DETECTION AND SURVEY OF BLACKEYE COWPEA MOSAIC VIRUS IN ZARIA AND ENVIRONS

BONIFACE DAVID KASHINA
B. AGRIC. TECH. (HONS), AKURE, NIGERIA

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DEPARTMENT OF CROP PROTECTION
FACULTY OF AGRICULTURE
AHMADU BELLO UNIVERSITY
ZARIA, NIGERIA

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DECLARATION

I hereby declare that this thesis has been written by me and that it is a record of my own research work. It has not been presented before in any previous application for a higher degree.

.....
Boniface David KASHINA
M.Sc. Candidate

Date: 28-2-97

The above declaration is confirmed.

.....
Dr. Matthew Dada Alegbejo
Major Supervisor

Date: 3-3-97

CERTIFICATION

This thesis entitled "DETECTION AND SURVEY OF BLACKEYE COWPEA MOSAIC VIRUS IN ZARIA AND ENVIRONS" by Boniface David KASHINA meets the regulations governing the degree of Master of Science of Ahmadu Bello University, Zaria, and is approved for its contribution to scientific knowledge and literary presentation.

..... Date: 3-3-97
Dr. M.D. Alegbejo
(Chairman, Supervisory Committee)

..... Date: 2/03/97
Dr. I. Onu
(Member, Supervisory Committee)

..... Date: 3/3/97
Dr. C.I. Amatobi
(Member, Supervisory Committee)

..... Date: 4/03/97
Dr. I. Onu
(Head of Department)

..... Date: 22/09/97
(Dean, Postgraduate School)

DEDICATION

To my parents, Mr. and Mrs. David Kashina, and the entire Kashina family for their prayer, love and encouragement.

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ABSTRACT

A two-year survey of the incidence of blackeye cowpea mosaic virus (B1CMV) in Zaria and its environs revealed that the virus is widespread. Incidence levels and distribution of serotypes varied from location to location and year to year.

When forty cowpea lines were screened for resistance against the virus, results obtained from glasshouse screening were more consistent than those obtained from field screening. Twenty-one lines tested positive to both serotypes of the virus in the glasshouse while twenty and twenty one lines respectively tested positive during the field screening in 1993 and 1994 respectively. Assessment of the reaction of the forty cowpea lines to BICMV infection indicated that twenty were resistant, eighteen were moderately susceptible and only two were highly susceptible. Significant yield loss was recorded only in three cowpea lines: IT 86^{0}-715, TVU 1582 and KVX 402-5-2.

Infectivity of the virus was lost at temperatures above 60^{c}c; at a dilution of above 10 and after storage of virus sap for more than 54 hours at room temperature (25°c).

The virus was transmitted by Aphis craccivora Koch, in a non-persistent manner. It was acquired after 15 seconds of feeding on virus source. The virus was also transmitted after 15 second of feeding on susceptible cowpea plants. Similarly, preliminary fasting for 15 to 30 minutes was efficient in increasing the efficiency of transmission.

It is concluded that BICMV is widespread and therefore requires more study for incorporating its resistance into cowpea.

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CHAPTER ONE

INTRODUCTION

Cowpea, Vigna unguiculata (L.) Walp. is grown in all the continents of the world (Lana et al., 1988). It forms the major protein-producing food legume in tropical Africa (Rachie, 1972). In Nigeria, it is an important source of plant protein in many diets and is virtually the next economically important food legume to groundnut (Ladipo, 1976; Rossel, 1977). The seedlings, tender green leaves, immature and unripe green pods are cooked as vegetables whereas, the mature dry seeds are used in many preparations as a pulse. The seeds are highly nutritious and relatively free of antimetabolites and other toxins (Rachie, 1972).

In 1970, the major cowpea growing countries in tropical Africa - Nigeria, Burkina Faso, Niger and Uganda together produced 1.03 million metric tonnes on 2.5 million hectares of world production and area respectively (Rachie, 1972). Yields can be reasonably high but because the crop is susceptible to many pests and diseases (Thottappilly and Rossel, 1992), grain yields are often low particularly under farmers' field conditions (Rachie, 1972).

Of the numerous pathogens of cowpea, viruses constitute a major constraint to large scale production in several African countries (Thottappilly, 1992). A reduction in seed yield of 40% due to early infection by yellow mosaic virus (CYMV), was reported by Bliss and Robertson (1971) in New Era cowpea. Thottappilly and Rossel (1985; 1992) reported at least 20 viruses known to be infecting cowpea. Among these viruses, blackeye cowpea mosaic

virus (BlCMV) and cowpea aphid-borne mosaic virus (CAbMV) (Lana et al., 1988) are considered very important and widespread.

Cowpea aphid-borne mosaic and blackeye cowpea mosaic potyviruses, cause very important virus diseases of cowpea in Africa. In Iran, Kaiser and Mossahebi (1975), reported yield losses of 15 - 18% due to CAbMV infection. A complete loss of an irrigated cowpea crop in northern Nigeria previously attributed to CAbMV (Raheja and Leleji, 1974) was later proved to be due to combine infection by CAbMV and cowpea golden mosaic virus (Rossel, 1977).

Blackeye cowpea mosaic virus, a potyvirus, has been reported in various parts of the world. It was first reported in Florida, U.S.A. (Anderson, 1955a; Lima et al., 1979; Purcifull and Gonsalves, 1985). Since then, CAbMV/BlCMV has been reported in many African countries: Kenya (Bock, 1973), Nigeria (Ladipo, 1976), Morocco (Fischer and Lockhart, 1976a), Tanzania (Patel and Kuwite, 1982), Uganda (Anonymous, 1981), Zambia (Anonymous, 1981), Egypt (Anonymous, 1981), Togo (Gumedzoe et al., 1989) and Botswana (Burke et al., 1986).

Documented information on BlCMV is either sparingly available or completely lacking, particularly in Zaria. BlCMV which up till 1982 was considered a strain of CAbMV, is now known to be distinctly different from CAbMV. The two viruses vary in identity and in their reactions to cowpea varieties (Taiwo et al., 1982). Lima et al. (1979), suggested that BlCMV and CAbMV should be treated as distinct members of the potyvirus group owing to their

differences in host range and distant serological relationship. The suggestion was accepted by the International Committee on the Taxonomy of viruses (Taiwo et al., 1982).

BlCMV induces different reactions which are symptomatically manifested by different hosts. The symptoms can be systemic or local lesions. Systemic symptoms include severe mottling, distortion, yellowing, mosaic and vein necrosis (Thottappilly and Rossel, 1992). It is readily transmitted by sap inoculation and non-persistently by aphids (Anderson, 1955b; Mali and Kulthe, 1980). 1985). It is also seed transmissible depending on the virus strain and cowpea cultivar (Zettler and Evans, 1972; Mali et al, 1983).

Due to the inevitable need for proper understanding of the epidemiology of BlCMV; for a clear distinction between its pathogenic strains and unambiguous identification for resistance breeding, the following constitute the objectives of this study:

- Detection and identication of serotypes of the virus and their relative distribution using monoclonal antibodies.
- Assessment of the distribution and spread of the virus in farmers' fields in Zaria and environs
- Screening cowpea varieties in the glasshouse and field for resistance to BICMV
- The physical properties of the virus
- The vector-virus relationships

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 GENERAL

More than twenty viruses are known to infect cowpea in different cowpea-producing areas. Very many of them are of local importance, and others occasionally occur as natural infections of minor significance.

The major cowpea viruses that readily come to mind include:

2.1.1 COWPEA MOSAIC VIRUS.

cowpea mosaic virus (CpMV) which is considered one of the most important virus diseases in Africa owing to its widespread occurrence, epidemic potential and severity. Symptoms produced by the virus depend on the cowpea variety and virus isolate. Systemic symptoms in susceptible varieties range from an inconspicuous light-green mottle to a distinct yellow mosaic, leaf distortion with significantly reduced growth, and premature death of the plant (Bliss and Robertson, 1971). The virus is readily sap transmissible and by various beetles (Thottappilly and Rossel, 1985). CPMV is characterised by isometric particles averaging 24nm in diameter, having two kinds of nucleoprotein particles that are similar in size but differ in their single-stranded RNA content. In addition, particles without RNA are also produced by most isolates (Thottappilly and Rossel, 1985).

2.1.2 COWPEA SEVERE MOSAIC VIRUS.

Cowpea severe mosaic virus (CSMV) on inoculated leaves of

cowpea develop chlorotic or necrotic lesions. Portions of the major veins turn red and become necrotic. Trifoliate leaves show severe mosaic with blistering, distortion and necrosis. Plants inoculated as young seedlings show necrosis of the epicotyl just below the primary leaves and then collapse. However, Agrawal (1964) and Shepherd (1964) observed that isolates of the Arkansas-type do not induce systemic necrosis, mosaic or distortion but induce pronounced vein-clearing on lower leaves. About ten species of beetles have been implicated by many workers as vectors of the virus (Thottappilly and Rossel, 1985). Haque and Persad (1975) have recorded 3.3% and 5.8% seed transmission in four of seven cowpea selections in Trinidad. The virus is characterised by isometric particles of approximately 25nm in diameter. It has two kinds of morphologically identical nucleoprotein particles containing different single-stranded RNA molecules whose presence is necessary for infection to occur (De Jager, 1979).

2.1.3 COWPEA APHID-BORNE MOSAIC VIRUS.

Symptoms induced by cowpea aphid-borne mosaic virus (CAbMV) include severe mosaic, the severity being dependent on the interactions between host cultivar and virus strain. Infected plants show dark-green vein-banding, leaf distortion, blistering and stunting (Bock and Conti, 1974). The European strain (type) causes a severe distorting mosaic in cowpea (Lovisolo and Conti, 1966). The African stain (neo-type) induces irregular, angular broken mosaic, and the African vein-banding strain induces a broad dark-green vein-banding (Bock, 1973). CAbMV is readily transmitted

by sap inoculation and by aphids in a non-persistent manner (Atiri, 1982). It is characterised by flexuous, filamentous particles about 750nm long.

2.1.4 COWPEA CHLOROTIC MOTTLE VIRUS.

Cowpea plants infected with cowpea chlorotic mottle virus (CCMV), usually develop bright yellow mottling which can be distinct chlorotic rings, mosaic-like areas or complete and uniform yellowing of leaflets. The virus is sap and beetle-transmitted (Hobbs and Fulton, 1979). CCMV particles are isometric and about 25nm in diameter (Bancroft et al., 1968).

2.1.5 COWPEA MOTTLE VIRUS.

Symptoms induced on cowpea by cowpea mottle virus (CMeV) include distortion and reduction in leaf size and a witches' broom syndrome. It is readily transmitted by sap and by insects, especially beetles. Shoyinka et al., (1978) recorded up to 10% seed transmission and 7% from naturally and artificially inoculated plants respectively. CMeV particles are isometric and measure about 30nm in diameter. They contain single-stranded RNA.

2.1.6 CUCUMBER MOSAIC VIRUS-COWPEA STRAIN.

The cowpea strain of cucumber mosaic virus, CuMV-CS, produces poorly developed chlorotic areas or reddish necrotic rings on cowpea. Systemic symptoms are mild mottle and distortion. A few varieties develop severe mottle, distortion and considerable reddish vein necrosis. Anderson (1955b), noted that symptoms in most varieties are mild or partial. CuMV-CS is seed-borne to the range of 4-26% in cowpea (Fischer and Lockhart, 1976b), and seed

transmission varies with cultivar in the range of 3.5 - 31.5% (Mali et al., 1983). The virus is also transmitted by aphid vectors (Fischer and Lockhart, 1976b). It has isometric particles about 28nm in diameter containing single-stranded RNA with tripartite genome (Francki et al., 1979).

2.1.7 COWPEA MILD MOTTLE VIRUS.

Cowpea plants infected naturally by cowpea mild mottle virus (CMMV), exhibit either mild systemic mottle or they are symptomless, but inoculated plants of susceptible cultivars develop necrotic lesions on primary leaves and severe systemic chlorosis and necrosis on trifoliate leaves (Thottappilly and Rossel, 1985). CMMV isolates are transmitted by the whitefly vector, Bemisia tabaci Genn. either in a semi-persistent manner (Iwaki et al., 1982) or non-persistent manner (Muniyappa and Reddy, 1983). CMMV is seed-borne in cowpea, soyabean and French bean but not in Nicotiana clevelandii, the level being higher in cowpea and soyabean (90%) than in French bean (15%) (Brunt and Kenten, 1973). The virus has straight to slightly flexuous, fragile filamentous particles (650 x 13nm in diameter).

2.1.8 SUNHEMP MOSAIC VIRUS.

Symptom expression by cowpea varieties due to sunhemp mosaic virus (SHMV), varies among isolates. SHMV has rigid rod-shaped particles 300nm long and contains single-stranded RNA (Kassanis and Varma, 1975). No potential vector has been reported.

2.1.9 SOUTHERN BEAN MOSAIC VIRUS.

Primary leaves inoculated with southern bean mosaic virus

(SBMV) are symptomless; occasionally chlorotic lesions appear and ring spots may form. Systemic symptoms include vein-clearing followed by mild to severe mottling or coarse mosaic pattern on the leaves. The cowpea strain of SBMV is seed-borne in the range of 3 to 4% (Shepherd and Fulton, 1962). The bean leaf beetle, Cerotoma trifurcata Forster has been reported to be an efficient vector of the virus (Walters and Henry, 1970). SBMV has isometric particles 28-30nm in diameter and contains a single-stranded RNA (Shepherd, 1971). It is readily sap transmissible but infectivity is confined to the leguminosae family.

2.1.10 **GOLDEN YELLOW MOSAIC VIRUS.**

Cowpea plants infected with cowpea golden yellow mosaic virus (CGYMV), have large bright yellow patches on their leaves and, under severe infection, the entire leaf surface turns bright yellow. The virus is transmitted by whitefly. So far, no information is available on virus particles (Thottappilly and Rossel, 1985).

2.1.11 **COWPEA STUNT VIRUS.**

Cowpea stunt virus (CSV) causes severe stunting of field-infected plants; leaves are small, mottled, blistered and malformed (Pio-Ribeiro et al., 1978). The virus is transmissible by sap inoculation and by aphid vectors. However, the CSV reported from Indonesia is distinct from that of U.S.A. or Morocco because it is transmitted by aphid vectors only and not by sap inoculation; and the particles have not been observed (Iwaki, 1979).

2.1.12 BLACKEYE COWPEA MOSAIC VIRUS

Blackeye cowpea mosaic virus (BlCMV) causes both local and systemic symptoms on cowpea. Local symptoms include large reddish lesions that spread along the veins. Systemic symptoms include severe mottling, distortion, yellowing, mosaic and vein necrosis. BICMV is readily sap transmissible and is transmitted by Macrosiphum solanifolii Ashom, Aphis gossypii Glov., Aphis craccivora Koch and Myzus persicae Sulzer (Anderson, 1955a; Mali and Kulthe, 1980; Pio-Ribeiro et al., 1978). BlCMV is also seed transmitted; the percentage ranging from 3.5 to 55 and is dependent upon the cowpea cultivar and virus isolate interaction (Anderson, 1955b; Gay and Windstead, 1970; Zettler and Evans, 1972; Mali et al., 1983). The virus is characterised by flexuous filamentous particles of about 750nm (Lima et al., 1979; Pio-Ribeiro et al., 1978; Taiwo et al., 1981) and resistance has been found to be conditioned by a single recessive allele (Walker and Chambliss, 1981).

2.2 DIAGNOSIS

Quite a number of criteria for diagnosing of BICMV infection exist. Notable among these are:

2.2.1 VARIETAL REACTIONS

A knowledge of varietal reactions and symptoms expressed by different hosts is an essential tool used in the identification of plant viruses (Bos, 1970).

Symptoms produced by BlCMV infection vary with the host cultivar. Symptoms can be local or systemic. Local symptoms are

usually large reddish lesions that spread along the veins while systemic symptoms include severe mottling, distortion, yellowing, mosaic and vein necrosis (Anderson, 1955b). According to Owolabi (1985), BlCMV infections in some Nigerian commercial cultivars can be so severe as to cause complete loss in yield.

2.2.2 HOST RANGE

Blackeye cowpea mosaic virus (BlCMV) has a wide experimental host range, including species in the families Leguminosae, Amaranthaceae, Caesalpinaceae, Chenopodiaceae, Labiatae and Solanaceae (Mali and Kulthe, 1980). Systemic infection was observed in plants of genera Vigna sp., Phaseolus sp., Vicia sp., Crotalaria sp., Desmodium sp. and local lesion in Glycine maximum (L.) Merrill, Dolichos sp., Cassia sp., Zinnia sp. and Chenopodium sp. (Raychandhuri, 1977).

2.2.3 PHYSICAL PROPERTIES OF CRUDE EXTRACTS

That a virus is stable in sap depends upon the virus source, vector, host plant and the conditions both physical and chemical, of the infective sap (Bos, 1970). Though many potyviruses have similar physical properties, there are distinctions that by and large aid their identification.

The physical properties of BlCMV as reported by Raychandhuri (1977) are; thermal inactivation point 50-60^{0}c, dilution end-point 1:10,000 and a longevity in-vitro of 2-3 days. In undiluted sap from infected plants, Taiwo et al, (1982), found that BlCMV had a thermal inactivation point of 57-60^{0}c, longevity in-vitro of 24-48 hours at 22^{0}c and a dilution end-point of between 10^{-4} and 10^{-5}.

2.2.4 SEROLOGY

Serological reactions indicate relationships of viruses to each other because of their high specificity and sensitivity. A well chosen serological test will edge out ambiguous identification of a virus or its strains, that may arise from closer relationships.

Taiwo and Gonsalves (1982) found in reciprocal SDS-immunodiffusion tests that BlCMV was closely related to bean common mosaic virus (BCMV) and that this relationship was closer than that between BlCMV and CAbMV. Lima et al (1979) in expanded reciprocal double immunodiffusion tests with SDS-treated antigens showed that BlCMV is serologically related to, but distinct from BCMV-BV-1, dasheen mosaic virus (DMS), lettuce mosaic virus (LMV), BCMV-siratro isolate, bean yellow mosaic virus (BYMV), PVY, SoyMV, TEV and water melon mosaic virus-2 (WMV-2).

2.3 VIRUS EPIDEMIOLOGY

2.3.1 Vector-Virus Relationship

Generally, transmission of plant viruses is through plant material and by vectors. Phytophagous insect species constitute major vector groups of plant viruses. Aphids are known to transmit by far, a larger number of plant viruses than any invertebrate vectors (Kennedy et al., 1962).

Variations in transmissibility of plant viruses has been generally observed by many workers. It has been shown that some aphid species quickly lose the ability to transmit viruses while others keep on transmitting viruses as feeding progresses all

through their life-span (Watson and Roberts, 1939; Watson, 1946). Watson and Roberts (1939) introduced the terms non-persistent and persistent viruses respectively to identify the two groups. Later Kennedy et al. (1962) introduced the terms stylet-borne, circulative and propagative viruses based on route of travel and location of virus in vector. Viruses transmitted by aphids are mainly non-persistent and semi-persistent.

Rapidity of acquisition and transmission of viruses is a common feature in non-persistent relationships owing to the high concentration of the virus in the plant (Bradley, 1964). The duration between acquisition and inoculation access periods, and the possible transmission of viruses is positively correlated in a persistent vector-virus relationship.

Some investigators (Kassanis, 1941; Nariani and Sastry, 1962; Proeseler and Weidling, 1975) noted that the inoculative ability of the vector declines rapidly with feeding in non-persistent relationships and that only another access feeding to virus source can ensure further transmission. However, pre-acquisition fasting increases the efficiency of the vector (Proeseler and Weidling, 1975). Conversely, post-acquisition fasting decreases the virus transmission ability of the vectors (Nariani and Sastry, 1962). With aphids, virus transmission is efficient if they probe briefly on virus source and test plants - a tendency readily observed in aphids subjected to fasting (Bradley, 1961). Interestingly, Nariani and Sastry (1962) observed that in a serial transfer of a single viruliferous aphid to healthy test plants, the aphid

transmitted the virus to only one test plant exhibiting a loss of viruliferous ability thereafter.

2.3.2 Vector-host relationship

The reproductive potentials and populations of insect vectors are influenced by the availability of suitable hosts on which they spend the unfavourable period of the season. The condition of the host also affects the condition of the culture of the vectors, particularly aphids. Matthews (1970) noted that both the vector species and plant variety used as either a virus source or test plant affect the frequency by which a vector transmits a virus. Old hosts produce passive and sluggish aphid colonies whereas an active colony is produced by a young vigorous host plant (Alegbejo, 1978, 1986). Some vector species may avoid certain plant varieties in favour of others for faster breeding (Broadbent, 1959).

Different workers at different times have come up with results showing positive correlations between vector numbers and infested plants. Broadbent (1950) found a positive correlation between potato leaf-roll incidence and alate M. persicae. A positive correlation between the total number of A. craccivora, infested plants of groundnut and rosetted plants was reported in Mokwa (Booker, 1963). The same relationship was observed by Zitter and Ozaki (1973) between pepper viruses and M. persicae. Alegbejo (1986) found a positive correlation between the numbers of M. persicae and Hysteroneura setariae, and transmission rate of pepper veinal mottle virus. Such has not been reported in the case of BlCMV.

2.3.3 Host-Virus Relationship

Viruses like any other organism, would wish to survive and spread continuously. This is however, prevented by the checks and balances meted out by nature. Viruses in hosts that cause mild and moderate diseases which allow the hosts to effectively survive and reproduce, are more likely to survive than those that rapidly kill their hosts. Viruses that multiply to high concentrations and remain stable in the hosts are more likely to survive than unstable viruses. Similarly, mutating viruses produce strains or mutants that enhance their survival and dispersal.

Resistance in cowpea to BlCMV has been reported (Lima et al., 1979) and has been found to be conditioned by a single recessive allele (Walker and chambliss, 1981). Lana et al (1988), observed resistance in some cowpea varieties tested and various symptoms in the susceptible ones.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 DETECTION AND IDENTIFICATION OF BLACKEYE COWPEA MOSAIC VIRUS.

Cowpea varieties (Ife brown and TVU 1582) were sown in a field near the glasshouses within the premises of the Institute for Agricultural Research, Samaru. The cowpea plants were observed for typical blackeye cowpea mosaic virus (BlCMV) symptoms. Plants showing symptoms of the virus were tagged and samples of their leaves were taken to the Virology Laboratory for detection using monoclonal antibodies as described by Huguenot, C., Furneaux, M.T., Thottappilly, G., Rossel, H.W. and Hamilton R.I. (1992). Samples which tested positive for both serotypes of the virus were identified and used as the Samaru isolates for this study.

3.2 VIRUS CULTURE MAINTENANCE, INOCULUM PREPARATION AND INOCULATION PROCEDURES

The BlCMV isolate used for the study was maintained in the glasshouse. Serial transfer of the isolate to fresh cowpea maintenance host plants was done by sap inoculation at regular intervals - the resulting infected plants being used as virus source for subsequent inoculation.

The inoculum for mechanical inoculation was prepared from the Samaru isolate by grinding infected leaves in a sterilized mortar and diluting with 0.01M phosphate buffer (p^H 7.0) at the rate of 1g infected tissue to 10ml of phosphate buffer (1:10 w/v).

The homogenate was pressed through a piece of cheese cloth to remove the plant material. The tip of the finger was dipped

periodically in the inoculum and used to gently streak the carborundum (600 mesh) - dusted leaves. The inoculated leaves were immediately washed with distilled water using a wash bottle.

3.3 PHYSICAL PROPERTIES

3.3.1 Thermal inactivation point

Glass tubes containing sap from cowpea leaves infected with the same isolate of BlCMV were heated to temperature of 30^0c and thereafter at intervals of 5^0c. This was done by securely holding the tubes with attached thermometer and dipping it into a beaker containing water, and heating it to the corresponding temperature for 10 minutes. The heated sap was cooled in running water and used to mechanically inoculate three replicates of four susceptible cowpea plants. The inoculated plants were maintained in the glasshouse for observation of symptoms. As a control, two cowpea plants were inoculated with unheated sap.

3.3.2 Longevity in-vitro

Prepared sap from cowpea leaves infected with the BlCMV isolate was used to inoculate four susceptible cowpea plants immediately and thereafter at intervals of 6 hours i.e. 6, 12, 18, 24, 30, 36, 42, 48, 54 and 60 hours. The four susceptible plants which were replicated three times, were inoculated with infected sap stored for a particular time interval. They were maintained in the glasshouse for observations.

3.3.3 Dilution end-point

Different dilutions of the Samaru isolate of BlCMV 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} and 10^{-9} were used to inoculate four

susceptible cowpea plants replicated three times. Glass tubes containing 9ml of distilled water were used. One millilitre of the virus isolate was pipetted into the first tube and shaken properly to give dilution 10^{-1}. One ml from this tube was pipetted into the second tube giving dilution 10^{-2} and this continued progressively until dilution 10^{-9} was attained. The contents of each tube and undiluted sap (control) were used for the mechanical inoculation of susceptible cowpea plants which were later maintained in the glasshouse for observation.

3.4 VECTOR-VIRUS RELATIONSHIPS

3.4.1 Acquisition feeding time.

To determine the effect of acquisition feeding time on virus transmission, a number of apterous adult A. craccivora individuals collected from a healthy colony (reared on groundnuts) were starved for 30 minutes and allowed to feed on BlCMV-infected cowpea plants for varying periods of 15, 30, 45 and 60 seconds, 5, 15, 30 and 60 minutes. After each acquisition feeding period batches of ten aphids were each transferred using a camel hair brush to four susceptible cowpea plants (replicated three times) and allowed to feed for 30 minutes. The aphids were killed by spraying with Karate EC (a.i. Lamdacyhalothrin), 2ml/l of water using a handgrip applicator.

3.4.2 Pre-acquisition fasting time.

The effect of pre-acquisition fasting time on virus transmission was determined by exposing a large number of apterous

adult aphids to various fasting periods: 0, 1, 5, 15, 30 and 60 minutes, 6, 12 and 24 hours. Batches of ten aphids were allowed acquisition feeding time of 30 minutes on a BlCMV-infected cowpea plant, and 30 minutes of inoculation feeding time each on four susceptible cowpea test plants replicated three times. The plants were maintained in the glasshouse for observation after killing the aphids with Karate EC (2ml/l of water) using a hand grip applicator.

3.4.3 Inoculation feeding time.

To determine the effect of inoculation feeding time on virus transmission, non-viruliferous apterous adult A. craccivora individuals from a healthy colony were starved on petri dishes for 30 minutes and allowed acquisition feeding time of 30 minutes. Viruliferous aphids in batches of ten were allowed inoculation feeding periods of 15, 30, 60 seconds, 5, 15, 30, 60 minutes, 6, 12 and 24 hours respectively. After these periods, the aphids were killed with Karate EC (2ml/1 of water) and the plants were maintained in the glasshouse for observation. Four plants each were used and replicated three times.

3.5 SURVEY

Collection of samples was carried out in both researchers' and farmers' fields during the 1993 and 1994 wet seasons (August - September). Samples were randomly collected from four quadrats, 4m x 4m in size each randomly chosen on each of the fields visited. Collected samples were placed in small plastic bags, labelled using appropriate codes, brought to the Virology laboratory and kept in

the freezer overnight for subsequent serological tests. Areas visited for sample collection are shown in Figure 1.

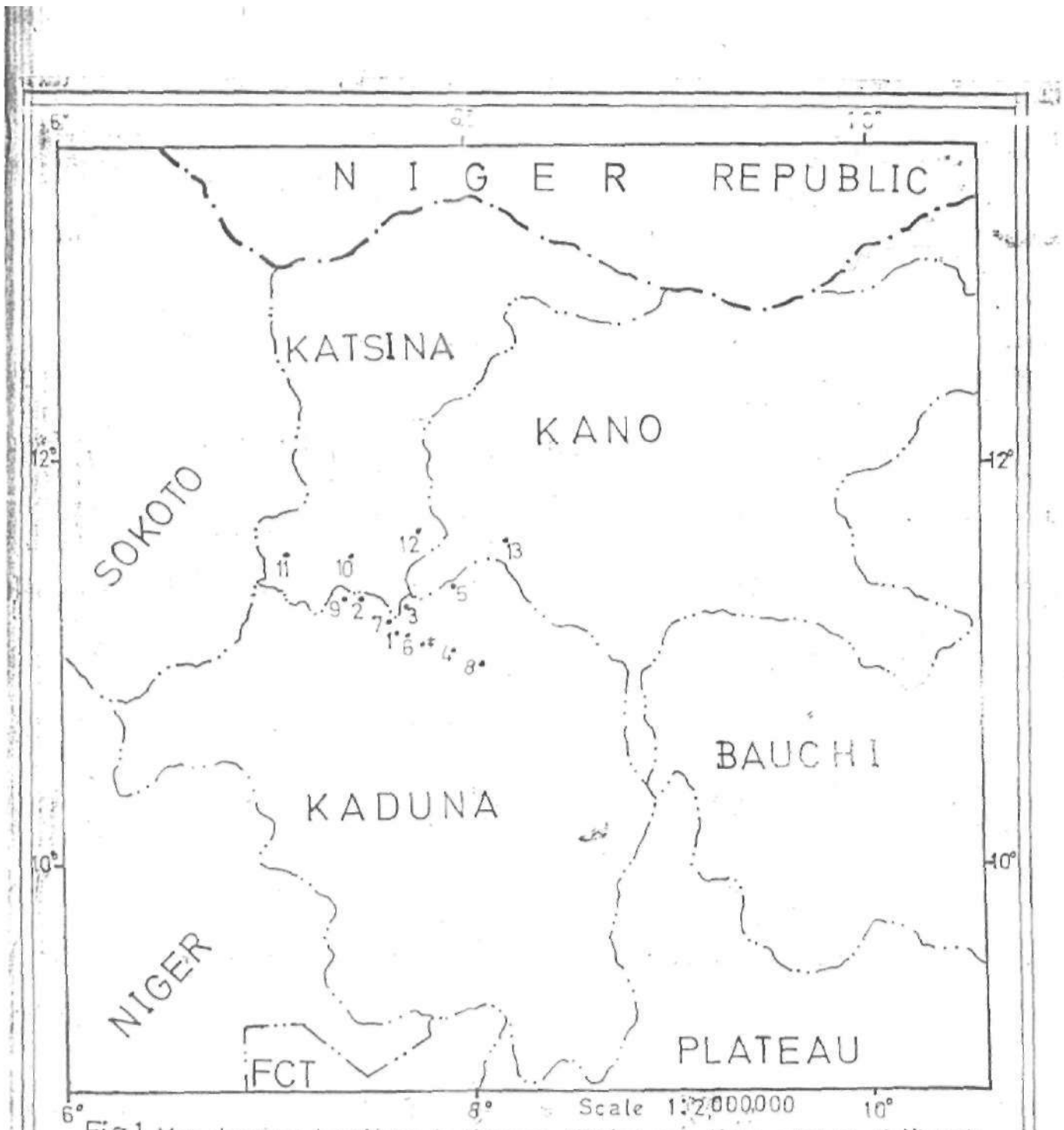


Fig.1 Map showing locations in Kaduna, Katsina & Kano states of Nigeria where virus samples were collected

KEY :

1	Biye	6	Samaru	11	Funtua
2	Giwa	7	Shika	12	Malumfashi
3	Hunkuyi	8	Soba	13	Dangora
4	Maigana	9	Yakawada	≠	Zaria
5	Makarfi	10	Bakori		

3.6 SCREENING FOR RESISTANCE

3.6.1 Field screening

Forty cowpea lines were sown in the Institute for Agricultural Research (IAR), Samaru field in three replicates using the randomised complete block experimental design. Each line was planted in a plot of three ridges prepared 1 x 3m long at an intra-row spacing of 30cm. Two plants were maintained per stand giving an average of 60 plants per plot. A gap of 0.5m was maintained between plots and one ridge between replicates - Glasshouse potted plants were artificially inoculated with the virus and infested with A. craccivora before being transplanted into the field to serve as sources of inoculum. On inoculated plant each was planted at the beginning of the middle ridge and by the side of each plot two weeks after sowing to ensure inoculum build-up.

The plots were manually weeded four weeks after sowing and fertilizer [NPK 15:15:15 (133 Kg/ha) and P_2O_5 (40 Kg/ha)] as supplemental was applied two weeks after germination. Observation of plants for evaluation of symptoms was done weekly starting from two weeks after germination. The following scoring scale was used for rating disease severity.

1: no visible symptoms
2: mild and quite inconspicuous mosaic and/or mottle symptoms.
3: fairly conspicuous mosaic and/or mottle symptoms, but no, or very little noticeable effect on leaf

shape and plant growth.

4: moderately severe mosaic/mottle symptoms, including leaf deformation and reduced growth of plant.

5: very severe symptoms, including leaf distortion/ deformation, severely stunted growth and, sometimes, die-off.

3.6.2 Glasshouse screening

The same forty cowpea lines used for the field screening were sown in plastic pots (30cm in diameter). A total of five plants per variety (one as control) were screened. These were replicated three times. Inoculation was done at the trifoliate stage of the plants using sap and aphid as stated earlier. Observation for symptom evaluation was done one week after inoculation. Using the same 1-5 scoring scale, disease severity scores were recorded.



Cages used for glasshouse screening

3.7 SEROLOGICAL TESTS

All samples collected from survey and screening were subjected to serological test using the double antibody sandwich - enzyme-linked immunosorbent assay test (DAS-ELISA) as described by Huguenot *et al.*, (1992). The ELISA plates were first coated with either monoclonal antibody 16G5 or 15E6 diluted in PBS-Tween (pH 7.4) at 1:10,000 and 1:1,000 UL respectively. The coated plates were incubated at 37°c for 2 hours and washed with PBS-T thrice. The washed plates were blocked with 1% fat-free bovine serum albumin (BSA) powder dissolved in PBS-T and incubated for 30 minutes at 37°c. The plates were emptied and the antigen ground in PBS-T 1g to 10ml was introduced into the wells. Incubation was again done at 37°c for 2 hours. The plates were removed, washed thrice with PBS-T and coated with detecting antibody-biotin combination 10G5 diluted in PBS-T (1:10,000). Plates were refridgerated overnight, removed, washed with PBS-T three times and coated with the conjugated streptavidin-alkaline phosphatase (1:1,000). These were incubated for 2 hours at 37°c, washed thrice and coated with the substrate tablets p-nitrophenyl phosphate (pNPP) dissolved in substrate buffer (pH 9.8) diluted at 10mg to 10ml. After incubating for 1 hour at 37°c, the plates were removed, observed for colour change and then read in the "Dynatech Minireader II". Table 1 summarises the procedures adopted.

3.8 STATISTICAL ANALYSIS

Data obtained from screening and survey were subjected to the analysis of variance with the means separated using the Duncan's Multiple Range Test (DMRT).

Table 1: Basic procedure of double antibody sandwich enzyme-linked immunosorbent assay (DAS - ELISA)

STEP	PROCEDURE	TIME	RINSE
Coating	Primary coating with trapping antibody (MAb) at suitable dilution in coating buffer	2 hrs, 37°c	With PBS-T
Blocking	Block all uncoated sites with 1% BSA in PBS-T	½ hr, 37°c	None
Antigen	Add antigen (Plant sap) at suitable dilution in PBS-T	2 hrs, 37°c	With PBS-T
Antibody	Add second antibody (MAb. Biot) at the suitable dilution in PBS-T	Overnight, 4°c	With PBS-T
Substrate	Add substrate (pNNP) 1mg/ml in substrate buffer (2 tablets of 5mg per plate)	1 hr, 37°c	

CHAPTER FOUR

4.0 RESULTS

4.1 Thermal Inactivation Point

Heating the sap to temperatures of between 30°c and 40°c produced significantly higher effect on infectivity of the virus than the other temperatures. This was followed by temperature of 45°c which produced significantly higher effect than 55°c and 60°c, but the effect was not significantly different from that produced at 50°c (P=0.05). There was no significant effect on infectivity of the virus when the sap was heated between 55°c and 60°c. At 65°c, the virus did not infect any of the test plants. (Table 2).

4.2 Longevity in-vitro

Storage time had significant effect on infectivity of BlCMV (Table 3). When the sap was stored between 6 and 18 hours at room temperature, the effect on infectivity was significantly higher than the other storage times at P = 0.05. This was followed by storage time of between 24 and 36 hours which produced significantly higher effect than storage at between 42 and 54 hours. At 42 to 54 hours of storage the effect on infectivity of the virus was not statistically significant. None of the test plants was infected with the virus when the sap was stored beyond 54 hours at room temperature.

TABLE 2: Effect of temperature on infectivity of BlCMV

Temperature (^{0}c)	Number of plants infected out of four			Mean
	I	II	III	
30	4.0	4.0	4.0	4.00[a]
35	4.0	4.0	4.0	4.00[a]
40	4.0	4.0	4.0	4.00[a]
45	4.0	3.0	4.0	3.67[a]
50	3.0	2.0	3.0	2.67[b]
55	1.0	1.0	1.0	1.00[c]
60	1.0	-	1.0	0.67[c]
65	-	-	-	
70	-	-	-	

Means followed by the same letter(s) are not significantly different at P = 0.05 using the Duncan's Multiple Range Test.

TABLE 3: Effect of storage time (at room temperature) on infectivity of BICMV

Time(Hrs)	Number of plants infected out of four: I	II	III	Mean
6	4.0	4.0	4.0	4.00[a]
12	4.0	4.0	4.0	4.00[a]
18	3.0	4.0	4.0	3.67[a]
24	3.0	3.0	4.0	3.33[a,b]
30	2.0	3.0	3.0	2.67[b,c]
36	2.0	3.0	3.0	2.67[bc]
42	2.0	2.0	2.0	2.00[cd]
48	1.0	1.0	1.0	1.00[e]
54	-	1.0	-	0.33[e]
60	-	-	-	

Means followed by the same letter(s) are not Significantly different at P = 0.05 using the Duncan's Multiple Range Test.

4.3 Dilution end-point

Dilution of the infective sap of the virus up to 10^{-3} produced significantly higher effects on the infectivity of the virus than the other dilutions at P = 0.05. This was followed by dilution at 10^{-4} which was significantly higher in effect that dilutions 10^{-5} and 10^{-6}. the effect on infectivity of the virus was not statistically significant at dilutions of 10^{-5} and 10^{-6}. One of the test plants was infected at dilution of 10^{-7} (Table 4).

4.4 VECTOR-VIRUS RELATIONSHIPS

4.4.1 Acquisition feeding time

The effect of acquisition time on transmission of the virus was significantly higher at 5 and 15 minutes than the others. This was followed by acquisition feeding for 45 and 60 seconds, and 30 minutes which produced significantly higher effects than acquisition feeding for 15 and 30 seconds and 60 minutes. Acquisition feeding time of 45 , 60 secodnds and 30 minutes did not produce any significant effect. Neither did acquisition feeding for 15, 30 seconds and 60 minutes (Table 5).

4.4.2 Pre-Acquisition fasting time

Pre-acquisition fasting times of 5, 15 and 30 minutes produced significantly higher effects on transmission of BlCMV than the others (P = 0.05). this was followed by pre-acqusition fasting time of 1 hour which produced significantly different effect from that produced at other times. similarly, pre-acquisition fasting of 1 minute was significantly different effect from that at 6 hours which was in turn different significantly from the effect produced

by unstarved aphids and at 12 hours of fasting. No significant effect was produced when the aphids were starved for 12 and 24 hours (Table 6).

TABLE 4: Effect of dilution on infectivity of BlCMV

Dilution	Number of plants infected out of four: I	II	III	Mean
10^{-1}	4.0	4.0	4.0	4.00^{a}
10^{-2}	4.0	4.0	4.0	4.00^{a}
10^{-3}	3.0	4.0	3.0	3.33^{a}
10^{-4}	2.0	2.0	1.0	1.67^{bc}
10^{-5}	1.0	1.0	1.0	1.00^{d}
10^{-6}	1.0	-	-	0.33^{d}
10^{-7}	-	-	-	
10^{-8}	-	-	-	

Means followed by the same letter(s) are not significantly different at P = 0.05 using the Duncan's Multiple Range Test.

TABLE 5: Effect of acquisition feeding time of A. craccivora on transmission of BlCMV.

Acquisition feeding time	Number of plants infected out of four: I	II	III	Mean
15 Seconds	1.0	1.0	2.0	1.33^{c}
30 Seconds	2.0	2.0	2.0	2.00^{bc}
45 Seconds	3.0	2.0	2.0	2.33^{b}
60 seconds	3.0	2.0	3.0	2.67^{b}
5 minutes	4.0	4.0	4.0	4.00^{a}
15 minutes	4.0	4.0	4.0	4.00^{a}
30 minutes	3.0	3.0	2.0	2.67^{b}
60 minutes	1.0	2.0	1.0	1.33^{c}

Means followed by the same letter(s) are not significantly different at P = 0.05 using the Duncan's Multiple Range Test.

TABLE 6: Effect of pre-acquisition fasting time of *A. craccivora* on the transmission of BlCMV

Pre-acquisition feeding time	Number of plants infected out of four:			Mean
	I	II	III	
0	2.0	1.0	2.0	1.67^{d}
1 minute	2.0	3.0	3.0	2.67^{bc}
5 minutes	4.0	3.0	2.0	3.00^{a}
15 minutes	4.0	4.0	4.0	4.00^{a}
30 minutes	4.0	4.0	4.0	4.00^{a}
1 hour	3.0	3.0	4.0	3.33^{ab}
6 hours	2.0	2.0	2.0	2.00^{cd}
12 hours	2.0	1.0	1.0	1.33^{de}
24 hours	1.0	1.0	1.0	1.00^{e}

Means followed by the same letter(s) are not significantly different at P = 0.05 using the Duncan's Multiple Range Test.

4.4.3 Inoculation feeding time

Table 7 shows that inoculation feeding time of 30, 60 minutes, 6, 12 and 24 hours produced the highest mean of infected plants. This mean was not significantly different (P = 0.05) from the next highest mean produced at an inoculation time of 15 minutes. However, the mean at 5, and 15 minutes differed significantly (P = 0.05) with the next highest mean at 60 seconds which did not differ significantly with the mean produced at 15 and 30 seconds.

4.5 SURVEY

Inconsistent variations in incidence level of BlCMV were recorded in the areas surveyed. The highest incidence was recorded at Hunkuyi in 1993 which was significantly different (P = 0.05)

from the incidence levels at the other locations except at Maigana, Soba and Malumfashi. The least incidence in 1993 was recorded at Dangora and this was significantly different from the incidence recorded at the other locations. In 1994, the highest incidence was recorded at Soba while the least was recorded at Makarfi. There were no significant differences in the levels of incidence in all the locations in 1994. A combined analysis showed that the disease incidence in 1993 was generally higher in all the locations than in 1994. Table 8 provides the details.

Table 9 indicates the distribution of serotypes in samples collected from the locations surveyed. In 1993, serotype A was detected in thirty-nine samples, serotypes A and B in seventeen samples and only five samples had serotype B. On the other hand, thirty samples contained serotype A; forty-three contained serotype A and B and thiry-eight contained serotype B in 1994. Serotype A was not detected in samples collected from Bakori and Giwa in 1993, and Maigana and Makarfi in 1994. Serotype B was also not detected in some samples from all the locations in 1994. ELISA readings of test results for all the locations are shown in tables 10 - 22.

TABLE 7: Effect of inoculation feeding time of A. craccivora on transmission of BlCMV.

Inoculation time	Number of plants infected out of four: I	II	III	Mean
15 seconds	1.0	1.0	2.0	1.33^{d}
30 seconds	1.0	1.0	2.0	1.33^{d}
60 seconds	2.0	1.0	3.0	2.00^{cd}
5 minutes	3.0	3.0	3.0	3.00^{b}
15 minutes	3.0	4.0	4.0	3.67^{ab}
30 minutes	4.0	4.0	4.0	4.00^{a}
60 minutes	4.0	4.0	4.0	4.00^{a}
6 hours	4.0	4.0	4.0	4.00^{a}
12 hours	4.0	4.0	4.0	4.00^{a}
24 hours	4.0	4.0	4.0	4.00^{a}

Means followed by the same letter(s) are not significantly different at P = 0.05 using the Duncan's Multiple Range Test.

TABLE 8: Incidence of BlMV disease in Zaria and environs

Location	Incidence (%) 1993*	1994*	Mean 1993	1994
Bakori	293.92	18.49	73.48^{cde}	4.62^{h}
Biye	311.25	26.14	77.81^{bcd}	6.54^{h}
Dangora	9.76	4.26	2.44^{h}	1.07^{h}
Funtua	154.09	4.49	38.52^{g}	1.12^{h}
Giwa	200.91	3.69	50.22^{f}	0.92^{h}
Hunkuyi	400.0	14.29	100.00^{a}	3.57^{h}
Maigana	377.59	2.26	94.40^{ab}	0.57^{h}
Makarfi	308.22	0.91	77.08^{bcd}	0.23^{h}
Malumfashi	336.06	3.17	84.02^{abcd}	0.79^{h}
Samaru	206.33	21.11	51.58^{fg}	5.28^{h}
Shika	270.28	30.98	67.57^{def}	7.75^{h}
Soba	354.01	42.05	88.50^{abc}	10.51^{b}
Yakawada	217.07	9.91	54.27^{efg}	2.48^{h}

Means followed by the same letter(s) are not significantly different at P = 0.05 using the Duncan's Multiple Range Test.

* Total incidence from four fields.

TABLE 9: Distribution of BlCMV serotypes in samples tested.

SITE	Number that reacted positively					
	MAbs16G5+10G5		MAbs15E6+10G5		Both	
	1993	1994	1993	1994	1993	1994
Bakori	-	3	2	4	-	2
Biye	1	2	-	3	6	3
Dangora	5	2	-	7	1	1
Funtua	3	1	-	3	1	10
Giwa	-	4	1	3	1	6
Hunkuyi	7	4	-	1	1	-
Maigana	6	-	-	1	-	-
Makarfi	2	-	-	1	-	1
Malumfashi	2	1	-	4	1	9
Samaru	5	3	1	2	1	2
Shika	3	1	-	3	1	4
Soba	4	7	-	2	3	1
Yakawada	2	2	1	4	1	4
TOTAL	39	30	5	38	17	43

NOTE: MAbs 16G5 + 10G5 detect serotype A of BlCMV
MAbs 15E6 + 10G5 detect serotypes A and B of BICMV

TABLE 10: ELISA readings (at 405nm) of samples collected from Bakori

	READING			
	MAbs 16 5 + 10G5		MAbs 15E6 + 10G5	
Sample	1993[a]	1994	1993[a]	1994
1	0.07	0.05	0.06	0.06
2	0.08	0.06	0.06	0.07*
3	0.07	0.06	0.05	0.07*
4	0.05	0.06	0.05	0.06
5	0.07	0.06	0.09*	0.07*
6	0.07	0.06	0.09*	0.06
7	0.06	0.06	0.08	0.06
8	0.06	0.07*	0.05	0.07*
9	-	0.07*	-	0.06
10	-	0.05	-	0.06
11	-	0.06	-	0.05
12	-	0.10*	-	0.06
13	-	0.07*	-	0.06
14	-	0.06	-	0.06
15	-	0.07*	-	0.07*
16	-	0.06	-	0.12*
Healthy control	0.06	0.00	0.06	0.00
Infected control	0.09	0.07	0.09	0.07

* Positive reaction when compared with healthy control.

a = Eight samples were collected.

TABLE 11: ELISA readings (at 405nm) of samples collected from Biye

	READING			
	MAbs 16G5 + 10G5		MAbs 15E6 + 10G5	
SAMPLE	1993[a]	1994	1993[a]	1994
1	0.24*	0.04	0.24*	0.06
2	0.32*	0.05	0.19*	0.04
3	0.20*	0.05	0.21*	0.07*
4	0.17*	0.08*	0.11	0.06
5	0.21*	0.07*	0.16*	0.05
6	0.10	0.06	0.12	0.04
7	0.12	0.05	0.09	0.07*
8	0.21*	0.07*	0.16*	0.08*
9	0.19*	0.06	0.17*	0.05
10	-	0.08*	-	0.09*
11	-	0.04	-	0.05
12	-	0.05	-	0.07*
13	-	0.08*	-	0.07*
14	-	0.04	-	0.05
15	-	0.04	-	0.06
16	-	0.05	-	0.05
Healthy control	0.07	0.00	0.07	0.00
Infected control	0.16	0.07	0.16	0.07

* Positive reaction when compared with healthy control

a = Nine samples were collected

TABLE 12: ELISA readings (at 405nm) of samples collected from Dangora.

	READING			
	MAbs 16G5 + 10G5		MAbs 15E6 + 10G5	
SAMPLE	1993[a]	1994	1993[a]	1994
1	0.13*	0.04	0.10*	0.04
2	0.09*	0.06*	0.05	0.06*
3	0.08	0.06*	0.07	0.04
4	0.08	0.04	0.07	0.04
5	0.10*	0.05	0.08	0.04
6	0.09*	0.04	0.06	0.06*
7	0.10*	0.05	0.08	0.04
8	0.10*	0.05	0.07	0.06*
9	-	0.04	-	0.06*
10	-	0.05	-	0.05
11	-	0.06*	-	0.05
12	-	0.04	-	0.07*
13	-	0.05	-	0.06*
14	-	0.05	-	0.06*
15	-	0.05	-	0.05
16	-	0.04	-	0.07*
Healthy control	0.00	0.00	0.00	0.00
Infected control	0.09	0.06	0.09	0.06

* Positive reaction when compared with healthy control.

a = Eight samples were collected

TABLE 13: ELISA readings (at 405nm) of samples collected from Funtua

	READING			
	MAbs 16G5 + 10G5		MAbs 15E6 + 10G5	
SAMPLE	1993[a]	1994	1993[a]	1994
1	0.08	0.05	0.07	0.05
2	0.07	0.06*	0.05	0.05
3	0.08	0.05	0.07	0.05
4	0.09*	0.05	0.07	0.06*
5	0.09*	0.05	0.08	0.05
6	0.08	0.05	0.08	0.06*
7	0.09*	0.06*	0.09*	0.06*
8	0.11*	0.05	0.08	0.06*
9	-	0.06*	-	0.06*
10	-	0.06*	-	0.06*
11	-	0.07*	-	0.08*
12	-	0.08*	-	0.08*
13	-	0.08*	-	0.08*
14	-	0.08*	-	0.07*
15	-	0.07*	-	0.08*
16	-	0.07*	-	0.07*
17	-	0.08*	-	0.07*
Healthy control	0.06	0.00	0.06	0.00
Infected control	0.09	0.06	0.09	0.06

* Positive reaction when compared with healthy control.

a = Eight samples were collected

TABLE 14: ELISA readings (at 405nm) of samples collected from Giwa.

SAMPLE	READING			
	MAbs 16G5 + 10G5		MAbs 15E6 + 10G5	
	1993[a]	1994	1993[a]	1994
1	0.08	0.06	0.07	0.06
2	0.07	0.08*	0.07	0.06
3	0.08	0.11*	0.07	0.07*
4	0.07	0.05	0.07	0.07*
5	0.08	0.06	0.07	0.06
6	0.07	0.08*	0.08	0.07*
7	0.12*	0.08*	0.10*	0.07*
8	0.08	0.06	0.10*	0.06
9	-	0.07*	-	0.08*
10	-	0.06	-	0.07*
11	-	0.07*	-	0.07*
12	-	0.07*	-	0.06
13	-	0.07*	-	0.07*
14	-	0.07*	-	0.06
15	-	0.06	-	0.07*
16	-	0.07*	-	0.06
Healthy control	0.06	0.00	0.06	0.00
Infected control	0.10	0.07	0.10	0.07

* Positive reaction when compared with healthy control.

a = Eight samples were collected.

TABLE 15: ELISA readings (at 405nm) of samples collected from Hunkuyi.

	READING			
	MAbs 16G5 + 10G5		MAbs 15E6 + 10G5	
SAMPLE	1993[a]	1994	1993[a]	1994
1	0.18*	0.08*	0.11*	0.06
2	0.10*	0.06	0.09	0.06
3	0.12*	0.05	0.09	0.03
4	0.10*	0.09*	0.08	0.05
5	0.10*	0.07*	0.09	0.03
6	0.14*	0.02	0.08	0.03
7	0.13*	0.05	0.09	0.04
8	0.17*	0.05	0.09	0.03
9	-	0.04	-	0.06
10	-	0.06	-	0.07*
11	-	0.05	-	0.04
12	-	0.05	-	0.04
13	-	0.06	-	0.06
14	-	0.06	-	0.04
15	-	0.07*	-	0.04
16	-	0.05	-	0.05
Healthy control	0.01	0.00	0.01	0.00
Infected control	0.10	0.07	0.10	0.07

* Positive reaction when compared with healthy control.

a = Eight samples were collected.

TABLE 16: ELISA readings (at 405nm) of samples collected from Maigana.

	READING			
	MAbs 16G5 + 10G5		MAbs 15E6 + 10G5	
SAMPLE	1993[d]	1994	1993[d]	1994
1	0.18*	0.05	0.09	0.05
2	0.12*	0.05	0.07	0.06
3	0.10*	0.05	0.07	0.04
4	0.08	0.04	0.06	0.06
5	0.09	0.06	0.06	0.04
6	0.11*	0.05	0.08	0.05
7	0.12*	0.05	0.09	0.06
8	0.18*	0.06	0.08	0.07*
9	-	0.06	-	0.06
10	-	0.06	-	0.05
11	-	0.04	-	0.06
12	-	0.05	-	0.06
13	-	0.06	-	0.06
Healthy control	0.00	0.00	0.00	0.00
Infected control	0.10	0.07	0.10	0.07

* Positive reaction when compared with healthy control.

a = Eight samples were collected.

TABLE 17: ELISA readings (at 405nm) of samples collected from Makarfi.

	READING			
	MAbs 16G5 + 10G5		MAbs 15E6 + 10G5	
SAMPLE	1993[a]	1994	1993[a]	1994
1	0.09*	0.05	0.07	0.06
2	0.11*	0.06	0.05	0.05
3	0.06	0.07	0.05	0.07*
4	0.02	0.05	0.06	0.07*
5	0.08	0.05	0.06	0.06
6	0.07	0.05	0.07	0.06
7	0.07	0.03	0.06	0.04
8	0.07	0.06	0.06	0.06
9	-	0.06	-	0.06
10	-	0.06	-	0.06
11	-	0.04	-	0.05
12	-	0.05	-	0.03
13	-	0.04	-	0.05
14	-	0.04	-	0.04
15	-	0.05	-	0.05
16	-	0.04	-	0.05
Healthy control	0.00	0.00	0.00	0.00
Infected control	0.09	0.07	0.09	0.07

* Positive reaction when compared with healthy control.

a = Eight samples were collected.

TABLE 18: ELISA readings (at 405nm) of samples collected from Malumfashi.

	READING			
	MAbs 16G5 + 10G5		MAbs 15E6 + 10G5	
SAMPLE	1993[a]	1994	1993[a]	1994
1	0.10*	0.07*	0.08	0.07*
2	0.09	0.06	0.09	0.07*
3	0.08	0.06	0.08	0.07*
4	0.10*	0.07*	0.09	0.08*
5	0.09	0.05	0.09	0.06
6	0.09	0.05	0.09	0.06
7	0.08	0.07*	0.08	0.07*
8	0.12*	0.06	0.10*	0.07*
9	-	0.07*	-	0.07*
10	-	0.07*	-	0.07*
11	-	0.07*	-	0.07*
12	-	0.07*	-	0.08*
13	-	0.05	-	0.09*
14	-	0.07*	-	0.08*
15	-	0.08*	-	0.08*
16	-	0.07*	-	0.05
Healthy control	0.07	0.00	0.07	0.00
Infected control	0.10	0.07	0.10	0.07

* Positive reaction when compared with healthy control.

a = Eight samples were collected.

TABLE 19: ELISA readings (at 405nm) of samples collected from Samaru

SAMPLE	READING			
	MAbs 16G5 +10G5		MAbs 15E6 + 10G5	
	1993	1994	1993	1994
1	0.02	0.03	0.02	0.03
2	0.09*	0.04	0.00	0.06
3	0.03*	0.04	0.00	0.05
4	0.01	0.07*	0.00	0.04
5	0.00	0.06	0.03*	0.08*
6	0.00	0.07*	0.00	0.09*
7	0.00	0.07*	0.00	0.05
8	0.05*	0.06	0.00	0.08*
9	0.00	0.08*	0.00	0.07*
10	0.04*	0.03	0.02	0.05
11	0.01	0.08*	0.01	0.06
12	0.02	0.04	0.02	0.03
13	0.04*	0.06	0.02	0.04
14	0.02	0.04	0.02	0.05
15	0.03*	0.05	0.03*	0.05
16	0.02	0.06	0.01	0.04
Healthy control	0.00	0.00	0.00	0.00
Infected control	0.03	0.07	0.03	0.07

* Positive reaction when compared with healthy control.

TABLE 20: ELISA readings (at 405nm) of samples collected from Shika.

SAMPLE	READING			
	MAbs 16G5 + 10G5		MAbs 15E6 + 10G5	
	1993[a]	1994	1993[a]	1994
1	0.04	0.06	0.03	0.06
2	0.05	0.06	0.03	0.04
3	0.07*	0.05	0.03	0.04
4	0.04	0.06	0.04	0.06
5	0.04	0.06	0.05	0.06
6	0.08*	0.06	0.04	0.06
7	0.09*	0.06	0.10*	0.07*
8	0.10*	0.07*	0.05	0.07*
9	–	0.06	–	0.08*
10	–	0.06	–	0.07*
11	–	0.07*	–	0.06
12	–	0.06	–	0.05
13	–	0.07*	–	0.07*
14	–	0.07*	–	0.09*
15	–	0.08*	–	0.07*
16	–	0.06	–	0.03
Healthy control	0.01	0.00	0.01	0.00
Infected control	0.07	0.07	0.07	0.07

* Positive reaction when compared with healthy control.

a = Eight samples were collected.

TABLE 21: ELISA readings (at 405nm) of samples collected from Soba.

SAMPLE	READING			
	MAbs 16G5 + 10G5		MAbs 15E6 + 10G5	
	1993[a]	1994	1993[a]	1994
1	0.23*	0.05	0.15*	0.06
2	0.15*	0.07*	0.14*	0.05
3	0.12*	0.07*	0.09	0.06
4	0.14*	0.07*	0.09	0.06
5	0.11*	0.07*	0.09	0.06
6	0.09	0.07*	0.09	0.05
7	0.11*	0.06	0.09	0.06
8	0.12*	0.07*	0.10*	0.06
9	-	0.06	-	0.04
10	-	0.06	-	0.06
11	-	0.04	-	0.06
12	-	0.08*	-	0.05
13	-	0.07*	-	0.07*
14	-	0.05	-	0.07*
15	-	0.06	-	0.07*
16	-	0.05	-	0.05
Healthy control	0.01	0.01	-	0.01
Infected control	0.10	0.07	0.10	0.07

* Positive reaction when compared with healthy control.

a = Eight samples were collected.

TABLE 22: ELISA readings (at 405nm) of samples collected from Yakawada.

	READING			
	MAbs 16G5 + 10G5		MAbs 15E6 + 10G5	
SAMPLE	1993[a]	1994	1993[a]	1994
1	0.07	0.07*	0.08*	0.07*
2	0.08*	0.07*	0.06	0.07*
3	0.07	0.07*	0.06	0.07*
4	0.07	0.08*	0.06	0.06
5	0.07	0.05	0.07	0.06
6	0.07	0.05	0.07	0.10*
7	0.08*	0.05	0.07	0.06
8	0.08*	0.07*	0.09*	0.07*
9	-	0.07*	-	0.06
10	-	0.07*	-	0.05
11	-	0.06	-	0.06
12	-	0.06	-	0.07*
13	-	0.06	-	0.06
14	-	0.06	-	0.06
15	-	0.05	-	0.06
16	-	0.06	-	0.07*
17	-	0.06	-	0.07*
Healthy Control	0.05	0.00	0.05	0.00
Infected Control	0.08	0.07	0.08	0.07

* Positive reaction when compared with healthy control.

a = Eight samples were collected.

4.6 RESISTANCE SCREENING

4.6.1 Field screening

ELISA results of the forty field-screened cowpea lines revealed the presence of the virus in twenty lines in 1993 while twenty-one lines tested positive in 1994. In 1993, ten of the lines that tested positive had serotypes A and B; four had only serotype A and six had only serotype B. On the other hand, seventeen lines had both serotypes A and B; three had serotype A and only one had serotype B in 1994 (Table 23).

TABLE 23: ELISA test results of field-screened cowpea varieties.

VARIETY	ELISA Reading at 405nm			
	MAbs 16G5 + 10G5		MAbs 15E6 + 10G5	
	1993	1994	1993	1994
IAR 48	0.05	0.06	0.03	0.05
83^{D} - 442	0.03	0.05	0.04	0.04
85^{F} - 2805	0.05	0.06	0.04	0.06
$IT86^{KD}$ - 245	0.07*	0.09*	0.05	0.08*
$IAR\ 176^{B}$	0.08*	0.08*	0.07*	0.07*
1696/TVX 3000 - 33	0.05	0.06	0.04	0.05
IAR 341	0.05	0.10*	0.08*	0.09*
TVX 3000/K59-14	0.04	0.05	0.06	0.06
$IT\ 86^{D}$ -715	0.10*	0.12*	0.08*	0.09*
TVU 2657	0.03	0.05	0.04	0.05
85^{F} - 3139	0.07*	0.05	0.06	0.08*
IAR 353	0.06	0.07*	0.08*	0.05
$IT\ 89^{KD}$ - 434	0.04	0.04	0.06	0.04
TVX 3000/K59-58	0.03	0.08*	0.07*	0.07*
KVX 402-5-2	0.08*	0.09*	0.06	0.08*
48W	0.04	0.10*	0.06	0.08*
83^{S} - 472	0.06	0.04	0.03	0.06
1696/TVX 3000-46	0.05	0.07*	0.07*	0.05
Ife Brown	0.04	0.08*	0.07*	0.06
84^{D} - 448	0.05	0.06	0.05	0.05
$IT82^{F}$ - 16	0.04	0.03	0.05	0.04
K - 39	0.07*	0.09*	0.07*	0.08*
85^{F} - 2687	0.05	0.05	0.03	0.06
81^{D} - 1137	0.06	0.04	0.03	0.04

TABLE 23 Contd.

VARIETY	MAbs 16G5 + 10G5		MAbs 15E6 + 10G5	
	1993	1994	1993	1994
ART 91 - 2	0.09*	0.07*	0.10*	0.08*
IAR[4]/48/15-1	0.11*	0.09*	0.09*	0.10*
88[D] - 867-11	0.03	0.06	0.05	0.03
L - 72	0.07*	0.07*	0.09*	0.07*
TVU 1582	0.08*	0.08*	0.06	0.07*
90[KD] - 245-1	0.06	0.05	0.06	0.04
IT82[D] - 889	0.03	0.06	0.04	0.03
85[F] - 867-5	0.04	0.06	0.04	0.04
IAR 355	0.07*	0.10*	0.08*	0.09*
IAR[2]/180-4-9	0.09*	0.08*	0.07*	0.10*
19/48-18	0.07*	0.07*	0.09*	0.07*
83[S] - 818	0.03	0.06	0.04	0.03
85[F] - 1380	0.04	0.04	0.05	0.03
84[S] - 2135	0.06	0.04	0.04	0.05
84[D] - 449	0.05	0.08*	0.07*	0.08*
IAR 339-1	0.10*	0.07*	0.08*	0.09*
Healthy Control			0.01	
Infected Control			0.07	

* Positive reaction when compared with healthy control.

4.6.2 Glasshouse screening

Results of the glasshouse screening by sap and aphid inoculation were consistent. Twenty-one lines tested positive indicating the presence of the virus. Serotype A and B were detected in all the twenty-one lines that tested positive (Table 24).

TABLE 24: ELISA results of glasshouse-screened cowpea varieties.

VARIETY	ELISA reading at 405nm			
	MAbs 16G5 + 10G5		MAbs 15E6 + 10G5	
	Sap Inoc.	Aphid Inoc	Sap Inoc.	Aphid Inoc.
IAR 48	0.06	0.03	0.05	0.05
83^{D} - 442	0.04	0.06	0.04	0.05
85^{F} - 2805	0.05	0.04	0.06	0.03
$IT86^{ID}$ -245	0.08*	0.09*	0.08*	0.07*
$IAR\ 176^{B}$	0.10*	0.09*	0.11*	0.08*
1696/TVX-3000-33	0.04	0.03	0.04	0.06
IAR 341	0.08*	0.09*	0.07*	0.10*
TVX 3000/K59-14	0.05	0.06	0.03	0.05
$IT86^{D}$ -715	0.07*	0.10*	0.09*	0.08*
TVU 2657	0.04	0.06	0.06	0.06
85^{F} - 3139	0.08*	0.07*	0.09*	0.07*
IAR 353	0.07*	0.09*	0.08*	0.08*
$IT89^{KD}$ - 434	0.05	0.06	0.04	0.06
TVX 3000/K59-58	0.09*	0.07*	0.10*	0.07*
KVX 402-5-2	0.07*	0.08*	0.10*	0.11*
48W	0.08*	0.07*	0.08*	0.09*
83^{S} - 472	0.03	0.06	0.06	0.04
1696/TVX-3000-46	0.07*	0.09*	0.12*	0.09*
Ife Brown	0.08*	0.07*	0.09*	0.09*
84^{D} - 448	0.03	0.05	0.06	0.04
$IT82^{E}$ - 16	0.04	0.06	0.04	0.03
K - 39	0.07*	0.12*	0.09*	0.08*
85^{F} - 2687	0.06	0.06	0.05	0.04

TABLE 24 Contd.

VARIETY	MAbs 16G5 + 10G5		MAbs 15E6 + 10G5	
	Sap Inoc.	Aphid Inoc.	Sap Inoc.	Aphid Inoc
81^{D} - 1137	0.03	0.05	0.04	0.05
ART 91 - 2	0.09*	0.07*	0.08*	0.09*
IAR^{1}/48/15-1	0.10*	0.08*	0.12*	0.07*
88^{D} - 867-11	0.06	0.03	0.05	0.05
L - 72	0.07*	0.07*	0.09*	0.07*
TVU 1582	0.09*	0.09*	0.10*	0.07*
90^{MD} - 245-1	0.06	0.03	0.05	0.04
$IT82^{D}$ - 889	0.03	0.04	0.06	0.03
85^{F} - 867-5	0.03	0.05	0.05	0.04
IAR 355	0.08*	0.10*	0.12*	0.09*
IAR^{1}/180-4-9	0.11*	0.07*	0.10*	0.08*
19/48-18	0.08*	0.08*	0.07*	0.10*
83^{S} - 818	0.04	0.05	0.06	0.03
85^{F} - 1380	0.03	0.05	0.04	0.05
84^{S} - 2135	0.06	0.03	0.06	0.04
84^{D} - 449	0.07*	0.09*	0.10*	0.07*
IAR 339-1	0.09*	0.08*	0.09*	0.07*
Healthy Control	0.00			
Infected Control	0.07			

* Positive reaction when compared with healthy control.

Based on symptom expression as adjudged by a mean disease severity score, the forty cowpea lines were classified into three classes namely resistance/tolerance, moderate susceptibility and high susceptibility. Twenty lines proved resistant; eighteen were moderately susceptible whereas only two exhibited high susceptibility (Table 25).

TABLE 25: Reactions of cowpea varieties inoculated with BlCMV.

REACTION	SAP INOCULATION	APHID INOCULATION
Resistant/ Tolerant	IAR 48, 83^{D}-442, 85^{F}-2805, 1696/TVX3000-33, TVX3000/ K59-14, $IT89^{KD}$-434, 83^{S}-872, TVU 2657,84^{D}-448,$IT82^{E}$-16, 85^{F}-2687,81^{D}-1137,88^{D}-867-11 90^{KD}-245-1,$IT82^{D}$-889,85^{F}-867 -5,83^{S}-818, 85^{F}-1380,84^{S}- 2135,$IT86^{KD}$-245,$IAR176^{S}$, IAR 341, IAR 353,TVX 3000/ K59-58,48W.	- do -
Moderately Susceptible	1696/TVX 3000-46, Ife brown, K - 39, ART 91-2, IAR^{1}/48/ 15-1, L - 72, TVU 1582, IAR 355, IAR^{7}/180-4-9, 85^{F} - 3139, 19/48-18, 84^{D}-449, IAR 339-1.	- do -
Highly Susceptible	$IT86^{D}$-715, KVX 402-5-2	- do -

Resistance/Tolerance = Mean Severity Score of 1.0

Moderate susceptibility = Mean Severity Score of > 1.0 < 3.0

High susceptibility = Mean Severity Score of > 3.0

Variability in severity of BlCMV over the two year period was assessed. Cowpea lines that were highly susceptible to the virus invariably showed a high significant difference (P = 0.05) in disease severity when compared with the other lines showing moderate susceptibility and tolerance. Cowpea lines classified as moderately susceptible showed variations in severity of symptoms expressed. Lines with a mean severity score of 2.83 and 2.5 showed significant difference in BlCMV severity when compared with lines having a mean score of 2.17 and/or below at P = 0.05. There was no significant difference in BlCMV infection among lines having mean severity scores between 1.83 and 2.33. Cowpea lines having a mean severity score of between 1.33 and 1.83 were also not significantly different at P = 0.05. However, there were significant differences in BICMV infection among lines with mean severity scores of 1.5 and 1.0 but not between the lines with mean scores of 1.33 and 1.0 at P = 0.05. (Table 26).

TABLE 26: Variation in severity of BlCMV over a two-year period

Variety	Total disease severity score (three replicates)		Overall total	Mean
	1993	1994		
IAR 48	3	3	6	1.00[e]
83^{D}-442	3	3	6	1.00[e]
85^{F}-2805	3	3	6	1.00[e]
$IT86^{KD}$-245	4	5	9	1.50[d]
$IAR\ 176^{B}$	5	6	11	1.83[c,d]
1696/TVX 3000-33	3	3	6	1.00[e]
IAR 341	8	9	17	2.83[b]
TVX 3000/K59-14	3	3	6	1.00[e]
$IT86^{D}$-715	11	11	22	3.67[a]
TVU 2657	3	3	6	1.00[e]
85^{F}-3139	6	7	13	2.17[c]
IAR 353	4	5	9	1.50[d]
$IT\ 89^{KD}$-434	3	3	6	1.00[e]
TVX 3000/K59-58	4	4	8	1.33[d,e]
KVX 402-5-2	10	12	22	3.67[a]
48W	5	6	11	1.83[c,d]
83^{S}-472	3	3	6	1.00[e]
1696/TVX 3000-46	6	6	12	2.00[c]
Ife Brown	6	7	13	2.17[c]
85^{D}-448	3	3	6	1.00[e]
$IT82^{E}$-16	3	3	6	1.00[e]
K - 39	3	3	6	1.00[e]
85^{F}-2687	7	7	14	2.33[b,c]
81^{D}-1137	3	3	6	1.00[e]
ART 91-2	3	3	6	1.00[e]
IAR^{4}/48/15-1	7	6	13	2.17[c]
88^{D}-867-11	7	7	14	2.33[b,c]
L - 72	3	3	6	1.00[e]
TVU 1582	7	6	13	2.17[c]
90^{KD}-245-1	5	5	10	1.67[d]
$IT\ 82^{D}$-889	3	3	6	1.00[e]
85^{F}-867-5	3	3	6	1.00[e]
IAR 355	3	3	6	1.00[e]

TABLE 26 contd. : Variation in severity of BlCMV over a two-year period

Variety	Total disease severity score Total disease severity score (three replicates)		Overall total	Mean
	1993	1994		
IAR[J]/180-4-9	7	5	12	2.00[c]
19/48-18	8	7	15	2.50[b]
83[S]-818	7	8	15	2.50[b]
85[F]-1380	3	3	6	1.00[e]
84[S]-2135	3	3	6	1.00[e]
84[D]-449	4	5	9	1.50[d]
IAR 339-1	7	8	15	2.50[b]

Means followed by the same letter(s) are not significantly different at P = 0.05 using the Duncan's Multiple

When the effect of BlCMV infection on cowpea grain yield was assessed only three lines - IT86[D] - 715, KVX 402-5-2 and TVU 1582 had significant reductions in yield. Yield loss of the other cowpea lines was not significant (P = 0.05) when the grain yields of the inoculated and uninoculated lines were compared (Table 27).

TABLE 27: Effect of infection with BlCMV on grain yield of cowpea

VARIETY	Grain yield per plant (g)	
	INOCULATED	UNINOCULATED
IAR 48	8.03	8.60
83[D] - 442	9.40	9.50
85[F] - 2805	8.20	8.20
IT86[KD] -245	6.10	8.00
IAR 176[B]	6.30	7.30
1696/TVX-3000-33	3.97	4.00
IAR 341	7.20	7.77
TVX 3000/K59-14	9.20	10.90
IT86[D] - 715	6.80	11.06*
TVU 2657	7.20	7.50
85[F] - 3139	8.20	9.10
IAR 353	3.60	3.90
IT 89[KD] - 434	12.03	12.10
TVX 3000/K59-58	3.30	4.00
KVX 402-5-2	3.20	7.60*
48W	8.40	10.60
83[S] - 472	11.20	11.50
1696/TVX-3000-46	9.10	10.40
Ife Brown	3.10	4.60
84[D] - 448	14.80	15.20
IT82[E] - 16	11.10	11.20
K - 39	3.20	3.50
85[F] - 2687	14.20	14.40
81[D] - 1137	4.10	5.30
ART 91-2	6.03	8.30
IAR[4]/48/15-1	8.20	9.50
88[D] -867-11	4.30	5.50
L - 72	4.80	6.60
TVU 1582	3.30	8.03*
90[KD] - 245-1	15.10	15.30
IT82[D]-889	25.30	25.40

TABLE 27 contd.

VARIETY	INOCULATED	UNINOCULATED
85[F]-867-5	9.50	9.50
IAR 355	2.60	2.80
IAR[2]/180-4-9	6.20	8.00
19/48-18	8.20	10.30
83[S]-818	14.10	14.30
85[F] - 1380	9.30	9.50
84[S]-2135	12.40	12.50
84[D]-449	7.10	8.30
IAR 339-1	6.50	7.50

* = Significantly different from inoculated treatment at P = 0.05 using the Duncan's Multiple Range Test.

[a]Values of means of 12 plants.

CHAPTER FIVE

5.0 DISCUSSION

The results of this investigation corroborated those of Raychandhuri (1977) who reported a thermal inactivation point of 60 - 65°c, dilution end-point of 10^{-4} and longevity in-vitro of 2-3 days. Taiwo et al.(1982) reported a thermal inactivation point of 57 - 60°c, longevity in-vitro of 24 - 48 hours at 22°c and a dilution end-point of 10^{-4} to 10^{-5}.

The virus was acquired even after 15 seconds of feeding on infected cowpea plants. However, transmission of the virus to susceptible cowpea was efficient at longer acquisition feeding time of between 5 and 15 minutes. Acquisition feeding time had significant effect on transmission of the virus at P = 0.05. Similarly, at 5% level of significance, varying the inoculation feeding time significantly affected the transmission of the virus to susceptible cowpea variety. Though the virus was transmitted even at 15 seconds of inoculation feeding, the transmission was most efficient at inoculation feeding experimental time of between 30 minutes and 24 hours.

Subjecting aphids to fasting before acquiring the virus has significant effect on virus transmission at P = 0.05. Preliminary fasting time of between 15 and 30 minutes was more effective in increasing the efficiency of transmission. Beyond this time, there was a decline in efficiency of transmission.

Viruses transmitted by aphids are classified into non-persistent and persistent viruses based on the time taken by the virus to survive in the aphid vector(s). Non-empirical and more stable criteria such as route of virus transport in the vector, location of virus in the vector and whether or not the virus is lost during ecdysis were proposed by Kennedy et al (1962). This results in the use of the terms stylet-borne, circulative and propagative viruses.

Stylet-borne viruses are picked by the aphid vector(s) after short period of feeding on the virus source and can be transmitted to susceptible plant species almost immediately (Smith, 1977). Short acquisition times are usually more efficient than long acquisition feeding time for the transmission of the virus. Again, the efficiency of transmission is increased by starving the aphid vectors before allowing for the acquisition of the virus (Proeseler and Weidling, 1975).

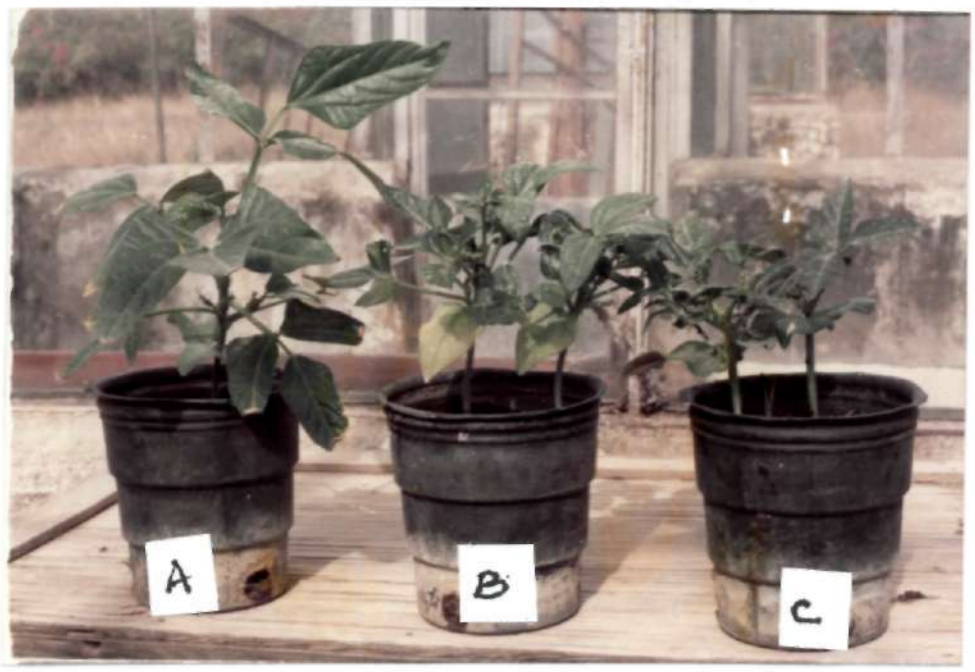
The BlCMV possesses the above features and therefore qualifies as a stylet-borne virus transmitted among other aphid vectors, by Aphid craccivora as revealed by the results of this study. The non-persistent manner of transmission of BlCMV by aphid vectors has been reported by many workers (Anderson, 1955b; Mali and Kulthe, 1980; Pio-Ribeiro et al., 1978).

Different incidence levels of BICMV were recorded for all the locations surveyed for both years. The distribution and spread of the virus was probably influenced by environmental factors, presence of suitable and susceptible hosts, and the presence and

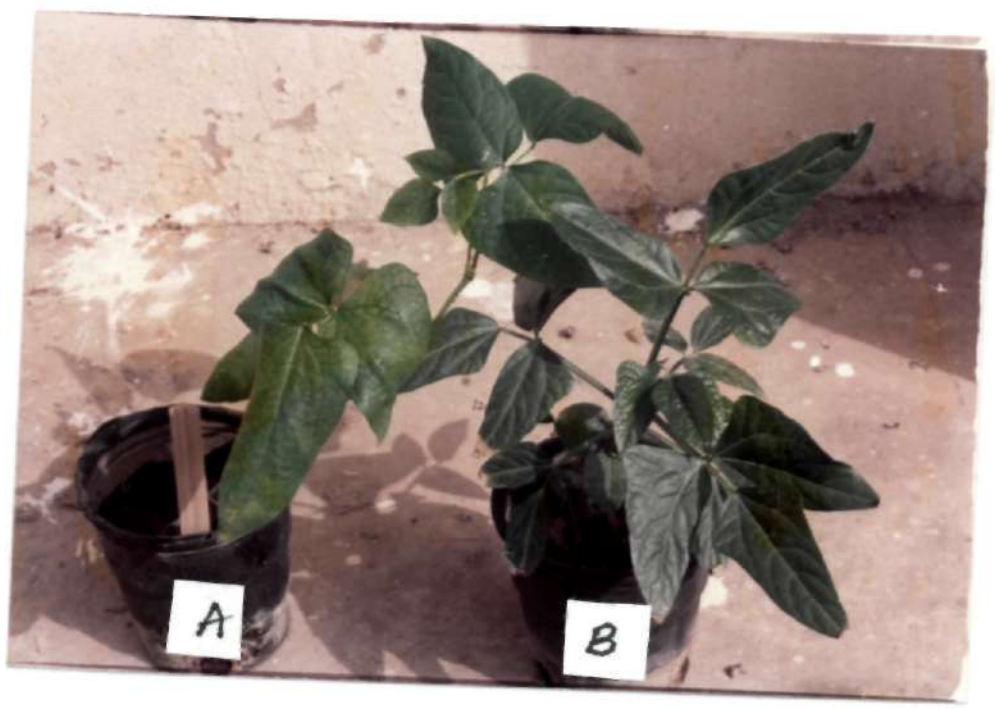


Typical BICMV symptons on cowpea variety IT82^{E} - 10 (81 - 11)

Typical BICMV symptons on cowpea variety IT82^{E} - 10 (IT - 16)



Severity of BICMV on cowpea line TVU 1582 (Healthy (A) vs infected (B and C)



Severity of BICMV on cowpea line 48 W (Healthy (B) vs infected (A)

activity of vectors. The biotic and/or environmental factors that can influence the development, distribution and activity of the vector can equally affect the distribution and spread of the virus. Infection by the virus in the various locations surveyed was natural and may have stemmed primarily from seed infection and weed hosts. Since the rate of seed transmission of the virus varies with cowpea cultivar and its interaction with virus isolate (Gay and Winstead, 1970; Zettler and Evans, 1972; Mali et al., 1983), variations in incidence levels of the virus were expected. Fields surveyed were small holdings and so farmers most probably used seeds obtained from very cheap sources from year to year. In most of the fields visited, the cowpea grown was mixed or intercropped with crops such as tomatoes, peppers, pumpkins, maize, sorghum and rice (Appendices 5.1 and 5.2). Movement of virus vectors under such cropping conditions can be affected leading to reduced spread of the virus in such fields. Barrier crops have been reported as capable of reducing the incidence of non-persistent pepper vein-banding virus (Simons, 1957) and pepper veinal mottle virus (Alegbejo and Uvah, 1986). Since non-persistent aphid-borne viruses are in general, retained for only few hours by the aphid vectors, tall crops probably mechanically deter aphids from landing on susceptible cowpea plants for some time after they have acquired the virus and thereby decrease the extent of effective virus transmission by the vectors. Alegbejo and Uvah (1986) reported that intercropping reduces the spread of the pepper veinal mottle

virus by acting as mechanical barriers and/or diversionary barriers to the spread of the virus. Intercrops might have afforded physical protection to cowpea plants by causing the aphid vectors to alight less frequently.

At survey time, cowpea plants were at different stages of growth for the different locations (Appendix 5.3). Virus concentration differ with the stages of growth and so are the incidence levels. Old hosts are said to produce passive and sluggish aphid colonies whereas an active colony is produced by young vigorous host plants (Alegbejo, 1978). Rose (1974) reported that epidemics occur at early stage in the growth of plants when they are most susceptible. Cowpea plants at the pre-flowering stage recorded higher incidence levels of the virus than those at the flowering and podding stages.

There was no particular trend in the distribution of serotypes in the test samples. Serotype constitution being an inherent anatomical feature of the virus could be modified during multiplicative and replicative processes of the virus. Host conditions could also actively influence such processes which are more active and vigorous at earlier stages of virus invasion of the host. Changing environmental factors could also affect the condition of the host to the detriment of the virus.

Results of field-screened cowpea showed inconsistent serotypes by the different cowpea lines. This is expected since the screening was carried out under natural field conditions where vector species, virus strains and vector populations were

uncontrolled. Since resistance to BlCMV by cowpea varieties is conditioned by a single recessive allele (Walker and Chambliss, 1981), variability in serotype possession and symptom expression especially, under natural conditions by different cowpea lines cannot be ruled out. Lana et al. (1988) observed various symptoms in susceptible cowpea varieties. The possibility of a number of cowpea genotypes reacting differently to different isolates of the virus was further confirmed by Rossel and Thottappilly (1988).

The relatively consistent results obtained by sap and aphid inoculation further highlights the comparative advantage and efficiency of sap and aphid inoculation procedures over field screening procedures. The use of single vector species, A. craccivora and single colony coupled with the controlled conditions of the glasshouse contributed to the consistent results obtained. Enhanced transmission efficiency and reduced possibility of escape contributed to the consistency in serotype possion and reactions by the cowpea test lines.

Aphis craccivora has been recognised as a very important vector of plant viruses (Lana et al., 1973; Whitam, 1974). The biology, feeding behaviour, rate of reproduction and worldwide distribution make them ideally suitable for transmitting plant viruses. This is particularly so in sole cowpea fields where the crop is a very suitable and readily available host for aphids.

Variation in symptoms induced on cowpea test lines by BlCMV was observed both in the field and in the glasshouse. Lima et al. (1979) reported that some of the symptoms induced by the viruses

vary with temperature and light conditions, and age at which the plants were inoculated. However, no variation was observed with respect to the resistance of the cowpea cultivars particularly under glasshouse conditions.

Yield reduction was observed between the inoculated and uninoculated cowpea lines in the glasshouse. Significant yield reduction was recorded in only three lines; the others being either resistant or tolerant. The controlled glasshouse conditions were probably responsible for the insignificant yield reductions in most of the lines. The tolerant mechanisms developed against the virus by the lines probably contributed to compensatory and/or over-compensatory growth thereby reducing the cummulative effect of the virus on grain yield.

CHAPTER SIX

SUMMARY AND CONCLUSION

A survey of areas in and around Zaria in the wet seasons of 1993 and 1994 (August to September), revealed the occurrence of blackeye cowpea mosaic virus in all the locations surveyed. There were inconsistent variations in incidence levels of the virus in both the areas surveyed and between the two seasons. Generally, higher incidence levels were recorded in 1993 than in 1994 in all the locations. While some significant differences in incidence levels were observed in some locations in 1993, incidence levels in 1994 were insignificantly different in all the locations surveyed.

The distribution of serotypes of the virus in samples collected from the locations surveyed, varied from location to location and from season to season. Serotype A was more prevalent than serotype B in 1993 while the reverse was the case in 1994. Serotype A was not detected in samples collected from Bakori and Giwa in 1993, and Maigana and Makarfi in 1994. Serotype B was also not detected in some samples from all the locations in 1993 and 1994.

Screening by aphid and sap inoculation in the glasshouse showed more consistent results than the field screening. Of the forty cowpea lines screened in the glasshouse, twenty-one tested positive to both serotypes of the virus. In the field twenty and twenty-one lines tested positive to the virus in 1993 and 1994 respectively. The presence of the serotypes in these lines did not maintain a consistent pattern of distribution for both years.

When the reaction of the forty cowpea lines to BlCMV infection was assessed, twenty lines proved resistant; eighteen lines showed moderate susceptibility while only two lines, IT86^{D} - 715 and KVX 402-5-2 exhibited high susceptibility. Significant cowpea grain yield losses were recorded only in IT86^{D} -715, KVX 402-5-2 and TVU 1582 compared to the moderately susceptible and resistant lines.

Temperature, dilution and storage time had significant effects on BlCMV infection of susceptible cowpea varieties. Infectivity is lost at temperature beyond 60^{0}c, at dilution beyond 10^{-6} and when virus sap is stored beyond 54 hours at room temperature.

The virus was acquired even after 15 seconds of feeding on source. However, 5 and 15 minutes acquisition times were most efficient in transmitting the virus to susceptible cowpea plants. Preliminary fasting time of A. craccivora individuals had significant effect on the transmission of the virus. Pre-acquisition fasting times of between 15 and 30 minutes were found to be very effective in increasing the efficiency of transmission. Similarly, transmission of the virus was most efficient at inoculation feeding times of between 30 minutes and 24 hours even though the virus was transmitted after 15 seconds of feeding on susceptible cowpea plants.

In the present study, it was discovered that the virus is widely distributed in the areas visited. This distribution was also related to that of the serotypes. The different levels in disease incidence and serotypes could be as a result of a gamut of factors bordering on the environment, climate, cowpea varieties

used, type and number of vectors, presence of other hosts, cropping pattern and the general management of the fields. A multifacetted and integrated approach is needed to reveal the desired details. Extensive and intensive work is further recommended to provide both quantitative and qualitative data on the spread and importance of this virus in and out of season and in many other locations. In view of the recognised role played by resistant plant materials in crop improvement, there is the need for further investigation into the possibility of incorporating resistance into the various cowpea lines considering the fact that strains of BICMV do occur.

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APPENDICES

APPENDIX 5.1: Cropping pattern of fields surveyed.

LOCATION	FIELD NUMBER	Cropping pattern	
		1993	1994
Bakori	1	Cowpea	Cowpea
	2	"	"
	3	"	"
	4	"	"
Biye	1	"	"
	2	"	"
	3	"	"
	4	"	"
Dangora	1	Cowpea/Tomatoes	Cowpea/Sorghum/ pepper
	2	Cowpea/pepper/ Maize	Cowpea/Maize
	3	Cowpea/pepper	Cowpea/Tomatoes/ okra
	4	cowpea/pepper	cowpea/rice
Funtua	1	Cowpea/Maize	Cowpea
	2	Cowpea/Maize	Cowpea/sorghum
	3	Cowpea	Cowpea/Maize/ sorghum
	4	Cowpea	Cowpea/Maize
Giwa	1	Cowpea	Cowpea
	2	Cowpea	Cowpea
	3	Cowpea	Cowpea
	4	Cowpea	Cowpea
Hunkuyi	1	Cowpea	Cowpea/Pumpkins
	2	Cowpea	Cowpea/Tomatoes
	3	Cowpea	Cowpea/Pepper
	4	Cowpea	Cowpea/Tomatoes
Maigana	1	Cowpea	Cowpea/Soyabeans
	2	Cowpea	Cowpea/Sorghum

APPENDIX 5.1 Contd.

LOCATION	FIELD No.	1993	1994
Maigana	3	Cowpea	Cowpea
	4	Cowpea	Cowpea
Makarfi	1	Cowpea	Cowpea/Tomatoes
	2	Cowpea	Cowpea
	3	Cowpea	Cowpea/Maize
	4	Cowpea	Cowpea/Sorghum
Malumfashi	1	Cowpea	Cowpea/Maize
	2	Cowpea	Cowpea/Maize
	3	Cowpea	Cowpea/Maize
	4	Cowpea	Cowpea/Maize
Samaru	1	Cowpea	Cowpea/Maize
	2	Cowpea/Maize	Cowpea/Maize
	3	Cowpea/Pepper	Cowpea
	4	Cowpea	Cowpea
Shika	1	Cowpea	Cowpea
	2	Cowpea	Cowpea
	3	Cowpea	Cowpea/Maize
	4	Cowpea/Maize	Cowpea
Soba	1	Cowpea/Maize	Cowpea
	2	Cowpea	Cowpea
	3	Cowpea/ Tomatoes	Cowpea
	4	Cowpea/ Soyabeans	Cowpea
Yakawada	1	Cowpea	Cowpea/Maize
	2	Cowpea/Maize/ Okra	Cowpea
	3	Cowpea/Maize/ Rice	Cowpea
	4	Cowpea/Maize/ Rice	Cowpea

APPENDIX 5.2: Cropping pattern and BlCMV incidence in the locations surveyed.

LOCATION		Copping Pattern		Incidence (%)	
	FIELD	1993	1994	1993	1994
Bakori	1	Sole	Sole	84.52	6.37
	2	"	"	86.25	6.78
	3	"	"	100.00	2.25
	4	"	"	23.15	3.09
Biye	1	"	"	77.33	6.32
	2	"	"	79.22	7.21
	3	"	"	77.78	5.32
	4	"	"	76.92	7.29
Dangora	1	Mixed	Mixed	0.00	0.00
	2	"	"	0.00	0.00
	3	"	"	9.76	4.26
	4	"	"	0.00	0.00
Funtua	1	Mixed	Sole	24.17	3.81
	2	"	Mixed	84.38	0.00
	3	Sole	"	45.54	0.68
	4	"	"	0.00	0.00
Giwa	1	"	Sole	9.68	0.86
	2	"	"	70.81	0.00
	3	"	"	47.80	2.83
	4	"	"	72.62	0.00
Hunkuyi	1	"	Mixed	100.00	14.29
	2	"	"	100.00	0.00
	3	"	"	100.00	0.00
	4	"	"	100.00	0.00
Maigana	1	Sole	Mixed	93.33	0.00

Appendix 5.2 Contd.

LOCATION	Field	Cropping Pattern 1993	Cropping Pattern 1994	Incidence(%) 1993	Incidence(%) 1994
Maigana	2	Sole	Mixed	92.59	2.26
	3	"	Sole	100.00	0.00
	4	"	"	91.67	0.00
Makarfi	1	"	Mixed	100.00	0.00
	2	"	Sole	50.63	0.91
	3	"	Mixed	57.69	0.00
	4	"	"	100.00	0.00
Malumfashi	1	"	"	75.00	0.00
	2	"	"	89.06	3.17
	3	"	"	75.00	0.00
	4	"	"	97.00	0.00
Samaru	1	"	"	47.79	5.30
	2	Mixed	"	56.43	6.25
	3	"	Sole	46.38	4.73
	4	Sole	"	55.73	4.83
Shika	1	"	"	66.10	30.48
	2	"	"	65.46	0.50
	3	"	Mixed	63.49	0.00
	4	Mixed	Sole	75.23	0.00
Soba	1	"	"	100.00	15.25
	2	Sole	"	90.91	0.00
	3	Mixed	"	71.43	0.00
	4	"	"	91.67	26.80
Yakawada	1	Sole	Mixed	80.70	2.44
	2	Mixed	Sole	52.24	1.05
	3	"	"	67.46	6.42
	4	"	"	16.67	0.00

APPENDIX 5.3: Developmental stages of cowpea plants at survey time.

LOCATION	Field	Developmental stage of the crop	
		1993	1994
Bakori	1	Pre-flowering	Flowering
	2	"	"
	3	"	"
	4	"	"
Biye	1	Pre-flowering	Flowering
	2	"	"
	3	"	"
	4	"	"
Dangora	1	Podding	Podding
	2	"	"
	3	"	"
	4	"	"
Funtua	1	Flowering	Podding
	2	"	"
	3	"	"
	4	"	"
Giwa	1	Flowering	Flowering/ Podding
	2	"	"
	3	"	"
	4	"	"
Hunkuyi	1	Pre-flowering	Flowering
	2	"	"
	3	"	"
	4	"	"

Appendix 5.3 Contd.

LOCATION	FIELD No.	1993	1994
Maigana	1	Pre-flowering	Podding
	2	"	"
	3	"	"
	4	"	"
Makarfi	1	Podding	Podding
	2	"	"
	3	"	"
	4	"	"
Malumfashi	1	Pre-flowering	Podding
	2	"	"
	3	"	"
	4	"	"
Samaru	1	Flowering	Flowering
	2	"	"
	3	"	"
	4	"	"
Shika	1	Flowering	Pre-flowering
	2	"	"
	3	"	"
	4	"	"
Soba	1	Pre-flowering	Pre-flowering
	2	"	"
	3	"	"
	4	"	"
Yakawada	1	Flowering/ Podding	Podding
	2	"	"
	3	"	"
	4	"	"