STUDIES ON PEPPER VEINAL MOTTLE VIRUS IN THE NORTHERN STATES OF NIGERIA

BY

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DECLARATION

The candidate hereby declares that this Dissertation is the result of his own individual work and no part has been carried out in collaboration with anyone else except where reference is made to published literature and where assistance is acknowledged.

Candidate's Signature

DEDICATION

This work is dedicated to my parents Late Mr. Michael Alegbejo and Mrs. Ruth Alegbejo who laboured to give me the foundation of education.

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INTRODUCTION

Peppers (Capsicum annuum L. and Capsicum frutescens L.) were known since prehistoric times from burial sites at Ancon and Huaca Prieta in Peru. The viability of the seeds and the ease with which they can be transported assisted in its rapid spread in the tropics and subtropics from the New World by Spanish and Portuguese explorers (Purseglove, 1968). Its long history of cultivation led to the establishment of a large number of varieties differing in habit, mode of growth and especially in the size, shape, colour and pungency of the fruits (Purseglove, 1968., Phillips, 1973).

Capsicum annuum, an annual, is probably the more important of the two species economically. The sweet peppers, which are harvested in the unripe state to provide salad vegetables or in the ripe state to produce mild condiments, belong to this species. They are also stuffed with meat, used as flavouring or colouring material and pimiento in cookery (Cobley, 1965). Capsicum frutescens on the other hand is a perennial plant often treated as an annual in cultivation but with a longer growing season than C. annuum. The fruits are generally smaller, about 7.5 to 10.0 cm. in length and are more pungent than C. annuum. The fruit is used in the manufacture of sauces, and curry powders and in the

preparation of pickles. It also has great medicinal value. Peppers are rich in vitamins A, C, and E. Consumption of pepper stimulates digestive organs to produce an increased flow of saliva and gastric juices. The oil distilled from its fruit is mild in taste and is used in perfumery (Purseglove, 1968).

Widely grown, Capsicum spp. are called by different local names in Nigeria, such as Ata(Yoruba), Ose(Igbo), Barkono (Hausa). It is a major vegetable and cash crop for many farmers in Nigeria, particularly the northern States. The Nigerian Trade Summary shows that the country exported a total of 700,038 kg of peppers worth ₦827,686 in 1975 (Nigerian Trade Summary, 1975).

The most important area of pepper production in Nigeria is around Funtua in Kaduna State. Its peppers are well known and accepted internationally. Early work on peppers in Nigeria was carried out in the 1960's when some collection of local types was made. By 1974, the Horticultural Section of the Institute for Agricultural Research, Ahmadu Bello University, Zaria began investigations on the agronomy, breeding and selection of available local cultivars and the introduction of some promising exotic lines. Disease problems of the crop have not been fully investigated.

Many fungal and bacterial diseases have been reported to be associated with peppers all over the world and some of them

constitute major constraints to pepper production. Virus diseases are reported to be even more devastating / (Chupp and Sherf, 1960).

A number of viruses such as tobacco mosaic virus, tobacco etch virus, tobacco rattle virus, potato viruses X and Y and pepper veinal mottle virus have been reported from various parts of the world, to cause mottle or mosaic on pepper (Anderson, 1959., McLean, 1962., Cook and Anderson, 1959., Laird et al., 1964., Steepy et. al, 1967., Brunt and Kenten, 1971., Zitter, 1971, 1972 and 1973., De Wijs, 1973., Lockhart and Fischer, 1974., Lana et. al., 1975., Kamra and Dubey, 1975., Villalon, 1975., Weinbaum and Milbrath, 1976., Soh et. al., 1977). The above named viruses are also reported to cause considerable reduction in yields depending on the time of infection during the growing season (Soh, et. al., 1977., Zitter, 1972 and 1973). However, pepper veinal mottle virus appears to be the major virus on pepper throughout West Africa. Brunt and Kenten (1971) working in Ghana, were the first to describe this new virus which belongs to the potato virus Y group. De Wijs (1973) described a virus on pepper in Ivory Coast which was similar to the one described in Ghana. Later the same virus was also reported in Nigeria (Lana et. al., 1975).

Work on pepper viruses was initiated in this laboratory in 1974 with a virus causing mottling symptoms on pepper. This has been identified as pepper veinal mottle virus (PVMV).

Studies on host range, physical properties, transmission, serological reactions and electron microscopy revealed similarities to the virus described elsewhere in West Africa. Thin sections for electron microscopy study, showed typical pin wheel inclusions characteristic of the members of potato virus Y group. However, the virus had a very restricted host range. During regular field surveys, it was noticed that the virus is present throughout northern Nigeria and the incidence of the disease was quite high. Greenhouse and field experiments conducted for 3 to 4 seasons, consistently indicated considerable loss in yield due to the virus disease (Subbarayudu, unpublished).

Although the identity of the virus and the damage it can cause to pepper crop was known, information on other essential aspects was meagre. Therefore, the need for a thorough understanding of the distribution pattern of the virus in northern Nigeria, the presence of any other virus on pepper or strains of the same virus, and the identification of any wild host(s) which might harbour the virus, was strongly felt.

Although some aphid species were known to transmit the virus in a stylet-borne manner, it was thought useful to work out a detailed vector-virus relationship. Ultimately, it became very important to screen for source of resistance to the disease from various pepper cultivars that were introduced into the country.

In order to understand the above problems, investigations were therefore carried out on the following aspects of the virus disease:

Vector-virus biological relationship,

Screening for disease resistance,

Distribution of the virus disease in northern Nigeria, and

Survey for wild hosts that might harbour the virus.

LITERATURE REVIEW.

Many viruses cause mottle or mosaic symptoms on pepper. These include: Tobacco mosaic virus (TMV) which malforms the leaves, causes general stunting, reduction in flowering and yield. The virus particles are rod-shaped and is serologically related to type TMV. Transmission is by sap or contact but generally not by insects (Smith, 1972).

Gracia and Feldman (1974) reported that tobacco streak virus (TSV) causes mosaic or mottling, slight leaf deformation, systemic necrosis, dark green streaks on stems and petioles of pepper and sometimes terminal necrosis. The virus particles are isometric and measure about 32.8 nm.

Pepper plants infected with tobacco etch virus (TEV) show vein clearing, darkgreen veinbanding, followed by mild mottle and reduced size of terminal leaves. Infected fruits are roughened, distorted and develop chlorotic spots or streaks. The virus particles are flexuous rods measuring 705 to 730 nm in length (Mclean, 1962., Laird et. al., 1964., Weinbaum and Milbrath, 1976).

Tobacco rattle virus (TRV) has been reported by Kitajima and Costa (1969) to infect pepper in Brazil. The virus is transmitted by parasitic nematodes, Trichodorus spp. and the particle length ranges from 188 to 197 nm (Smith, 1972).

Potato virus X (PVX) causes severe necrosis of leaves and stems and eventually defoliation of infected pepper plants.

It is sap transmissible and occasionally mechanical contamination by grasshoppers may occur. The particle is a flexuous rod of about 515 nm long and 12 nm wide. (Walters, 1952., Laird et. al., 1964., Steepy, et. al., 1967).

Potato virus Y (PVY) is the most widely reported virus on pepper. It gives rise to mosaic symptoms in infected plants which then become stunted and develop fewer branches. The virus is mechanically transmitted by many aphid species, a mite (Tetranychus telarius), by sap and grafting. Its various strains are serologically related. The particle is a flexuous rod of about 730 nm in length and 10.5 nm wide (Cook, 1962., Schultz, 1963., Laird et. al., 1964., Lockhart and Fischer, 1964., Villalon, 1975).

Cucumber mosaic virus (CMV) infected pepper plants become curled along the midrib and basal portion of the leaf is lighter green than the tip. Leaves become mottled. Generally the plant is stunted and leaves may become filiform. Transmission is by sap and many aphid species. The particle is isometric and measures 28 to 30nm in diameter. It has many serologically related strains (Solymosy, 1959., Aillaud, 1971).

Chilli (C. frutescens L.) mosaic virus causes typical mosaic mottling symptoms on pepper. Puckering, blistering, and wavy margins of leaves result, followed occasionally by small chlorotic ring spots. It is aphid and sap transmissible and is

widely distributed in India. The particles are rigid rods varying from 102 to 399 nm in length (Nariani and Sastry, 1962., Kamra and Dubey, 1975).

Zitter (1975) reported pepper mottle virus, a member of the PVY group to cause typical mottle symptoms on pepper and general stunting. It is sap and aphid transmissible. Purciful et. al. (1975) working on the same virus observed that the particles were flexuous rods of about 729 to 749 nm in length.

Pepper veinal mottle virus was first reported by Brunt and Kenten (1971) as a new member of the PVY group. Infected pepper plants develop interveinal chlorosis and are often small and severely distorted. It is transmitted by sap, grafting and many aphid species. The particles are flexuous filaments about 750 nm long. (Brunt and Kenten, 1971., De Wijs, 1973., Lana et. al., 1975).

Virus diseases of peppers were reported to be exceptionally severe during the 1970-71 growing season in Florida. Losses to pepper growers in Palm Beach County alone were estimated to be close to $2 million. These viruses caused 100% infection of pepper plants in commercial fields 6 weeks after the first diseased plants were found and crop abandonments were also reported in some areas due to diseases caused by PVY and TEV (Zitter, 1972., 1975., Zitter and Cook, 1973).

Pepper veinal mottle virus is widely distributed in West Africa causing mottle on peppers. The first description of this virus was made by Brunt and Kenten (1971) from the eastern region of Ghana. They described it as a distinct virus from the rest of the viruses known to occur on pepper. Following this discovery cases of PVMV began to appear in other West African countries. De Wijs (1973) isolated PVMV from chillies (Capsicum frutescens) in Ivory Coast and found it to be serologically related to onion yellow dwarf virus and Columbian Datura virus. Although this virus was serologically related to the Ghana isolate, some differences in host range were reported. Lana et. al. (1975) reported PVMV in the South Western part of Nigeria but it was not transmitted to any solanaceous species other than pepper when tested by mechanical inoculation, grafting, or by aphid vectors.

A virus disease on pepper has been identified in Samaru as pepper veinal mottle virus (PVMV) from the Studies made on its host range, physical properties transmission, serological reactions and electron microscopy (Subbarayudu, unpublished).

Maduewesi (1967) working in eastern Nigeria observed a mosaic disease of pepper which infected only C. annuum var. Neopolitan, Solanum nigrum and Nicotiana glutinosa on which it produced systemic symptoms. However, no serological tests were carried out to identify the virus.

The most recent report of PVMV in West Africa was made by Ladipo and Roberts (1977) at Ile-Ife in the south-west of Nigeria on tomato. Unlike the one described by Lana et. al. (1975) it infected 13 Solanaceous species. These are probably strains of the same virus (PVMV).

CRITERIA FOR DIAGNOSIS

A number of criteria such as symptomatology, host range, varietal reactions, physical properties of crude extracts, transmission and serology are employed in routine virus identification. Some of these criteria employed for the identification of PVMV are discussed here.

Symptomatology.

Although different viruses often produce similar symptoms in the same host plant, symptomatology or host plant reactions of several different hosts are still useful in identification of plant viruses (Bos, 1970).

Brunt and Kenten (1971) observed that the leaves of pepper infected with PVMV developed interveinal chlorosis followed by

green veinbanding along the main veins. The leaves were often small and severely distorted. Heavily infected plants were prematurely defoliated and produced fewer and smaller fruits than healthy ones. De Wijs (1973) working in Ivory Coast reported no differences in symptoms between the Ghana and the Ivory Coast isolates of PVMV when they were separately inoculated onto C. frutescens "Ferke" and C. annuum "Poivron doux d'amerique". The symptoms observed on pepper due to PVMV by Lana et. al. (1975) were similar to the Ghana isolate reported by Brunt and Kenton (1971) except for the rugosity of the new foliage of the Nigerian isolate. Soh et. al. (1977) working in Malaysia noted the symptoms of PVMV on pepper. Essentially they were not different from those reported in West Africa.

Host range.

Host range is a list of the hosts of a parasite. The concept is essential to understanding the role of additional hosts in the perpetuation or establishment of a plant pathogen. The host range of a given virus is dependent on a number of factors including environment, experimental conditions, host variety, virus strain and

the concentration of the virus in the host at the time of harvest for assay.

Brunt and Kenten (1971) reported that PVMV infected eleven members of the Solanaceae and five species from three other families. The infected Solanaceous plants included: Capsicum annuum "Yolo Wonder", "Pimento" and Long Red", C. frutescens "Tobasco",, Nicotiana clevelandii Gray., N. tabacum L. "Dutch A" and White Burley"., N. rustica L., N. glutinosa L., N. megalosiphon Arg., Petunia hybrida Vilm., Solanum capsicastrum L., and Hyoscyamus niger L. The other species from three families are Chenopodium amaranticolor Coste and Reyn., C. quinoa Willd., C. murale L., C. capitatum,, Tetragonia expansa and Amaranthus candatus L.

De Wijs (1973) observed a similiarity in the host range of the Ivory Coast (PVMV - CI) and the Ghana (PVMV - Gh) isolates. Lana et. al. (1975) working in Ibadan (Nigeria) observed that apart from Capsicum annuum cv., "Yolo Wonder" and C. frutescens, none of the other thirty-nine herbaceous species and varieties in the Amaranthaceae, Chenopodiaceae, Cucurbitaceae, Leguminosae and Solanaceae were infected, but the possibility of an alternate host was not ruled out by them. On the other hand, the work of Ladipo and Roberts (1977) pointed out a similiarity in the host range of the

PVMV isolated from tomato at Ife-Ife (Nigeria) with the Ghana isolate reported by Brunt and Kenten (1971).

An isolate of PVMV under study in this laboratory, could infect only cultivars of C. annuum and C. frutescens but not any other host (Subbarayudu, unpublished).

Physical properties of crude extracts.

As discussed by Bos (1970), virus stability in sap depends upon virus source, test plant, physical and chemical conditions of infective sap as well as actual virus itself.

Physical properties are important in identification, but must await development of satisfactory purification techniques because many potyviruses have similar dilution endpoints (DEP) thermal inactivation points (TIP) and longevities in vitro (LIV) (Afansiev and Morris, 1952).

The physical properties of PVMV described in various parts of West Africa are tabulated below.

Researchers	Physical properties		
	DEP	TIP (^{o}C)	LIV (Days)
1. Brunt and Kenten	10^{-3} to 10^{-4}	55-60	7
2. Lana et. al.	10^{-3} to 10^{-4}	62	3
3. Ladipo and Roberts	10^{-3} to 10^{-4}	55	5
4. Subbarayudu	10^{-3} to 10^{-4}	62-64	3

Serology

Much of the potential usefulness of serology may depend upon adequate purification method which would enable the preparation of highly-specific antisera and allow quantifying of antigen concentration for serological tests. Serological reactions are highly specific and sensitive. An appropriate serological test in conjuction with other studies helps in identifying virus and virus strains. They are used to distinguish closely related viruses which appear identical by all other available tests.

Brunt and Kenten (1971) made serological tests of PVMV in precipitin tubes by mixing 1 ml of each suitably diluted antigen and antiserum and incubating the mixture at 37°C for 6 hours but failed to ge a positive result by agar double diffusion test. However, the broken particles of PVMV showed positive reaction in agar double diffusion tests.

De Wijs (1973) used microprecipitin test successfully with PVMV. Lana et al. (1975) had a positive result by both the tube precipitin, and the agar double diffusion method (by breaking the virus particles).

Immunodiffusion tests with rod-shaped viruses such as PVY often fail because the viruses are too long to diffuse readily into agar gels (Van Regenmortel, 1966., Wetter, 1965). To overcome this problem, several rod-shaped viruses are chemically degraded into diffusible, immuno-reactive components (Purcifull and Shepherd, 1964). In recent years, sodium dodecyl sulphate (SDS) and a variety of other degrading agents have been used for this purpose (Gooding and Bing, 1970., Purcifull et. al., 1973). Sodium dodecyl sulphate (SDS) facilitates dissociation of antigens and also helps to minimize problems with non-specific precipitates.

VECTORS & EPIDEMIOLOGY

Virus-vector biological relationships.

In general plant viruses are transmitted through plant material and by vectors. Among the vectors, insects are the most important. The well-known insect vectors belong to the phytophagous species in the orders: Hemiptera, Thysanoptera, Coleoptera and Orthoptera. The suborder Homoptera (order Hemiptera) which includes aphids, leafhoppers, whiteflies and mealybugs is the most important group having plant virus vectors. The number of plant viruses transmitted by aphid is larger by far than the number transmitted by any other comparable invertebrate taxon. Kenedy et. al. (1962) listed 192 aphid species as vectors of plant viruses.

Early investigators showed that some aphid vectors rapidly lose their ability to transmit viruses as their feeding progresses while others retain and transmit the virus for many days or frequently throughout their entire life span (Watson, 1936., 1938., 1946., Watson and Roberts, 1939). These led to the classification of aphid transmitted viruses into two groups viz: Nonpersistent and persistent (Watson and Roberts, 1939). Kennedy et. al. (1962) introduced the terms stylet-borne, circulative and propagative viruses in order to describe the location and route followed by the virus in the vector. Stylet-borne viruses are usually non-persistent, carried on the stylet tip and are transmitted mainly by aphids while circulative and propagative viruses are of the persistent type. Both virus types are ingested, absorbed, translocated and finally added to the salivary secretions and from here they are transmitted to plant tissues during feeding. Propagative viruses in addition to being circulative can also multiply within the vector. Where the virus-vector relationship is of a persistent nature, there is a positive correlation between the duration (up to a point) of both the acquisition and inoculation access periods and the probability of virus transmission.

Semipersistent viruses are transmitted in an intermediate manner. Efficiency of transmission is not affected by pre-acquisition starvation or by long acquisition feeding.

The non-persistent relationship is characterised by rapidity of acquisition and transmission because the viruses usually occur in high concentration in plants even in the epidermis (Bradley, 1964).

The inoculative capacity of the vector declines rapidly with feeding (Kassanis, 1941., Nariani and Sastry, 1962., Proesele and Weidling, 1975) and further transmission will occur only if the vector is given another access to the virus source. The efficiency of the vectors increases if they are allowed pre-acquisition fasting (Watson, 1938., Bradley, 1953., Proesele and Weidling, 1975). Aphids as a rule, transmit virus most efficiently if they probe briefly and readily on both the source and test plants, fasted aphids have the tendency to make brief probes readily when returned to the plant (Bradley, 1961). This seems to indicate that the process is purely a mechanical one. Post-acquisition fasting on the other hand decreases the ability of the vector to transmit the virus (Nariani and Sastry, 1962). In serial transfer of a single viruliferous aphid to healthy test plants, the aphid can transmit the virus to only one test plant, thereafter the viruliferous ability is lost. Both alate and apterous forms of aphids are efficient in virus transmission (Nariani and Sastry, 1962).

Vectors

A number of aphid species are reported to transmit viruses that cause mottle or mosaic symptoms on pepper.

Tobacco mosaic virus (TMV) was reported to be transmitted by Melanoplus differentialis - a large species of grasshopper (Walters, 1952). Orlob (1963) reported that the potato fleabeetle, Epitrix cucumeris was a weak vector. The transmission of TMV by insects with biting and chewing mouthparts is purely by mechanical means.

Tobacco rattle virus (TRV) is easily transmissible by mechanical means. The vectors are parasitic nematodes, Trichodorus spp. (van Hoof, 1968).

Tobacco etch virus (TEV) is transmitted by several aphid species. These include Myzus persicae, Aphis fabae, and Macrosiphum euphorbiae (Kassanis, 1941., Mclean, 1962., Laird and Dickson, 1963., Villalon, 1975).

Potato virus X (PVX) was reported to be transmitted by grasshoppers (Walters, 1952) and the fungus, Synchytrium endobioticum (Nienhaus and Stille, 1965).

The vectors of potato virus Y (PVY) are various species of aphids. These include: Myzus persicae, Aphis gossypii, Macrosiphum solanifolli, M. pisi, A. spiraecola (Bradley, 1963., Bradley, 1954., Laird and Dickson, 1963., Lockhart and Fischer, 1974., Proeseler and Weidling, 1975).

The aphids M. persicae, A. gossypii and A. rumicis have been reported by various workers as vectors of cucumber mosaic virus (CMV), (Simons,1955,, Simons, 1959., Castillo and Orlob,1966., Megahed and Pirone, 1966., Praslicka, 1967., Suguira, et. el. 1975).

Pepper mottle virus (PeMV) is transmitted by the green peach aphid M. persicae (Zitter, 1975).

Many species of aphid have been reported by various workers as vectors of pepper veinal mottle virus. Brunt and Kenten (1971) recorded transmission with M. persicae and Aphis gossypii. De Wijs (1973) recorded two additional aphid species these are Aphis spiraecola and Toxoptera citricidus. Lana et. al. (1975) reported transmission by two more aphids — Aphis craccivora and an unidentified Aphis sp.

The transmission of these viruses by their various vectors were in a non-persistent (stylet-borne) manner.

Vector - Host factors.

In the presence of a suitable host some insect vectors of plant viruses particularly aphids have an enormous reproductive potential by the standard of the class insecta. The vector reproduction and population depends to a large extent on the number of host plants on which an insect-vector can spend unfavourable periods. This has a direct influence on the number available in the favourable season.

The condition of the aphid culture is affected by the condition of the host. A young vigorous host produces active individuals while old hosts produce stale colonies of aphids. The species and even the variety of plant used as the virus source plant or the test plant affects the frequency of virus transmission by the vector (Matthews, 1970). Vectors may prefer to feed and sometimes multiply faster on one variety of plant than another, and this may affect virus spread (Broadbent, 1959).

Vector population and environmental factors.

Environmental factors that influence insect population (multiplication and development) include temperature, relative humidity, photoperiodism, cataclysmic weather conditions, parasites and predators (Carter, 1973). Viruses spread fast under conditions optimal for insect multiplication and activity (Broadbent, 1959).

Aphids produce polymorphic forms due to the changes in environmental factors (for example photoperiodism).

Cataclysmic weather conditions such as hurricanes, severe and prolonged harmattan reduce the vector population so drastically that their feeding damage may not have economic consequence. Such an event could bring vector population down to an extremely low level and could leave some viruses vectorless (Carter, 1973).

Wind, temperature, light, rainfall and relative humidity affect insect movement and hence the virus spread (Carter, 1973). Aphids are by nature not very long distance travellers because they have tender wings. They lose their flight ability a few days after continous flight because of autolysis of the flight muscles. They therefore depend on wind for long distance migration and spread of plant viruses.

Insect vectors of plant viruses have been reported caught at great heights. For example Myzus persicae were taken at a mean height of 3,000 feet by airplane trapping (Glick, 1939).

The production of migrant aphids on their host is due to crowding, deteriorating physiological condition of their host (for example water stress) or cessation of shoot growth of the host plant. The crowding effect is enhanced by factors such as temperature and photoperiodism. Increasing amount of physical contact between the aphids provides a stimulus for the formation of alate (Swenson, 1968., Shaw, 1973). Vector flights and infectivity are influenced by temperature. For example aphids will not fly in darkness or at temperatures below 13°C and their infectivity increases with increase in temperature up to 29°C. (Swenson, 1968).

Photoperiodism of flying aphids changes after a period of flight such that they are more attracted to green, yellow and orange light than to short wavelengths, while very high relative humidity retards flight activity drastically and vice versa.

Vector population in relation to virus spread

Broadbent (1950) found a positive correlation between potato-leaf roll incidence and the number of alate M. persicae (its vector) caught on sticky traps. Dickson et. al. (1956) reported a similar result with flying aphid populations and the tristeza virus in southern California citrus groves. Booker (1963), working in Mokwa, found a correlation between the total number of A. craccivora - infested plants during the growing period of groundnut and the number of rosetted plants. Peak numbers of alate A. craccivora captured in water-filled yellow pans and on sticky traps corresponded closely with significant increases in the number of peanut stunt virus-infected plants in North Carolina (Bloch, 1971). Zitter and Ozaki (1973) in their study with pepper viruses showed an excellent correlation between M. persicae activity and virus spread.

Host-virus relationship.

A virus that is stable both inside and outside the plant and which reaches a high concentration in tissues is more likely to survive and spread than one which is highly unstable. Viruses which kill their host plants with a rapidly developing systemic disease are much less

likely to survive than those which cause mild and moderate disease that allows the host plant to survive and reproduce effectively. Also those that easily mutate to produce strains that cope effectively with changes in the environment are more able to survive and disperse.

A number of workers have attempted to find resistance to pepper viruses. Resistance to virus diseases is generally thought of in terms of either immunity to infection or ability to withstand the yield reducing effects of infection. Where insect transmission is involved, however, resistance may be displayed in other ways.

Cook and Anderson (1959) reported P11 a variety of C. annuum which is resistant to strains of Tobacco mosaic virus, Tobacco etch virus and Potato virus Y. Lloyd George raspberries, a variety of raspberries was found to be highly susceptible to both black raspberry necrosis and rubus yellow - net viruses, but not to its principal vector Amphorophora agathranica Hottes. Marchoux et. al. (1965) working on the reaction of pepper to some viruses prevalent in southern France observed that susceptibility depends upon the virus, the variety, and the stage of development of the host. Cook (1966) reported a bell pepper with resistance to PVY and TMV. Zitter and Ozaki (1973) reported some tolerant pepper varieties to the pepper virus complex in south Florida. Zitter and Cook (1973) while testing a Brazillian cultivar of C. annuum (Avelar) to potato virus Y found it tolerant. Alegbejo (1974) working at IITA observed that screening for virus resistance

in pepper is better carried out using sap rather than aphid inoculation. Of the fourteen lines of pepper tested he established that three were tolerant to virus disease. Cook et. al. (1976) bred Florida VR-2, a bell pepper with resistance to tobacco mosaic virus, tobacco etch virus and potato virus Y.

Weed hosts of pepper veinal mottle virus.

The role of weeds in the occurrence and spread of plant virus diseases is an integral part of virus transmission (Duffus, 1971). Wild plants and weeds as well as cultivated plants may close unfavourable gaps and thus contribute to epidemics of plant diseases by bridging between seasons, between crops and between locations. Thus continuity in both time and space is established (Dinoor, 1971). Weeds are reservoirs of vectors, viruses, or both (Duffus, 1971). Wild plants are an unlimited source of viral diseases, (Chessin and Lesemann, 1972). Many wild hosts are either symptomless or show different symptoms from those on cultivated plants.

Simons (1956 1957) noticed veinbanding virus of chilli, a strain of PVY in south Florida was harboured by solanaceous weeds. Anderson and Corbett (1957) and Anderson (1959) reported that severe damage to chilli plants was associated with the proximity of various weed hosts and they isolated a strain of PVY from Solanum nigrum in Florida. Mariappan et. al. (1973) reported that Solanum nigrum, a common weed found around Capsicum sp. harbours chilli mosaic

virus. Whitam (1974) working in Louisiana observed that eighteen indigenous plant species (including Solanum nigrum) in the vicinity of bell pepper fields were infected with one or more of the four viruses TEV, PVY, CMV and tomato spotted wilt virus.

I. GENERAL MATERIALS AND METHODS

In this chapter, the materials and methods which were generally used throughout the investigations regarding the maintenance of plant, virus and vector cultures and inoculation procedures are described. However, specific variations in the techniques employed in various experiments are given in the relevant chapters.

I. Greenhouse and general sanitation.

As far as possible, a high standard of sanitation of the greenhouse, soil, pots and seedlings used for experiments was maintained to prevent any possible contamination. Seeds were germinated in wooden trays filled with sterilized soil (to which superphosphate was added) because the soil could be contaminated by soil-borne viruses, bacteria, fungi and nematodes. Three week old seedlings were transplanted (at four-leaf stage) into plastic pots containing a soil mixture of soil, sand and peat moss (4 : 2 : 1). After use, the pots were washed and dried in the sun to remove soil-borne organisms. The greenhouse and all test plants were routinely sprayed with menazon or audugatox following experimentation to prevent aphid build up. Before entering the greenhouse, the sole of shoes were cleaned of soil materials. The window openings of the greenhouse were covered with fine wire gauze to exclude insects.

Experimental plants were watered and top dressed with fertilizer whenever required to prevent nutrient deficiency and water stress which might complicate virus symptoms. All experimental plant materials were grown and

maintained under greenhouse conditions. A local variety of Capsicum annuum L5962-2, known to be highly susceptible to pepper veinal mottle virus was used throughout the study except where otherwise stated. Other species of plants used for host range studies were also grown similarly.

II. Aphid Collection, Maintenance and Identification

The hosts and the locations of collection and hosts for rearing aphids are tabulated in table 1. Adult apterous aphids (from which nymphs were later born parthenogenetically) were collected from their various host plants. Each species of aphid was collected from a single host to ensure homogeneity of individuals in each clone, so that clone variation may not affect their level of virus transmission. The aphids were collected from young vigorous colonies which provided active individuals. The aphids were taken from plants which had not yet noticeably suffered from the feeding injury of the aphids because the transmission of a few aphid-borne viruses is known to be affected by the physiological condition of the plant on which the aphids were reared (Swenson, 1963). Since aphids are frequently attacked by their parasites, care was taken to get parasite-free colonies by rejecting sick colonies whenever they were found. Each clone was kept isolated in insect-proof rectangular cages covered with fine wire netting.

The aphid host plants were replaced periodically with young healthy ones so that the clones continued to remain vigorous. New colonies were started using about fifteen viviparous adult aphids (which were selected from the existing colonies) per plant. This regular changing of host plants and inoculating them with vigorous aphid individuals kept the colonies healthy and the population at a satisfactory level without overcrowding.

The non-viruliferous aphid colonies were checked at regular intervals for PVMV contamination by transfering some aphids of each colony to healthy pepper plants. Only those aphid colonies that did not produce symptoms on healthy pepper plants were used for transmission studies. The handling of individual aphids was done with the help of the moistened tip of the camel's hair brush. The lifting of the aphid was done by first touching it gently so that it can withdraw its stylet from the plant and lifting it from the rear end. The alate forms of all species of aphids used were sent to "The Commonwealth Institute of Entomology" in Britain for identification.

III. Maintenance of Virus Culture, Inoculum Preparation and Inoculation Procedures.

The PVMV isolate used for vector-virus biological relationship was obtained from the already existing culture under study in this laboratory. The source of all other isolates studied are reported in

the relevant chapter. They were maintained by aphid transmission at regular intervals. Myzus persicae was allowed to feed on virus infected pepper plants and transferred to healthy pepper test plants. The resulting infected pepper plants were used as virus source plants. During the cold-dry season germination and symptom development were stimulated by providing additional lighting (Plate 2).

For mechanical inoculation the following precautions were taken against contamination by other viruses. Mortars and pestles were thoroughly washed with soap, rinsed with tap water and dried before sterilizing in a hot air oven at 160°C for about 90 to 100 minutes. Pipettes, funnels, test tubes, beakers and other glassware used were first immersed in chromic acid solution, rinsed and washed with soap water. They were again rinsed and air dried. Glasswares were also regularly sterilized in hot air oven at 160°C for 90 to 100 minutes. Before and after each inoculation, hands were thoroughly washed with soap solution. Further contamination of clean hands through touching door knobs and tap handles in the greenhouse was avoided. Minimal handling and touching of experimental plants was done throughout this study. Contact between plants infected with different isolates was avoided by adequate spacing in the greenhouse. Except where otherwise stated, all inocula were made by grinding infected leaves in a sterilized mortar and diluted (1:1) with 0.01 M phosphate buffer pH 7.0. The ground material was pressed through a piece of cheesecloth

to remove plant materials. The tip of the finger or a piece of cheesecloth was dipped periodically in the inoculum and used in applying it by gently rubbing all carborundum (600-mesh) dusted leaves. The leaves were immediately rinsed with water and observed regularly for virus symptoms. Young leaves showing typical symptoms were used as source of inoculum.

IV. Statistical analysis.

Greenhouse and field data were analysed for statistical significance using the 'F' and least significant difference (l.s.d.) tests. The standard error of the means and transformed means were calculated. Means less than 10 were subjected to square root transformation ($\sqrt{X + \frac{1}{2}}$, where X = mean less than 10) to make them normal. One asterisk (*) represented significance at 5% level while two asterisks (* *) represented significance at 1% level.

In some instances regression analyses were done to detect the type of correlation that existed between some variable factors. These were used to interprete experimental results and appropriate inferences and deductions made from them.

II. VECTOR-VIRUS BIOLOGICAL RELATIONSHIPS.

INTRODUCTION

Most common virus diseases of crops are spread by insects and control of such insects by using a range of insecticides does not always help in controlling virus diseases. An understanding of the virus-vector relationships is very important before attempting insecticidal application to control vectors and hence virus diseases. A detailed study of the insect-virus relationships was carried out to:

1). determine whether the vector species reported by earlier workers (Brunt et. al., 1971; Lana et. al., 1975) are also vectors of PVMV that are prevalent in the northern States of Nigeria,

2). identify other aphids species that might be potential vectors of PVMV, and

3). determine the aphid-virus biological relationships using the most efficient aphid species.

The virus isolate used for this study had only pepper and P. micrantha as its hosts.

MATERIALS, METHODS AND RESULTS

(a) Ability of various aphid species to transmit PVMV.

Seven aphid species were tested to compare their ability to transmit PVMV. A large number of aphids from each of healthy

colonies of different species were collected separately and starved in dry Petri dishes for a period of 30 minutes. Later they were allowed to feed on pepper plants showing typical symptoms of PVMV, for 10 minutes. The feeding behaviour of the aphids was carefully observed and only those individuals probing leaf tissues were used in the tests. Groups of six aphids were transferred immediately to each healthy pepper test plant and were allowed to feed on them for another 10 minutes. Six plants were used for each test and each test was replicated four times. In all, 24 plants were employed for each test. Plants were then sprayed with menazon and kept in the greenhouse for observations.

All the seven aphid species tested (Table 1), except Rhopalosiphum maidis, were found to transmit PVMV (Table 2). Two aphid species Myzus persicae and Hysteroneura setariae showed higher levels of transmission when compared with the other five species However, M. persicae appeared to be the most efficient from the analysis of raw (untransformed) data.

(b) Effect of vector numbers on the transmission of PVMV.

Seven species of aphids were employed in this experiment. The aphids were starved for one hour, given 10 minutes acquisition feeding time (a.f.t.) and 10 minutes inoculation feeding time (i.f.t.). Viruliferous apterous aphids in single as well as groups of 5, 10 and 20 were tested on single pepper plants to compare the efficiency of

their transmission in groups. Ten plants were used for each of the tests and repeated four times. Corresponding controls were maintained by allowing the aphids to feed on healthy plants.

The results (Table 3) indicated that only M. persicae and H. setariae could transmit the virus in singles. The other species needed more than one aphid for transmission. R. maidis could transmit the virus only in a group of at least ten. In all respects, M. persicae transmitted the virus at a very high level. The efficiency of transmission by all species tested increased with the number of aphids used. There was a significant difference at 1% level in the efficiency of the aphids and their group means. The increase in the number of infected plants did not increase proportionately with the number of viruliferous aphids.

(c) Effect of preliminary fasting of M. persicae on the transmission of PVMV.

Since M. persicae was found to be the most efficient vector of PVMV, it was employed for the rest of the experiments, to understand its biological relationships with the virus.

A large number of apterous aphids were allowed to starve for periods of 0, 1, 5, 15, 30 minutes, 1, 2, 6, 12 and 24 hours before releasing them on to infected plants to acquire the virus. Ten aphids were given a.f.t. of 10 minutes on infected plant and 10 minutes of i.f.t. on healthy test plant. Aphids which fed on healthy pepper plants and transferred onto healthy test plants similarly served as control.

Statistical analysis of the results (Table 4) reveal that the preliminary fasting of M. persicae had a significant effect on the rate of PVMV transmission at 1 and 5 percent level. Although the virus was transmitted to the extent of 70 percent without any pre-acquisition fasting, the rate of transmission increased with increase in fasting time up to 30 minutes. Starving aphids beyond 30 minutes gradually reduced their efficiency.

(d) Effect of acquisition feeding time on the transmission of PVMV

A number of M. persicae aphid individuals collected from a healthy colony were allowed to starve in dry petri dishes for 30 minutes. Groups of aphids were allowed to feed on diseased pepper plants for periods of 15, 30 seconds, 1, 5, 15, 30 minutes, 1, 12 and 24 hours.

Table 1 : Aphid species used in transmission experiments of pepper veinal mottle virus.

Common name	Specific name	Host of collection	Location	Rearing plant for aphids.
Green peach aphid	Myzus persicae (sulzer)	Sorghum vulgare	Faculty plot	Pepper (Capsicum sp.)
-	Hysteroneura setariae	Triticum aestivum	Near Plant Pathology Section	Triticum aestivum (wheat)
Cotton aphid	Aphis gossypii Glover	Gossypium sp.	Faculty plot	Gossypium sp. (cotton)
Bean aphid	Aphis craccivora Koch.	Arachis hypogea	Mr. Rossel's cages.	Arachis hypogea (Groundnut)
-	Aphis fabae	Solanum nigrum	Kadawa irrigation scheme	Solanum nigrum
-	Rhopalosiphum sp.	Abelmoschus esculentus	Near I.A.R. green houses	Hibiscus esculentum (Okra).
Com leaf aphid	Rhopalosiphum maidis (Fitch)	Pennisetum typhoides	I.A.R. irrigation field.	Pennisetum typhoides (Millet)

Table 2 : Ability of various aphid species to transmit PVMV.

Aphid	Number of infected plants				Mean ($\bar{x}$)	Transformed mean
	I	II	III	IV		
Myzus persicae	4.00	5.00	4.00	4.00	4.25	2.18
Hysteroneura setariae	4.00	3.00	3.00	4.00	3.50	2.00
Aphis gossypii	3.00	3.00	4.00	2.00	3.00	1.86
Aphis craccivora	3.00	2.00	2.00	2.00	2.25	1.65
Aphis fabae	2.00	3.00	1.00	2.00	2.00	1.56
Rhopalosiphum sp.	1.00	1.00	0.00	2.00	1.00	1.18
Rhopalosiphum maidis	0.00	0.00	0.00	0.00	0.00	0.71
S. E.					**0.45	**0.15
lsd.					0.94	0.31

Table 3: Effect of numbers of viruliferous aphid species on the transmission of PVMV.

Aphid species tested	Mean number of plants infected				S.E.	l.s.d.
	Number of aphis per test plant					
	1	5	10	20		
M. persicae	2.75	6.00	7.75	8.50	** 0.10	0.19
H. setariae	1.25	3.50	6.50	7.50	** 0.10	0.21
A. gossypii	0.00	4.25	5.00	6.00	** 0.073	0.16
A. fabae	0.00	2.00	4.00	6.00	* 0.12	0.26
A. craccivora	0.00	2.00	3.75	5.00	** 0.11	0.24
Rhopalosiphum sp.	0.00	0.25	1.75	3.25	** 0.12	0.26
Rhopalosiphum maidis	0.00	0.00	1.25	2.25	** 0.10	0.18
Group mean	0.57	2.57	4.29	5.50		
S.E.	** 0.19					
lsd	** 0.37					

Table 4 : Effect of Preliminary fasting of M. persicae on the transmission of PVMV.

Preliminary fasting	Number of infected plants				Mean ($\bar{x}$)	Transformed mean
	I	II	III	IV		
0	7.00	6.00	8.00	7.00	7.00	2.74
1 Min.	7.00	8.00	9.00	9.00	8.25	2.95
5 "	10.00	9.00	8.00	9.00	9.00	3.10
15 "	10.00	10.00	10.00	10.00	10.00	3.24
30 "	10.00	10.00	10.00	10.00	10.00	3.24
1 hr.	9.00	9.00	10.00	10.00	9.50	3.16
2 hrs.	9.00	9.00	8.00	8.00	8.50	2.00
6 "	5.00	6.00	6.00	4.00	5.25	2.39
12 "	4.00	3.00	3.00	3.00	3.25	1.90
24 "	2.00	2.00	2.00	2.00	2.00	1.58
S.E.					**0.64	**0.12
lsd					0.92	0.17

Immediately after such acquisition feeding periods, 10 aphids from each group were transferred to a healthy plant and allowed to feed for another 10 minutes. Ten plants were used for each test and each test was replicated four times.

The results (Table 5) indicate that 15 seconds a.f.t. was enough to transmit the virus. However, the efficiency of transmission went up with increased periods of acquisition to a certain point. Maximum percentage infections were obtained with 5 and 15 minutes of a.f.t. A rapid decline in the percentage of transmission was noticed with longer a.f.t. than 15 minutes. Analysis of the data showed that a.f.t. had a significant effect on the transmission rate at 1 per cent level.

(e) Effect of inoculation feeding time on the transmission of PVMV

Non-viruliferous M. persicae aphids from a healthy colony were first allowed to starve for 30 minutes and were given an acquisition feeding time of 15 minutes. Viruliferous aphids in batches of 10 were transferred to each test plant and the feeding time was artificially terminated after 15, 30 seconds; 1, 5, 15, 30 minutes; 1, 12, and 24 hours. Soon after corresponding inoculation feeding times, the aphids were physically removed with a/forcep from test plants and plants were sprayed immediately with pair of menazon to kill any aphid that might have been left unremoved.

Table 5: Effect of acquisition feeding time on the transmission of PVMV.

Acquisition feeding time	Number of infected plants				Mean ($\bar{x}$)	Transformed mean
	I	II	III	IV		
15 seconds	3.00	2.00	3.00	2.00	2.50	1.73
30 seconds	5.00	5.00	5.00	5.00	5.00	2.35
1 min.	8.00	7.00	8.00	9.00	8.00	2.91
5 mins.	10.00	10.00	10.00	10.00	10.00	3.24
15 mins.	10.00	10.00	10.00	10.00	10.00	3.24
30 mins.	8.00	9.00	7.00	8.00	8.00	2.91
1 hr.	4.00	5.00	4.00	4.00	4.25	2.18
12 hrs.	3.00	2.00	2.00	2.00	2.50	1.65
24 hrs.	2.00	2.00	2.00	2.00	2.00	1.58
S.E.					**0.49	**0.11
lsd.					0.71	0.25

Table 6 : Effect of inoculation feeding time on the transmission of PVMV.

Inoculation feeding time	Number of infected plants				Mean ($\bar{x}$)	Transformed mean
	I	II	III	IV		
15 seconds	3.00	2.00	3.00	4.00	3.00	1.86
30 seconds	3.00	4.00	5.00	5.00	4.50	2.17
1 min.	7.00	6.00	8.00	8.00	7.25	2.78
5 mins.	8.00	9.00	9.00	10.00	9.00	3.08
15 mins.	9.00	9.00	10.00	10.00	9.50	3.16
30 mins.	10.00	10.00	10.00	10.00	10.00	3.24
1 hr.	10.00	10.00	10.00	10.00	10.00	3.24
12 hrs.	10.00	10.00	10.00	10.00	10.00	3.24
24 hrs.	10.00	10.00	10.00	10.00	10.00	3.24
S.E.					** 0.62	** 0.13
lsd.					0.90	0.19

Ten plants were used for each i.f.t. test and the test was replicated four times.

The data in table 6 indicate that viruliferous M. persicae were capable of transmitting PVMV to healthy pepper test plants even after a short inoculation period of 15 seconds. From the raw data, it appears that the percentage of infected plants increased progressively with corresponding increase in the feeding period up to 30 minutes at which point 100 percent transmission was recorded. But the transformed data did not indicate any statistically significant difference between 5 minutes i.f.t. and thereafter. It appears therefore that 5 minutes of i.f.t. is probably good enough to attain the highest level of transmission.

(f) Persistency of PVMV in M. persicae.

In order to determine how long viruliferous aphids remain infective without further access to a fresh infection source, two sets of experiments were conducted.

(i) Effect of post-acquisition fasting on the retention of PVMV by M. persicae.

To find out how long PVMV could be retained by M. persicae, first healthy aphids were allowed to starve for 30 minutes and given a 15 minutes acquisition feeding time on the virus source. The viruliferous aphids were transferred into dry petri dishes for post-acquisition fasting periods of 0, 15, 30 minutes; 1, 2, and 6 hours.

Soon after each period of post-acquisition fasting, 10 test plants were/each inoculated with 10 viruliferous aphids and allowed 15 minutes of i.f.t. The test was conducted four times.

The results (Table 7) show clearly that viruliferous aphids which were transferred to test plants immediately after acquisition, transmitted the virus to the extent of 100 percent. But, there was a significant decline in the rate of transmission when aphids were starved for various periods after acquiring the virus. However, the virus was retained up to 6 hours by few aphids. Statistical analysis of the data (Table 7) showed that post-acquisition fasting had a significant effect on the rate of transmission of PVMV by M. persicae.

(ii) Serial transfers of viruliferous aphids to healthy test plants.

A number of M. persicae aphids were allowed to starve for 30 minutes and fed on infected plants for 10 minutes to acquire the virus. Viruliferous aphids singly and in groups of ten were transferred in succession to a series of four healthy pepper plants giving 5 minutes of inoculation feeding time on each plant. Such series were conducted 10 times.

When single aphids were employed, only five tests gave positive transmission out of 10. But the results (Table 8) showed that M. persicae in singles could transmit PVMV to only one plant in the series and not to the second plant in succession, indicating

Table 7 : Effect of post-acquisition fasting on the retention of PVMV by M. persicae.

Post-acquisition fasting period	Number of infected plants				Mean ($\bar{x}$)	Transformed mean
	I	II	III	IV		
0 Min.	10.00	10.00	10.00	10.00	10.00	3.31
15 Mins.	7.00	7.00	6.00	8.00	7.00	2.74
30 "	6.00	4.00	5.00	5.00	5.00	2.34
1 hr.	5.00	4.00	3.00	3.00	3.75	2.05
2 hrs.	4.00	4.00	3.00	3.00	2.75	2.00
6 "	2.00	1.00	1.00	0.00	1.00	1.18
S.E.					**0.74	**0.21
lsd					1.47	0.41

Table 8 : Serial transfers of viruliferous aphids to healthy test plants.

Replicates	Serial number of plants							
	Single aphids				Groups of 10 aphids			
	I	II	III	IV	I	II	III	IV
1	+	-	-	-	+	-	-	-
2	-	-	-	-	+	+	+	+
3	-	-	-	-	+	-	+	-
4	+	-	-	-	+	+	-	-
5	-	-	+	-	+	+	-	-
6	-	-	-	-	+	+	+	-
7	-	-	+	-	+	-	-	+
8	+	-	-	-	+	+	+	-
9	-	-	-	-	+	-	+	-
10	-	-	-	-	+	+	-	-

that the aphid lost the virus after feeding on a single test plant and was therefore no longer capable of infecting another one without going back to the source of the virus.

However, a group of ten viruliferous aphids, carried the virus to the second test plant in succession at least in 9 tests out of 10. In two instances the virus was carried in succession to the fourth serial plant, indicating that the virus is retained by aphids for 20 minutes.

(g) Relative efficiency of transmission of PVMV by various developmental forms of M. persicae.

Three developmental forms of M. persicae were chosen (young and old nymphs and winged adults) to compare their relative efficiencies in the transmission of PVMV. Aphids in the three developmental forms were collected separately and starved for 30 minutes. They were allowed to feed on diseased plants for 15 minutes to acquire the virus. Groups of ten viruliferous individuals were transferred to one test plant and ten plants were used in a test. Each test was conducted four times. Separate tests were simultaneously conducted using young nymphs, old nymphs and winged aphids. Transmission by winged aphids was conducted in cages (Plate 1).

From Table 9 it is clear that all the three aphid developmental forms were capable of transmitting the virus. There was no

significant difference in the transmission rates between young and old nymphs and similarly between old nymphs and winged aphids. However, young nymphswere significantly more efficient than winged aphids.

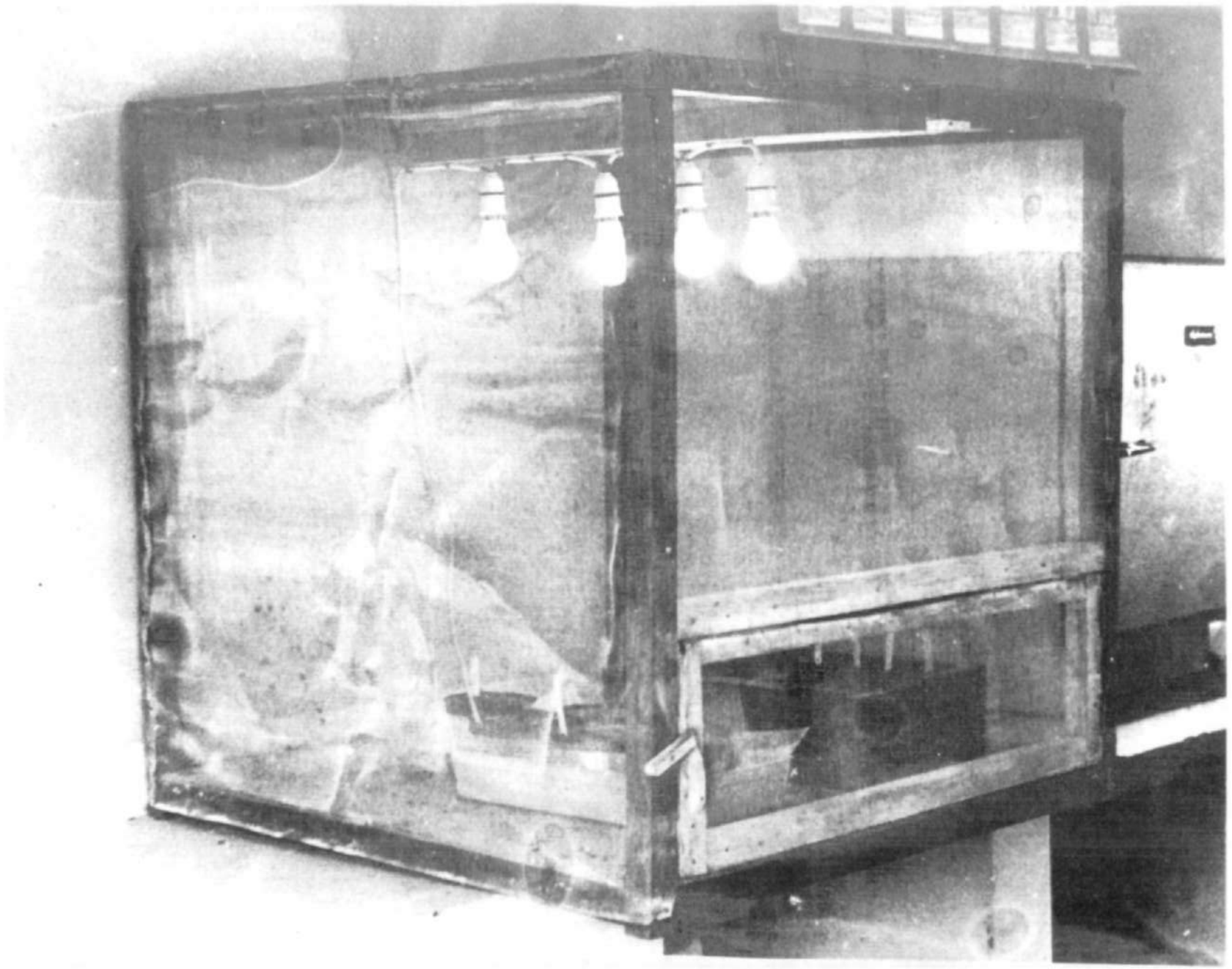
Table 9 : Relative efficiency of transmission of PVMV by various developmental forms of M. persicae.

Developmental form of aphid	Number of infected plants				Mean ($\bar{x}$)	Transformed mean
	I	II	III	IV		
Young nymphs	10.00	10.00	10.00	10.00	10.00	3.24
Old nymphs	9.00	8.00	9.00	10.00	9.00	3.08
Winged adults	9.00	8.00	8.00	7.00	8.00	2.91
S.E.					**0.67	**0.11
lsd					1.53	0.26

PLATE 1.

The set-up of PVMV transmission experiment with winged (alate) aphids. The cage prevented them from escaping, while the openings covered with netting material fascilitated aeration. The aphids were introduced through the corked opening.





DISCUSSION

Most virus diseases of crops are transmitted by insects and the control of such insects by spraying a range of insecticides does not always help in controlling viruses. A number of viruses (stylet-borne) are transmitted to healthy plants from diseased ones before insecticides start acting on their vector(s). In such cases, spraying insecticides is a wasteful and an expensive exercise. However, the spread of virus diseases could be effectively controlled by spraying insecticides against their vectors if the viruses are the circulative type. It is therefore, absolutely essential to know how a virus is transmitted by its vector(s) so that appropriate control measure(s) can be designed.

Watson (1938) and Watson and Roberts (1939, 1940) found that viruses transmitted by aphids fall into two groups viz 'nonpersistent' and'persistent' on the basis of their survival time in the aphid vector(s). Later on, by using more stable criteria such as actual routes of virus transport in the vector and whether or not the infectivity of the insect is lost following a moult, the terminology was changed to external or stylet-borne and internal or circulative viruses respectively. The terms stylet-borne and circulative proposed by Kennedy et. al., (1962)are more acceptable today.

The main features of stylet-borne type of transmission are that the virus can be picked up by aphid after a very short time of feeding on a diseased plant and can transmit it immediately to one or only a few healthy plants. Ability to transmit a virus is soon lost. The efficiency of transmission is often increased by starving the aphid before allowing it to feed on the virus source. Short acquisition feeding time on infective plants are more effective than long acquisition feeding time.

The studies made to determine the biological relationship of PVMV and its aphid vector/vectors revealed that starving aphids before they acquired the virus increased the efficiency of transmission, prolonged feeding on virus source plants decreased their efficiency (Table 4). Viruliferous aphids inoculated the virus to fresh plants within one minute. Furthermore, PVMV was not retained by M. persicae for a long time and the virus was lost by aphids soon after inoculation onto one plant without going to the second plant when single aphids were used (Table 8). The above results indicated clearly that PVMV was transmitted in a stylet-born manner.

When seven species of aphids were tested for the transmission of PVMV, all were found to transmit the virus. Thus it was found that all the 7 species used in this study are vectors of PVMV (Table 2). The result

indicated that PVMV has a low level of aphid specificity.

So far at least 2,7000 aphid species have been recorded in literature, out of these only 242 have been tested for their ability to transmit one virus or the other. About 80% of aphid species tested so far are vectors of viruses (Kennedy et. al., 1962). One can therefore expect the number of aphid vectors to go up when all known species are tested. Similarly, since PVMV showed a low level of specificity, it is also expected that the list of its aphid vectors would increase when more aphid species are tested.

There were significant differences in the ability of the aphids to transmit PVMV (Table 2). In all respects, it appeared as if M. persicae was the most efficient vector. M. persicae in singles could transmit the virus whereas R. maidis could do so only in a group of ten or more.

Studies on acquisition and inoculation of PVMV by M. persicae indicated that starving aphids for various periods increased their efficiency up to 30 minutes but a decline in transmission was observed when they were starved beyond this period (Table 2). Acquisition periods as short as 15 - 60 seconds were enough for transmission, although maximum level of transmission was obtained with 5 and 15 minutes of a.f.t. Longer feedings on virus source decreased transmission rate (Table 5). Short probes of 15 seconds by viruliferous aphids was enough to inoculate the virus, indicating that there was

practically no incubation period. The virus was acquired within a short time by the aphid and inoculated immediately to a fresh plant.

Classical work done by Watson (1938) with a stylet-borne virus showed two main features. These are (i) starving aphids before acquisition of the virus increased the efficiency of transmission. (ii) Short probes on virus source gave better transmission than long probes. Watson (1938) suggested that aphids produce a substance while feeding which makes the virus non-infective. However, there was no conclusive evidence to support this theory.

Following the work of Watson (1938) several workers showed that aphids improved their efficiency of transmission several times fold when they were starved before acquisition. Bradley (1964) attributed this to the behaviour of aphids which do not start feeding for a while when they are forcibly removed from the plant on which they were feeding. But when aphids are starved for a reasonable time they start feeding immediately.

The present study showed that PVMV was acquired within 15 seconds of feeding on a virus source. Bradley (1961) and Swenson and Welton (1966) tried to estimate the time needed for aphids to probe tissues by their stylets, but it was not easy to observe the insertion and retreat of the stylets. Zettler and Wilkinson (1966) while working on probing behaviour of M. persicae in the transmission of bean common mosaic virus came up with the view that

the short exploratory probes are important for stylet-borne transmission but long feeding probes are not. They showed that single short probes by aphids were better for transmission.

Bawden et. al. (1954) suggested that the reason why stylet-borne viruses are picked up more readily after short probes may be because such viruses occur in high concentration in the epidermal cells. However, Hitchborn, (1958), Van Hoof, (1958) and Namba, (1962) could not demonstrate the unequal distribution of the viruses in the tissues.

Viruliferous M. persicae transmitted PVMV within 15 seconds of inoculation feeding time. Such short inoculation period were reported by several other workers for non-persistent or stylet-borne viruses. Increase in the rate of transmission was obtained with increase in acquisition feeding time up to 5 minutes in this study but a.f.t. longer than this period did not increase the transmission rate. Bradley (1952) reported that the first probes longer than 15 - 30 seconds do not increase the probability of transmission.

Soon after acquisition, viruliferous M. persicae transmitted PVMV to healthy plants. This indicated that there was no incubation period. Studies made to determine how long the virus is retained by M. persicae, by (i) Starving the aphids after acquisition and (ii) by serially transferring the aphids to several pepper plants

(Table 8) revealed that the transmission rate declined progressively with increase in the period of post-acquisition fasting. The virus was retained by few aphids for about 6 hours. Similar results were obtained by Bradley et. al. (1962) using M. persicae for transmitting potato virus Y. The loss of infectivity was better demonstrated when single viruliferous aphids could not transmit PVMV to two plants consecutively. This further supports the view that the virus is stylet-borne.

Bradley and Ganong (1955) and Sylvester and Richardson (1964) failed to get transmission when the stylets of a vector carrying a supposedly non-persistent virus was treated with formalin or ultraviolet irradiation. This further supports the view that such viruses are stylet-borne.

During this study it was found that young nymphs of M. persicae were significantly more efficient than winged adults. Paine and Legg (1953) and Orlob (1962) reported that various developmental forms of an aphid differ in their efficiencies of transmission probably because the different developmental forms are adopted to different seasons and different host plants.

III. SCREENING FOR RESISTANCE TO PEPPER VEINAL MOTTLE VIRUS

INTRODUCTION

The resistance or susceptibility of a host plant to a virus is controlled by a single or many genes. Therefore the genetic make up of a host plant often has a profound effect on the outcome of infection by a given virus. There are several conditions such as immunity, resistance, hypersensitivity and tolerance in the development of infections. Among them resistance is probably the most common phenomenon in host plants. There are fewer instances where tolerance to virus infection were successfully used to control virus diseases (Williams, personal communication).

Further there are two aspects of host plant genetics that are important in the spread of virus diseases that are transmitted by vectors. One is the resistance of the host plant to the virus itself and the other aspect is the resistance or unattractiveness of the host plant to the insect vectors (Simons, unpublished). Although it is not easy to evaluate which aspect is dominant under field conditions, it is probably a combination of these two aspects of resistance that might work better in controlling virus diseases and their spread. Breeding for resistance is important for a given crop in a particular locality against a particular virus. Therefore, the transfer of resistance gene or genes into agriculturally satisfactory varieties is not easy.

Despite such difficulties, to-date breeding for resistance is the most effective measure for controlling virus diseases. The first step in breeding for resistance is the identification of source of resistance either in wild or cultivated species of plants.

Results of experiment in the last chapter indicated that PVMV is a non-persistent or stylet-borne virus. In general such viruses are difficult to control by the use of insecticides which kill their insect vectors since the transmission of the virus could be effected by the vectors before they are killed/ (Broadbent, 1957) It was therefore necessary to look for sources of resistance.

Efforts were made to screen a number of pepper varieties or selections to identify the source of resistance to PVMV. Experiments were conducted in the greenhouse by mechanical and aphid inoculation and in the field by natural infection. Attempts were also made to score the severity of PVMV symptoms on the varieties in the field. A score card was developed to compare the severity of the symptoms between the varieties. Since all the aphid species tried in the transmission experiment in the greenhouse were found to be vectors of PVMV, the relative prevalence of the various aphid species in the field was estimated by trapping the alate aphids visiting a pepper field, using yellow painted tray traps. Available meteorological data was used to discuss the influence of weather conditions on the prevalence of aphid vectors and the spread of the disease.

MATERIALS AND METHODS

Screening of pepper varieties for resistance to PVMV:

I. In the Greenhouse:

Seeds of thirty varieties of pepper were sown in the greenhouse and transplanted into 15.24 cm diameter pots after 3 to 4 weeks at 4-leaf stage. The seedlings were inoculated with PVMV by mechanical inoculation and by aphids.

(a) Mechanical inoculation.

Standard extract of diseased leaves was prepared by grinding leaf tissue in mortar (1.0 gm of leaf + 1 ml of 0.01 M phosphate buffer at pH 7.0). Large particles of plant material were removed by straining the jice through cheesecloth. The extract was applied gently on the upper surface of carborundum (600 mesh) dusted leaves, dipping the cheesecloth periodically in the extract. The leaves were then rinsed with distilled water and the plants kept in the greenhouse for observations. Six plants were inoculated per variety and six other uninoculated plants per variety were maintained as controls. The experiment was replicated four times.

(b) Inoculation by aphids.

The green peach aphid M. persicae was employed in the screening for resitance. A number of aphids were collected from a healthy

colony and allowed to starve for 30 minutes. Such aphids were allowed to feed on virus source for 15 minutes to acquire the virus. They were immediately transferred to healthy pepper varieties in groups of 10 aphids per test plant and allowed 15 minutes of inoculation feeding time. The plants were sprayed with menazon 1.75 ml a.i./litre to kill the aphids. Six plants were inoculated per variety. The experiment had four replicates and corresponding uninoculated plants were kept as controls.

In the greenhouse screening, all the varieties not showing symptoms due to mechanical inoculation were tested by sap inoculation on the pepper variety L5962-2 which was known to be very susceptible to PVMV, to find out if the virus was carried symptomlessly in them. A similar test was carried out in the case of aphid inoculation experiments.

II. In the Field:

(a) Land preparation and layout of experiment.

Seedlings of 30 varieties of pepper were raised in an insect-proof greenhouse as described in the general materials and methods and transferred to the field after four weeks of growth.

A fairly uniform field in I.A.R. farm ($S1O_b$) was prepared and a standard basal application of superphosphate was applied. The whole field was ridged at 60 cm interval. The field was divided

into 3 major blocks which represented 3 replicates. Each block was further divided into 30 smaller plots of two ridges of 600 cm length which accommodated 20 plants of one variety. The varieties were randomly allotted to the plots using the complete randomize block design (Plate 3).

Seedlings were transplanted on the ridges at 60 cm distance. The crop was top-dressed twice, two weeks and six weeks after transplanting with a compound fertilizer N.P.K. (1:1:1) at the rate of 200 kg/ha. Weeding was done 4 times at 2-weeks interval.

(b) Observations and disease rating.

Weekly observations of number of plants showing symptoms was made, starting from the eighth week after transplanting, for 9 weeks.

Attempts were made to evaluate the severity of symptoms in the various varieties due to PVMV infection. The following score card was developed for scoring (rating) the severity of the symptoms (Plate 6):

Score 0 = No visible symptoms.

Score 1 = No stunting of plant, mild mottling and no leaf distortion.

Score 2 = Slight stunting of the plant, mottling and slight leaf distortion.

Score 3 = General stunting of the plant, severe mottling and leaf distortion.

Score 4 = General stunting of the plant, very severe mottling and leaf distortion.

Observations were recorded at weekly intervals for 4 weeks towards the end of the growing season when the spread of the virus had almost ceased. The scores of individual plants in each variety were added up and the mean was calculated by dividing the total number of scores by the total number of plants scored. Only the last set of scores were finally used for disease rating.

(c) Meteorological data.

The daily weather reports supplied by the agro-climatological unit of the Institute for Agricultural Research, Samaru, on temperature, relative humidity, sunshine, wind-run and rainfall were used to discuss the influence of weather on the prevalence of aphid species which are vectors of PVMV and the spread of the disease.

(d) Aphid traps in the field.

Six rectangular metal trays painted bright yellow were set up in the field (Plate 4) two in each major block (Plate 5), 45.72 cm above ground level against a contrasting background of bare soil (Heathcote, 1958., Evans and Medler, 1966). The trays were filled with a solution

containing water (98%), teapol (detergent: 1.5%) and formalin (preservative: 0.5%).

The insects trapped in the trays were collected every week and sorted out discarding insects other than aphids. The total number of aphids trapped were recorded. Further, aphids were sorted into various species on the basis of external appearance using a binocular microscope and the number of each species recorded.

RESULTS

Screening of pepper varieties for resistance to PVMV by mechanical and aphid inoculations:

I. Greenhouse results.

The mean number of plants showing symptoms are shown in Table 10. The varieties were classified into resistant, moderately susceptible and highly susceptible on the basis of mean number of plants showing symptoms out of 6 plants inoculated. The following scale was used:

Mean No. of Plants showing Symptoms	Classification of variety
0.00	Resistant
1.00 to 4.00	Moderately susceptible
5.00 to 6.00	Highly susceptible

(a) Mechanical inoculation.

From Tables 10 and 11 it is clear that except the variety TCa 14xxx76, all varieties tested were susceptible to PVMV. Eight varieties were moderately susceptible while 21 were highly susceptible. Pepper veinal mottle virus was recovered by sap inoculation from symptomless plants of TCa 14xxx76 to a very susceptible variety L5962-2 indicating that the virus was symptomlessly carried by the variety. However, the virus was not recovered from the variety by aphids (M. persicae).

(b) Inoculation by aphids.

Two varieties, TCa 14xxx76 and California mild were resistant, six were moderately susceptible and twenty two were highly susceptible (Tables 10 and 11). Aphids (M. persicae) were unable to recover the virus from the symptomless plants of both varieties. However, California mild was moderately susceptible when mechanically inoculated.

Statistical analysis of the raw and transformed data using the "F" and l.s.d. tests at 1% level showed that there was a significant difference in the level of infection of the varieties by both mechanical (sap) and aphid inoculation.

Table 10: Screening for virus (PVMV) resistance in pepper by aphid and sap inoculation.

Code Number	Variety	Mean of infected plants	
		Sap inoculation	Aphid inoculation
1	Red cherry	3.00	3.00
2	L2170 (Tatasai)	3.00	5.00
3	L2289	1.00	1.00
4	California mild	1.00	0.00
5	Cayenne long slim	5.00	3.25
6	Kimba (S.B.E.C.)	1.00	1.25
7	L2190 (S.B.E.C.)	4.00	3.00
8	Kashim Bargu (S.B.E.C.)	4.00	4.00
9	L2096	6.00	4.25
10	Red Chilli	6.00	4.50
11	L2025 (S.B.E.C.)	4.00	1.75
12	Dantsiga (S.B.E.C.)	2.00	3.00
13	Serrano Chilli	6.00	4.25
14	L2188 (S.B.E.C.)	4.00	3.00
15	Sakarho (S.B.E.C.)	2.00	2.75
16	Ex-Hunkuyi	5.00	4.00
17	Danmeyere (S.B.E.C.)	2.00	1.00
18	L3874	2.00	2.75
19	Cayenne Large Red Thick	3.00	4.00
20	L2191 (Tatasai)	1.00	2.00
21	L2164 (Tatasai)	4.00	3.00
22	L5962-2 (Tatasai)	5.25	5.00
23	Caloro	5.25	6.00
24	Anahem	6.00	6.00
25	Yolo Wonder	6.00	4.75
26	Hungarian Yellow wax	5.00	4.75
27	TCa 14xxx76	0.00	0.00
28	Green leaf tabasco	6.00	5.00
29	Nihort Rodo	5.25	5.00
30	Nihort Tatasai	6.00	5.00
		S.E. = $\overset{**}{0.29}$	S.E. = $\overset{**}{0.21}$

Table 11: Classification of thirty varieties of pepper screened for resistance to PVMV by sap and aphid inoculations.

Classification	Sap inoculation	Aphid inoculation
Resistant varieties	TCa 14xxx76	TCa 14xxx76 California mild
Moderately Susceptible Varieties	L228 Kimba L2190 Red Cherry Kashim Bargu Danmeyere L3874 L2191 L2164	L2289 Kimba L2190 Red Cherry Kashim Bargu Danmeyere L3874 L2191 L2164

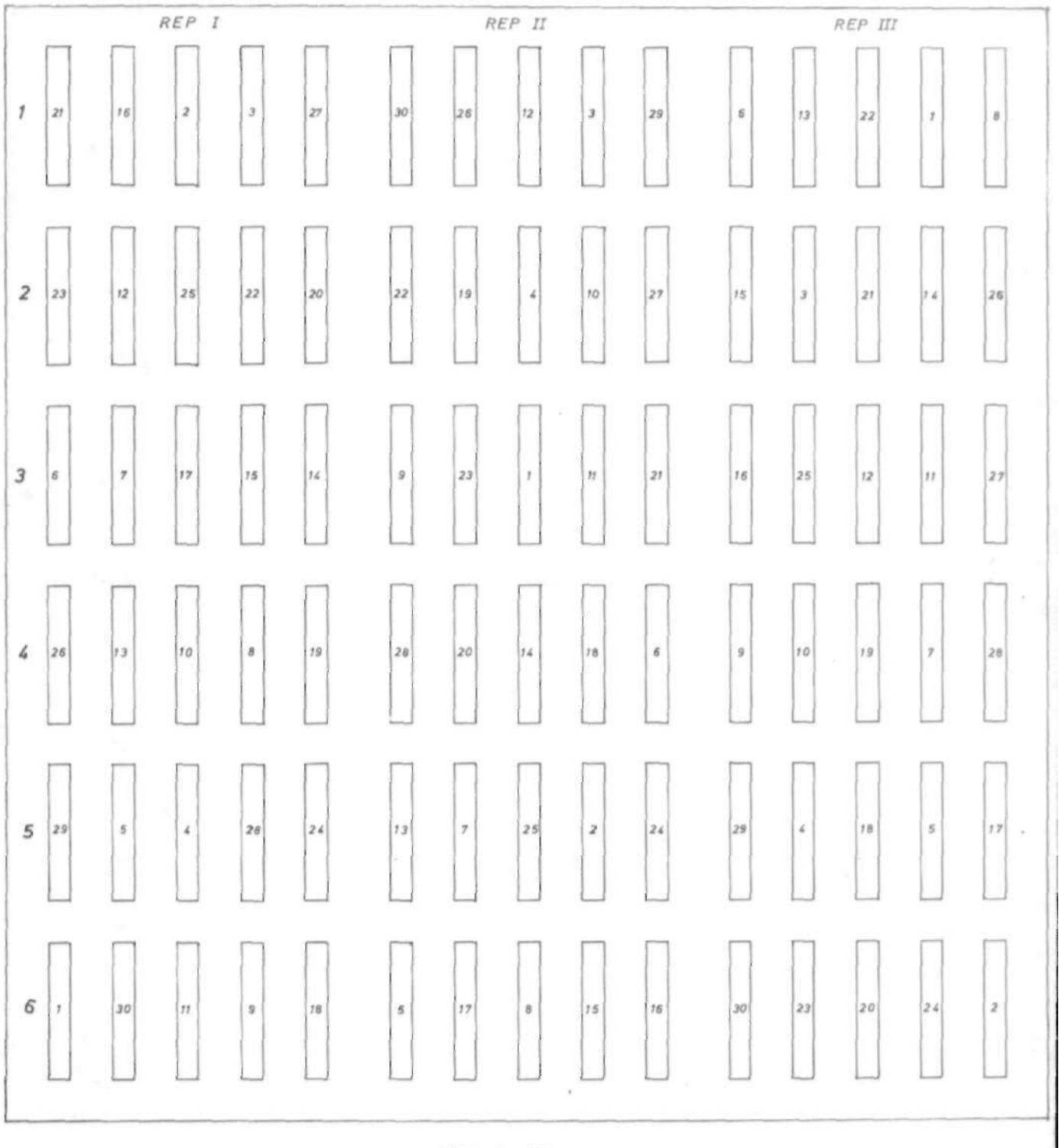
II. Screening pepper varieties for resistance to PVMV in the field:

Table 12 gives the mean number of infected plants out of a mean of 20. The following arbitrary scale was developed for classifying the number of infected plants per variety:

Mean number of plants showing symptoms	Classification of variety
0.00	Resistant
1.00 to 4.00	Moderately susceptible
5.00 and above	Highly susceptible

The data in Table 13 clearly indicates that none of the varieties tested was entirely resistant. The varieties that showed resistance in the greenhouse screening were found to be moderately susceptible under field condition. However, all the varieties showed two categories of susceptibility-moderately and highly susceptible.

FIELD PLAN: SCREENING FOR VIRUS RESISTANCE IN PEPPER



SCALE :- 1cm = 10cm







0



1



2



3



Statistical analysis of the cumulative number of infected plants during the first reading - 13th Sept. (Table 12) showed that there was no significant difference at 1% level between the mean number of infected plants in the 30 pepper varieties.

On the other hand, statistical analysis of the 3rd and last readings (27th Sept. and 8th Nov. respectively) showed that there was a significant difference in the number of infected plants in the varieties at 1% level. When block means were subjected to the "F" test, there was no significant difference between them indicating that soil fertility gradient and other factors that create block differences were probably non-operational and the treatments were randomly distributed.

Severity of symptoms due to PVMV infection on pepper varieties.

Observations of symptom picture were made inorder to find out if the severity of symptoms corresponded with the degree of susceptibility of the varieties. The score card for disease severity is shown as Plate 6. The varieties were categorized accordingly.

From Table 14 it is interesting to note that majority of the varieties grouped as resistant and moderately susceptible showed milder symptoms compared with the highly susceptible ones.

When the severity means were subjected to the "F" and l.s.d. tests, there was a significant difference in the severity means of the 30 varieties at 1% level. No significant differences were recorded between the block means.

On the basis of greenhouse, field screening and disease severity, an attempt was made to summarise the performance of the pepper varieties. Out of the 30 varieties tested, 9 of them (arranged in order of tolerance in Table 15) appeared to be promising. Generally, TCa 14xxx76, Kimba (S.B.E.C.), California mild and Danmeyere were outstanding but only California mild is a sweet pepper (C. annuum) among them while others are hot pepper (C. frutescens).

Correlation between aphid population and disease incidence.

Transmission studies revealed that PVMV is transmitted in a stylet-borne manner by several species of aphids. When the virus was transmitted by aphids to pepper plants, it took about 7 days for symptoms to appear (inoubation period). Therefore disease appearance due to the aphids trapped in a given week was expected in the following week. This was taken into consideration when plotting the graph

Tabl

Recording date
11th Sept.
20th Sept.
27th Sept.
4th Oct.
11th Oct.
18th Oct.
25th Oct.
1st Nov.
8th Nov.

for resistance to PVMV in the field.

Classification	Variety
Resistant	None
Moderately susceptible	California mild Kimba (S.B.E.C.) Dantsiga Danmeyere L3874 L2191 (Tatasai) L2164 (Tatasai) TCa 14xxx76
Highly susceptible	Red cherry L2170 (Tatasai) L2289 Cayenne long slim L2190 (S.B.E.C.) Kashim Bargu (S.B.E.C.) L2096 Red Chilli L2025 (S.B.E.C.) Serrano Chilli L2188 (S.B.E.C.) Sakarho (S.B.E.C.) Ex-Hunkuyi Cayenne Large Red Thick L5962-2 Caloro Anahem Yolo Wondor Hungarian Yellow wax Greenleaf tabasco Nihort Rodo Nihort Tatasai

Table 14: Severity of PVMV on thirty pepper varieties at Samaru on 22/11/77.

Variety	Scores			Mean ($\bar{x}$)
	I	II	III	
Kimba (S.B.E.C.)	0.20	0.00	0.00	0.07
TCa 14xxx76	0.20	0.00	0.22	0.14
Danmeyere	0.00	0.00	0.70	0.23
California mild	0.20	0.60	0.20	0.33
Dantsiga (S.B.E.C.)	0.50	0.00	0.50	0.33
L2164 (Tatasai)	0.00	0.50	0.50	0.33
L2025 (S.B.E.C.)	0.70	0.00	0.36	0.35
L2191 (Tatasai)	0.60	0.70	0.00	0.43
L3874	0.30	1.10	0.00	0.47
Sakarho (S.B.E.C.)	0.40	0.00	1.10	0.50
Caloro	1.00	0.30	1.00	0.77
L2289	1.20	0.80	0.40	0.80
Greenleaf tabasco	0.80	1.10	0.50	0.80
Anahem	1.33	0.30	0.80	0.81
Ex-Hunkuyi	1.50	0.00	1.00	0.83
Hungarian Yellow wax	0.66	1.30	1.00	0.99
Yolo Wonder	1.20	1.33	0.50	1.01
L2190 (S.B.E.C.)	1.40	1.00	1.10	1.17
Red cherry	1.90	1.10	1.20	1.40
Nihort Rodo	1.40	1.10	2.20	1.57
L2170 (Tatasai)	1.30	2.00	1.70	1.67
Cayenne Large Red Thick	2.20	1.40	1.70	1.77
Nihort Tatasai	2.20	1.70	1.80	1.90
Kashim Bargu (S.B.E.C.)	3.10	3.20	2.00	2.77
L2096	3.00	2.70	3.00	2.90
L2188 (S.B.E.C.)	3.10	3.10	3.00	3.07
L5962-2 (Tatasai)	2.77	3.40	3.10	3.09
Cayenne long slim	3.50	3.50	2.50	3.17
Red Chilli	3.20	3.30	3.10	3.20
Serrano Chilli	2.90	3.50	3.30	3.23

S.E. = $\overset{**}{0.23}$
l.s.d. = 0.84

Table 15: Pepper varieties that showed promising signs of resistance to PVMV at Samaru.

Variety	Greenhouse Screening		Field Screening	
	No. of infected plants out of 6		No. of infected plants out of 60	Disease severity
	Mechanical inoculation	Aphid inoculation		
TCa 14xxx76	0.00	0.00	2.00	0.14
Kimba (S.B.E.C.)	1.00	1.25	2.00	0.07
California mild	1.00	0.00	2.00	0.33
Danmeyere (S.B.E.C.)	2.00	1.00	2.00	0.23
Dantsiga (S.B.E.C.)	2.00	3.00	3.00	0.33
L2191 (Tatasai)	1.00	2.00	3.67	0.43
L2164 (Tatasai)	4.00	3.00	2.33	0.33
L3874	2.00	2.75	4.33	0.47
Sakarho (S.B.E.C.)	2.00	2.75	4.67	0.50

KEY

Tatasai = Hausa word for sweet pepper.

S.B.E.C. = Small bird eye chilli.

The data in Table 16 clearly indicate that the aphid population was highest (during the recording period) on 20th September and the disease incidence recorded the following week was the highest indicating that the higher the population of aphids in a particular week the higher the number of infected plants the following week. The delay of 7 days, accounted for the incubation period of the virus in the plant. It was also noticed that with a decrease in aphid population, the number of plants infected also decreased.

A significant difference at 1% level was recorded when the total number of aphids trapped per week was subjected to the "F" and least significant difference tests. There was a highly positive correlation between the number of aphids trapped per week and the rate of infection of pepper plants with PVMV.

Relative prevalence of aphid species.

Regular weekly counts of alate aphids trapped in yellow trays indicated the consistent presence of 5 aphid species - Aphis craccivora, M. persicae, R. maidis, A. fabae and A. gossypii. In the earlier studies on vetor-virus relationships, all the five aphid species were found to be vectors of PVMV. The aphids that were not identified were called 'Others' (Table 17).

Table 16: The relationship between aphid population and the incidence of PVMV on pepper at Samaru in the 1977 wet season.

Recording date	Age of pepper plants in weeks	No. of infected plants	No. of aphids trapped
13/9	12	1	29
20/9	13	93	40
27/9	14	181	31
4/10	15	64	29
11/10	16	53	28
18/10	17	42	22
25/10	18	46	17
1/11	19	42	9
8/11	20	4	6

Table 17: Species of aphids trapped in a pepper field at Samaru season.

Recording date	A. craccivora	M. persicae	R. maidis	A. fabae	A. gossypii
16/8	20	5	0	8	4
23/8	28	27	22	4	19
30/8	11	12	5	6	7
6/9	2	5	7	0	0
13/9	6	9	-		
20/9					
27/9					
4/					
11					

The data in Table 17 clearly indicate that the aphid species A. craccivora, M. persicae and R. maidis were the most prevalent. They showed significantly higher numbers in weekly counts. However, there were no significant differences between these 3 species.

From the data it was rather difficult to compare the peaks of these three aphids. Therefore it was not possible to point out which one of these 3 species was the most important. Looking at the counts made on 23/8/77 when the highest number of aphids were recorded, it seems as if all the 3 species occurred in the field more or less in the same strength, indicating that they were probably more important in the spread of PVMV.

Analysis of the weekly aphid counts indicated that there was a significant difference between the number of aphids trapped on different dates (Appendix I). The total number of aphids trapped decreased from 195 in August to 115 in September, 96 in October and finally to 15 in mid-November when reading was terminated.

Table 18: Relationship between weather and the number of alate aphids trapped in a pepper field at Samaru in the 1977 wet season.

Period	Average temp. (min & max.) (X_1)	Mean Relative Humidity (%) (X_2)	Mean Sunshine (Hours) (X_3)	Mean Wind Run (Km) (X_4)	Mean Rainfall (mm) (X_5)	No of Alate aphids trapped (Y)
9-15th August	23.14	80.10	3.60	91.70	18.10	41.00
16-22nd August	24.90	77.30	7.50	134.00	5.8	101.00
23-29th August	23.10	82.90	3.10	163.60	16.7	48.00
30th August - 5th Sept.	24,60	78.30	6.70	103.60	5.80	15.00
6-12th Sept.	23.70	75.00	7.80	85.10	13.30	29.00
13-19th Sept.	25.00	62.60	8.80	98.10	2.40	40.00
20-26th Sept.	25.00	68.90	6.90	105.30	5.90	31.00
27th Sept. - 3rd Oct.	25.20	64.40	6.80	99.90	2.40	29.00
4-10th Oct.	25.20	66.30	7.00	93.30	0.00	28.00
11-17th Oct.	23.10	60.10	8.70	103.30	0.62	22.00
18-24th Oct.	24.40	45.50	9.70	95.30	0.00	17.00
25-31st Oct.	20.40	55.20	10.40	141.20	0.00	9.00
1-7th Nov.	20.20	24.60	10.50	118.00	0.00	6.00

Influence of weather on aphid population in the field.

The relationship between the number of alate aphids trapped and climatic factors is shown on Table 18. There was a positive correlation between the number of aphids trapped and relative humidity (Appendix II). Periods of high relative humidity generally corresponded with high population of aphids. The next climatic factor that showed a little correlation with the number of aphids trapped was temperature. But this relationship was not significant at 1% level (Appendix II).

Generally there was no significant correlation between the number of alate aphids trapped and the following climatic factors: temperature, sunshine, wind-run and rainfall.

DISCUSSION

The result of the greenhouse screening of thirty pepper varieties for resistance to PVMV indicated that the sap inoculation method was slightly more efficient than aphid inoculation (Table 10). A similar result was obtained by Alegbejo (1974) at IITA. In the sap inoculation method, the virus concentration was high, the inoculum was conditioned by adjusting the pH, non-lethal wounds were made on the leaf surface by rubbing it with carborundum for a direct entry of the virus into the leaf tissue. These factors might have made the sap more efficient than the aphid inoculation method.

Two varieties viz: TCa14xxx76 and California mild were found to be resistant to PVMV in the greenhouse. Controlled conditions such as a single isolate of PVMV and aphids of one species (*M. persicae*) and one colony were used. The same varieties did not react similarly in the field where they were tested under natural conditions. Here aphid density, prevalence of a particular species and the strain(s) of the virus were not controlled. These two varieties were only moderately susceptible in the field and could therefore only be considered as promising varieties.

It is also interesting to note that the moderately susceptible varieties showedless severe symptoms compared with the susceptible ones.

Resistant varieties are normally expected to produce less severe symptoms than susceptible ones where the concentration of the virus is normally higher. In the present study it was not possible to assay the virus concentration in the various varieties because the virus had no local lesion host (Holmes, 1929).

Field screening and aphid trap results showed that the incidence of PVMV is caused from the primary spread of the virus by viruliferous aphids viz: Aphis craccivora, A. fabae, A. gossypii M. persicae and R. maidis migrating into pepper fields (Table 16, Fig. 8). Since the incubation period of the virus in pepper is about 7 days, the disease incidence of a any given week was correlated with the aphid population of the previous week. High population of aphids consistently corresponded with high PVMV incidence. Similar observations were made by Bloch (1971), Zitter and Ozaki (1973) and Whitam (1974) with the viruses they were working with.

In the previous chapter, 7 species of aphids were found to be vectors of PVMV although they differred in their efficences of transmission. It is not uncommon to find less efficient species being very important vectors in a given locality because of their presence in large numbers (Van Emden, 1972). A similar situation was encountered in this study where A. craccivora and R. maidis which were less

efficient than M. persicae were trapped in large numbers. H. setariae which was one of the most efficient vectors of PVMV in the greenhouse was not trapped in the field.

A craccivora, M. persicae, R. maidis, A. fabae and A. gossypii colonize groundnut, guinea corn, millet, Solanum nigrum and cotton respectively. These crops are normally grown at Samaru in the wet season and provide breeding grounds for these aphids. Out of the five species of aphid trapped, A. craccivora, M. persicae and R. maidis were trapped in higher numbers. These species are probably the most important vectors of PVMV at Samaru.

M. persicae may probably be very important because its population peaks corresponded with the peaks of PVMV incidence and it was the most efficient vector in the laboratory studies. This aphid was reported by Brunt and Kenten (1971), Ladipo and Roberts (1977) as a vector of PVMV. Dickson (1949) and Simons (1958) thought that M. persicae is a very efficient spreader of a number of virus diseases in the field because of its habit of short flight.

The importance of A. craccivora in the spread of different viruses including PVY and PVMV was recognised by Mariappa et al. (1973)., Lana et al. (1975)., and Whitam (1974). It was probably very important in the field spread of PVMV at Samaru where its host-groundnut is widely grown.

Although Rhopalosiphum maidis was not an efficient vector of PVMV (relatively) in the laboratory studies, nevertheless, it might be important in spreading PVMV. Its hosts maize and millet are grown by most farmers in Samaru.

Aphis fabae and A. gossypii whose hosts are Solanum nigrum and cotton respectively, though less abundant than the other aphids trapped, were fairly efficient vectors of PVMV in the laboratory studies. They might be important field vectors. The latter aphid was reported by Brunt and Kenten (1971) De Wijs (1973) and Lana et al. (1975) as a vector of PVMV.

Weather factors such as temperature, relative humidity, sunshine and wind influence the movement of aphids. In this study, only relative humidity had a significant influence on aphid population. As the relative humidity decreased the population of aphids also decreased and hence the number of pepper plants infected with PVMV.

The need to use resistant pepper varieties is likely to be the most valuable answer to the control of PVMV. It is therefore suggested that farmers use TCaxxx76 and California mild or the moderately susceptible pepper varieties until resistant varieties are bred by a team of breeders and plant virologists.

IV. THE DISTRIBUTION OF PEPPER VEINAL MOTTLE VIRUS IN THE NORTHERN STATES OF NIGERIA.

INTRODUCTION

Virus diseases affecting peppers (Capsicum spp.) are of world wide importance. Workers from various parts of the world have made numerous reports on pepper virus problems. Viruses such as TMV, CMV, TEV, PVY and TNV are known to cause losses in yield of pepper crop. Pepper veinal mottle virus is probably the most important virus on pepper in West Africa. It has been reported in Nigeria by Lana et. al. (1975). Since then the virus has been reported in other parts of the country (Subbarayudu, unpublished., Ladipo and Roberts, 1977).

An understanding of the distribution of a virus or viruses in a particular crop and locality is very important before attempting control measures. A systematic survey in the northern states of Nigeria was attempted to locate the presence of pepper virus or viruses. Attempts were also made to see if there were strain relationships between the isolates collected. The survey was conducted in Kaduna, Kano, Sokoto, Niger, Kwara, Plateau and Benue States. Bauchi, Borno and Gongola States were not included because of limited funds. Identification of the viruses and their strains was based on studies such as host-range, physical properties and serology.

MATERIALS AND METHODS

I. Survey of virus diseases.

Survey was conducted in 7 states. From Kaduna and Kwara States, samples were collected from more than one place. In each locality at least 3 representative samples were collected. Pepper plants showing typical mosaic or mottle symptoms were spotted and leaves showing symptoms were plucked and packed in small polythene bags, labelled and brought to the laboratory.

The sap extracts of all samples were inoculated separately onto 4 week old pepper seedlings of C. annuum cv. L5962-2.
Regular transfers were made to new seedlings by using aphid vectors.
The isolates were continuously maintained in the greenhouse.
Care was taken to separate the various isolates by keeping wooden planks or glass sheets in between isolates and spraying regularly with aphicides.

Table 19: The abbreviations used for the isolates collected from various locations.

STATE	PLACE	ABBREVIATION USED FOR THE ISOLATE
KADUNA	Samaru	SAM
	Angwan Sambo	AS
	Pambegua	PAMB
	Kaduna	KAD
	Funtua	FU
	Hunkuyi	HU
KANO	Kadawa	K
SOKOTO	Gusau	GU
NIGER	Mokwa	MK
KWARA	Ilorin	IL
	Kabba	KAB
BENUE	Makurdi	MAKD
PLATEAU	Jos	J

II. Identification of the virus isolates.

a) Host range studies.

Thirty plant species belonging to six different families were employed. Various virus inocula were prepared from their respective isolates maintained on C. annuum cv. L5962-2. Standard extracts of virus were obtained by triturating the leaves showing symptoms with 0.01M phosphate buffer at pH 7.0 in pestle and mortar.

The extract was strained through two layers of cheesecloth. Thirty species of plants were inoculated with each inoculum on ..carborundum (600 mesh) dusted leaves, with a piece of cheesecloth. The inoculated leaves were rinsed immediately with water from a wash bottle. Five test plants of each species were inoculated while 5 other uninoculated plants were kept as controls. Plants were left in the greenhouse for observation. Plant species that did not show symptoms after 4 weeks were tested on C. annuum cv. L5962-2, to find out if they were carrying the virus symptomlessly. Back inoculations were also conducted to pepper plants by sap. and aphid inoculations from the hosts showing symptoms.

b) Physical properties

Determination of physical properties was done only for the isolates that were collected from Kano and Kaduna States. Thermal inactivation point, dilution end point and longevity in vitro were studied for these isolates.

Inocula of the isolates for these studies were obtained from leaves of pepper plants cv. L5962-2 showing mottling symptoms. Inoculations were made on leaves of the above cultivar and rinsed soon after inoculation. Five plants were used for each treatment and replicated 4 times. The plants were kept in the greenhouse and observations recorded.

Dilution-end point

Crude extracts of the isolates were prepared from pepper leaves showing distinct mottling symptoms. The following dilutions were made from the crude extracts: Undiluted, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7}. Test plants were inoculated with each inoculum.

Thermal Inactivation Point

Crude extracts of the isolates were distributed into thin walled test tubes which were exposed to various temperatures in water baths. The temperatures of the sap inside the test tubes were taken into account. Test tubes containing 2 ml of crude extracts were exposed to temperatures of 50, 54, 56, 58, 60, 62, 64, 66, 68 and $70^{\circ}C$ for ten minutes in a water bath. The contents were cooled and each inoculated onto five pepper test plants.

Longevity in vitro

Crude extracts of the isolates were put in test tubes and separately stored at 7 and $25^{\circ}C$. Each of the above inocula were inoculated onto five pepper test plants per day for 10 days.

III. Purification of the virus isolates

Several methods have been published on the purification of viruses belonging to the potato virus Y group (Delgado-Sanchez and Gregan, 1966., Damirdagh and Shepherd, 1970., Gillaspie, 1971). However, complete purification of these viruses was not very successful. The primary problem in their purification is the irreversible aggregation

of the virus particles during high speed and loss of the virus during low speed centrifugation steps. Whitam (1974), suggested that any method that could partially purify the virus, might be used to achieve necessary results, if enough host debris could be removed.

Brunt and Kenten (1971) used chloroform and 2-mercaptoethanol in the initial extraction to prevent aggregation and sedimentation of virus during low-speed centrifugation. The yellowish aqueous phase was filtered through Whatman No. 4 filter paper to remove the plant material and the virus precipitated at 0 to 4°C by adding 50 g of polyethylene glycol (600 M.W) per litre. The precipitate (mainly virus particles) was collected by centrifugation at 10,000 g for 10 min., dispersed in 0.5 M borate buffer and after 2 hours plant material was further removed by another cycle of low centrifugation. Borate buffers (pH 7.8 and 7.5) were effective in resuspending the pelleted virus after low-speed and rate zonal density gradient centrifugation respectively.

Whitam (1974) working with PVY used the Damirdagh and Shepherd (1970) method with a slight modification. He used n-butanol to remove the plant material. But unlike Brunt and Kenten (1971) phosphate buffer was used in place of borate and a reasonable concentration of the virus was obtained.

Isolates collected from all locations were purified for the purpose of serological test. Whitam (1974) method, a slight modification of the method suggested by Damirdagh and Shepherd (1970)

for the purification of viruses of the potato virus Y group, was used.

a) Extraction.

The sap extract was prepared by triturating diseased leaves in a sterilized mortar and pestle kept at a temperature of 4°C. With 0.5 M phosphate buffer, pH 7.1 (1 g : 1 ml) containing 1% 2-mercaptoethanol.

b) Clarification.

The homogenate was pressed through two layers of cheesecloth and n-butanol was added slowly into the extract until it was 8% of the solution. This process was done tow times.

c) Isolation.

The virus was precipitated from the clarified sap by dissolving 4.00 g of polyethylene glycol (PEG molecular weight 6,000) per 100 ml. of the extract. This was incubated for 90 minutes and the precipitated virus was centrifuged at a low speed of 10,000 g for 15 minutes. The pellets were resuspended in 30 ml of 0.025 M phosphate buffer, pH 7.4. and left overnight. The virus was again precipitated by adding PEG and NaCl to make a 4% solution. The mixture was left for 90 minutes and the pellets were collected by low speed centrifugation. The pellets were again resuspended in 15 ml of 0.025 M phosphate buffer pH 7.4 and used for serological tests.

The isolates used in this study were purified from the following plants :-

Group	1	from	pepper cv. L5962-2
"	2	"	Physalis floridana
"	3	"	Petunia hybrida
"	4	"	Solanum nigrum
"	5	"	Nicotiana tabacum

Serological Tests.

Antisera to PVMV, PeMV, TMV, CMV, TEV and PVY were obtained from several sources. Antisera of PVMV were obtained from A. A. Brunt of the glasshouse crops Research Institute, Sussex, England., J.J. De Wijs of Ciba-Geigy AG, Agrochemical division Switzerland., A.O. Lana, Department of Agricultural Biology, University of Ibadan.

Initially Ouchterlony agar double diffusion test was used for the determination of serological relationships of the isolates, but most of the later tests were conducted by using micro-precipitin test with drops between slides (Noordam, 1973).

(a) Ouchterlony agar double diffusion test.

Majority of pepper viruses described so far belong to the potato virus Y group, which are rod-shaped. Therefore, Sodium dodecyl sulphate (SDS) was used to degrade the long particles to facilitate their movement through agar gels. Further SDS minimizes

non-specific reactions. Gooding and Bing (1970) medium which contained 0.8% Bactoagar, 0.5% SDS and 1% Sodium azide, all w/v in water was used. The medium was poured into Petri dishes and allowed to settle. Wells were cut by using gel cutter with seven tubes of 4mm diameter and 4mm apart. Antibodies were placed in the central wells and antigens in the surrounding wells. The plates were observed for one week for the development of precipitation bands.

(b) Microprecipitin test between slides (Noordam, 1973)

Plate 23 shows the arrangement of materials in the precipitin test with drops between slides. Undiluted sap of about 1, $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$, to 1/8192ml. were put in fourteen centrifuge tubes. 0, $\frac{1}{2}$, $\frac{3}{4}$, $\frac{7}{8}$ to 8191/8192ml. of saline buffer solution were added to the undiluted sap respectively. Similar dilutions were prepared with the antiserum. The drawing (Plate 23) was laid on the table and sap dilutions indicated on the top starting on the left with saline-buffer solution. The slide with thickness 6mm was put on the drawing. A pasteur pipette was filled with saline buffer and the drops placed on the upper and left row. The pipette was emptied and filled with the lowest concentration of sap. Seven drops of sap were put on the second vertical row and so on up to a vertical row no. 12. A new pipette was filled with the lowest antiserum concentration and a drop added to each of the 12 drops and so on up to the last horizontal row. A new pipette was used for each row. This procedure was repeated on another

slide with antiserum and sap concentrations from 1:256 to 1:8192. The 1mm frame was then put on the slide with drops. The slide with thickness 5mm was put exactly on top, without shifting when the drops made contact with the upper slide. Four pieces of adhesive tape were fixed onto 4 slides of the slides. The slides were put in polythene bags and kept at a temperature of about 35°C. This prevented evaporation of the drops. After 24 hours, the reactions were read with a binocular microscope. After each test, the slides were cleaned with water then with a detergent. They were again rinsed with water and cleaned with a soft tissue papper.

RESULTS

I. Survey of virus diseases

Plate 7 shows the locations in the northern states of Nigeria where virus samples were collected from infected pepper plants. These are the areas well known for pepper production.

II. Identification of the virus isolates

(a) Host range studies and symptomatology:

A total of 28 virus isolates were inoculated separately onto 30 plant species belonging to 6 families. The isolates were found to vary in their host range. Only some members of two families viz: Solanaceae and Chenopodiaceae were found to be susceptible to all the

isolates tested (Table 21a). The list of the hosts that did not react positively to any of the 28 isolates is given in Table 21(b). On the basis of their host range, the isolates were classified into 5 groups (Table 22). Many isolates fell in group 1, which infected only two hosts viz: Pepper and Physalis micrantha. It is interesting to note that only P. micrantha apart from pepper was a common host of the isolates in groups 1 to 4.

Only isolate PAMB$_1$ (group 5) had host range plants quite different from those of groups 1 to 4, indicating that it could be a distinct virus. Further, the symptoms produced by this group on pepper was different from those produced by the other groups.

able 20a: The description of the symptoms produced by the isolates in groups 1 to

Group	Host	Description of Symptoms
1 to 4	Pepper *C. annuum*	Mottling of leaves, distortion along veins. Wrinkling and outward curling of leaves, fruits distorted and reduced in size. (Plates 8 to 10).
	Physalis micrantha.	Chlorosis of leaves followed by vein-banding, blistering, abaxial and adaxial curling of leaves (Plates 11 & 12).
2 to 4	*P. floridana*	Dark-green mosaic patterns one week after inoculation. Leaves blistered, variously distorted and reduced in size. Plants generally stunted. (Plates 13 & 14).
3 & 4	*Petunia hybrida*	Severe stunting resulting from shortened internode. Leaves mottled and reduced in size. (Plate 15).
4	*Solanum nigrum*	Leaf mottling one week after inoculation. Followed by blistering and severe distortion (Plates 16 & 17)
	S. incanum	Mosaic symptoms followed by blistering, distortion and outward curling of leaves at the apex. (Plate 18).

Table 20(b): The description of the reaction of plants infected with virus group 5 (PAMB).

Host	Description of Symptom
Pepper (C. annuum)	Systemic mosaic 8 days after inoculation. Reduced leaf size, shortened internode giving rise to stunted plants.
Lycopersicum esculentum	Necrotic spots on inoculated leaves. Terminal necrosis of plants which were then stunted.
Petunia hybrida	Systemic mosaic of leaves which were later on reduced in size and slightly distorted. Internodes shortened.
Nicotiana tabacum (cv. White Burley)	Alternate pale and dark-green patches on leaves. Later, leaves were blistered, distorted, curled outwards and reduced in size. (Plate 19)
N. glutinosa	Necrotic local lesions two days after inoculation No systemic infection (Plate 20).
N. clevelandii	Systemic mosaic symptoms 10 days after inoculation.
N. rustica	- do -
N. tabacum (Samsum NN)	"
Chenopodium quinoa	Chlorotic lesions on inoculated leaves after 3 days (Plate 21).
C. amaranticolor	Necrotic local lesions on inoculated leaves 3 days after inoculation (Plate 22).

Back Inoculations

When back inoculations were made onto a very susceptible pepper variety (C. annuum cv. L5962-2) by means of sap and aphids from the hosts which did not show visible symptoms 3-4 weeks after inoculations, the pepper plants did not develop symptoms of infection indicating that the symptomless plants were free from the virus.

Back inoculations made onto the same pepper variety by means of sap and aphid inoculations from hosts which developed symptoms of infection using inocula of virus group 4 (as a representative inocula for groups 1 to 4 since it infected all the hosts of groups 1 to 3) and group 5, showed that the virus was recovered from these hosts to pepper by sap inoculation. Virus group 4 was also recovered from these hosts by aphids (M. persicae) except from Petunia hybrida (Table 23). The aphid consistently failed to recover the virus from this host.

The isolate of group 5 ($PAMB_1$) was not recovered from any of its hosts to pepper by aphid inoculation. This gave a further evidence that $PAMB_1$ was probably a distinct virus from those of groups 1 to 4.

Zitter (1972) in Florida, U.S.A. used some pepper cultivars (EarlyCalwonder, Avelar, Agronomico 8 and PI 342947), as differential hosts to distinguish the viruses on pepper. Similar attempts were made to differentiate between the 5 virus groups encountered in this study. When six cultivars of pepper were separately inoculated with

Table 21(a): Host range of mosaic virus isolates of pepper collected from different locations in northern Nigeria.

Isolate	H O S T													
	CHENOPODIACEAE		SOLANACEAE											
	Chenopodium amaranticolor	C. quinoa	Capsicum annuum L. cv. L5962-2.	Lycopersicum esculentum	Nicotiana clevelandii	N. glutinosa	N. rustica	N. tabacum cv. White Burley	N. tabacum cv. "Samsum NN"	Petunia hybrida	Physalis floridana	P. micrantha	Solanum incanum	S. nigrum
AS_1	-	-	+	-	-	-	-	-	-	+	+	+	+	+
2	-	-	+	-	-	-	-	-	-	+	+	+	-	-
3	-	-	+	-	-	-	-	-	-	-	-	-	-	-
4	-	-												
FU_1	-	-												
2	-													
HU														
SAM														
IL_1														
2														
3														
KAB_1														
2														
MK_1														
2														

Table 21(b): Plant Species that did not develop symptoms when inoculated with 28 virus isolates.

Plant species	Symptoms
AMARANTHACEAE	
Celosia argentea	
Gomphrena globosa L.	
CRUCIFERAE	
Brassica pekenensis	
B. pentamusi	
COMPOSITAE	
Zinnea elegans	
LEGUMINOSAE	No visible symptom and virus was not recovered from symptomless plants.
Phaseolus vulgaris Beka	
" " Imuna	
" " Pinto	
Vicia faba L.	
Vigna unguigulata	
V. sinensis	
Voandzia subterranea	
SOLANACEAE	
Datura stramonium	
Petunia peruviana	
Solanum macrocarpon	
S. tuberosum	

Table 22: Grouping of 28 virus isolates of pep
in northern Nigeria.

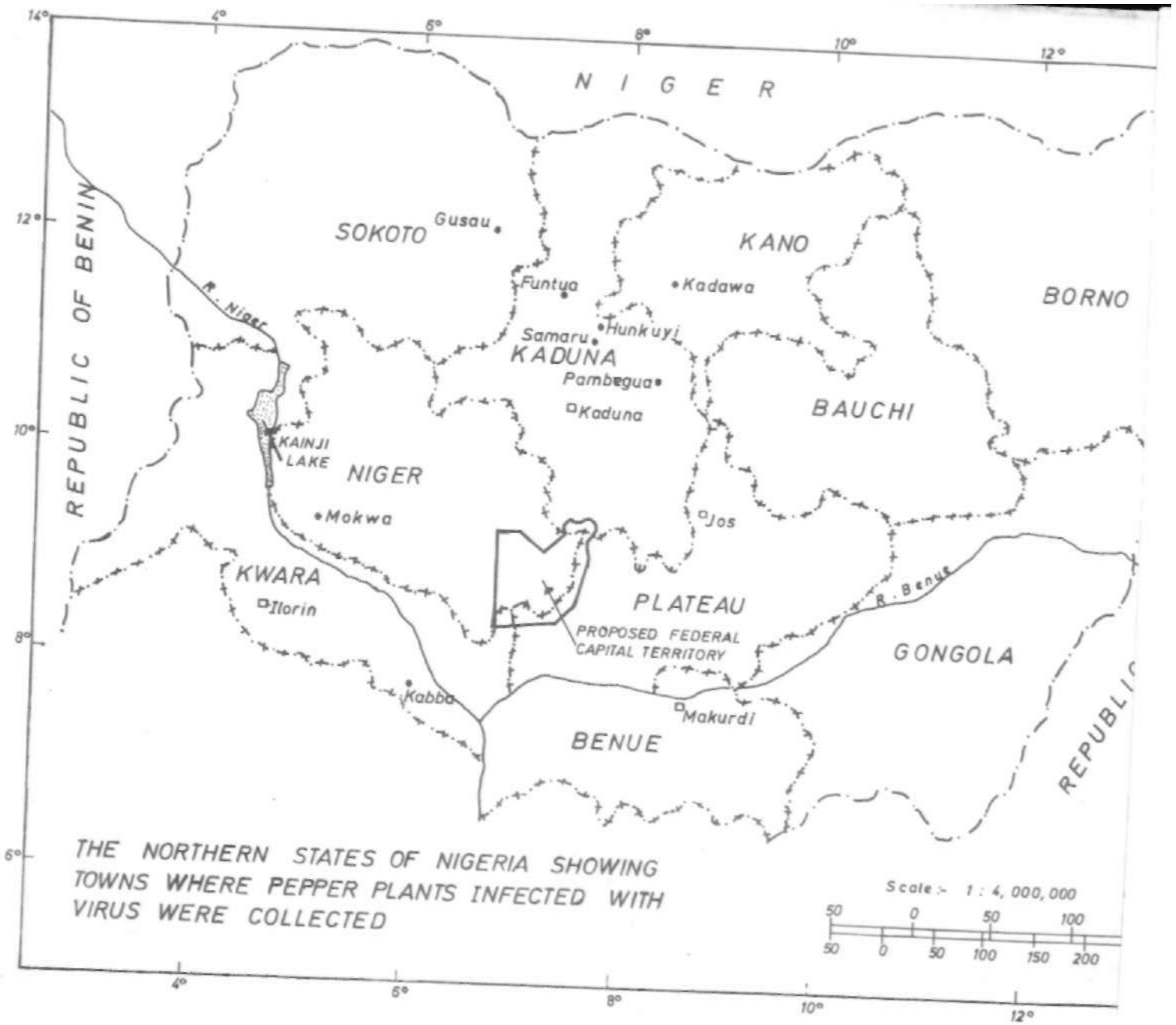
	Group 1.	Group 2.
Artificial host range.	1. Pepper (Capsicum spp.) 2. Physalis micrantha	1. Pepper (Capsicum 2. Physalis florida 3. P. micrantha
Virus isolates	SAM KAD FU_1 & 2 K_1 K_4 AS_3 JOS $PAMB_2$ $MAKD_1$ & 2 AIK_2 GU_1 & 2	K_2 HU AS_4 IL_1 MK_1

Table 23 Back inoculations of virus isolates from susceptible hosts to pepper.

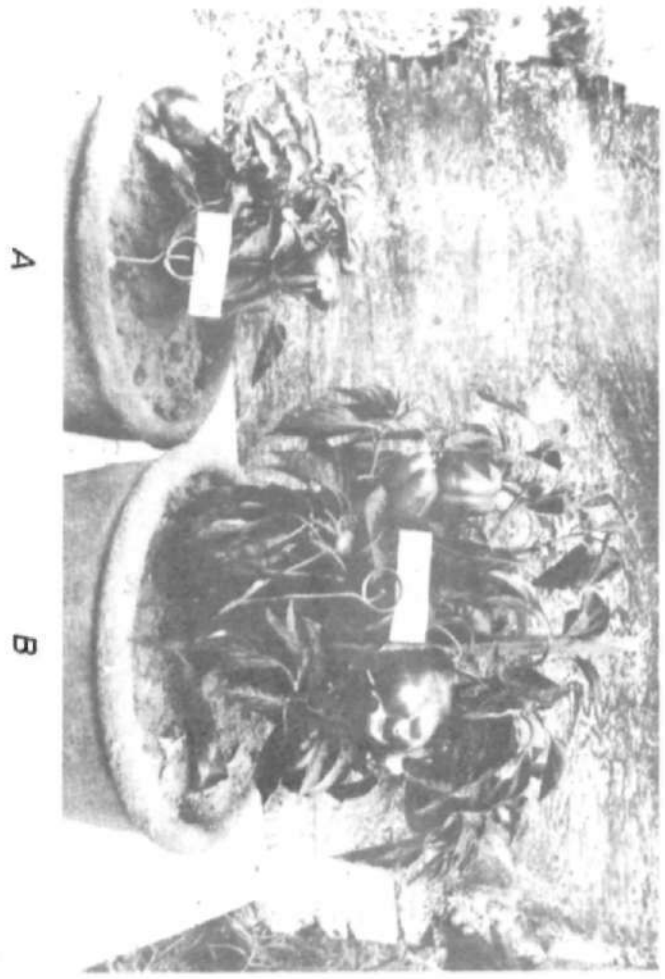
Host plant		Mean of number of infected plants	
		Sap inoculation	Aphid inoculation
Physalis floridana	Group 4.	5	5
P. micrantha		5	4
Petunia hybrida		4	0
Solanum nigrum		5	5
S. incanum		4	3
Nicotiana clevelandii	Group 5.	5	0
N. glutinosa		4	0
N. rustica		4	0
N. tabacum cv. White Burley		5	0
"Samsun NN"		5	0
Petunia hybrida			

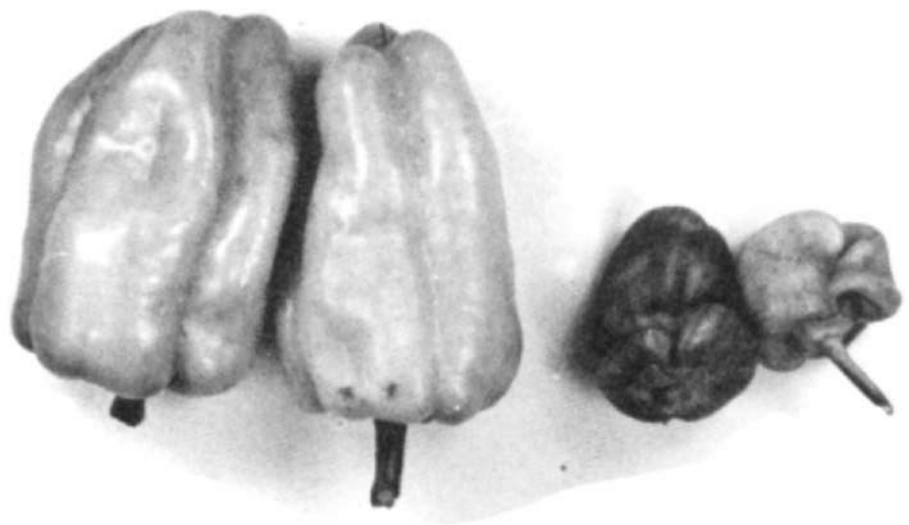
Table 24: Reaction of mosaic virus groups on

Mosaic virus Groups	Pepper culti		
	Yolo wonder	Green leaf tabasco	Agro
1 to 4	Mottling from the base of leaves progressed to the apex. No distortion of leaves.	Severe mottling followed by abaxial and adaxial curling of leaves. Stunting of whole plant.	Very sev and blis leaves. severely
5.	Systemic mosaic symptom. Plants severely stunted. Leaves often blistered.	Similar to Yolo wonder	Similar wonder.

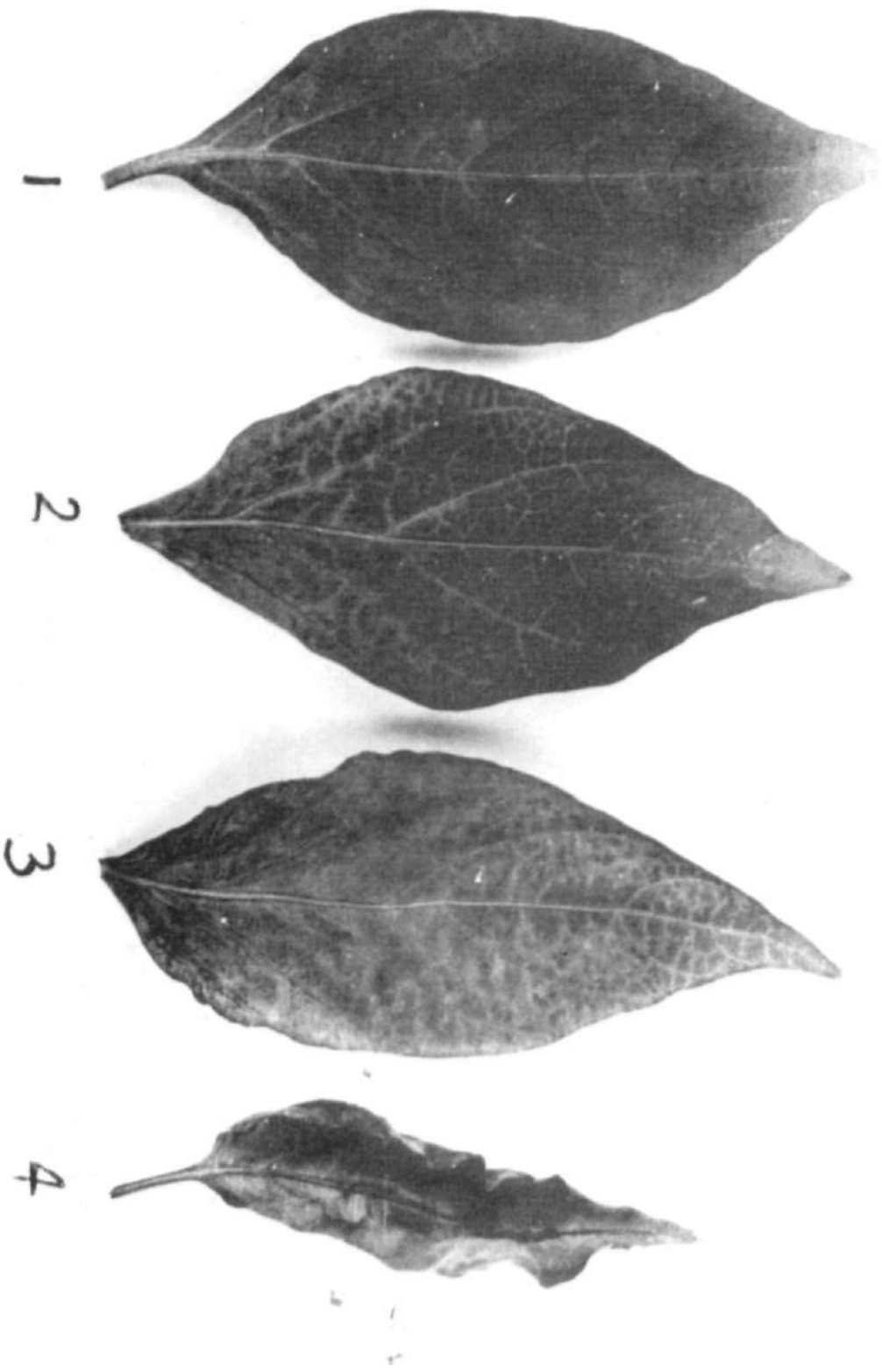


THE NORTHERN STATES OF NIGERIA SHOWING TOWNS WHERE PEPPER PLANTS INFECTED WITH VIRUS WERE COLLECTED

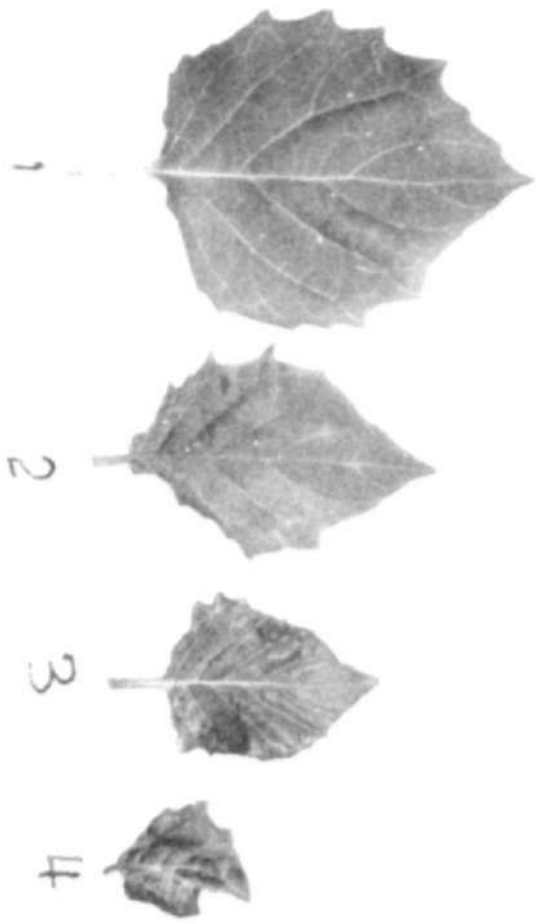

A
B

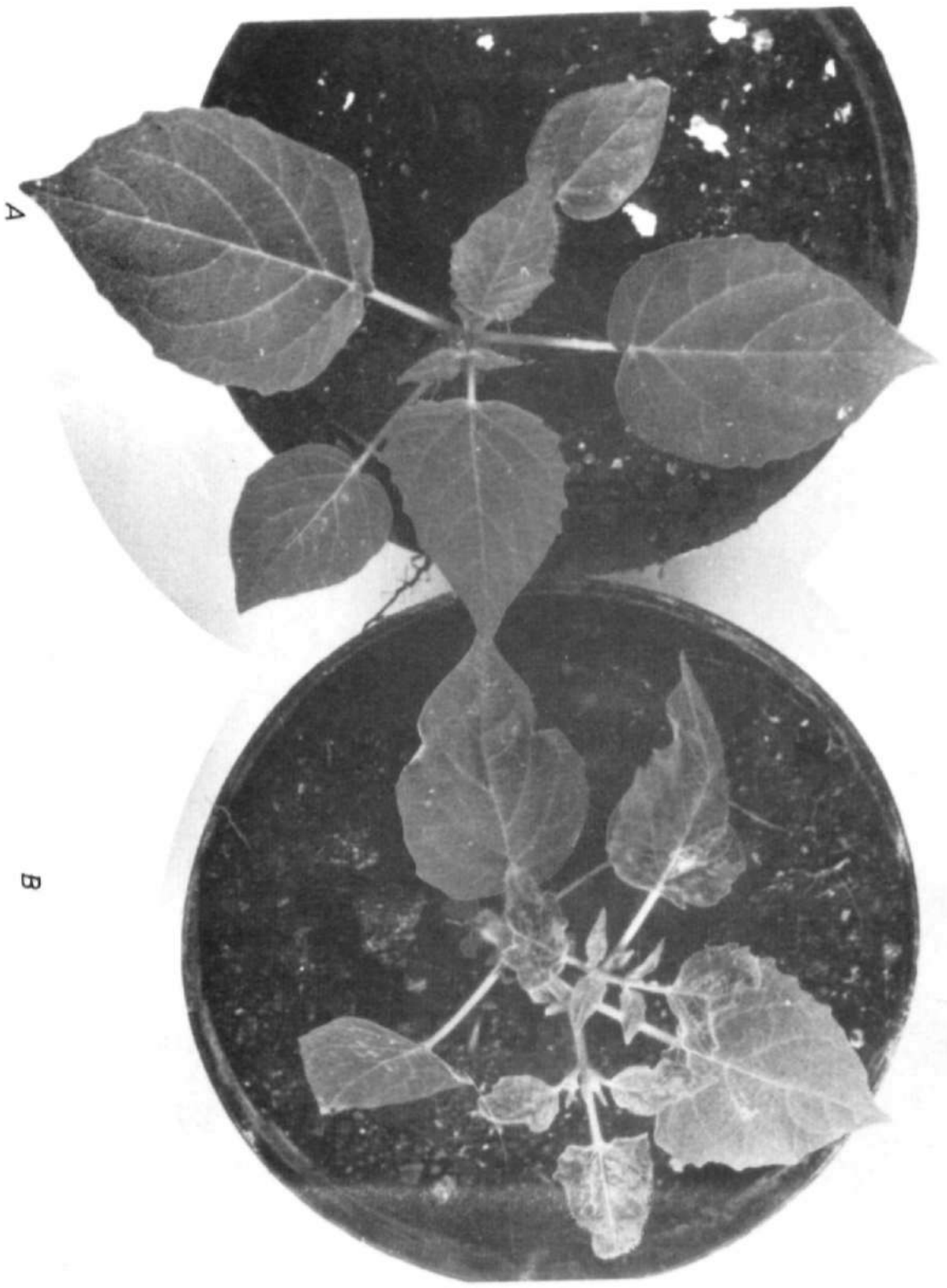


A B


1
2
3
4

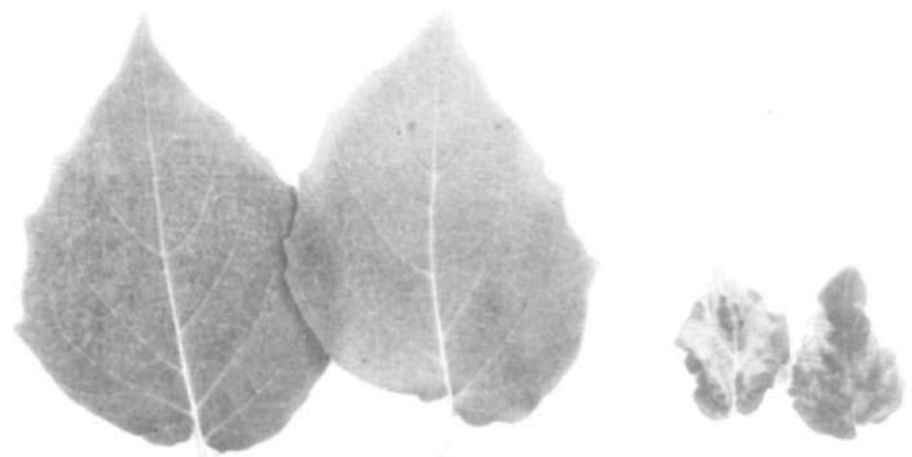

A
B


1
2
3
4



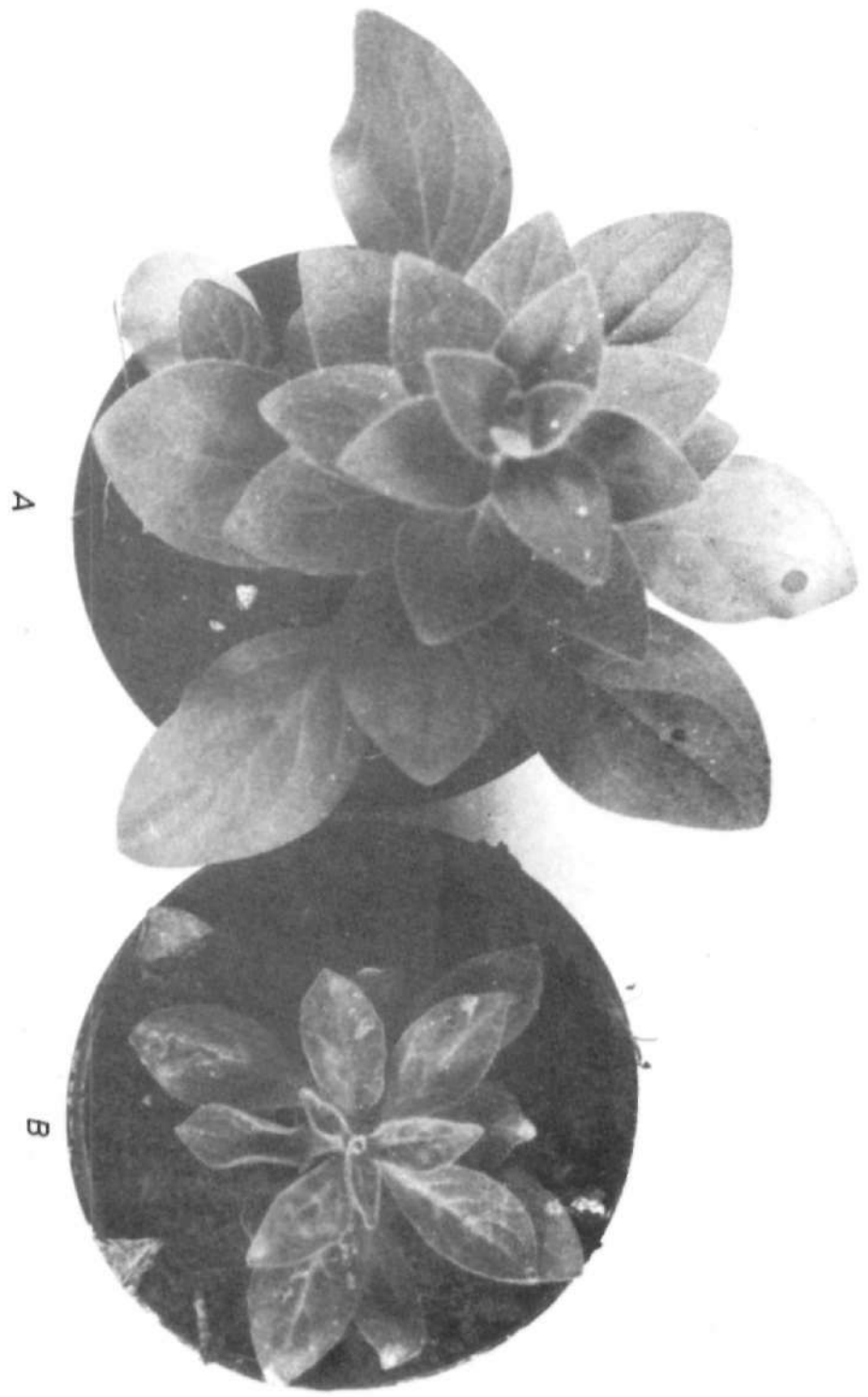
A

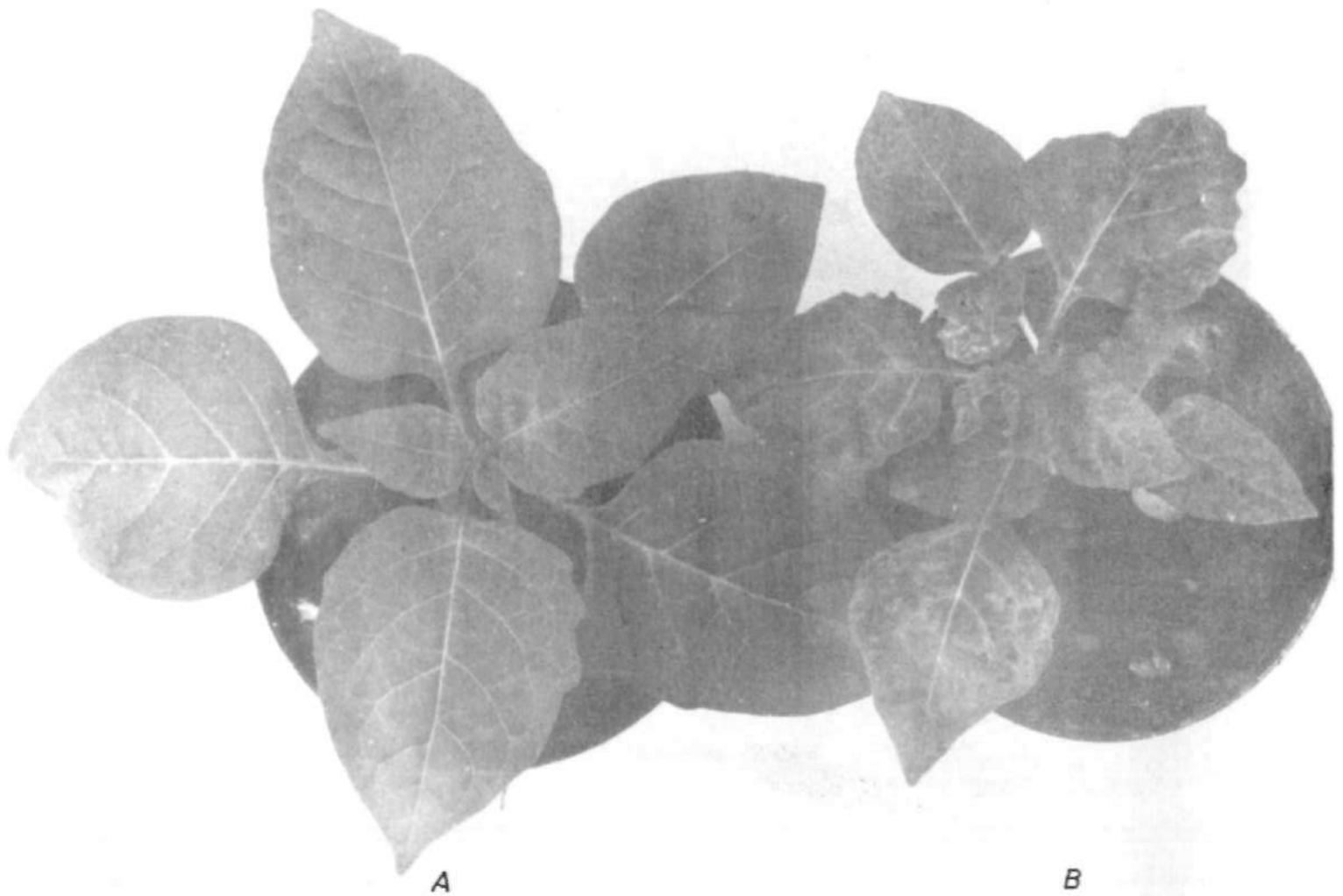
B



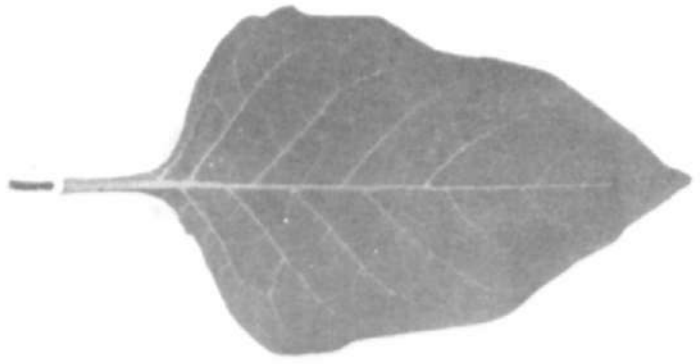
HEALTHY DISEASED

A B


A
B



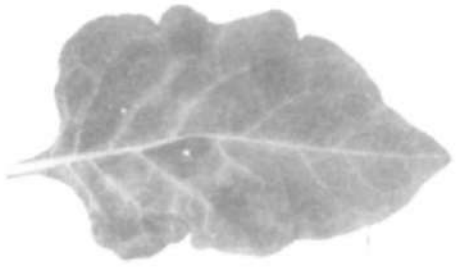
A B



2

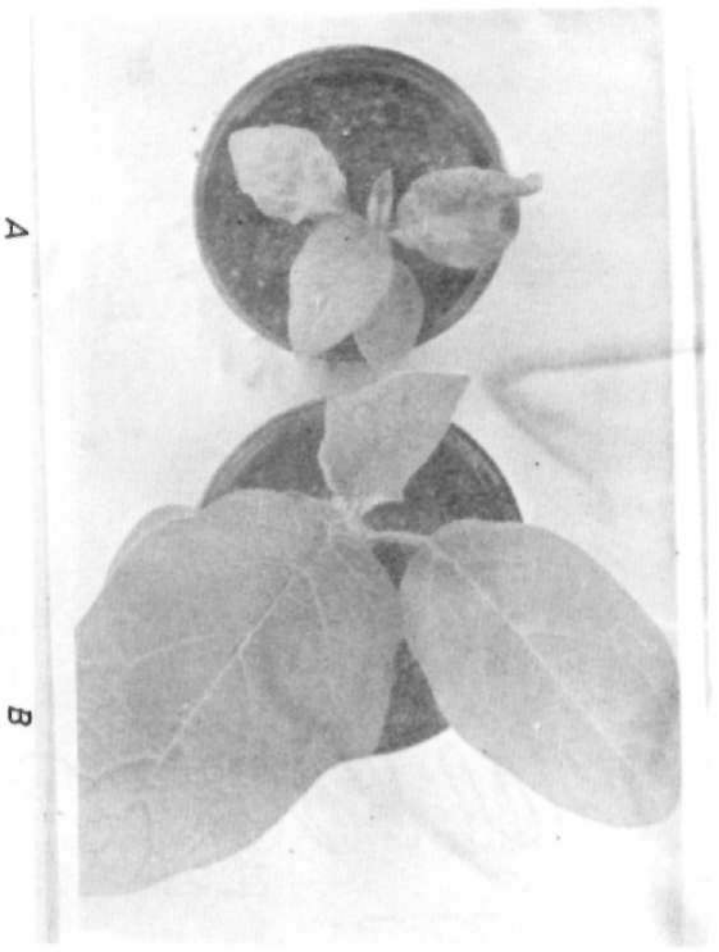


3



1

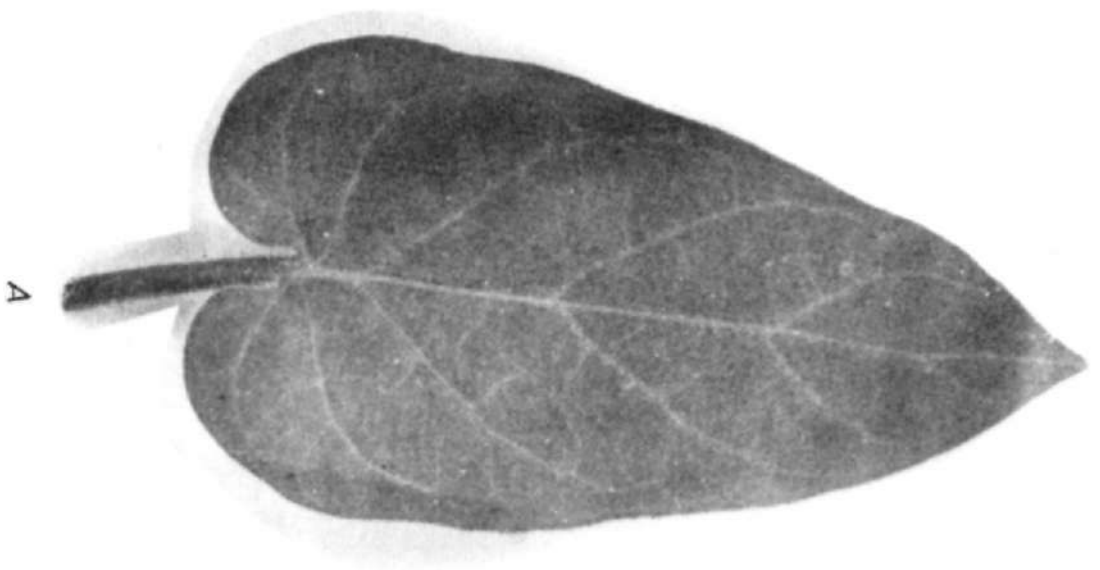



A
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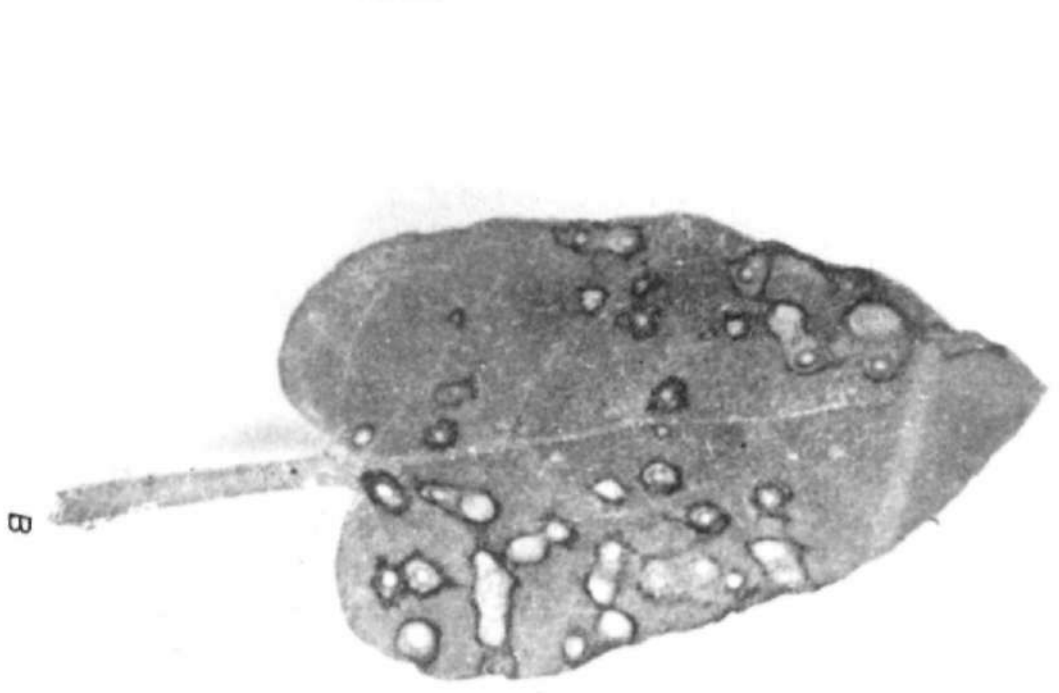


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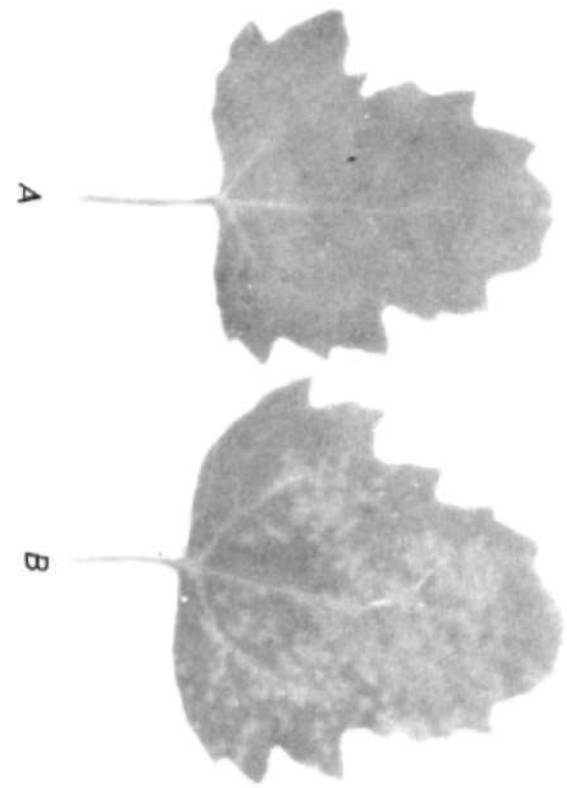
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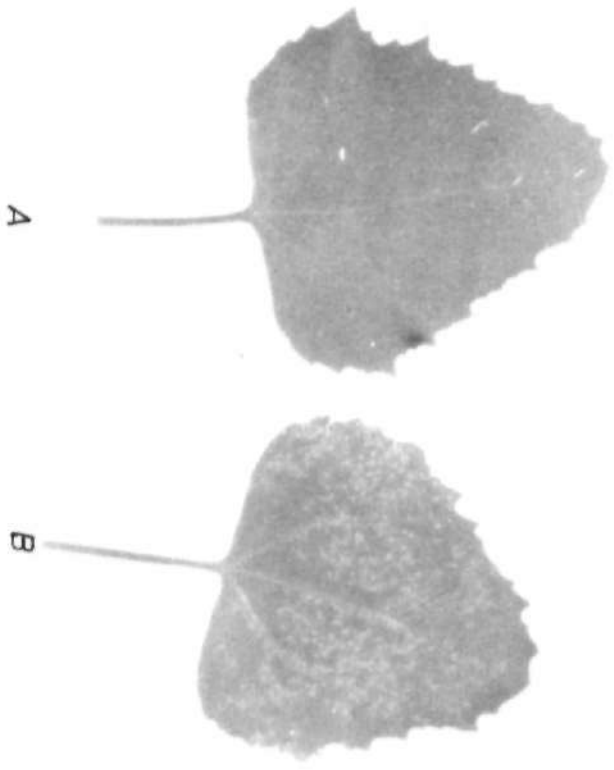


A



B


A
B


A
B

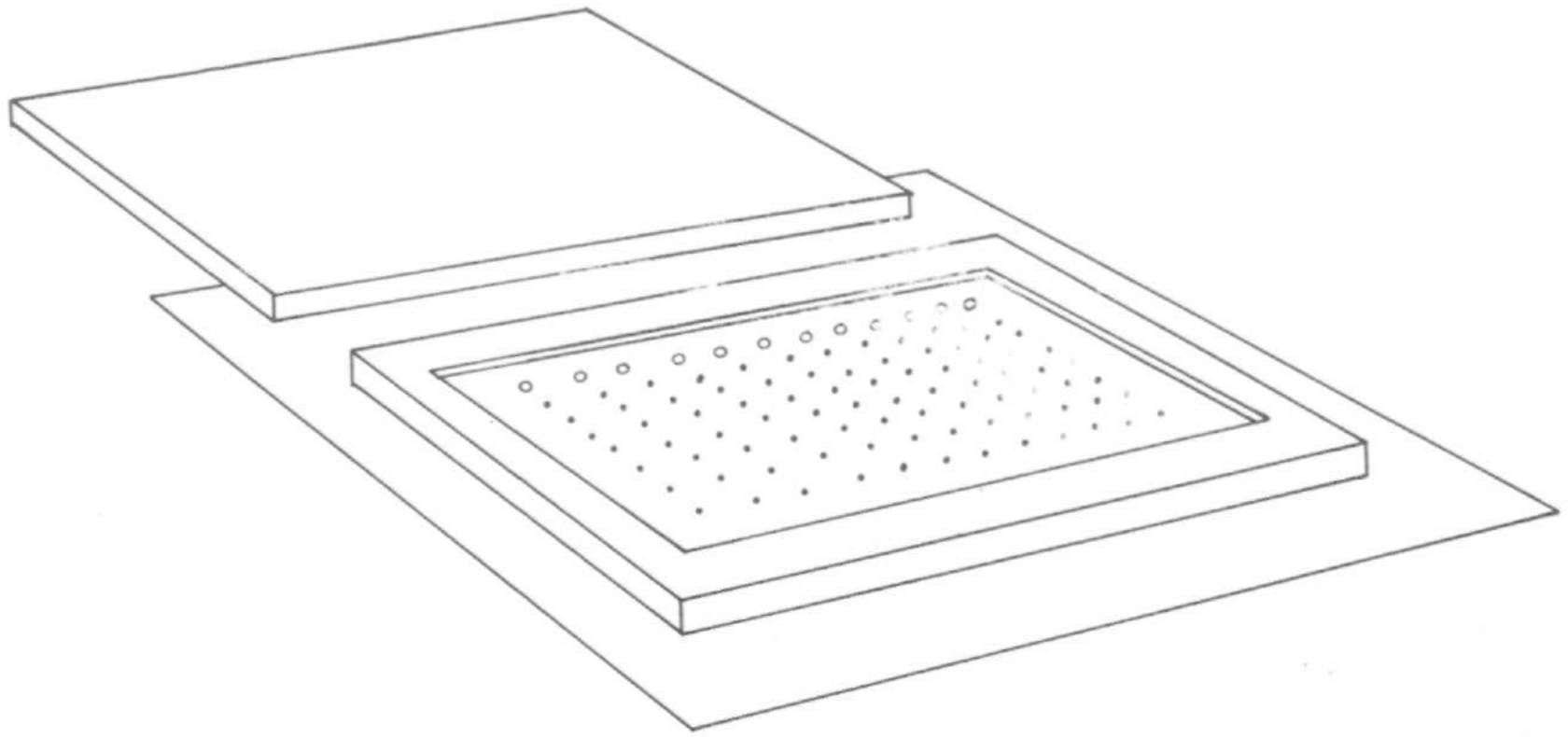


Plate 23. *Arragement of materials in the precipitin test with drops between slides. The thickest slide with the frame on top is put on a scheme with 8 x 12 dots and one row of drops is laid down. After the last drop has been added, the slide in the background is put on the slide with frame and drops.*

5 representative inocula of the virus groups, the members of groups 1 to 4, produced very similar symptoms which were not distinguishable (Table 24). But the isolate of group 5 was distinct in its reactions on the pepper cultivars when compared with the other 4 groups. The symptoms produced were more severe and stunting was more pronounced. This reaction probably gives further indication that the virus classified as group 5 was quite distinct from the rest. However, from the symptoms produced on six pepper cultivars, the members of groups 1 to 4 were indistinguishable.

III. Physical Properties.

(a) Dilution end-point

The dilution end-point (D.E.P.) of most of the virus isolates was between 10^{-3} and 10^{-4} (Table 25) except for AS_1, AS_4 and $PAMB_1$. AS_1 and AS_4 both had a D.E.P. between 10^{-4} and 10^{-5}. However, the isolate $PAMB_1$ of group 5 was infective at a dilution of 10^{-6} but not at 10^{-7}.

(b) Thermal inactivation point

The temperature required for the complete inactivation (T.I.P.) of most of the virus isolates in crude sap after 10 minute exposure was $62^{\circ}C$ except K_4 and $PAMB_1$. The T.I.P. of K_4 and $PAMB_1$ were found to be 60° and $94^{\circ}C$ respectively (Table 26).

(c) Longevity in vitro.

All the virus isolates tested for their longevity in vitro at room temperature (25°C) as well as at 7°C were found to be infective even after 9 days (Table 27).

IV. Purification

The modified Damirdagh and Shepherd's (1970) method used by Whitam for purifying PVY was successfully used for purifying the virus isolates causing mosaics on pepper in northern Nigeria. Table 28 shows the flow chart of this method. The purified virus preparations were used for serological tests.

V. Serological tests

Twenty eight isolates belonging to 5 groups (Table 22) on host-range basis, were purrified and tested against antisera of TMV, CMV, PVY, PVMV and TEV using the Ouchterlony agar double-diffusion and microprecipitin tests.

The results showed that no precipitation bands were observed in all tests with all isolates against all the antisera by using the Ouchetelony agar double-diffusion test. However, when microprecipitin tests were conducted with all isolates against the 5 antisera, positive reactions were obtained with PVMV in 27 instances against isolates of groups 1 to 4. Group 5 antigen reacted positively with only TMV. These reactions indicated that 27 isolates were

Table 25: Dilution end-point of pepper virus isolates collected from different locations in Kaduna and Kano States.

Dilution	Virus groups														
	1								2			3		4	5
	AS_3	FU_1	FU_2	K_1	K_4	KAD	$PAMB_2$	SAM	AS_4	HU_1	K_2	AS_2	K_3	AS_1	$PAMB_1$
Undiluted	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10^{-1}	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10^{-2}	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10^{-3}	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10^{-4}	-	-	-	-	-	-	+	-	+	-	-	-	-	+	+
10^{-5}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
10^{-6}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
10^{-7}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

KEY

+ = Infection

- = No infection

Table 26: Thermal inactivation point of pepper virus isolates collected from different locations in Kaduna and Kano States.

Temperature (°C)	Virus groups 1								2			3		4	5
	AS_3	FU_1	FU_2	K_1	K_4	KAD	PAMB	SAM	AS_4	HU_1	K_2	AS_2	K_3	AS_1	$PAMB_1$
50	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
54	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
56	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
58	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
60	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+
62	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
64	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
66	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
70	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
75	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
80	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
85	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
90	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
92	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
94	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
96	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

KEY

+ = Infection

- = No infection

Table 27: Longevity in vitro of pepper virus isolates collected from different locations in Kaduna and Kano States kept at 25°C.

Time in days	Virus groups														
	1								2			3		4	5
	AS_3	FU_1	FU_2	K_1	K_4	KAD	PAMB	SAM	AS_4	HU_1	K_2	AS_2	K_3	AS_1	$PAMB_1$
1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

KEY

+ = Infection

- = No infection.

Table 28: Damirdagh and Shepherd's (1970) modified method of purifying PVY.

Fresh diseased leaves homogenised at 4°C in a mortar with 0.5 M phosphate buffer (pH 7.1) containing 1.0% 2-mercaptoethanol and strained through cheesecloth.

↓

Clarified at 4°C by dripping n-butanol slowly into the extract until 8% was added.

↓

Centrifuged at low speed for 15 min at 10,000 *g* to remove plant material.

} 2 times.

↓

Virus precipitated from the clarified sap by dissolving 4.0 g P.E.G. (mol wt. 6,000) per 100 ml of extract.

↓

Incubated for 90 min and Centrifuged at low speed, 10,000 *g*, for 15 min.

↓

Pellets resuspended in 30 ml of 0.025 M phosphate buffer (pH 7.4) overnight.

↓

Damirdagh and Shepherd's method up to this point →

↓

Virus precipitated a second time by adding PEG and NaCl to make a 4% solution.

↓

Mixture allowed to stand for 90 min.

↓

Pellets collected and centrifuged at low speed, 10,000 *g* for 15 min.

↓

Pellets resuspended in 0.025 M phosphate buffer (p H 7.4).

} Whitam

Table 29: Precipitation reaction of an antiserum against an Ivory Coast isolate of pepper veinal mottle virus against a clarified sap of the virus inducing mosaic symptoms on pepper in northern Nigeria.

Antiserum dilution	Dilution of sap containing mosaic inducing virus													
	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	1:2048	1:1496	1:8192
1:1	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1:2	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1:4	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1:8	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1:16	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1:32	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1:64	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1:128	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1:256	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1:512	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1:1028	+	+	+	+	+	+	+	+	-	-	-	-	-	-
1:2048	+	+	+	+	+	+	+	+	-	-	-	-	-	-
1:4096	+	+	+	+	+	+	+	+	-	-	-	-	-	-
1:8192	+	+	+	+	+	+	+	+	-	-	-	-	-	-

KEY

+ = Precipitate

- = No precipitate

serologically related to PVMV and the only isolate of group 5 ($PAMB_1$) was serologically related to TMV.

Detailed serological tests conducted by using the microprecipitin test with drops between slides (Noordam, 1973) to determine the dilution end-point of the antigen and the antiserum titre showed that all the 27 antigens tested had similar reactions. Table 29 which shows the end-points of one of the isolates also represents those of the other 26 isolates. The antiserum titre against the 27 isolates of PVMV was found to be up to 1/8192. Similarly, the virus end-point was up to 1/8192. However, positive reactions were obtained up to 1/1028 dilution of the antiserum when the virus dilution was 1/128. Also positive reactions were obtained up to 1/8192 virus dilution when the antiserum dilution was 1/512. Dilution of antiserum higher than 1/512 when mixed with dilutions of antigen higher than 1/128 did not give any precipitate.

DISCUSSION

Studies on host range, physical properties and serological reactions of the isolates collected, revealed that pepper veinal mottle virus is the most predominant virus of wet season pepper in the northern states of Nigeria. The other virus which was serologically related to tobacco mosaic virus was collected from Pambegua - a tobacco growing area of Kaduna State. It is therefore not surprising that TMV was isolated from pepper grown in this area. Since TMV has no known specific insect vector, it is presumed that the virus was picked up by the plants in the nursery from a contaminated soil. The other isolate collected from Pambegua was identified as PVMV. This indicated the presence of two viruses in the same locality. But their relative importance in this area was not studied.

All the other 27 isolates were serologically related to an Ivory Coast isolate of PVMV. Except for slight variation in the physical properties of the other 27 isolates, their dilution end-point was between 10^{-3} and 10^{-4}, thermal inactivation point was between 60-62°C and longevity *in vitro* was more than 10 days (Tables 25-27). Similar ranges in the physical properties of PVMV have been reported in West Africa by Brunt and Kenten (1971)., Lana *et al.* (1975)., Ladipo and Roberts (1977) and Subbarayudu (unpublished).

The slight variations in few of the isolates (AS_1, AS_4, $PAMB_2$ and K_4) could be due to experimental error and/or host variations at the time of inoculation or some other unknown factors.

The isolates when tested serologically using the microprecipitin test had similar endpoints. But they distinctly and consistently differed in their host range. On the basis of their ability to infect certain hosts, they were divided into four groups / Table 22. One is inclined to regard them as strains of PVMV. However, more work needs to be done in this direction. Many strains of a virus may have similar host ranges, while others may differ considerably. McRitchie and Alexander (1963) differentiated strains of TMV by inoculating a number of species and selections of tomato when they were unable to distinguish them on the basis of symptoms produced on tobacco and tomato. Cook (1967) isolated a strain of PVY from pepper with specific virulence for each of the 3 pepper varieties (differential varieties) which were resistant to field strains of PVY.

Zitter (personal communication) observed host differences in serologically related virus samples (such as the situation encountered in this study) and thought they may be different strains.

Serological reactions were successfully demonstrated with PVMV by Brunt and Kenton (1971) and Lana et. al. (1975) in agar double diffusion tests by using sodium dodecyl sulphate to degrade the virus particles. Similar attempts made in this study using the Purciful and Batchelor (1977) method was not successful. This is probably due to the fact that the antiserum used was preserved in glycerin. If the reaction were positive, there would have been an o portunity to observe cross-reaction by means of spur formation, which indicate strain differences. Antisera to the four virus groups need to be prepared to study their cross-reactions.

This study clearly show that PVMV is the predominant virus causing virus disease of pepper in the northern States of Nigeria. The presence of a number of strains of the virus is a possibility. A more extensive survey and more samples might reveal the presence of other viruses that produce mosaic symptoms.

V. SURVEY FOR WEED HOSTS OF PEPPER VEINAL MOTTLE VIRUS

INTRODUCTION

In order to survive a virus which is an obligate parasite must have a host plant in which it can multiply, an effective means of transmission to fresh host plants and a continuous supply of suitable healthy host plants to which it can spread. Viruses vary greatly in their ability to infect various species of host plants. Those viruses which have a wide host range have greater chances of survival and spread than viruses with very narrow host-range which survive because their major host is a perennial, their hosts are vegetatively propagated or because they are seed transmitted.

Pepper veinal mottle virus reported on pepper in this laboratory has a very narrow host range, the crop is not vegetatively propagated, the host (pepper) is an annual and the virus is not seed transmitted. A number of aphid species transmit the virus in a stylet-borne manner, the disease is endemic and incidence may be up to 80 per cent by the end of the cropping season. Hence it was suspected that there may be certain other hosts, apart from dry season pepper cultivated under irrigation and fadama areas, which might perpetuate the virus.

A survey was therefore, undertaken of the borders of infected irrigated pepper fields to find out if there are weeds that carry the virus with or without symptoms.

MATERIALS AND METHODS

I. Survey for weed hosts of PVMV

Ten weed species growing around pepper fields (under irrigation) at Kadawa and Samaru were tested for the presence of PVMV in them, during the 1976/77 and 1977/78 dry seasons. The leaves of the weed plants were randomly collected to make a representative sample of each species. Sap and aphid inoculations were made onto a susceptible pepper cultivar - L5962-2.

Identification of the virus was attempted on the basis of transmission, physical properties, host range and serological studies. The materials and methods used for the above studies were the same as those in the last chapter.

RESULTS

I. Survey for weed hosts of PVMV

Out of the ten weed species tested, only Solanum nigrum was found to harbour a virus (Table. 30). Both sap and aphid inoculations gave positive results. The virus produced a typical mosaic sysptom. The plants were generally stunted, with fewer branches and reduced lamina. Leaves showed bright mosaic with darkgreen blisters and veinbanding (Plate 24).

II. Host-range of the isolate

Out of the 30 species of plants from 5 different families tested, only 6 species from a single family, Solanacese, were found to be the hosts of the virus. All the 6 species produced systemic mosaic or mottling symptoms on their leaves (Table 32).

III. Physical properties

The physical properties of the isolates from Kadawa and Samaru were identical.

The T.I.P. of the isolates was 62°C (Table 33) while the dilution end point was between 10^{-4} and 10^{-5} (Table 33). The virus was infective even after 10 days of storage at temperature 25° and 7°C (Table 33).

IV. Serological Tests

Microprecipitin test was employed in this study. The antigen gave a positive reaction to only PVMV antiserum but not to the antisera of TMV, TEV and CMV. The virus titre was 1:128 and the antiserun end point was as high as 1:8,192 (Table 34).

Table 30: Weeds tested for the presence of PVMV.

Weeds	Virus isolated
Solanum macrocarpon Linn.	None
Solanum nigrum Linn.	PVMV
Physalis micrantha Link	None
Portulaca oleracea Linn	None
Vernonia pauciflora (Willd) Less.	None
Acanthosperum hispidum DC	None
Leucas martinicensis (Jacq.) Ait.	None
Ageratum conyzoides Linn.	None
Borreria verticillata (Linn.) G.F.W. Mey	None
Euphorbia heterophylla Linn	None

Table 31 Aphid transmission of a virus isolated from Solanum nigrum.

Aphid	No. of infected plants				Mean
	I	II	III	IV	
Myzus persicae	8.00	9.00	9.00	7.00	8.25
Aphis fabae	8.00	7.00	8.00	6.00	7.25

10 test plants and 10 aphids/plant were used for each replicate

Table 32: Host-range of a mosaic virus isolated from *Solanum nigrum*

Family and species of test plant	Type of sympton
AMARANTHACEAE	
Celosia argentea	None
Gomphrena globosa L.	"
CHENOPODIACEAE	
Chenopodium amaranticolor	None
C. quinoa L.	"
CRUCIFERAE	
Brassica pekenensis	None
B. pentamusi	"
LEGUMINOSAE	
Physeolus vulgaris Beka	None
" " Imuna	"
" " Pinto	"
Vicia faba L.	"
Vigna unguigulata	"
Vigna sinensis	"
Voanzia subterranea	"
SOLANACEAE	
Datura stramonium L.	None
Capsicum annuum cv. L5962-2	Systemic mottling
Lycopersicum esoulentum Mill	None
Nicotiana clevelandii	"
N. glutinosa	"
N. rustica	"
N. tabaoum cv. White burley	"

Table 32 Contd.

Family and species of test plant	Type of symptom
SOLANACEAE	
N. tabacum "Samsun NN"	None
Petunia bybrida Vilm.	Systemic mottling
P. peruviana	None
Physalis floridana	Systemic mottling
P. micrantha	"
Solanum incarnum	Systemic mottlings & distortion of leaves
S. macrocarpon	None
S. nigrum L.	Blistering & leaf distortion
S. tuberosum	None
COMPOSITAE	
Zinnea elegans	None

Table 33: Physical properties of a virus isolated from Solanum nigrum.

Thermal Inactivation Point			Dilution end point			Longivity in Vitro				
Temp. (°C)	No. of plant infected Out of 4		Dilution	No of plant infected Out of 4		No. of Days	At 7°C		At 25°C	
	Isolate			Isolate			Isolate		Isolate	
	Samaru	Kadawa		Samaru	Kadawa		Samaru	Kadawa	Samaru	Kadawa
50	4	4	10^{-1}	4	4	1	4	4	4	4
54	4	4	10^{-2}	4	4	2	4	4	4	4
56	4	4	10^{-3}	4	4	3	4	4	4	4
58	4	4	10^{-4}	2	2	4	4	4	4	4
60	3	2	10^{-5}	0	0	5	4	4	4	4
62	0	0	10^{-6}	0	0	6	3	4	4	4
64	0	0	10^{-7}	0	0	7	4	4	3	4
66	0	0	Control	4	4	8	4	4	3	4
68	0	0				9	3	4	3	3
70	0	0				10	3	3	2	2
Control (22°C)	4	4								



A B

Table 34: Micro-precipitin reaction of an antiserum against an Ivory Coast isolate of PVMV against a clarified sap of a virus isolated from Solanum nigrum.

Antiserum dilution	Dilution of sap containing virus isolated from Solanum nigrum													
	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	1:2048	1:1496	1:8192
1:1	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1:2	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1:4	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1:8	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1:16	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1:32	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1:64	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1:128	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1:256	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1:512	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1:1024	+	+	+	+	+	+	+	+	-	-	-	-	-	-
1:2048	+	+	+	+	+	+	+	+	-	-	-	-	-	-
1:4096	+	+	+	+	+	+	+	+	-	-	-	-	-	-
1:8192	+	+	+	+	+	+	+	+	-	-	-	-	-	-

KEY: + = Precipitate, - = No precipitate

DISCUSSION

Plant viruses are obligate parasites and therefore, must have a living host plant in which they can multiply so that they can survive and spread from plant to plant, season to season and year to year. Their survival depends on physical and biological factors and their interactions. An understanding of the ecology of a virus in a particular crop and locality is essential for the development of reasonable control measures. In most obligate parasites such as viruses, the dominant ecological factors to be considered are the way they spread from plant to plant and the ways the other factors influence such spread.

Pepper veinal mottle virus is a fairly stable virus, transmitted by several aphid species in a stylet-borne manner. Greenhouse studies showed that the isolates used in this study have very limited host range. Practically no other cultivated crop other than pepper is its host. Viruses with narrow host range survive because they are seed transmitted or their chief host is a perennial. However, PVMV is not seed transmitted and pepper is generally grown as an annual.

In the northern States of Nigeria, pepper is grown mainly as a wet season crop. But the disease is endemic and the incidence may be

up to 80 to 100% towards the end of the cropping season. Since PVMV has a narrow host-range, the survival and continuity of the virus was a perplexing problem.

The survey for weed hosts of PVMV in the 1976/77 and 1977/78 dry seasons indicated that Solanum nigrum from which a virus was isolated might play an important role in bridging the gap between the wet seasons. The artificial host range of the virus was limited to six Solanaceous species viz: Capsicum spp., Petunia hybrida, Physalis floridana, P. micrantha, Solanum incarnum and S. nigrum. The concept of host range is essential to the understanding of the role of additional hosts in the perpetuation or establishment of a disease (Dinoor, 1971). Where wild plants are concerned, the hosts are variable populations rather than uniform entities, and listing botanical species as hosts, as is done in greenhouse work, does not necessarily mean that all individuals of the species are indeed hosts.

Only S. nigrum was found to harbour PVMV in the field (Table 30 and Plate 24). Although this weed is known to be an annual, it thrives perennially along river banks (Fadama areas) in northern Nigeria and hence it appears to be important in closing the gap between the wet seasons, crops and locations. This is further

supported by the fact that Aphis fabae one of the vectors of PVMV was constantly found associated with S. nigrum. Symptomless carriers are also important epidemiologically, although none was reported in the course of this study. They may still be a source of constant supply of inoculum in the northern States of Nigeria. A more extensive study may reveal the presence of symptomless carriers.

Anderson and Corbett (1957) and Anderson (1959) observed that severe damage to pepper plants was associated with the proximity of various weed hosts. They isolated a strain of potato virus Y from S. nigrum in Florida.

Simons (1955; 1956 and 1957) observed veinbanding virus of pepper a strain of PVY in south Florida harboured by Solanaceous weeds. The transmission of the virus PVY from the natural weed host to pepper crop in the field was reported to be mainly through the aphid vectors Aphis gossypii and Myzus persicae. The weed S. nigrum was found to grow throughout the year in garden land areas and hence it was thought to serve as a potential source of the virus infecting C. annuum. Mariappan et. al. (1973) reported S. nigrum as an important weed in the spread of PVY to pepper crop in Tamil Nadu State in India. De Wijs (1973) isolated PVMV from indigenous Datura metel in Ivory Coast. Whitam (1974)

reported eighteen indigenous host species infected with one or more pepper viruses prominient among which was S. nigrum.

The virus isolated from S. nigrum in northern Nigeria was found to be PVMV and similar to the one described as group 4 in the last chapter on the basis of host range, physical properties and serological reactions. Because of increasing public demand, pepper is now grown in Fadamas and irrigation schemes where S. nigrum is a common weed. Therefore, the role of S. nigrum in the survival of PVMV in the dry season is likely to increase.

Although the virus was not isolated from S. incarnum and Physalis micrantha laboratory work on host range studies did show that they are susceptible hosts. The possibility of these two serving as additional hosts cannot be ruled out as they were found growing in some of the surveyed areas.

Extensive survey of all fadamasand irrigation schemes in the northern States of Nigeria might reveal additional weed hosts of PVMV.

STUDIES ON PEPPER VEINAL MOTTLE VIRUS IN THE NORTHERN STATES OF NIGERIA.

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ABSTRACT

Studies on the aphid transmission of pepper veinal mottle virus (PVMV) revealed the vector-virus relation to be of the non-persistent or the stylet-borne type. All seven aphid species tested were found to be vectors of this virus. Two of them - Myzus persicae and Hysteroneura setariae showed higher levels of transmission compared with the other five species. However, M. persicae appeared to be the most efficient. Transmission rates increased (but not linearly) with increase in the number of viruliferous aphids. Fasting M. persicae for various periods up to 30 minutes before acquiring PVMV, increased the transmission rate. Starving the aphid beyond this period decreased the rate of transmission. Transmission rate increased with increase in the acquisition and inoculation feeding times but not indefinitely. Fifteen seconds of acquisition and inoculation feeding times were sufficient for M. persicae to transmit the virus. Single viruliferous aphids transmitted the virus to only one test plant.

There was a significant decline in the rate of transmission with increase in post-acquisition fasting. However, the virus was retained up to 6 hours by few aphids. Young nymphs of M. persicae were significantly more efficient than winged adults.

Out of the thirty varieties of pepper screened for resistance to PVMV in the greenhouse, only TCa14xxx76 and California mild were found to be resistant. Under field conditions however, no variety was resistant. Many of the varieties grouped as resistant and moderately susceptible showed milder symptoms when compared with the highly susceptible ones. On the whole nine varieties seemed to be tolerant. A positive correlation was established between the number of alate aphids trapped and the number of infected plants in the following week. Of all the aphids trapped, A. craccivora, M. persicae and R. maidis were the most predominant. Among the weather factors, only relative humidity was found to have a significant influence on the number of aphids trapped at various times.

Pepper veinal mottle virus was the most prevalent virus disease of pepper in the northern States of Nigeria during the wet season. It was found in all the thirteen locations where isolates were collected while tobacco mosaic virus was found in only one location - Pambegua, a tobacco growing area of Kaduna State. The virus isolates collected in all locations were classified into four groups on host range basis, but they were indistinguishable physically and serologically.

Out of the ten weed species collected from Kadawa and Samaru irrigation schemes and "fadama" areas during the 1976/77 and 1977/78 dry seasons, only Solanum nigrum was found to harbour PVMV. This weed is probably responsible for the survival and continuity of PVMV between the wet seasons.

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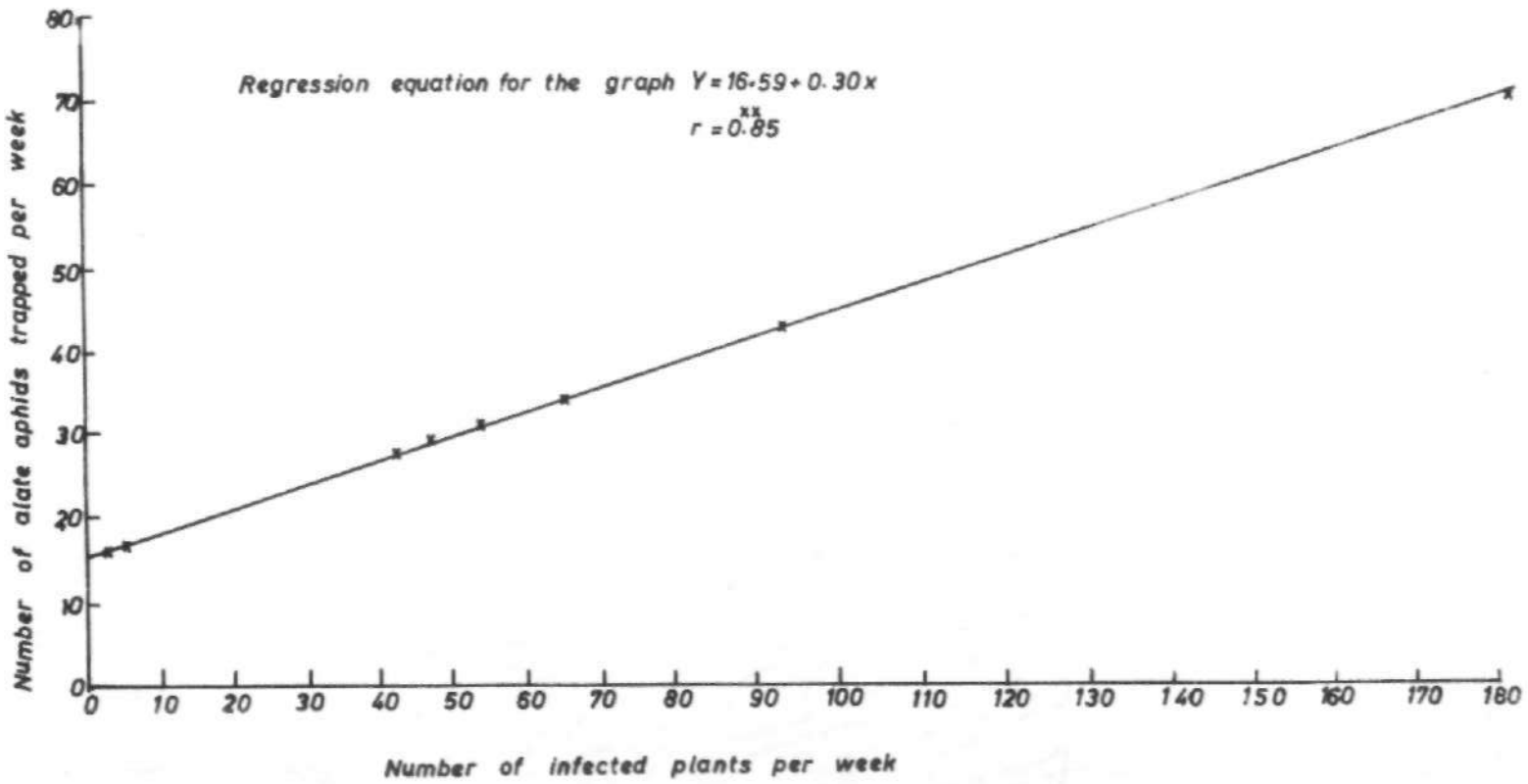
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APPENDICES

APPENDIX I

STATISTICAL ANALYSIS OF THE RELATIONSHIP BETWEEN THE RATE OF INFECTION OF PEPPER BY PVMV AND THE NUMBER OF ALATE APHIDS TRAPPED IN YELLOW TRAYS AT SAMARU DURING THE 1977 WET SEASON



APPENDIX II

Statistical analysis of the relationship between weather and the number of alate aphids trapped in a pepper field at Samaru during the 1977 wet season.

(Correlation coefficients)

Variables	X_1 Average temp. (°C)	X_2 Mean relative humidity (%)	X_3 Mean sunshine (Hours)	X_4 Mean wind run (Km.)	X_5 Mean rainfall (mm.)	Y No. of alate aphids trapped
X_1 Average temp. (°C)		-0.46	-0.89	0.53		
X_2 Mean relative humidity (%)			0.80	-0.34	-0.79	
X_3 Mean sunshine (Hours)				-0.06	-0.42	0.07
X_4 Mean wind run (Km.)					-0.32	0.13
X_5 Mean rainfall (mm.)						-0.81
Y No. of alate aphids trapped						