was a gradual increase from an average value of 20.39 at 3 weeks to 23.0 at 6 weeks after mock inoculation. Infected plants had fluctuating crude fibre content with the highest average value of 21.86 at 4 weeks while the least value of 17.02 was observed at 5 weeks. Except at 5 weeks the crude fibre content was not significantly higher than for infected plants (Fig. 7.2c).

7.3.3 EFFECT OF CuMV ON THE POTASSIUM, SODIUM, PHOSPHORUS AND NITROGEN CONTENTS OF *CUCURBITA MOSCHATA*

The result which are similar to those of *C. argentea* previously reported showed a comparatively higher amount of K, Na, P and N in infected than in healthy plants (Appendix 22).

The K content was significantly higher at all sampling times except at 3 weeks after virus infection (Fig. 7.3a). The average K content of infected plant was 3.47 at 3 weeks compared to 2.65 for the healthy plant. At 5 weeks when infected plants had 4.75 the value for the control was 3.01, the differences between the values for the healthy and infected plants ranged from 23.6 to 36.6%.

Similarly, the amount of Na was higher in infected than in healthy plants at all sampling times. Its content

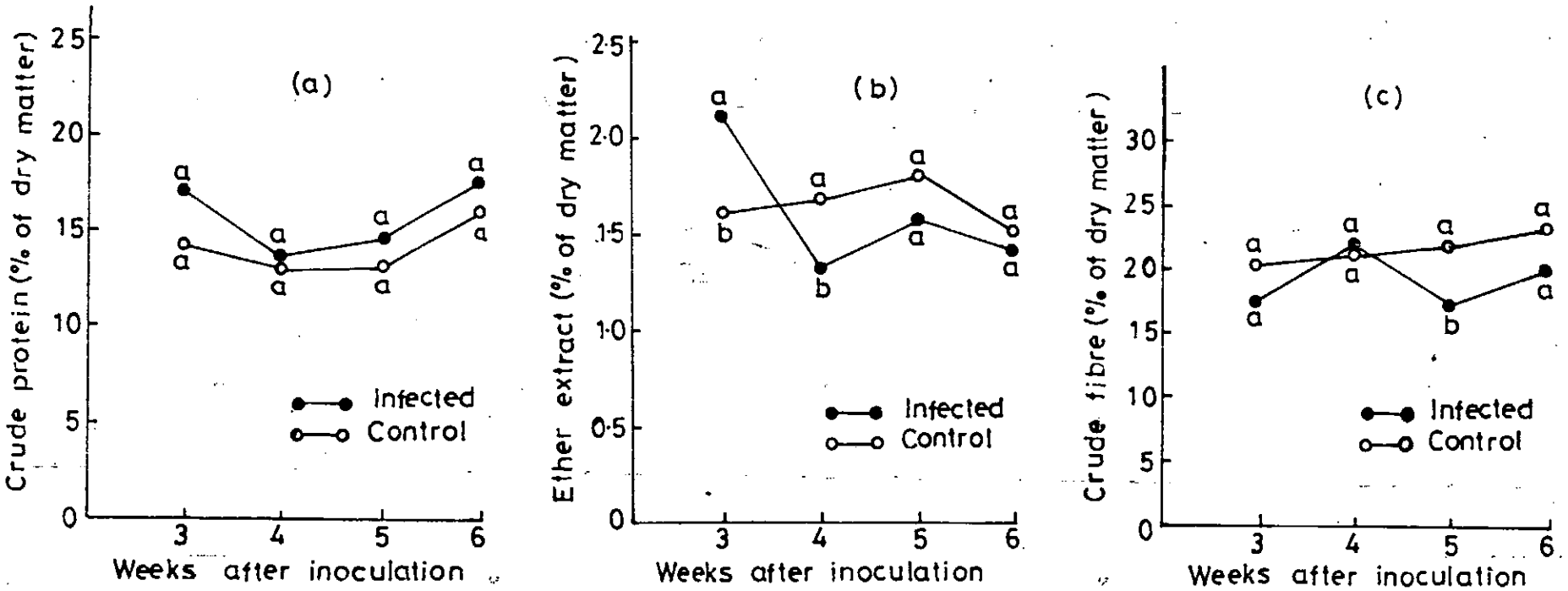


Fig 7.2 Changes in the crude protein (a), ether extract (fat) (b) and crude fibre (c) contents of Celosia argentea inoculated with Celosia leaf curl virus at various times after inoculation. Points followed by the same letter are not significantly different ($P = 0.05$) using Student's t-test.

in infected plants ranged from 0.34 at week 4 to 0.39 at 5 weeks after inoculation whereas the maximium and the least values for the healthy were 0.35 and 0.30 at 5 and 3 weeks after inoculation respectively. These increases were however not significant (Fig.7.3b).

At 3 and 4 weeks after virus infection the P content of infected plants averaged 0.61 and 0.44 respectively. These were significantly higher than the average values of 0.36 and 0.29 for healthy plants at the same period. The values obtained at 5 and 6 weeks for the infected plants did not differ significantly from the corresponding values for the controls (Fig.7.3c).

The N content of infected plants ranged from 0.67 at 3 weeks to 1.12 at 6 weeks after inoculation. For the control, a value of 0.58 was observed at 3 weeks while the highest value of 1.02 was recorded at 6 weeks after inoculation. The amounts of N in the healthy and infected plants were only significantly different at weeks 4 and 5after inoculation (Fig.7.3c) when percentage differences were 22.7 and 20.6 (Appendix 22).

7.3.4 EFFECT OF CuMV INOCULATION ON CRUDE PROTEIN, ETHER EXTRACT (FAT) AND CRUDE FIBRE CONTENTS OF *CUCURBITA MOSCHATA*

Virus inoculation of *C. moschata* led to higher amounts of crude protein in infected plants. The control plants generally contained more fat and crude fibre than CuMV infected plants (Appendix 23).

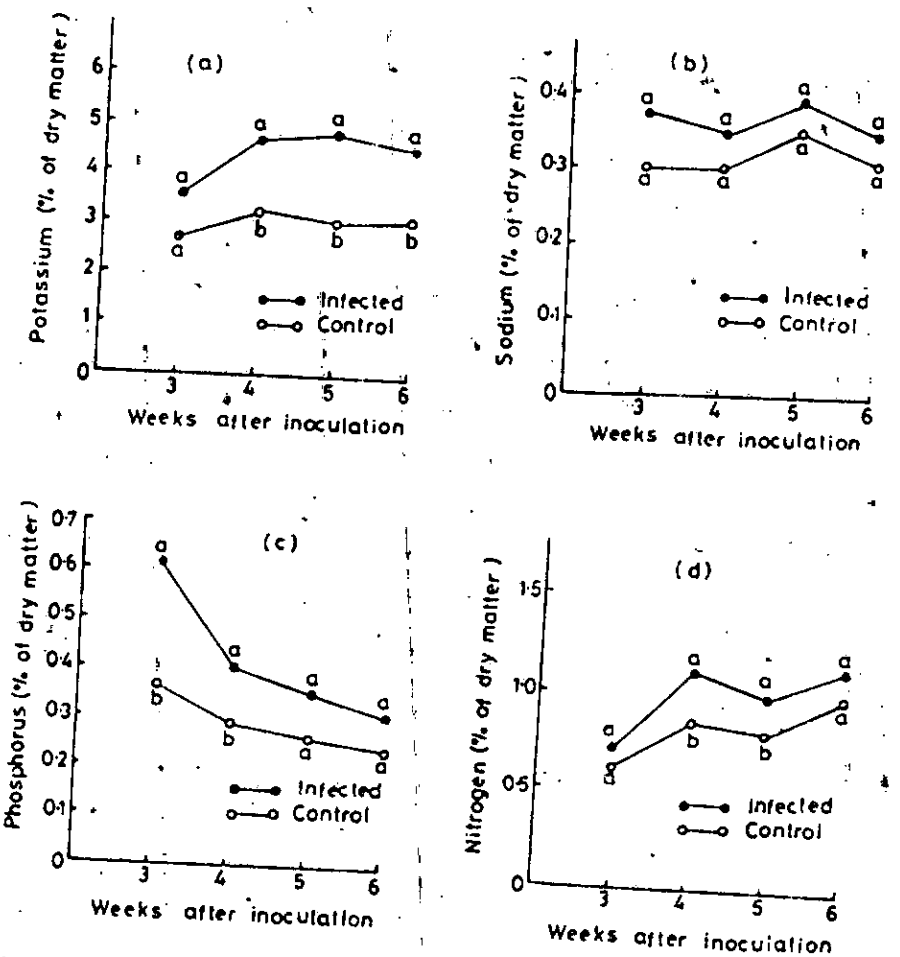


Fig. 7.3 Changes in the potassium (a), sodium (b), phosphorus (c) and nitrogen (d) contents of Cucurbita moschata inoculated with Cucurbita mosaic virus at various times after inoculation. Points followed by the same letter are not significantly different ($P = 0.05$) using Student's t-test.

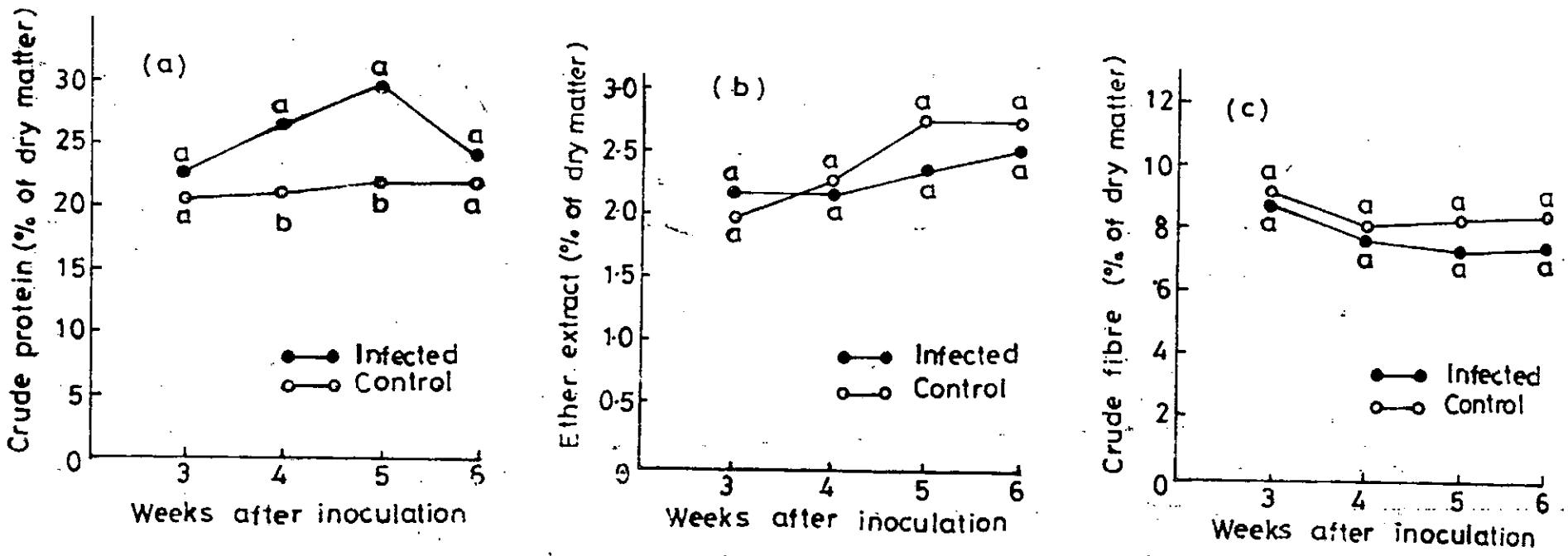


Fig. 7.4 Changes in the crude protein (a) ether extract (fat)(b) and crude fibre (c) contents of Cucurbita moschata inoculated with Cucurbita mosaic virus at various times after inoculation. Points followed by the same letter are not significantly different (P = 0.05) using Student's t-test.

The crude protein in infected plants increased from an average value of 22.6 at 3 weeks to a peak value of 29.6 at 5 weeks after inoculation. This was followed by a decline to 24.5 at 6 weeks. For the control, the crude protein content remained relatively unchanged. Values ranged from 20.7 at 3 weeks to 21.9 at 6 weeks after mock inoculation.

The crude protein content of infected plants was significantly higher than that of control plants at 4 and 5 weeks (Fig.7.4a) when the percentage differences were 20.1 and 25.3 repectively.

In respect of the fat content, infected plants had lesser amount at almost all the sampling times compared to healthy plants. At 4,5 and 6 weeks for example, the average values for virus infected plants were 2.19, 2.45 and 2.55 repectively whereas the controls had corresponding averages of 2.34, 2.82 and 2.80. However, the differences were not significant (Fig.7.4b).

Healthy control plants had insignificantly higher amounts of crude fibre at all sampling times than virus inoculated plants (Fig.7.4c). For instance, the average crude fibre content at 3 and 4 weeks for healthy plants were 9.21 and 8.11 respectively while infected plants had corresponding values of 8.86 and 7.89.

7.4 DISCUSSION

In this study, inoculation of *C. argentea* and *C. moschata* with CLCV and CuMV repectively resulted in increases in the potassium, sodium, phosphorus and nitrogen contents of infected plants as compared to buffer inoculated plants.

These results seem to confirm previous reports by Matthews *et al.* (1963), Moline and Ford (1974 a,b,c), Orellana *et al.* (1963) and Suresh *et al.* (1990) who observed increases in the levels of potassium, total nitrogen and phosporus in virus infected plants in comparison with healthy controls. These results however, differ from those of Leal and Lastra (1984) who reported a reduction in the content of nitrogen in tomato plants infected by tomato yellow mosaic virus and Singh *et al.* (1976) who had earlier reported a similar observation for potato infected with a geminivirus, tomato leaf curl virus.

The role of mineral nutrients in plant nutrition has long been recognised. Nitrogen and phosphorus form part pf the structural components of proteins, nucleic acids, and coenzymes (Epstein, 1972). The principal role of potassium is that of an activator of numerous enzymes (Evans and Sorger, 1966). Evidence has also been obtained for the direct involvement of potassium in nucleic acid and protein synthesis (Pandey and Sinha, 1981). The role of sodium is little known in plants, but it is believed to be generally

essential in plant species with C_4 photosynthetic pathway (Brownwell and Crossland, 1972).

The relatively higher content of these mineral elements, particularly nitrogen and phosphorus in infected plants was mostly detected between 4-5 weeks after virus inoculation. These increases probably coincided with the period of maximum virus synthesis and apprearance of more severe disease symptoms. During this period, nitrogen and phosphorus would be required for incorporation as structural components of new virions while potassium would probably be needed as an activator of some virus - specific enzymes required for virus replication.

Inoculation of *C. argentae* and *C. moschata* with CLCV and CuMV respectively led to increases in the crude protein content of infected relative to healthy plants. In contrast, healthy plants of both vegetable crops had higher amount of fat and crude fibres than in infected plants.

One common characteristic of members of the potyvirus group is the induction of virus specific proteins such as `pinwheel' inclusion bodies and helper components (Edwardson, 1974; Pirone and Thornbury, 1984; Shoyinka *et al.*, 1987; Ladipo *et al.*, 1988b). Virus infection of plants also results in the production of virus - specific enzymes necessary for virus multiplication (Matthews, 1970; White and Blakke, 1982). Also, infection of plants by viruses and viroids have been reported to cause accumulation of pathogenesis-related (PR) proteins (Garcia

- Breijo *et al.*, 1990; Nasser *et al.*, 1990; Stintzi *et al.*, 1991). It may be that CLCV and CuMV, both of which are potyviruses, induced some non-structural proteins in infected cells of their respective hosts. These proteins in addition to the capsid proteins of the viruses were probably responsible for the observed increases in the protein contents of infected plants of both *C. argentea* and *C. moschata* relative to the healthy plants. White and Blakke (1982) attributed increased relative amount of protein in barley infected with wheat streak and barley stripe mosaic viruses to high level of RNA polymerases and the viral coat proteins in expanding systematically infected leaves.

The higher crude fat content of control plants of both viruses, relative to the infected plants constrasts with the results of Best (1968) who reported significantly greater amount of lipid in *N. glutinosa* leaves infected with tomato spotted wilt virus (TSWV). The difference in the results could be attributed to the viruses involved. Tomato spotted wilt virus is isometric in shape and the particles are enclosed within a lipoprotein envelope (Walkey, 1985). The virus probably caused the synthesis of extra lipid materials in its host which is necessary for its lipoprotein envelope. Potyviruses such as CLCV and CuMV lack lipids in their protein coat.

The results of this study seem to suggest that infected plants of both vegetables would be more nutritious

than healthy plants. This is because weight for weight infected plants will supply more nitrogen, phosphorus, potassium, sodium and protein but possibly less ascorbic acid in the diet since virus infection generally causes a reduction in the ascorbic acid content of infected plants (Milo and Santilli, 1967; Suresh *et al.*, 1990). However, the various symptoms induced by CLCV and CuMV in *C. argentea* and *C. moschata* respectively might make them unattractive to buyers. Also, the yield per hectare of infected plants would be much less than those of uninfected plants and it would therefore not be economical to cultivate infected plants.

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APPENDIX 1

INCIDENCE OF LEAFY VEGETABLE VIRUS INFECTION AT AMUWO ODOFIN FARM.

VEGETABLES	AMARANTHUS			CELOSIA			COCHORUS			'CUSBARA'			SOLANUM		
MONTH	NO. SAMPLED	NO. INFECTED	% INFECTION*	NO. SAMPLED	NO. INFECTED	% INFECTION	NO. SAMPLED	NO. INFECTED	% INFECTION	NO. SAMPLED	NO. INFECTED	% INFECTION	NO SAMPLED	NO. INFECTED	% INFECTION
1989															
OCTOBER	1570	80	5.48	308	20	6.49	α[b]	-[c]	0	α	-	0	600	1	0.17
NOVEMBER	3620	176	4.86	988	32	3.24	α	-	0	α	-	0	945	-	0
DECEMBER	4779	214	4.48	1660	35	2.11	α	-	0	α	-	0	1351	-	0
1990															
JANUARY	2859	257	8.99	856	28	3.27	α	-	0	α	-	0	759	-	0
FEBRUARY	4324	194	4.49	1503	25	1.66	α	-	0	α	-	0	1256	-	0
MARCH	4232	196	4.63	1675	31	1.85	α	-	0	α	-	0	1085	-	0
APRIL	5686	181	3.18	1174	103	8.77	α	-	0	α	-	0	363	3	0.83
MAY	2578	226	8.77	1510	270	17.88	α	-	0	α	-	0	111	-	0
JUNE	5905	494	8.37	791	214	27.05	α	-	0	α	-	0	2101	60	2.86
JULY	6843	436	6.37	553	10	1.81	α	-	0	α	-	0	842	11	1.31
AUGUST	3250	623	19.17	1202	94	7.82	α	-	0	α	-	0	710	-	0
SEPTEMBER	4847	692	14.28	1364	99	7.26	α	-	0	α	-	0	835	5	0.60

* Percentage infection was determined by dividing the number of plants showing viral-like symptoms by the total number of plants examined multiplied by 100.

[b] α = Number of plants sampled innumerable.

[c] - = No infected plants were recorded.

APPENDIX 2

INCIDENCE OF LEAFY VEGETABLE VIRUS INFECTION AT TEJUOSO FARM.

VEGETABLES	1989			1990								
	OCT.	NOV.	DEC.	JAN.	FEB.	MAR.	APR.	MAY	JUN.	JUL.	AUG.	SEP.
AMARANTHUS												
NO. SAMPLED	4571	7069	4801	5168	5487	7468	8784	6548	5086	2422	5489	4426
NO INFECTED	15	24	29	35	19	5	37	20	6	12	7	15
% INFECTION	0.33	0.34	0.60	0.68	0.35	0.07	0.42	0.31	0.12	0.50	0.13	0.34
CELOSIA												
NO SAMPLED	572	3661	1454	3155	2991	1631	2628	2388	2358	636	1517	2816
NO INFECTED	3	3	8	-[b]	9	1	16	7	-	9	3	9
% INFECTION	0.52	0.08	0.55	0	0.30	0.06	0.61	0.29	0	1.43	0.20	0.32
COCHORUS												
NO SAMPLED	α[c]	α	α	α	α	α	α	α	α	α	α	α
NO INFECTED	-	-	-	-	-	-	-	-	-	-	-	-
% INFECTION	0	0	0	0	0	0	0	0	0	0	0	0
'CUSBARA'												
NO SAMPLED	α	α	α	α	α	α	α	α	α	α	α	αα
NO INFECTED	-	-	-	-	-	-	-	-	-	-	-	-
% INFECTION	0	0	0	0	0	0	0	0	0	0	0	0
SOLANUM												
NO SAMPLED	846	2305	1164	1505	2127	1245	NC	786	1458	480	620	1105
NO INFECTED	-	-	-	-	-	-	-	-	-	-	-	-
% INFECTION	0	0	0	0	0	0	0	0	0	0	0	0
CUCURBITA												
NO SAMPLED	5	5	NC[d]	NC	NC	NC	18	18	18	18	18	18
NO INFECTED	3	3					5	9	11	11	11	11
% INFECTION	60	60					27.77	50.0	61.11	61.11	61.11	61.11
TELFAIRIA												
NO SAMPLED	31	31	NC	NC	NC	NC	NC	NC	27	27	27	27
NO INFECTED	9	9							5	5	8	12
% INFECTION	29	29							18.5	18.5	29.6	44.4
BRASSICA												
NO SAMPLED	500	NC	NC	NC	NC	NC	848	NC	NC	NC	NC	NC
NO INFECTED	459						2					
% INFECTION	91.6						0.24					

[a] Percentage infection was determined by dividing the number of plants showing viral-like symptoms by the total number of plants examined multiplied by 100.

[b] no infected plants were recorded.

[c] α = Number of plant sampled immerable.

[d] NC = Vegetable not in cultivation.

APPENDIX 3

INCIDENCE OF LEAFY VEGETABLE VIRUS INFECTION AT ABULE ADO FARM.

VEGETABLES	AMARANTHUS			CELOSIA			COCHORUS			'CUSBARA'			SOLANUM		
MONTH	NO. SAMPLED	NO. INFECTED	% INFECTION	NO. SAMPLED	NO. INFECTED	% INFECTION	NO. SAMPLED	NO. INFECTED	% INFECTION	NO. SAMPLED	NO. INFECTED	% INFECTION	NO SAMPLED	NO. INFECTED	% INFECTION
1989															
OCTOBER	960	12	1.25	1025	0	0	α[c]	-	0	α	-	0	691	-	0
NOVEMBER	2448	6	0.25	1312	2	0.15	α	-	0	α	-	0	1790	-	0
DECEMBER	5152	10	0.19	1925	8	0.42	α	-	0	α	-	0	925	-	0
1990															
JANUARY	2726	7	0.26	1642	14	0.82	α	-	0	α	-	0	1160	-	0
FEBRUARY	5859	9	0.15	2469	7	0.28	α	-	0	α	-	0	1255	-	0
MARCH	7355	-[b]	0	2729	10	0.37	α	-	0	α	-	0	1745	-	0
APRIL	5889	10	0.17	2239	16	0.71	α	-	0	α	-	0	1470	-	0
MAY	5631	-	0	2206	7	0.32	α	-	0	α	-	0	2958	-	0
JUNE	5028	22	0.44	1863	25	1.34	α	-	0	α	-	0	950	-	0
JULY	4236	25	0.59	1531	19	1.24	α	-	0	α	-	0	816	-	0
AUGUST	5454	4	0.07	2496	12	0.48	α	-	0	α	-	0	655	-	0
SEPTEMBER	4210	19	0.45	1480	15	1.01	α	-	0	α	-	0	1195	5	0

[a] Percentage infection was determined by dividing the number of plants showing viral-like symptoms by the number of plants sampled multiplied by 100.

[b] _ = no infected plants were recorded.

[c] α = Number of plant innumerable.

APPENDIX 4

MEAN AND PERCENTAGE REDUCTION IN LEAF SIZE OF *CELOSIA ARGENTEA* INOCULATED WITH CELOSIA LEAF CURL VIRUS AT DIFFERENT AGES.

TREATMENT (plant age at) (inoculation)	LEAF SIZE[a] (cm^2)		
	INOCULATED	CONTROL	%REDUCTION[b]
3 WK	22.77	39.53	42.4
4 WK	22.18	35.08	36.8
5 WK	22.78	38.04	13.8
6 WK	31.67	36.24	12.6
7 WK	27.32	28.83	5.2

[a]Each value is a mean of five replicates

[b]Percentage reduction was calculated by expressing the difference between inoculated and control as percentage of the value for the control.

APPENDIX 5(a)

MEAN PLANT HEIGHT[a] (CM) OF *CELOSIA ARGENTEA* INOCULATED WITH CELOSIA LEAF CURL VIRUS AT DIFFERENT AGES.

TREATMENT (Plant age at age at inoculation)	REPLICATION I	II	III	IV	V	TREATMENT TOTAL	TREATMENT MEAN	% REDUCTION[b]
3WK	73.9	80.9	86.7	75.5	73.9	391.1	78.2	19.1
4WK	74.2	91.8	88.6	76.2	75.5	406.3	81.3	15.1
5WK	81.1	104.9	86.6	87.2	99.2	459.3	91.8	5.1
6WK	77.9	102.9	91.0	86.7	106.8	465.3	93.1	3.7
7WK	87.4	97.8	97.6	93.1	101.8	477.7	95.5	1.2
Control	88.2	97.3	98.4	100.4	99.2	483.5	96.7	-
Block Total (Tb)	**482.7**	**575.6**	**548.9**	**519.3**	**556.4**	**2682.9 - Σx**		

[a] Values recorded are means of five replicates.

[b] Percentage reduction was calculated by expressing the difference between inoculated and control values as percentage of the value for the control

APPENDIX 5 (b)

ANALYSIS OF VARIANCE OF MEAN PLANT HEIGHT OF *CELOSIA ARGENTEA* INOCULATED WITH CELOSIA LEAF CURL VIRUS AT DIFFERENT AGES.

Sources of variation	df	SS	MS	Obs.f.	Req.f.5%
Total	29	3068.7			
Block	4	878.14	219.53	6.42	
Treatment	5	1506.96	301.39	8.82**	2.71
Error	20	683.6	34.18		

** Significant at 0.05 probability.

APPENDIX 6(a)

MEAN PLANT NUMBER[a] OF *CELOSIA ARGENTEA* INOCULATED WITH CELOSIA LEAF CURL VIRUS AT DIFFERENT AGES.

TREATMENT (Plant age at inoculation)	REPLICATION I	II	III	IV	V	TREATMENT TOTAL	TREATMENT MEAN	% REDUCTION[b]
3WK	32.5	33.0	31.8	35.4	35.3	168.0	33.6	17.0
4WK	33.3	36.0	35.4	31.4	42.4	178.5	35.7	11.9
5WK	33.0	35.5	35.2	36.4	40.0	180.1	36.0	11.1
6WK	32.2	40.2	37.5	38.2	37.0	185.1	37.0	8.6
7WK	39.8	39.5	32.8	40.4	43.4	195.9	39.2	3.2
Control	38.4	41.4	39.2	40.6	43.1	202.6	40.5	-
Block Total (Tb)	**209.2**	**225.6**	**211.9**	**222.4**	**241.1**	**1110.2 - Σx**		

[a] Values recorded are means of five replicates.

[b] Percentage reduction was calculated by expressing the difference between inoculated and control values as percentage of the value for the control

APPENDIX 6 (b)

ANALYSIS OF VARIANCE OF MEAN LEAF NUMBER OF *CELOSIA ARGENTEA* INOCULATED WITH CELOSIA LEAF CURL VIRUS AT DIFFERENT AGES.

Sources of variation	df	SS	MS	Obs.F.	Req.F.5%
Total	29	371.7			
Block	4	107.3	26.83	4.99	
Treatment	5	156.77	31.35	5.83**	2.71
Error	20	107.63	5.38		

** Significant at 0.05 probability.

APPENDIX 7(a)

MEAN TOP FRESH WEIGHT[a] (g) OF *CELOSIA ARGENTEA* INOCULATED WITH CELOSIA LEAF CURL VIRUS AT DIFFERENT AGES.

TREATMENT (Plant age at inoculation)	REPLICATION I	II	III	IV	V	TREATMENT TOTAL	TREATMENT MEAN	% REDUCTION[b]
3WK	63.6	58.5	63.2	60.8	64.9	311.0	62.2	34.0
4WK	65.4	68.6	67.5	64.0	66.0	332.0	64.4	29.7
5WK	90.2	93.0	90.3	89.9	92.4	455.8	91.2	3.5
6WK	80.6	94.0	91.8	87.1	95.3	448.8	87.8	7.1
7WK	95.9	90.6	94.7	93.5	94.0	468.5	93.7	0.8
Control	94.0	92.8	94.3	96.7	94.8	472.6	94.5	-
Block Total (Tb)	**489.5**	**497.5**	**501.8**	**492.0**	**507.9**	**2488 - Σx**		

[a] Values recorded are means of five replicates.

[b] Percentage reduction was obtained by expressing the difference between inoculated and control values as percentage of the value for the control

APPENDIX 7 (b)

ANALYSIS OF VARIANCE OF MEAN TOP FRESH WEIGHT OF *CELOSIA ARGENTEA* INOCULATED WITH CELOSIA LEAF CURL VIRUS AT DIFFERENT AGES.

Sources of variation	df	SS	MS	Obs.F.	Req.F.5%
Total	29	5551.91			
Block	4	36.77	9.19	1.04	
Treatment	5	5338.36	1067.67	120.78**	2.71
Error	20	176.78	8.84		

** Significant at 0.05 probability.

APPENDIX 8(a)

MEAN PLANT DRY WEIGHT[a] (g) OF *CELOSIA ARGENTEA* INOCULATED WITH CELOSIA LEAF CURL VIRUS AT DIFFERENT AGES.

TREATMENT (Plant age at inoculation)	REPLICATION I	II	III	IV	V	TREATMENT TOTAL	TREATMENT MEAN	% REDUCTION[b]
3WK	4.3	3.6	4.2	4.6	4.1	20.8	4.2	28.3
4WK	4.4	5.8	5.5	4.4	5.6	25.7	5.1	29.6
5WK	6.1	6.4	6.0	5.9	6.4	30.8	6.2	8.7
6WK	5.1	6.5	5.9	5.5	6.5	29.5	5.9	5.0
7WK	6.6	6.1	6.4	6.4	6.3	31.8	6.4	5.0
Control	6.3	6.2	6.5	6.8	6.5	32.3	6.5	-
Block Total (Tb)	**32.8**	**34.6**	**34.5**	**33.6**	**35.4**	**170.9 - Σx**		

[a] Values recorded are means of five replicates.

[b] Percentage reduction was obtained by expressing the difference between inoculated and control values as percentage of the value for the control

APPENDIX 8 (b)

ANALYSIS OF VARIANCE OF MEAN PLANT DRY WEIGHT OF *CELOSIA ARGENTEA* INOCULATED WITH CELOSIA LEAF CURL VIRUS AT DIFFERENT AGES.

Sources of variation	df	SS	MS	Obs.F.	Req.F.5%
Total	29	24.23			
Block	4	0.67	0.17	0.89	
Treatment	5	19.75	3.95	20.79**	2.71
Error	20	3.81	0.19		

** Significant at 0.05 probability.

APPENDIX 9(a)

MEAN FRESH WEIGHT[a] (g) OF LEAVES OF *CELOSIA ARGENTEA* PLANTS INOCULATED WITH CELOSIA LEAF CURL VIRUS AT DIFFERENT AGES.

TREATMENT (Plant age at inoculation)	REPLICATION I	II	III	IV	V	TREATMENT TOTAL	TREATMENT MEAN	% REDUCTION[b]
3WK	27.7	29.1	26.2	25.1	27.2	135.3	27.1	35.4
4WK	26.6	27.9	28.1	24.7	25.9	133.2	26.6	21.5
5WK	35.3	33.6	33.2	35.9	34.6	172.6	34.5	4.6
6WK	32.1	36.6	37.1	35.6	37.6	179.7	35.9	9.2
7WK	36.0	35.1	36.1	34.6	37.6	179.4	35.9	1.5
Control	38.4	35.5	37.4	38.5	38.8	188.6	37.8	-
Block Total (Tb)	**196.1**	**197.8**	**198.1**	**194.4**	**202.4**	**988 - Σx**		

[a] Values recorded are means of five replicates.

[b] Percentage reduction was calculated by expressing the difference between inoculated and control values as percentage of the value for the control

APPENDIX 9 (b)

ANALYSIS OF VARIANCE OF MEAN LEAF FRESH WEIGHT OF *CELOSIA ARGENTEA* INOCULATED WITH CELOSIA LEAF CURL VIRUS AT DIFFERENT AGES.

Sources of variation	df	SS	MS	Obs.F.	Req.F.5%
Total	29	643.31			
Block	4	5.95	1.49	0.58	
Treatment	5	586.25	117.25	45.8**	2.71
Error	20	51.11	2.56		

** Significant at 0.05 probability.

APPENDIX 10(a)

MEAN PLANT DRY WEIGHT[a] (g) OF *CELOSIA ARGENTEA* INOCULATED WITH CELOSIA LEAF CURL VIRUS AT DIFFERENT AGES.

TREATMENT (Plant age at inoculation)	REPLICATION I	II	III	IV	V	TREATMENT TOTAL	TREATMENT MEAN	% REDUCTION[b]
3WK	2.1	1.4	1.3	1.8	2.0	8.6	1.7	37.0
4WK	1.2	2.5	2.1	1.6	2.2	9.6	1.9	29.6
5WK	2.9	2.7	2.2	2.6	2.8	13.2	2.6	3.7
6WK	1.8	2.4	2.2	2.4	3.6	12.4	2.5	7.4
7WK	2.2	2.2	2.6	2.3	3.3	12.6	2.5	7.4
Control	2.8	2.5	2.7	2.5	3.2	13.7	2.7	-
Block Total (Tb)	**13.0**	**13.7**	**13.1**	**13.2**	**17.1**	**70.1 - Σx**		

[a] Values recorded are means of five replicates.

[b] Percentage reduction was calculated by expressing the difference between inoculated and control values as percentage of the value for the control

APPENDIX 10 (b)

ANALYSIS OF VARIANCE OF MEAN LEAF DRY WEIGHT OF *CELOSIA ARGENTEA* INOCULATED WITH CELOSIA LEAF CURL VIRUS AT DIFFERENT AGES.

Sources of variation	df	SS	MS	Obs.F.	Req.F.5%
Total	29	9.19			
Block	4	2.03	0.51	3.64	
Treatment	5	4.31	0.86	6.14**	2.71
Error	20	2.85	0.14		

** Significant at 0.05 probability.

APPENDIX 11 (a)

NUMBER[a] OF STAMINATE FLOWERS PRODUCED BY *CUCURBITA MOSCHATA* INOCULATED WITH CUCURBITA MOSAIC VIRUS AT THREE GROWTH STAGES (DATA WERE COLLECTED 6 WEEKS AFTER PLANTING)

Treatment number	Growth stage at incoulation	REPLICATION I	II	III	Treatment total (Tt)	Treatment mean
1	First true leaf	4.0	7.8	4.8	16.6	5.5
2	Vegetative	4.8	6.6	7.6	19.0	6.3
4	Control	4.9	7.4	6.5	18.8	6.3
Block Total (Tb)		**13.7**	**21.8**	**18.9**	**54.2** → **Σx**	

[a] Each value is a mean of three replicates

Data were not collected for treatment 3 (first perfect flower stage) so as not to disturb newly set fruits.

APPENDIX 11 (b)

ANALYSIS OF VARIANCE (ANOVA) OF STAMINATE FLOWERS PRODUCED PER PLANT OF CUCURBITA MOSCHATA INOCULATED WITH CUCURBITA MOSAIC VIRUS.

Sources of variation	df	ss	ms	obs. F	Reg. F (5%)
Total	8	16.44			
Block	2	11.23	5.62	2.33	6.94
Treatment	2	1.18	0.59	0.24**	6.94
Error	4	9.64	2.41		

*** Not significant at 0.05 probability.**

APPENDIX 12 (a)

NUMBER[a] OF STAMINATE FLOWERS PRODUCED BY *CUCURBITA MOSCHATA* INOCULATED WITH CUCURBITA MOSAIC VIRUS AT THREE GROWTH STAGES (DATA WERE COLLECTED 7 WEEKS AFTER PLANTING)

Treatment Treatment number	Growth stage at incoulation	REPLICATION I	II	III	Treatment total (Tt)	Treatment mean
1	First true leaf	8.3	13.0	8.6	29.9	10.0
2	Vegetative	9.4	11.8	12.6	33.8	11.3
4	Control	13.2	12.5	11.7	37.4	12.5
Block Total (Tb)		**30.9**	**37.3**	**32.9**	**101.1**	→ **Σx**

[a]Each value is a mean of three replicates

Data were not collected for treatment 3 (first perfect flower stage) so as not to disturb newly set fruits.

APPENDIX 12 (b)

ANALYSIS OF VARIANCE (ANOVA) OF STAMINATE FLOWERS PRODUCED PER PLANT OF *CUCURBITA MOSCHATA* INOCULATED WITH CUCURBITA MOSAIC VIRUS.

Sources of variation	df	SS	MS	Obs. F	Reg. F (5%)
Total	8	29.9			
Block	2	7.15	3.58	1.07	6.94
Treatment	2	9.38	4.69	1.40**	6.94
Error	4	13.37	3.34		

** **Not significant at 0.05 probability.**

APPENDIX 13 (a)

NUMBER[a] OF STAMINATE FLOWERS PRODUCED BY CUCURBITA MOSCHATA INOCULATED WITH CUCURBITA MOSAIC VIRUS AT THREE GROWTH STAGES (DATA WERE COLLECTED 8 WEEKS AFTER PLANTING)

Treatment number	Growth stage at incoulation	REPLICATION I	II	III	Treatment total (Tt)	Treatment mean
1	First true leaf	12.0	19.0	13.5	44.5	14.8
2	Vegetative	14.4	15.6	17.0	47.0	15.7
4	Control	14.0	16.8	16.2	47.0	15.7
Block Total (Tb)		**40.4**	**51.4**	**46.7**	**138.5**	→ **Σx**

[a]Each value is a mean of three replicates

Data were not collected for treatment 3 (first perfect flower stage) so as not to disturb newly set fruits.

APPENDIX 13 (b)

ANALYSIS OF VARIANCE (ANOVA) OF STAMINATE FLOWERS PRODUCED PER PLANT OF *CUCURBITA MOSCHATA* INOCULATED WITH CUCURBITA MOSAIC VIRUS.

Sources of variation	df	SS	MS	Obs. F	Reg. F (5%)
Total	8	36.29			
Block	2	20.31	10.16	2.85	6.94
Treatment	2	1.39	0.70	0.24**	6.94
Error	4	13.99	3.57		

** **Not significant at 0.05 probability.**

APPENDIX 14 (a)

NUMBER[a] OF STAMINATE FLOWERS PRODUCED BY CUCURBITA MOSCHATA INOCULATED WITH CUCURBITA MOSAIC VIRUS AT THREE GROWTH STAGES (DATA WERE COLLECTED 9 WEEKS AFTER PLANTING)

Treatment number	Growth stage at incoulation	REPLICATION I	II	III	Treatment total (Tt)	Treatment mean
1	First true leaf	12.8	21.6	16.5	51.0	17.0
2	Vegetative	17.8	20.4	21.4	59.6	19.9
4	Control	17.4	20.3	20.0	57.7	19.2
Block Total (Tb)		**48.0**	**62.3**	**58.0**	**168.3**	→ **Σx**

[a]Each value is a mean of three replicates

Data were not collected for treatment 3 (first perfect flower stage) so as not to disturb newly set fruits.

APPENDIX 14 (b)

ANALYSIS OF VARIANCE (ANOVA) OF STAMINATE FLOWERS PRODUCED PER PLANT OF *CUCURBITA MOSCHATA* INOCULATED WITH CUCURBITA MOSAIC VIRUS.

Sources of variation	df	SS	MS	Obs. F	Reg. F (5%)
Total	8	64.56			
Block	2	35.89	17.94	4.76	6.94
Treatment	2	13.61	6.81	1.81**	6.94
Error	4	15.06	3.77		

** **Not significant at 0.05 probability.**

APPENDIX 15 (a)

NUMBER[a] OF STAMINATE FLOWERS PRODUCED BY CUCURBITA MOSCHATA INOCULATED WITH CUCURBITA MOSAIC VIRUS AT THREE GROWTH STAGES (DATA WERE COLLECTED 8 WEEKS AFTER PLANTING)

Treatment number	Growth stage at incoulation	REPLICATION I	II	III	Treatment total (Tt)	Treatment mean
1	First true leaf	0.8	0.8	0.2	1.8	0.9
2	Vegetative	0.8	0.8	0.6	2.2	0.7
4	Control	0.6	1.1	1.0	2.7	1.4
Block Total (Tb)		**2.2**	**2.7**	**1.8**	**6.7**	→ **Σx**

[a]Each value is a mean of three replicates

Data were not collected for treatment 3 (first perfect flower stage) so as not to disturb newly set fruits.

APPENDIX 15 (b)

ANALYSIS OF VARIANCE (ANOVA) OF STAMINATE FLOWERS PRODUCED PER PLANT OF *CUCURBITA MOSCHATA* INOCULATED WITH CUCURBITA MOSAIC VIRUS.

Sources of variation	df	SS	MS	Obs. F	Reg. F (5%)
Total	8	0.54			
Block	2	0.13	0.065	0.93	6.94
Treatment	2	0.13	0.065	0.93**	6.94
Error	4	0.28	0.07		

** **Not significant at 0.05 probability.**

APPENDIX 16 (a)

NUMBER[a] OF STAMINATE FLOWERS PRODUCED BY CUCURBITA MOSCHATA INOCULATED WITH CUCURBITA MOSAIC VIRUS AT THREE GROWTH STAGES (DATA WERE COLLECTED 9 WEEKS AFTER PLANTING)

Treatment number	Growth stage at incoulation	REPLICATION I	II	III	Treatment total (Tt)	Treatment mean
1	First true leaf	1.4	1.2	0.8	3.4	1.1
2	Vegetative	1.0	1.1	1.0	3.1	1.0
4	Control	1.3	1.9	1.6	4.8	1.6
Block Total (Tb)		**3.7**	**4.2**	**3.4**	**11.3** →	**Σx**

[a]Each value is a mean of three replicates
Data were not collected for treatment 3 (first perfect flower stage) so as not to disturb newly set fruits.

APPENDIX 16 (b)

ANALYSIS OF VARIANCE (ANOVA) OF STAMINATE FLOWERS PRODUCED PER PLANT OF *CUCURBITA MOSCHATA* INOCULATED WITH CUCURBITA MOSAIC VIRUS.

Sources of variation	df	SS	MS	Obs. F	Reg. F (5%)
Total	8	0.92			
Block	2	0.11	0.06	0.86	6.94
Treatment	2	0.55	0.27	3.86	6.94
Error	4	0.26			

** **Not significant at 0.05 probability.**

APPENDIX 17 (a)

MEAN FRUIT NUMBER[a] PER PLANT OF CUCURBITA MOSCHATA INOCULATED WITH CUCURBITA MOSAIC VIRUS AT THREE GROWTH STAGES

Treatment number	Growth stage at incoulation	REPLICATION I	II	III	Treatment total (Tt)	Treatment mean	%b Reduction
1	First true leaf	1.33	1.66	1.00	3.99	1.33	24.9
2	Vegetative	2.00	1.00	2.00	5.00	1.67	5.6
3	First perfect flower	1.66	1.66	1.33	4.65	1.55	12.4
4	Control	1.66	2.33	1.33	5.32	1.77	-
Block Total (Tb)		**6.65**	**6.65**	**5.68**	**18.96**	→ **Σx**	

[a]Each value is a mean of three replicates

[b]Percentage reduction

APPENDIX 17 (b)

ANALYSIS OF VARIANCE (ANOVA) OF FRUIT NUMBER PER PLANT OF *CUCURBITA MOSCHATA* INOCULATED WITH CUCURBITA MOSAIC VIRUS.

Sources of variation	df	SS	MS	Obs. F	Reg. F (5%)
Total	11	1.80			
Block	2	0.16	0.08	0.36	5.14
Treatment	3	0.32	0.11	0.5**	4.76
Error	6	1.32	0.22		

** **Not significant at 0.05 probability.**

APPENDIX 18 (a)

MEAN FRUIT WEIGHT[a] PER PLANT OF CUCURBITA MOSCHATA INOCULATED WITH CUCURBITA MOSAIC VIRUS AT THREE GROWTH STAGES

Treatment number	Growth stage at incoulation	REPLICATION I	II	III	Treatment total (Tt)	Treatment mean	%b Reduction
1	First true leaf	0.41	0.36	0.47	1.24	0.41	38.8
2	Vegetative	0.41	0.38	0.37	1.16	0.37	44.8
3	First Fruit Flower	0.53	0.42	0.55	1.50	0.50	25.4
4	Control	0.65	0.66	0.70	2.01	0.67	-
Block Total (Tb)		**2.00**	**1.82**	**2.09**	**5.91**	→ **Σx**	

[a]Each value is a mean of three replicates

[b]Percentage reduction

APPENDIX 18 (b)

ANALYSIS OF VARIANCE (ANOVA) OF FRUIT WEIGHT PER PLANT OF *CUCURBITA MOSCHATA* INOCULATED WITH CUCURBITA MOSAIC VIRUS.

Sources of variation	df	SS	MS	Obs. F	Reg. F (5%)
Total	11	1.17			
Block	2	0.01	0.005	5.1	5.14
Treatment	3	0.15	0.05	50.1**	4.76
Error	6	0.0059	0.001		

** **Significant at 0.05 probability.**

APPENDIX 19

ANALYSIS OF VARIANCE (ANOVA) OF PERCENTAGE OF TOTAL NUMBER OF FRUIT PER PLANT IN EACH SIZE CLASS PRODUCED BY FIELD GROWTH *CUCURBITA MOSCHATA* INOCULATED WITH CUCURBITA MOSAIC VIRUS AT THREE GROWTH STAGES.

Sources of variation	df	SS	MS	Obs. F	Reg. F (5%)
Total	19	5597.13			
Block	4	4277.4	1069.35	9.72*	3.26
Treatment	3	0.004	0.0013	0.0001	3.49
Error	12	1319.73	109.98		

** **Significant at 0.05 probability.**

APPENDIX 20

MEAN VALUES (%) OF POTASSIUM, SODIUM, PHOSPHORUS AND NITROGEN IN CELOSIA ARGENTEA INOCULATED WITH CELOSIA LEAF CURL VIRUS

	POTASSIUM			SODIUM			PHOSPHORUS			NITROGEN		
Week after inoculation	Infected	Control	% difference[a]	Infected	Control	% difference[a]	Infected	Control	% difference[a]	Infected	Control	% difference[a]
3	8.0	7.97	0.4*	0.52	0.60	13.3**	0.90	0.86	4.4*	1.27	0.40	68.5*
4	6.26	4.78	23.6*	0.39	0.31	20.5*	0.76	0.75	1.3*	1.82	0.90	50.5*
5	6.54	4.04	38.2*	0.41	0.39	4.8*	0.58	0.42	16.0*	1.75	0.50	71.4*
6	3.93	4.05	3.0**	0.42	0.36	14.3*	0.32	0.31	3.1	2.87	1.20	50.2*

Each value is a mean of 3 replicates.

[a] Values were obtained by expressing the difference between inoculation and control as percentage of the value for the control (the higher value)

* Value for infected greater than control

** Value for control greater than infected

APPENDIX 21

MEAN VALUES (%) OF CRUDE PROTEIN, ETHER EXTRACT (FAT) AND CRUDE FIBRE IN CELOSIA ARGENTEA INOCULATED WITH CELOSIA LEAF CURL VIRUS.

Week after inoculation	CRUDE PROTEIN			ETHER EXTRA (FAT)			CRUDE FIBRE		
	Infected	Control	% difference[a]	Infected	Control	% difference[a]	Infected	Control	% difference[a]
3	16.92	14.0	17.3*	2.11	1.60	24.2*	17.62	20.39	13.6**
4	13.48	12.9	14.3*	1.13	1.70	23.5**	21.86	20.99	0.4*
5	14.58	13.12	10.1*	1.58	1.78	11.2**	17.02	22.3	23.7**
6	17.5	16.23	7.1*	1.36	1.54	10.5**	19.67	23.0	14.5**

Each value is a mean of 3 replicates.

[a] Values were obtained by expressing the difference between inoculation and control as percentage of the value for the control

* = Value for infected greater than control

** = Value for control greater than infected

APPENDIX 22

MEAN VALUES (%) OF POTASSIUM, SODIUM, PHOSPHORUS AND NITROGEN IN CUCURBITA MOSAIC VIRUS *CUCURBITA MOSCHATA*

Week after inoculation	POTASSIUM			SODIUM			PHOSPHORUS			NITROGEN		
	Infected	Control	% difference[a]	Infected	Control	% difference[a]	Infected	Control	% difference	Infected	Control	% difference[a]
3	3.47	2.65	23.6*	0.37	0.30	18.9*	0.61	0.36	41.0*	0.67	0.58	13.4*
4	4.61	3.25	31.6*	0.34	0.30	11.8*	0.40	0.29	27.5*	1.10	0.85	22.7*
5	4.75	3.01	36.6*	0.39	0.35	10.5*	0.35	0.28	20.0*	1.02	0.81	20.6*
6	4.52	3.10	31.4*	0.35	0.31	11.4	0.31	0.24	22.6*	1.12	1.02	8.9*

Each value is a mean of 3 replicates.

[a] Values were obtained by expressing the difference between inoculation and control as percentage of the value for the control.

* = Value for infected greater than control

APPENDIX 23

MEAN VALUES (%) OF CRUDE PROTEIN, ETHER EXTRACT (FAT) AND

Week after inoculation	CRUDE PROTEIN % Infected	% Control	difference[a]	ETHER EXTRA (FAT) % Infected	% Control	difference[a]	CRUDE FIBRE % Infected	% Control	difference[a]
3	22.6	20.7	8.4*	2.24	2.04	8.9*	8.86	9.21	3.8*
4	26.8	21.4	20.1*	2.19	2.34	6.4**	7.89	8.11	2.7**
5	29.6	22.1	25.3*	2.45	2.82	13.2**	7.55	8.38	9.9**
6	24.5	21.9	10.6*	2.55	2.80	8.9**	7.62	8.41	9.4**

Each value is a mean of 3 replicates.

[a] Values were obtained by expressing the difference between inoculation and control as percentage of the value for the control.

* = Value for infected greater than control.

** = Value for control greater than infected.