OCCURRENCE AND DISTRIBUTION OF SOME TOBACCO VIRUSES IN SOUTHERN, CENTRAL AND LUSAKA PROVINCES OF ZAMBIA.

BY

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A Dissertation submitted to the Graduate School of the University of Zambia in partial fulfilment of the requirements for Master of Science in Agronomy (Crop Science).

THE UNIVERSITY OF ZAMBIA

LUSAKA.

1998

DECLARATION

I, DAVID SAMUEL MAYUNGA declare that this dissertation represents my own work and that it has not previously been submitted for a degree at this or any other university.

Signature_____

Date_____

APPROVAL

This dissertation of Mr. DAVID SAMUEL MAYUNGA is approved as fulfilling part of the requirements for the award of the degree of Master of Science in Agronomy (Crop Science) by the University of Zambia.

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ABSTRACT

Nine tobacco fields of small and large scale farmers in Southern, Central and Lusaka provinces of Zambia were surveyed for the occurrence and distribution of virus diseases during tobacco growing season in 1997. Three tobacco fields in each province were selected for virus detection and sample collection. During this survey, infected tobacco plants were examined and disease symptoms recorded. Virus infected weeds occurring in or near the tobacco crop were also included to determine if they served as virus reservoir along with potential insect vectors.

Virus infected tobacco leaf samples were inoculated on a panel of differential hosts to identify the commonly occurring viruses on tobacco in Zambia. Sap from field infected and symptomatic greenhouse plants were tested serologically using DAS-ELISA. Viruses were identified if they gave positive reaction against antisera from Agdia Inc., USA. Virus particle morphology was also studied in some cases by electron microscopy. The results indicated that at least four viruses - tobacco mosaic (TMV), potato virus Y (PVY), alfalfa mosaic (AMV) and tobacco ringspot (TRSV) occur in tobacco in Zambia.

Tobacco mosaic virus (TMV) was recovered in 77% of diseased samples while PVY was recovered in 66.6%, AMV in 33.3% and TRSV in 22.2%. Obviously TMV and PVY are of more common occurrence.

The prevalence of TMV and PVY in all the three provinces indicate that these viruses could be responsible in limiting tobacco production in Zambia. It is therefore important that the scope of virus diseases occurring in Zambia should be broadened to identify all the viruses and their impact on tobacco if satisfactory management strategies are to be worked out.

DEDICATION.

To my parents and relatives for encouraging me . I love you all.

ACKNOWLEDGEMENT

I would like to extend my sincere gratitude to my supervisor Professor R. G. Kapooria for his guidance, tolerance, great understanding, constructive criticism and concern during the period of this work.

May I also extend my thanks to Mr. G .Kaitisha, Mount Makulu Research Station, Chilanga and Ms I. Nawa, Department of Crop Science for co-supervising, directing and finding time from their busy schedule to assist me in this work.

I would like to take this opportunity to thank Dr. G. Pietersen and Mr. G. G. F. Kasdorf of Plant Protection Research Institute, Virology and Electron Microscope Unit, Pretoria for helping me with the identification of some viruses by electron microscopy.

Special thanks to my sister E.P. Mayunga and special friends for encouragement and patience throughout the course of my studies.

My sincere appreciation goes to SACCAR/GTZ for having accorded me the scholarship to study under this programme and my employers in Tanzania (Tanzania Tobacco Co-operative Apex) for granting me the study leave.

I must confess that this work was done under poor conditions marked by lack of laboratory facilities and absence of necessary instrumentation which had to be sought from outside. Despite many problems and difficulties encountered, I have been able to do the research and write this dissertation.

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LIST OF ACRONOMY

AMV	Alfalfa mosaic virus
CMV	Cucumber mosaic virus
CSAITGA	Central and Southern African region of the International Tobacco Growers' Association
cv(s)	Cultivar(s)
DAS-ELISA	Direct double antibody sandwich enzyme-linked immunosorbent assay.
ITGA	International Tobacco Growers' Association
nm	Nanometer
PBST	Phosphate buffered saline Tween
PVY	Potato virus Y
TAZ	Tobacco Association of Zambia
TEV	Tobacco etch virus
TLCV	Tobacco leaf curl virus
TMV	Tobacco mosaic virus
TRSV	Tobacco ringspot virus
TRV	Tobacco rattle virus
TSV	Tobacco streak virus
TSWV	Tomato spotted wilt virus
TVMV	Tobacco vein mottle virus
TVNV	Tobacco vein necrosis virus
USA	United States of America

1.0 INTRODUCTION

1.1. General

Tobacco (*Nicotiana tabacum* L.) originated as a natural hybrid in Central America and has been under cultivation for many centuries. By the time explorers from Europe came to the Americas in 1499, tobacco cultivation was well spread in North, Central and South America and since then it has been grown all over the World (Akehurst, 1981).

Tobacco is a high value crop of wide demand throughout the world for the production of cigarettes, cigars and other tobacco products (Akehurst, 1981). It is a high value crop that is worth more than a billion dollars annually to the US farmer. In addition, federal, state and municipal tobacco taxes in the US total more than 5.5 billion dollars annually while the governments of Western Europe obtain about sixth (or 15%) of their operating revenues from profits of tobacco monopolies and tobacco tariffs and taxes (Lucas, 1975).

Leaf tobacco is produced in more than 100 countries (78 being developing countries) and almost every country is involved in the international trade of tobacco leaf products (FAO, 1989). The estimated total number of people dependent on income from tobacco as a whole is 8 million (ITGA, 1989).

Tobacco has shown itself to be an excellent cash crop and significant earner of foreign exchange for developing countries. Over 70% of foreign exchange in the case of Malawi, 25% in Zimbabwe and 12% in Tanzania is earned by tobacco (CSAITGA, 1992)

In Africa, tobacco is grown in large quantities in East, Central and South Africa, which encompass Kenya, Malawi, Mozambique, South Africa, Tanzania, Uganda, Zaire, Zambia and Zimbabwe (Akehurst, 1981).

In Zambia, tobacco has for many years been regarded as one of the most promising export crops and has provided a livelihood for a large number of people in areas of the country unsuitable to other crops (CSAITGA-1992). Tobacco is estimated to employ some 50,000 people and sustain around 75,000 to a reasonable living standard in Zambia. It earns US $ 10 million in foreign exchange and contributes almost 200 million Kwacha to government revenue in excise duty and sales tax (Wallace, 1993).

1.2 Tobacco production trends in Zambia

Burley and virginia tobacco are produced in Zambia and flue-cured tobacco accounts for virtually all the output. Virginia tobacco is grown mostly by large scale farmers along the line of the rail from Livingstone to Kabwe and Mkushi. Burley, and an increasing amount of virginia, is also grown by small scale farmers in Eastern province (ITGA, 1992).

On a global scale the levels of tobacco produced in Zambia are insignificant. The figures for 1996 show the total for unmanufactured leaf at 3,841 tonnes. The quantity had varied between 2,762 tonnes in 1985 and 6,740 tonnes in 1993 and dropped to 2,806 tonnes in 1994 (Appendix i).

1.3 Factors limiting tobacco production

Despite the developed skills in producing a high yield and desirable tobacco crop, Zambian farmers face numerous disease problems from bacteria, fungi, nematodes and viruses. (Wallace G, 1996 - Personal communication). The diseases degrade the quality of tobacco and reduce the value of the crop as measured by market prices (Akehurst 1981).

With the exception of viruses, most other diseases are generally identified in the field and are easily manageable (Wallace G, 1996 - Personal communication). Detection and proper identification of virus requires the use of modern technology such as serology and electron microscopy, although much can be done by simple commercial simple tests. The viruses occurring on tobacco in Zambia need to be identified and characterized in order to properly assess their potential to limit production; such information is of vital importance in the development of disease management practices and in breeding tobacco varieties for disease resistance. Accordingly, despite the lack of sophisticated facilities at the University of Zambia, it was decided to embark on a project to:

(i) isolate and identify the commonly occurring tobacco viruses in Southern, Central and Lusaka Provinces of Zambia; and

(ii) determine the occurrence and prevalence of viruses.

2.0 REVIEW OF LITERATURE

2.1 Geographical Spread And Economic Significance Of Common Tobacco Viruses

Virus diseases have a disastrous effect on crop yields and threaten the tobacco producing potential of Africa and other parts of the world (Thottappilly, 1992). Although many viruses infect tobacco, only a few cause economic damage (Reilly, 1983). Reduction in growth, abnormal growth forms and eventual yield losses may be due to alterations in the synthesis, translocation and effectiveness of various growth stimulants such as auxin, cytokinins and giberillins (Diener, 1963; Mathews, 1970).

Tobacco mosaic virus (TMV) is widespread in all tobacco growing countries (Lucas, 1975). It has been reported to cause serious losses in tobacco in North Carolina (Todd, 1980). Estimates of annual disease losses caused by TMV ranges from 0.03 to 0.88% (Todd, 1980). Wolf and Moss (1933) in their early work, reported losses due to mosaic of 30% and 42% in yield and value respectively. More recent research has determined less severe losses of 24% in yield and 29% in value (Johnson *et al.*, 1983). However, Diallo and Mulchi (1981) reported reductions in yields, value, average price and quality index values of cured tobacco due to TMV, compared to healthy plants of 25.8, 28.2, 3.4 and 8.5 % respectively.

Cucumber mosaic virus (CMV) also infects tobacco worldwide and reduces both the quality and weight of the crop. It is also associated with lower concentration of calcium, phosphorous, petroleum ether extractions and waxes in the leaves (Srivastava and Bhaskar, 1987).

Symptoms of CMV can easily be mistaken for tobacco mosaic virus and this may be a reason for it to be seldom reported on tobacco, although it probably occurs frequently, particularly in areas where vegetables are grown (Mink, 1969). In Africa, the virus has been reported to infect tobacco in Morocco (Thottapilly, 1992) and South Africa (Swanepoel and Nel, 1995). Large crop losses caused by CMV have been reported from time to time in Japan and the value per hectare may be reduced by more than 50%, if the plants are infected within one month of planting (Lucas, 1975)

The cause of tobacco leaf curl was not known until 1931 when Storey in Tanzania and Thung in Java (Lucas 1975) independently announced that it was caused by tobacco leaf curl virus (TLCV). Tobacco leaf curl is common in the tropics and subtropics and is also reported from the U.S.A, Europe and Japan (Agrios, 1988). In Africa, the virus has been reported from Tanzania, Nigeria, Sudan and South Africa (Akehurst, 1981; Thottapilly, 1992). Tobacco losses due to TLCV have been estimated at 5 - 10 % in Venezuela and the virus has prevented tobacco cultivation in certain areas of Pakistan (Lucas, 1975).

Potato virus Y (PVY) has caused significant losses on tobacco in several countries (Lucas, 1975) and has been known to cause serious problems in Chile, Hungary, Spain, and South Africa for tobacco growers (Gooding *et al.,* 1985); Morocco and Kenya (Thottapilly, 1992). Losses could average 25 - 45 % of the cash value in tobacco crop (Lucas, 1975). However, Sievert (1978) demonstrated that the value per hectare of some cultivars and hybrids of burley tobacco infected by PVY can be reduced by 11 to 83 %. Potato virus Y has also caused

significant damage in the United States of America, and several of its strains are of concern because of the lack of effective control measures (Gooding *et al.*, 1985)

Etch, a disease of tobacco caused by tobacco etch virus (TEV), was first described in Kentucky (Valleau and Johnson 1928). Since then the disease has been found to be of general occurrence in the U.S.A , Canada (Stover, 1951; Smith, 1972) and has been reported from other countries (Lucas, 1975). Reductions in yield and value of cured tobacco by TEV have been reported to be 37.0 and 39.8% respectively (Diallo and Mulchi, 1981).

Tobacco vein mottle virus (TVMV) was first reported by Gooding and Sun (1972). It was identified on burley tobacco in Kentucky and Tennessee (Akehurst, 1981). Incidence of TVMV and TEV in North Carolina in 1984 was approximately 2 and 1 % respectively, causing an estimated loss of US $ 2 million (Main and Nusser, 1985). In 1986, incidence was 0.7 and 2% for TMV and TEV respectively, causing a combined loss of less than US $ 1 million (Main and Byrne, 1986).

Tomato spotted wilt virus (TSWV) was first observed on tobacco in South Africa in 1906 and thought to be caused by *Fusarium sp.* (Lucas, 1975). It is now accepted universally that the disease is caused by tomato spotted wilt virus (TSWV). The virus was first identified in flue-cured tobacco in Georgia (Arnett, 1986). TSWV has been recognized as a serious problem of tobacco in many parts of the world (Lucas, 1975). It has also been reported to cause severe losses in tomato (*Lycopersicon esculentum* Mill.) and pepper (*Capsicuum annuun* L.) (Bond *et al.*, 1983; Greenough *et al.*, 1985). Surveys have shown that TSWV incidence occasionally

reaches 60% in commercial fields and 100% in home gardens (Greenough *et al.,* 1985). However the incidence of TSWV in tobacco has been shown to increase but not at levels high enough to cause significant losses of plant stand or yield (Bertrand and Csinos,1989).

Tobacco ringspot virus (TRSV) has been found in tobacco in all production areas and is the second only to TMV in prevalence in North Carolina (Rush and Gooding, 1970). It is now widespread in the tobacco growing areas of the U.S.A and has been reported from Australia, Canada, Europe, Japan, Malawi, New Zealand, Russia, South Africa and Sumatra (Thornberry, 1966; Lucas, 1975). The virus is less prevalent and rarely epiphytotic, but nevertheless, can cause severe losses on individual farms (Reilly, 1983). Some fields may have as high as 90% plant infection and suffer severe injury whereas certain fields may have only a few infected plants (Akehurst, 1981).

Alfalfa mosaic virus (AMV) is prevalent worldwide (Crill *et al.,* 1970). Although numerous *Nicotiana spp.* may serve as host of AMV (Mollings, 1959; Silber and Heggestad, 1965; Thornberry, 1966), reports on natural infections in field-grown tobacco have been limited (Lucas, 1975).

Other viruses which infect tobacco include tobacco rattle virus (TRV), tobacco streak virus (TSV), tobacco veinal necrosis virus (TVNV), tobacco yellow vein virus (TYVV), rosette and bushy top virus and pepper veinal mottle (PVMV). TRV is a soilborne virus which has been reported to occur in Europe, North America and Japan (Harrison, 1970). TSV is a disease which is widely distributed in U.S.A and has been found in various countries (Smith, 1972; Lucas, 1975). Vein banding (also called tobacco veinal necrosis) has become widespread and

serious in Europe since 1953 particularly in those tobacco producing regions where potato is an important crop (Lucas, 1975). Adams and Hull (1972) reported the occurrence of TYVV in Malawi while rosette and bushy top viruses were firstly reported to infect tobacco in Zimbabwe (Lucas, 1975). These viruses have very serious potential for damage to tobacco crops of East and Central Africa (Akehurst, 1981).

2.2. Symptomatology

The effects of viruses may be severe and easily noticeable or they may be mild and easily overlooked. The most obvious symptoms of virus infected plants are usually those appearing on the leaves, but some viruses may cause striking symptoms on the stem (Agrios, 1988). Infection of seedlings or transplants may cause complete crop failure where as infection of fully grown plant produce a minimal effect on returns (Akehurst, 1981)

The primary symptoms on tobacco mosaic virus (TMV) infected plants are:

(i) clearing of the veins of the young leaves in which the tissue immediately adjacent to the vein fades from normal to light green thus outlining the veins in contrast to the surrounding tissue (Diallo and Mulchi, 1981) and downward curling of leaves (Walker, 1969).

(ii) The development of local lesions is generally expressed as spots or rings of necrotic or chlorotic tissue. A few days after the primary symptoms, the characteristic blotching or mottling of the immature leaves develops (Diallo *et al.*, 1981). The dark green areas usually are associated with the veins (Reilly, 1983) and thicker somewhat elevated in a blister-like fashion over the thinner chlorotic light green areas. In young plants the leaves often show marked distortion and irregularities of growth (Lucas, 1975).

Typical mottling and mosaic patterns appear when tobacco is infected by CMV, sometimes accompanied by stunting, narrowing and leaf distortion. Severe strains may cause interveinal discoloration and the 'oak-leaf' pattern of necrosis on the lower leaves. "Mosaic burn" or sun-scald frequently appear on the upper leaves of infected plant. Mild strains cause only a faint mottling of the leaves (Lucas, 1975).

The symptoms of tobacco leaf curl virus (TLCV) vary according to the virulence of the strain. In Africa, the typical puckering of the infected leaf causes it to resemble that of 'savoy' cabbage. The veins on the underside become thickened and twisted to give a knotted appearance (Akehurst, 1981). Other symptoms are leafy outgrowth (enations) from the veins on the lower surface of the curled, twisted leaves which are smaller than normal, the greening of the veins and severe stunting of plant (Smith, 1972; Lucas, 1975). The flowering parts are curled, deformed and apical dominance is lost, so that the plant assumes a bloom-like rosette appearance (Lucas, 1975; Akehurst, 1981).

The first symptom to be noted on infected tobacco plant with potato virus Y (PVY) is a clearing of the veins of the youngest leaves 7 days after infection (Smith, 1972). With burley tobacco the disease displays a general yellowing and often premature death of lower leaves. In flue-cured tobacco the symptoms are similar except that one strain of the virus causes necrosis in cultivars resistant to the root-knot nematodes and another strain causes necrosis on all flue-cured cultivars (Gooding and Tolin, 1973). Field symptoms on plants infected by only necrotic strain of PVY show systemic vein-clearing, faint mottling, green vein banding and brown discoloration of the veins, which is especially noticeable on the lower surfaces of older

leaves. Numerous small, buff-colored necrotic lesions are frequently observed on some infected plants (Lockhart and Fischer, 1976).

The symptoms of tobacco etch virus (TEV) vary according to the strain of the virus present, the type of tobacco and condition of the plant growth (Lucas, 1975). Generally, however the symptoms of the disease include vein clearing, necrosis, chlorosis, stunting and etching (Stover, 1951; Lucas, 1975; Akehurst, 1981). Also chlorotic spots can be observed on the upper leaves in the green tobacco. The etching may be described as shallow necrosis or collapse of tissue due to local desiccation. The upper leaves of TEV infected plants exhibit a mild mottling of the upper leaves with an alternating light and dark green colors (Diallo and Mulchi, 1981).

Tobacco vein mottle virus (TVMV) shows vein clearing and banding which could be confused with PVY infection (Sun *et al.*, 1974). However, the irregular vein mottling symptom can be noted on the ruffles of older leaves and full expression of the vein banding shows it to be discontinuous hence the fairly descriptive name of the disease. The severe stage which follows, some two weeks after inoculation, involves heavy necrotic spotting, but without the distortions common to infection by virulent strains of PVY (Akehurst, 1981).

The typical symptoms of tomato spotted wilt virus (TSWV) vary with the age of the infected plant. Foliar symptoms include small concentric necrotic rings and chlorotic spots that coalesce, veinal necrosis, severe distortion and necrosis of levels in the terminal bud in young

plants (Greenough *et al.*, 1990, Culbreath *et al.*, 1991). Death of the entire plant following appearance of symptoms can also occur (Culbreath *et al.*, 1991).

The symptoms of tobacco ringspot virus (TRSV) are continuous with concentric lines of chlorotic and necrotic tissue. They can be either circular or centered around major veins and parallel to the veins or occur as irregular wavy lines or streaks (Lucas, 1975). Reilly (1983) reported the occurrence of light yellow rings with a tan dot in the center of the inoculated leaf, but 'oak leaf' patterns are clearly identifiable at first, but eventually fade as the season progresses. Infection can occur from the seedbed stage onwards. Early infections can lead to severe symptoms and result in typical virus effects of stunting and poor leaf quality (Akehurst, 1981).

Alfalfa mosaic virus (AMV) induces primary yellow lesions with or without necrotic centers and greyish-white necrotic rings or flecks. These are followed by systematic vein clearing and mottling, sometimes with oak leaf pattern. On older plants symptoms are usually milder with little or no necrotic flecking (Smith, 1972).

In the field, the symptoms of tobacco yellow vein virus on tobacco have been reported to be similar to those of tobacco bushy top virus or tobacco rosette disease (Smith, 1946; Gates, 1962).The young leaves show bright chlorosis of the veins. Leaves that develop subsequently are distorted, frequently with the tip bent downwards and with bright chlorosis of the veinal areas and green blisters between veins. Leaf size is reduced and the plant becomes stunted and rosetted (Adams and Hull, 1972).

As the new leaves in plants infected with the rosette and bushy top virus are formed, they curl inwards and the resulting rosette gets lighter and the center of the plant becomes a ball of compressed leaves with the interveinal tissues building outwards (Smith, 1972). As the plant grows upwards, the tissues start to split. The splits of tissue develop longitudinally in the stem, in the petioles, in all the veins of the leaves and even in the flower most of which fall without opening. Sometimes enations develop on the surface of the leaves (Smith, 1946).

Both tobacco necrosis and stunt viruses are diseases occurring in the seedbeds. The symptoms are necrotic spots which can coalesce and kill young seedlings but which on older plants affect only the lower leaves, with the upper ones apparently healthy. Affected leaves may be crinkled and have downward-turning margins. In the case of the stunt virus there is some form of necrosis and buds turn yellow. Leaf tips turn downward have some crinkling of the leaf surface. Shortening of internodes and a ring of necrotic tissue in the plant stalk, just above the ground leaves, are also visible (Smith, 1972; Lucas, 1975; Akehurst 1981).

2.3 Characteristics and Symptoms Induced by Tobacco Virus Strains

Strains are groups of virus isolates that are serologically and pathogenically similar. Very few plant viruses, particularly those of the sap-transmissible mosaic type, are single entities and consist of several strains. The differences between strains are usually less than the differences between different viruses (Noordam, 1973). Rare strains may become widespread as the ordinary strains disappear because of growing resistant varieties and therefore strains are of much importance in work on breeding for virus resistance (Pelham, 1970).

Many strains of tobacco mosaic virus (TMV) have been recognized that originally were isolated from tobacco (Henning and Wittman, 1972*)*. Although the chemical, physical and genetic properties of different strains of virus have been studied, very few recent studies have been conducted with the strains of (TMV) currently found in field grown tobacco (Ford and Tolin, 1983*)*. The common strain of TMV (TMV-C) is endemic in flue-cured tobacco growing areas of Virginia (Ford and Tolin, 1983*)* and North Carolina (Gooding, 1971) and the strain TMV-C has been reported to cause reduction in yield and loss of market quality resulting in millions of dollars in losses each year (Lucas, 1975; Todd, 1980). TMV-75 is another strain of tobacco mosaic virus which infect different tobacco cultivars in the United States of America. It induces both necrotic and chlorotic local lesions on tobacco cultivars ' NC 95', 'Speight G 28,' 'Coker 319' and 'Coker 347', where as TMV-C induces only chlorotic local lesions on the host (Ford and Tolin, 1983). On the same cultivars TMV-75 induces more severe systemic symptoms, including stunting of the plant, and leaf puckering and narrowing (Ford and Tolin, 1983*)*. Mild strains of TMV have also been reported from flue-cured tobacco and exceed those caused by common strains where disease incidence is low (Gooding, 1981).

De Bokx (1964) proposed a classification of PVY into three groups of strains based on their occurrence on potato and tobacco. In this system, PVY° comprised the common strain, based on the severity of systemic symptoms produced. PVY^N comprises the group of strains producing veinal necrosis on certain tobacco cultivars. Similarly, Gooding and Tolin (1973) found that isolates of PVY, occurring in flue-cured tobacco in the Southeastern United States, could be grouped into three distinct strains based on the reaction they induce. However, necrotic strains of PVY have been reported to cause severe losses on tobacco in several

countries (Lucas, 1975) and continue to be a serious threat in many areas of the tobacco production and where they become established (Gooding *et al.*, 1982).

There are numerous strains of CMV that vary in symptoms, virulence, ease of transmission, stability and dilution endpoint (DEP) and thermal inactivation point (TIP) (Scott, 1968). Recently, site-directed mutagenesis in the coat protein genes of CMV-M and CMV-Fny strains confirmed that alteration of the amino acid at position 129 of the coat protein reciprocally changes the phenotype from chlorosis to green mosaic or vice-versa on tobacco (Shintaku *et al.*, 1992). Induction of severe yellow chlorotic spots on tobacco leaves inoculated with two nuclear-coded recessive host genes (Takakhashi and Ehara, 1993). Other strains include CMV-O, CMV-P6, CMV-Q and CMV-D (Hayakawa *et al.*, 1989).

There are at least 6 major strains of tobacco ringspot virus (TRSV) and many minor variants can be distinguished by symptomatology, host susceptibility and serology (Lucas, 1975). Gooding (1970) reported six natural serological strains of TRSV. He found no correlation between serotype and symptoms caused on tobacco or cucumber and postulated that serological strains may have evolved from host or vector selection pressure.

At least 44 strains of alfalfa mosaic virus (AMV) have been recognized and they vary in host range, symptoms, aphid transmission and chemical characteristics. Various strains of AMV cross-protect against each other; cross protection starts about 5-7 days after inoculating the protecting strains. The amount of various components varies with the strain and growing conditions (Lucas, 1975).

2.4 Factors Affecting Symptom Expression

A variety of factors influence symptom development or expression on the infected plants. Symptoms are greatly influenced by temperature, length of day, light intensity, age of plant, nutrient excesses or deficiencies, genetic disposition of the cultivars and the virus strains. The same virus can produce a range of symptoms, depending on the environment and the host genotype. However, a lack of symptoms does not necessarily mean that no viruses are present. It may simply mean that the infection is latent (Green, 1991).

Lucas (1975) noted that the interval between infection and symptom appearance of TMV is influenced more by the age and rate of plant growth than any other factor, if the plant is growing under normal conditions of temperature and moisture. Symptoms on young, actively growing tobacco plants will appear within 2 to 3 days after inoculation where as for slow growing plants it may take 6 to 10 days or more. Other factors that influence symptom appearance in host include strain virulence, illumination, temperature, host resistance and nutrition.

Temperature, nutrition, light intensity and plant leaf age have been reported to influence symptom development of CMV-infected plants (Stimman and Swenson, 1967). High temperature, longer day length, high light intensity and excess nitrogen favour multiplication of most strains of CMV. The older leaves, which have been infected for several weeks, may have low virus titre (Lucas, 1975). Some strains of CMV failed to multiply and cause

symptoms in tobacco plants kept above 28-30°C whereas strains infected and caused severe symptoms at 36°C (Badami, 1959).

Cool temperature and low light intensity increase severity of symptoms of tobacco necrosis virus. Kassanis (1968) and co-workers showed that strains infected less readily at 30 °C than 20 °C and symptoms failed to develop if plants were held at 36 °C for 24 hours or more, soon after inoculation. Tobacco necrosis virus (TNV) inoculated on parts subjected to low light intensity developed severe necrotic symptoms but those grown in bright light produced only a few scattered leaf spots.

Mild symptoms of tobacco ringspot virus with low virus concentration developed when plants were grown at low N-levels, however symptom severity and virus concentration increased with increase in N concentration. Phosphorus exerts similar but has less pronounced effects (Helms and Pound, 1955).

Symptom expression of vein banding caused by PVY is dependent not only on the virus strain but also on environmental conditions, cultivar characteristics and prior passage of the virus through other host plants. Usually younger plants suffer more damage than older plants. Maximal concentration of PVY occurs at 20°C, and is low at 30°C. Symptoms are more severe on tobacco plants grown in shade (Lucas, 1975).

2.5 Transmission And Epidemiology Of Tobacco Viruses

Plant viruses rarely, if ever, come out of the plant spontaneously (Agrios, 1988). For this reason, viruses, generally do not cause infections even when they are carried in plant sap or debris unless they come in contact with the contents of a wounded living cell. Viruses are transmitted from plant to plant in a number of ways such as vegetative propagation plant sap, seed, pollen, insect, mites, nematodes, dodder and fungi (Agrios, 1988).

Natural spread of tobacco mosaic virus has been considered to be primarily by mechanical means (Zaitlin, 1975). Several reports suggest transmission of TMV by various sucking insects such as aphids, leafhoppers, mealybugs, thrips and whiteflies (Brachak 1959, Fulton and Scott, 1977). Chewing insects such as grasshopper, the potato flea beetle and tobacco flea beetle have also been implicated in the transmission of TMV (Mercer *et al.*, 1982).

Cucumber mosaic virus (CMV) is reported to be transmitted by mechanical means and aphids (Lucas, 1975); *Myzus persicae* and *Aphis gossypii* appear to be the principal vectors (Kennedy *et al.*, 1962).

Tobacco leaf curl (TLCV) is transmitted by whitefly (*Bemisia spp).* In Northern Province (South Africa) *Trialeurodes natalensis* and in Venezuela *Bemisia tuberculata* and *Aleurotrachelus socialis* have been identified as vectors for TLCV (Smith, 1972).

Tobacco etch virus (TEV) is spread principally by aphids including species of *Aphis, Aulacorthium, Macrosiphum* and *Myzus* (Kennedy *et al.*, 1962). Other viruses transmitted by aphids include tobacco vein mottle (TVMV), but with a very high efficiency much higher, for example, than that recorded for etch (Sun *et al.*, 1974; Akehurst, 1981). Insect vectors are the most important means of dissemination of tomato spotted wilt virus (TSWV), although the virus may be transmitted mechanically. The species so far known as vectors are onion thrips *(Thrips tabaci,* Lind*)* and three species of black carnation thrips (*Frankliniella Schultzei* Tryb, *Frankiniella fusca* Hurds and *Frankiniella occidentalis* Perg (Sakimura, 1963; Greenough *et al.*, 1990).

Nematode *(Xiphinema americana)* has been reported to transmit tobacco ringspot virus (TRSV) from source plant within 24 hours and can remain infective at 10°C for at least 49 weeks (Sherf and Macnab, 1986). Other vectors are stated to be thrips (*Thrips tabaci),* grasshoppers (*Melanophus differentials,* spider mites (*Tetranychus sp.)* and tobacco flea beetle (*Epitrix hirtipennis).* However, it can also be easily transmitted mechanically (Smith, 1972).

Potato virus Y (PVY) is spread by several genera of aphids including species of *Acyrthosiphon, Aphis, Myzus* and *Neomyzus* (Kennedy *et al.*, 1962). It is also transmitted readily by mechanical means but not apparently by the true seed (Smith, 1972).

Infection of tobacco with alfalfa mosaic virus (AMV) most likely results from aphid transmission of the virus from other host species; given the extent of aphid infestations in tobacco, although seed transmission of AMV has been shown in other crops (Froshieser,

1974; Kaiser and Hannan, 1983). However, AMV can also be transmitted mechanically, by grafting and dodder (angiosperm parasite). Other viruses transmitted mechanically are tobacco stunt and necrosis viruses (Lucas, 1975)

2.6 Detection And Identification Techniques

Since viruses are too small to be detected with the naked eye or seen through the light microscope, their presence has been detected primarily by the symptoms exhibited by the host plants and by symptoms induced in indicator plants after the virus was transmitted by grafting, mechanical inoculation or by one of the vectors and by electron microscopy. Serological tests such as ELISA (enzyme linked immunosorbent assay) has also proved to be a reliable technique in virus identification (Noordam, 1973; Hill, 1984). For complete characterization and identification of virus, it is necessary to use more modern methods such as nucleic acid analysis, coat protein analysis and molecular hybridization (Walkey, 1985). However, procedures required to carry out these analyses require a suitably equipped laboratory (Coutts *et al.*, 1988).

2.6.1 Infectivity Bioassay

Infectivity tests employing indicator plants that specifically react to certain viruses have the advantage of demonstrating the presence of virus irrespective of symptoms in the tested material and they often distinguish between viruses. Many viruses can be transmitted mechanically but those that persist in the vector cannot be usually transmitted in this way. In principle, each susceptible plant can be used as a test plant, but a number of species and cultivars are now used internationally (Noordam, 1973).

Mechanical transmission involves successful extraction of the virus from the host material and transfer of the virus-bearing sap to the surface of the leaves of the test plant in such a way that virus can enter cells (Hill, 1984). This is usually done by dusting the leaves with fine carborundum powder and rubbing the leaf gently with inoculum. The carborundum powder (or Celite) greatly increases the points of entry of the virus. Pin pricking causes too much damage to be an efficient method of inoculation, but sometimes it can be useful (Noordam, 1973; Hill, 1984).

(a) Diagnostic Plant Species

Diagnostic host species for TMV include *Nicotiana tabacum* cv. Xanthi-nc (and various cultivars) which develop systemic mosaic, *Chenopodium quinoa, Nicotiana glutinosa, Nicotiana tabacum* cv., *Datura stramonium* and *Capsicum frutescence* cv. tabasco develop local lesion after inoculation (Sherf and Macnab 1986; Brunt *et al.*, 1990). However Ford and Tolin (1983) demonstrated the differences in symptom developed in differential hosts between TMV-C and TMV-75. They noted that on *Capsicum annuum* L. cv. 'California wonder', TMV-C alone induced local lesions. Necrosis of the shoot apex on *Nicotiana rustica* L.,cvs NC 95 and Burley -21 were characteristic only of infection with TMV-75. Systemic symptoms in *Gomphrena globosa* were induced by TMV-75 alone.

Chenopodium amaranticolor, C. quinoa develop chlorotic lesions when inoculated with cucumber mosaic virus (CMV) while *Cucumis sativus* and *Vigna unguiculata* develop systemic mosaic and necrotic local lesions respectively (Brunt *et al.*, 1990).

Nicotiana tabacum cv. Havana 425 can be used to differentiate tobacco vein mottle (TVMV) from tobacco etch (TEV), potato virus Y (PVY) and cucumber mosaic virus (CMV) commonly found on burley tobacco. The cultivar Havana 425 develops vein clearing, mosaic, mottling or vein banding symptoms when inoculated with TEV, PVY or CMV, but is symptomless host of TVMV. The virus can be also readily distinguished from TEV on its inability to induce wilt on tabasco pepper and from PVY on the basis of inability to infect *Solanum demissum* (Lucas, 1975).

Sun *et al.*, (1974) noted vein-banding symptoms on *Lycopersicon esculentum* Mill. cv. 'Homestead'; *Nicotiana glutinosa* L., *Physalis floridana* Rydb. and *Solanum carolinense* L. after inoculation with TVMV. *Solanum carolinense* and *Nicotiana glutinosa* showed a slight yellowing and stunting on young leaves. *Datura metel* was a symptomless host.

Tobacco ring spot virus (TRSV) induces well defined local lesions with no systemic infection on *Chenopodium amaranticolor* and *Chenopodium quinoa* while *Vigna sinensis* develops primary lesions in the form of solid necrotic spots followed by systemic mottling (Smith, 1972).

Potato virus Y (PVY) can be identified by its inability to infect *Chenopodium amaranticolor* and by its inability to systemically infect *Datura stramonium* (Sherf and Macnab, 1986). It has been reported to induce symptoms on *Datura metel, Nicotiana clevelandii, N. glutinosa* and *N. tabacum* cultivars 'Samsum' and 'White burley' (Agranovsky, 1993).

Brunt *et al.* (1990) reported systemic vein clearing and dark green leaf mottling on *Nicotiana tabacum* and *N. glutinosa;* systemic transient leaf mottling on *Lycopersicon esculentum* and systemic vein clearing followed by leaf mottling and distortion on *Datura stramonium* when the plants were inoculated with tobacco mottle virus.

Gates (1962) in his study of virus causing axillary bud sprouting (tobacco bushy top) of tobacco in Rhodesia (Zimbabwe) and Nyasaland (Malawi) revealed that *Nicotiana alata* Link Otto and *Nicotiana rustica* L. gave a bright pale yellow and very coarse vein clearing tending to complete chlorosis. Other plants such as *Nicotiana glutinosa* L., *Petunia hybrida* Vilm., *Solanum nigrum* and *N. rustica* developed vein clearing, pale leaves and axillary shoots, as in tobacco. *Lycopersicon esculentum* Mill., cv. 'Pearson' showed only stunting, chlorosis and sometimes necrosis; while *Nicotiana clevelandii* Gray and *Solanum melongena* L. showed no definite symptoms.

Phaseolus vulgaris is known to develop necrotic lesion or no lesion at all by some strains and systemic mottle, vein necrosis and leaf distorting when it is inoculated with alfalfa mosaic virus (AMV). *Chenopodium amaranticolor* and *Chenopodium quinoa* can be used to distinguish AMV from CMV since they develop chlorotic and necrotic flecks when inoculated with AMV. *Pisum sativum*, on the other hand, develops local lesions or wilting of inoculated leaves (most cultivars), systemic stem necrosis and plant death. *Nicotiana tabacum* produces necrotic or chlorotic local lesions, no local reactions by some strains, systemic mild mottle, bright chlorotic vein banding, coalescing ringspot and rarely deformation and / or enations by some strains (Brunt *et al.*, 1990).

Tobacco yellow vein virus induces bright chlorotic vein banding in systemically infected leaves, followed by distortion (severe strain) on *Nicotiana tabacum, N. clevelandii, N. glutinosa* and *N. rustica. Lycopersicon esculentum, Nicandra physaloides* and *Petunia hybrida* provide light and dark green systemic leaf mottling when inoculated with tobacco yellow vein virus. Leaves of *Arachis hypogea* develop systemic pale green rings and pale green areas along midribs while *Glycine max* gives systemic leaf mottling (Adams and Hull, 1972).

The reaction of tobacco streak virus (TSV) on different indicator plants has been described (Brunt *et al.*, 1990) Inoculated *Phaseolus vulgaris* cv. Manteiga and *Beta patellaris* develop necrotic local lesions. *Vigna unguiculata* produces chlorotic or necrotic lesions while *Nicotiana tabacum* cv. Turkish upper leaves become toothed (Brunt *et al* ., 1990). The virus causes necrotic spots on inoculated leaves of *Gomphrena globosa, Nicandra physaloides* and *Capsicum frutescens* followed by systemic mosaic, leaf deformation or top necrosis (Salazar *et al.*, 1981). It induces local lesion on *Chenopodium quinoa* (Cook *et al.*, 1997).

Diagnostic host species for tomato spotted wilt virus (TSWV) include *Cucumuis sativus.* The cotyledons develop chlorotic local spots with necrotic centres, without any systemic infection. *Petunia hybrida* cvs Pink Beauty and Ministreal develop local lesions; systemic patterns and leaf deformation (Brunt *et al.*, 1990)

2.6.2 Serology

Of the possible ways to detect plant viruses, serological techniques are frequently favoured because of their specificity, speed and the scope they provide for standardization (Clark and Adams, 1977). However, for many important viruses conventional serological techniques cannot be used because of limitations such as low virus concentrations, unsuitable particle morphology, or the presence of plant extracts of virus inactivators or inhibitors. These limitations can largely be overcome by use of the microplate method of enzyme-linked immunosorbent assay (ELISA) (Clark and Adams, 1977). For plant viruses, the double antibody sandwich form of ELISA has been found to be suitable. A report on the application of the method to virus detection has been made by Voller *et al.*, 1976.

Tobacco mosaic virus, TSV, TVMV, TNV and TRSV have been reported to be strongly immunogenic with particles which react in standard gel diffusion tests. However, TMV, TRSV, TVMV, AMV and CMV are reported to be detected by ELISA (Brunt *et al.*, 1990).

Detection of AMV on burley tobacco cultivars and certain *Nicotiana spp* has been accomplished successfully by direct (double antibody sandwich) DAS-ELISA three weeks after inoculation. Negative and positive controls consisting of extract from non inoculated burley tobacco plants and dry leaf tissue from original AMV isolates were used respectively (Tedford and Nielsen, 1990).

Both PVY and TEV have been reported to be serologically related to pepper vein mottle virus (PVMV) (Sherf and Macnab, 1986). Serological comparisons have been made to identify different strains of PVY by using partially purified antigens and different antisera (Fribourg and Nakashima, 1984; Fakhfakh *et al.*, 1996)

2.6.3 Electron Microscopy

Electron microscopy using the negative stain procedure or shadow casting (Noordam, 1973) has been reported to be one of the fastest ways to assign a particular virus to a taxonomic group (Horne, 1967). Depending on particle morphology and size, a virus can be tentatively assigned to a taxonomic group (Hill, 1984).

Shapes and sizes of TMV, CMV and AMV have been reported (Smith, 1972; Lucas, 1975; Sherf and Macnab, 1986). Electron microscopy has so far given an overall picture of TMV as rod shaped particle of 300 x 18nm and negative staining technique has confirmed the presence of the central hole (Smith, 1972; Lucas, 1975 Brunt *et al.*, 1990). Also CMV has isometric particles and AMV virions are pleimorphic spheres and bacilliform particles ranging from 18nm in diameter to 30nm, and bacilliform particles up to 60nm in length (Sherf and Macnab, 1986).

Potato virus Y is the type member of the potyvirus group. Potyvirus particles are flexous rods about 680-900nm in length (Hollings and Brunt, 1981). Dossar and Mungur (1982) reported the existence of elongated particles estimated to be 750nm long, which have been confirmed

by immunomicroscopic tests to be PVY. However Green (1991) indicated the particle size to be 730nm long.

Flexous rods with a normal size of 765 x 13 nm have been found in electron micrographs of partially purified TVMV preparations as well as from leaf dips (Sun *et al.*, 1974).

The spherical particles of TLCV which were isolated from formalized sap of infected tobacco plant had a mean diameter of 39nm. Two decades later it was partially purified from sap of infected tobacco leaves by butanol clarification and differential centrifugation in which the results indicated that its particle size was 18 x 30nm (Lucas, 1975).

Finlay and Teakle (1969) have reported that aldehyde fixation for electron microscopy on identification of TNV is necessary for some strains. Size of TNV is 26nm in diameter (Brunt *et al.*, 1990).

Black *et al.* (1963) were the first to obtain electron micrographs of TSWV which they found to measure 85nm in diameter. Van Kammen *et al.* (1966) published micrographs showing the virus to carry between 68nm and 102 nm in diameter, to be essentially spherical and to be surrounded by a membrane, some particles appeared to have a tail.

The particles of TRSV are isometric and 29 nm in diameter with 42 morphological subunits (Lucas, 1975), particles may also swell at pH 8.0 (Brunt *et al.*, 1990).

2.7 Management Of Tobacco Viruses

Virus diseases are hard to manage directly. Once a plant is infected with a virus, it becomes a source of contamination for other plants. However, indirect control remains the only practical method of controlling viruses. These methods include avoidance and elimination of virus sources, avoidance and control of vectors, and use of resistant cultivars.

2.7.1 Avoidance and elimination of virus sources

Virus infection can be minimized by removal of infected plants and of certain weeds that harbour the viruses early in the season. This reduces the subsequent spread of the virus to other plants during the various cultural practices (Agrios, 1988). For example the rate of infection of TLCV can be reduced by destroying the susceptible weeds and cultivated host near seedbeds (Lucas, 1975).

Crop rotation breaks the crop cycle and provides a host free period. This method is particularly useful with viruses which have limited host range (Green, 1991). A high percentage of mosaic plants should not be planted the following year; at least 2 years rotation should be practiced (Lucas, 1975).

For seed borne viruses, planting of virus-free seed is an obvious measure, which can be applied by using certified seed that has been tested properly for virus infection (Green, 1991).

TMV and CMV are mechanically transmitted and can be reduced by washing hands and tools before working with plants, handling plants as little as possible and by increasing distance between rows to reduce chance of plants touching each other (Sherf and Macnab, 1986).

Since there is poor tobacco grower acceptance to the use of milk as a hand wash for TMV control, an abrasive hand soap is an equally effective alternative that has proved more acceptable (Krausz and Fortnum, 1982). Reilly (1979) indicated that hand wash with a strong phosphate detergent (1%) solution is an acceptable alternative. Therefore abrasive hand soap, reconstituted dry milk and a strong detergent have been recommended for control of TMV due to their effectiveness. The use of latex rubber gloves and milk spray prior to pulling for transplanting have also been reported to be effective in controlling virus transmission by contact. (Krausz and Fortnum, 1982).

2.7.2 Avoidance and control of vectors

Spread by vectors can be avoided or their effects reduced when cropping is done in an area free of or with low incidence of the vector. Also changing in cropping practice may reduce the number of vectors.

Control of insect virus vectors has been reported to reduce or prevent infection of virus in tobacco seedbeds and fields (Green, 1991). TLCV is transmitted by whitefly while CMV, PVY and TEV are transmitted by aphids. Nematodes, thrips and fleabeetle are reported to transmit TRSV. TSWV is transmitted by thrips alone.(Lucas, 1975; Akehurst, 1981). The control of vectors can be achieved through use of insecticides such as spraying with 40% Dimethoate is recommended for the control of TLCV in Zimbabwe (Lucas, 1975). The use of insect traps has also been reported (Green, 1991). Success in controlling aphid-transmitted

viruses has been obtained by applying a film of oil to plant surface that interferes with virus transmission (Zitter and Simmons, 1980). Gooding and Lapp (1981) reported reduction of PVY incidence by application of JMS Stylet-oil® to flue-cured tobacco in a field.

2.7.3 Use of resistant cultivars

Several TMV resistant varieties of tobacco have been developed, but generally of low quality (Johnson and Main, 1983). Incorporation of resistance into acceptable flue-cured varieties has proved difficult, but it has now been achieved through the development of varieties such as coker 86, NC 628, Sc 71, Sc 72 virginia 770 and VA 080 (Koom, 1982; Johnson and Main, 1983). Tobacco cultivar NC-744 has been reported to be resistant to PVY. It is tolerant to the mild strain and has apparent immunity to all known isolates of the severe strain. In addition to PVY, NC-744 is resistant to TEV and TVMV (Chaplin *et al.,* 1980). Cultivars Virgin A mutant, TN 86 and PBD6 have also been reported to be resistant to PVY necrotic strain (Brandle *et al.*, 1995).

Because the control of aphid vectors of viruses and elimination of overwintering weed hosts have so far been impractical, introduction of resistance to TEV and TVMV into the standard burley varieties has been emphasized as the major control measure (Edwardson, 1974). The backcross method has been used in improving burley tobacco varieties with resistance from T 1406 (Collins *et al.*, 1975).

3.0. MATERIALS AND METHODS

3.1. Field Study

A field survey was carried out in Southern, Central and Lusaka Provinces during the tobacco growing season in 1997, when the crop was ready for topping. Flue-cured and air-cured tobacco fields (3 fields from each province) located in the predominantly tobacco growing areas in Zambia (Fig. 1) were selected in cooperation with Tobacco Association of Zambia field staffs (Table.1). The main criteria for field selection were representative crop production practices, access to field and cultivar.

Survey sampling procedures were adapted from those of Main and Proctor (1980). The area of the field surveyed ranged from 4-8 hectares depending on the size of the field. The surveyed part of the field was divided into quadrants of equal size. Within each quadrant, two sample blocks were selected by a random procedure and marked. Each block consisted of 10 rows with 10 plants each. In each sample block observed, data were recorded on virus symptoms observed, weed species available, virus insect vectors and the number of virus-infected plants. Percentage of virus-infected plants (Incidence = I) in each block was estimated by

$$I = \frac{D}{N}$$

Where D is number of diseased plants out of a total of N plants in each row.(Culbreath *et al.*, 1991)

Two to three young leaves with virus symptoms were removed from each diseased plant observed in each sample block. These leaves were mixed thoroughly to constitute a sample from one block. Infected leaves from weeds located in and around tobacco fields were also

collected for serological test. Therefore eight tobacco samples were collected from each field to make a total of 72 leaf samples. These leaf samples were kept in separate labeled plastic bags placed on ice in a cooler box and transported to the Laboratory of Department of Crop Science, at the University of Zambia.

3.2. Host range studies

A host range study was conducted under insect proof screen house condition at 18 - 26°C for 7 weeks utilizing the species listed in Table 2. Indicator hosts were grown in 4 inch plastic pots and plastic seedling sleeves containing a mixture of sterilized soil, sand and peat. Seeds of indicator hosts were kindly provided by Mount Makulu Research Station, Plant Virology section. Indicator plant species were arranged in 9 groups completely separated from each other. Each indicator host species was replicated four times in each group.

Inoculum from nine fields was prepared separately by homogenizing infected leaves in a 0.01M Na_2HPO_4-KH_2PO_4 buffer at pH 7.2 with a mortar and pestle at a rate of 1g of tissue per 2 ml of buffer.

Mechanical inoculations were made by rubbing the adaxial surface of leaves dusted with 22-μm (600-mesh) carborundum with sap inoculum using the forefinger followed by rinsing with distilled water. Two plants were inoculated per species in each group leaving the other two plants as controls. Indicator plants were inoculated at 3 to 5 leaf stage. After inoculation, symptoms were visually monitored and recorded for a period of 4-7 weeks.

3.3. Serology

The double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA) procedure, as described by Clark and Adams (1977), was followed. All samples for DAS-ELISA testing were ground in a mortar and pestle with extraction buffer, PBST at a ratio of 1:10 (Leaf tissue weight : Extraction buffer). The PBST buffer contained 0.05% Tween-20, 2% polyvinylpyrrolidone MW 40,000, Powdered egg (Chicken) albumin grade II, sodium sulphite and sodium azide.

Each test sample was placed in two adjacent precoated wells (with antibodies) of an ELISA plate (Agdia Inc. USA). Following loading diagram, 100 µl of prepared sample was dispensed into sample wells. 100µl of positive control was dispensed into control wells, and 100µl of extraction buffer into buffer wells. Following incubation of plates overnight at 4°C, the plates wells were washed with 1 x PBST buffer 3 times. After washing and drying the wells, 100µl of enzyme conjugate (diluted 1:100 in ECI buffer) were added to each well on the ELISA plate. The plates were incubated for 2 hours at room temperature. After incubation, the plates were washed again with 1 x PBST buffer 3 times. 100µl of *p*-nitrophenyl phosphate substrate (PNP) solution was dispensed per well. Approximately 60 minutes after the addition of the substrate solution at room temperature, reactions were arrested with 3M sodium hydroxide. The antisera used were against TMV-C, PVY, AMV, TSWV, CMV and TRSV were purchased from Agdia Inc., USA

ELISA reaction for test samples and for appropriate control were evaluated visually by two independent observers. Reactions were considered positive only if detected by both observers (Rosenberger *et al.*,1989)

3.4. Electron microscopy

Size and shape determination of virions could not be done at the University of Zambia Teaching Hospital as it was planned, since the electron microscope developed a fault in the course of the experiment. Samples were prepared and mounted on the carbon coated grids and sent to Plant Protection Research Institute (PPRI), Virology and Electron Microscopy Unit, Pretoria, South Africa for virus particle measurements only. No photographs were provided by the PPRI for financial constraints.

Leaf tissues were crushed in chilled mortar and pestle in 0.01M Na_2HPO_4-KH_2PO_4 buffer (pH 7.2) at a rate of 1g to 5ml. The suspensions were centrifuged at low speed (5000 x 2min). Drops (3 x30µl) from the supernatant were placed on dental wax in a Petri dish containing moist filter paper. On each droplet a carbon/neocollodion -coated grid (300 mesh) with coated side down was placed and let to stand for 5min. Carefully the excess plant sap was washed with 10 drops of double distilled water on clamped grid before staining. Freshly prepared 2% uranyl acetate (pH 4.3), ammonium molybdate (pH 5.4) and 2% phoshotungstic acid (pH 7.0) were used as stains. Two grids were prepared for each stain. Excess stain was removed from the grid using a small piece of filter paper touched to the grid. Grids stored in a grid storage box were sent to Plant Protection Research Institute, Virology and Electron Microscopy Unit, Pretoria, South Africa for electron microscope examination.

3.5 Statistical Analysis

Statistical evaluation of the data to compare virus disease incidence, involved analysis of variance and means separation and was done according to the complete randomized design with one factor. Data were analyzed by computer using the Mstatc package(Gomez and Gomez,1984).

Table.1 Tobacco farms surveyed for the presence of virus diseases in Southern, Central and Lusaka Provinces.

Farms	Cultivars	Location	Provinces
Popota Tobacco College	K35	Choma	Southern
Charles Itamoya	K35	Sibanyati	Southern
Petergreen PRC	NC 346	Kachenje	Southern
Zanje Estates	Banket A1	Kasavasa	Central
Mkushi Coffee Estates	NC 346	Mkushi Farming Block (Masansa)	Central
Green Leaf	K35	Chibwe Farming Block	Central
Lukali	RK3	Kabile tobacco Scheme	Lusaka
Bonanza	K35	Ngwerere	Lusaka
Kasondi Ranch	BRK 102	Kasisi	Lusaka

Table. 2: List of indicator plant species used for host range studies.

Capsicum annuum L. cv. California wonder

Capsicum annuum L. cv. Yoloy

Capsicum frutescence L. cv. Tabasco

Chenopodium amaranticolor Coste & Reyn

Chenopodium quinoa Willd.

Cucumis sativus L. cv. Pepino

Datura stramonium L.

Glycine max (L.) Merr. cv. Kaleya

Gomphrena globosa L.

Lycopersicon esculentum Mill. cv. Heinz

Nicotiana clevelandii Gray.

Nicotiana glutinosa L.

Nicotiana rustica L.

Nicotiana tabacum L. cvs Xanthi-nc, Samsun, KM10.

Pisum sativum L. cv. Green east

Phaseolus vulgaris. L. cv. Top crop

Physalis floridana Rybd

Solanum melongena L. cv. Black beauty

Vigna radiata

Vigna unguiculata (L.) Walp. cv. Bubebe (IT-328-16)

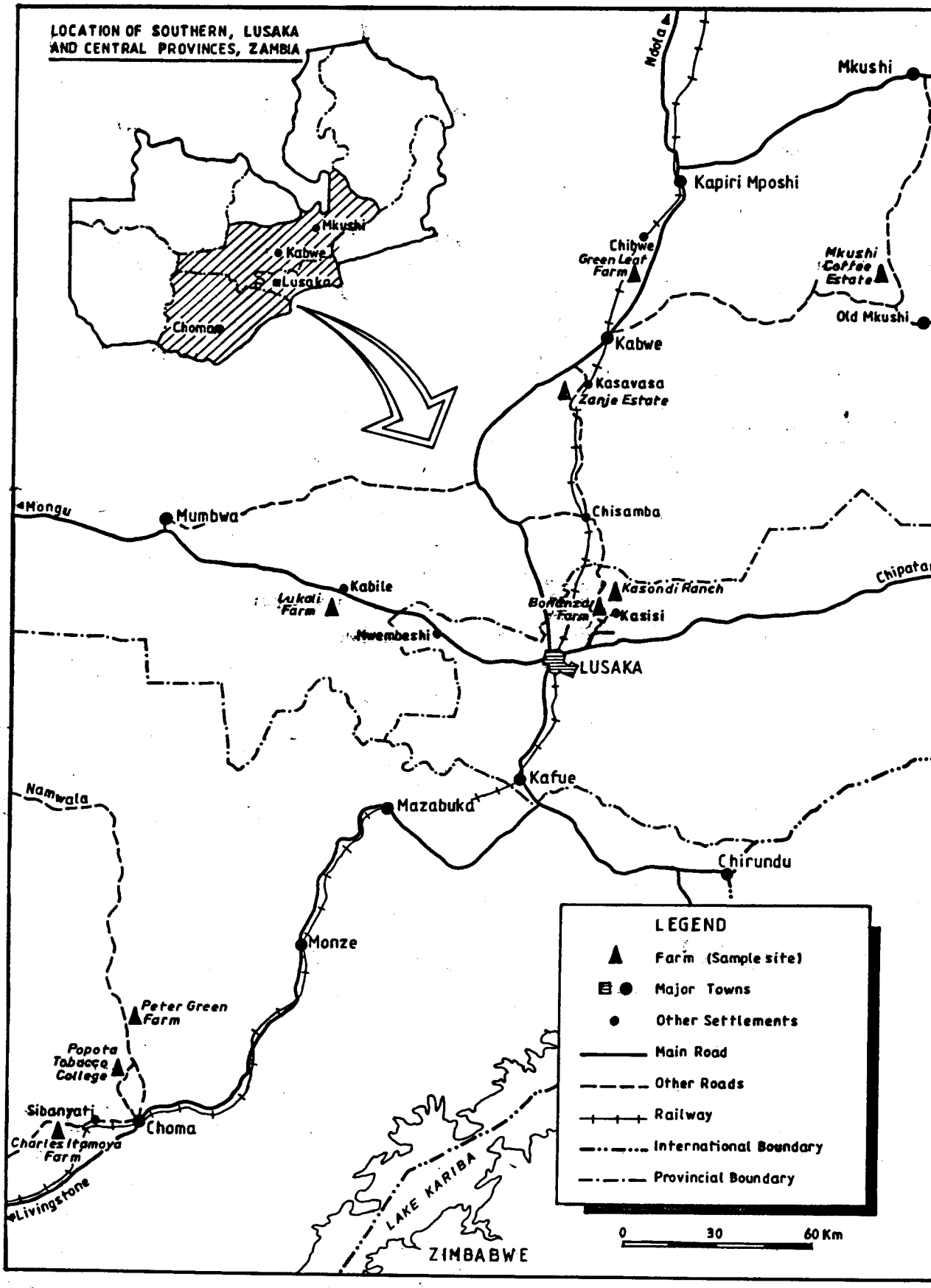

Fig. 1. SAMPLED AREAS OF TOBACCO PRODUCTION

4.0.0 RESULTS

4.1.0 SOUTHERN PROVINCE

Three tobacco fields were inspected for virus disease symptoms in early February 1997. Among these fields one was a large scale farm (Petergreen PRC tobacco farm) and the rest were small scale farms (Popota tobacco college farm and Charles Itamoya farm).

4.1.1 Popota tobacco college farm

(a) Field Symptoms

A flue cured tobacco farm of 4 hectares planted with cultivar K35 was surveyed. A number of plants in the field showed mosaic, chlorotic irregular patches on the foliage in addition to a milder, systemic mottle, interveinal chlorosis with dark green vein banding and downward curling of the leaves (Fig. 2a). Plants appeared to be infected as they approached maturity and showed mottling symptoms only in the topleaves (Fig.2b). The disease infection was determined to be 25% of the whole field (Table 3)

(b) Host range reactions

The sap extract from infected leaves of field sample produced local necrotic lesions on *Capsicum annuum* cvs California wonder and Yoloy; *Capsicum frutescens* cv. Tabasco, *Cucumis sativus* cv. Pepino, *Datura stramonium* and *Gomphrena globosa.* Systemic mosaic and mottling were observed on *Nicotiana tabacum* cvs Samsun, Xanthi-nc ; *Nicotiana glutinosa* and *Vigna unguiculata* cv Bubebe (IT-328-16). The systemic mosaic on *Nicotiana glutinosa* was followed by vein yellowing and stunted growth. *Physalis floridana* reacted with mosaic followed by leaf yellowing puckering and systemic stem necrosis (Table.4).

(c) Serology and Electron Microscopy

Sap extract from infected leaves of both field sample and indicator plants gave positive reaction with antisera against TMV. However, there was no reaction observed with antisera against PVY, AMV, CMV, TRSV and TSWV. Grids prepared from infectious crude sap and stained negatively showed rigid rod-shaped particles in electron microscope. Some particles appeared to be broken into shorter lengths. Estimated lengths of the particles were between 156-308nm.

4.1.2 Charles Itamoya Farm

(a) Field symptoms

Four hectares of flue-cured tobacco (cv. K35) in Sibanyati village , north west of Choma town were inspected. A pronounced mosaic, yellowing, mottling and stunting was observed on the affected plants (Fig.3a). Gross field symptoms showed mosaic with an overall yellowing appearance to the field, interveinal chlorosis, necrotic spots, leaf puckering, stunting and reduced leaf size (Fig.3b). The symptoms were always strongest on edges of the field. Some weeds in the field such as *Nicandra physaloides* and *Bidens pilosa* with the same symptoms were observed. The percentage of virus disease infection was significantly higher (30%) than those of Popota tobacco college and Petergreen PRC farms($P \leq 0$) (Table 3).

(b) Host range reactions

The crude sap extract produced local necrotic lesions on *Chenopodium amaranticolor*, *C. quinoa, Solanum melongena* cv. Black beauty, *Capsicum annuum* cvs California wonder, *Cucumis sativus* cv. Pepino; *Gomphrena globosa* (Fig.3c), *Nicotiana clevelandii*, *N. tabacum* cvs Samsun, KM10, and *Nicotiana glutinosa*. Systemic mosaic was observed on *Capsicum*

annuum cvs California wonder, Yoloy on which it started as mild mosaic followed by interveinal chlorosis and necrotic spots. As the plants grew older, the mosaic symptom started disappearing. Other test plants with systemic mosaic or mottling included *Lycopersicon esculentum* cv. Heinz, *Datura stramonium*, *Nicotiana clevalandii*, *N. rustica, N. tabacum* cvs Samsun KM10 and *Physalis floridana*. *Pisum sativum* cv.Green east developed no symptoms (Table 4).

(c) Serology and Electron Microscopy

Crude sap extract from field samples and symptomatic test plants gave positive test with antisera against PVY and TMV. Antisera against CMV, AMV, TRSV and TSWV gave negative results. Potato virus Y and TMV were also detected serologically on weeds (*Nicandra physaloides*) showing virus like symptoms collected from the field. Results of electron microscopy failed to resolve virus particle morphology.

4.1.3 Petergreen PRC tobacco farm.

(a) Field symptoms

Petergreen PRC tobacco farm is a commercial large scale farm situated north west of Choma town along Namwala road. It had 41 hectares planted with Virginia tobacco cvs K35 and NC 346. Seeds for cultivar NC 346 were imported from Zimbabwe. A total of eight hectares planted with cultivar NC 346 (field 1) was inspected. The prominent symptoms observed on tobacco plants were green vein banding, mild mottling, yellowing, downward leaf curling, necrotic lesions on the veins and stalks. Leaf malformation and dwarfing, especially on young

leaves, were also observed (Fig. 4a). The disease incidence was significantly low (7%) as compared to other fields surveyed in three provinces (Table 3).

(b) Host range reactions

Virus isolate from Petergreen tobacco farm produced necrotic lesion on *Chenopodium quinoa, C. amaranticolor, Solanum melongena* and *physalis floridana.* Systemic mild mosaic or mottling was observed on *Capsicum annuum* cvs California wonder (Fig. 4b), Yoloy and *Nicotiana tabacum* cvs Xanthi-nc, Samsun, KMN10. *Datura stramonium, Glycine max* cv. Kaleya, and *Pisum sativum* cv. Green east were symptomless hosts (Table 4).

(c) Serology and electron Microscopy

Crude sap extracted from both test plants and field sample reacted positively with the antiserum against PVY. However, no definite positive reaction was observed on antisera against TMV, CMV, TSWV, AMV and TRSV. Few slightly flexous particles were detected when the crude extract was viewed in electron microscope. The estimated particle lengths ranged from 728-784nm.

Table 3 Mean virus disease incidence in farms surveyed in Southern, Central and Lusaka Provinces

Provinces	Farms	Cultivars	Virus detected	Virus disease Incidence (%)*
Southern	Petergreen PRC	NC 346	PVY	7.0 f
	Popota Tobacco College	K35	TMV	25cd
	Charles Itamoya	K35	PVY, TMV	30bc
Central	Mkushi Coffee Estate	NC 346	AMV, TMV, TRSV	31b
	Zanje Estate	Banket A1	TMV, TRSV	25cd
	Green Leaf	K35	PVY, TMV	17e
Lusaka	Kasondi Ranch	BRK 102	TMV, PVY	30bc
	Lukali	RK3	TMV, PVY, AMV	40a
	Bonanza	K35	TMV, PVY	20de
CV 16.3%				

*Means followed by the same letter in a column were not significantly different at 0.01 probability level according to Duncan Multiple range test.

Table 4. Host range symptoms induced by samples from Southern Province

Indicator plant species	Popota Tobacco College Farm Symptoms		Petergreem PRC Farm Symptoms		Charles Itamoya's Farm Symptoms	
	Local	**Systemic**	**Local**	**Systemic**	**Local**	**Systemic**
Capsicum annuum cv. California wonder	Nl	CM	0	M, Vb	Nl	Ivc, M, Lc
Capsicum annuum cv. Yoloy	NL	M, Ivc, Lc	0	Mo	0	Mo
Capsicum frutescence cv. Tabasco	Nl	0	0	M	0	M
Chenopodium amaranticolor	Nl	0	Nl	0	Nl	0
Chenopodium quinoa	Nl	0	Nl	0	Nl	Cb
Cucumis sativus cv. pepino	Nl	Lne	0	Ivc, Mo	Cl, Nl	Cs
Datura stramonium	Nl	Ivc ,Ns, Lc	0	0	0	Mo
Glycine max cv. Kaleya	0	Cb, Cs	0	0	Ns	Lne, Cb, Lc
Gomphrena globosa	Cl, Nl	Mo,	0	Mo	Nl, Lne	Mo,
Lycopersicon esculentum cv. Heinz	Cb	Mo, St	0	0	0	Mo, Cb
Nicotiana clevelandii	0	Mo, St	0	M, Ld	Nl	M. Ld, St
Nicotiana glutinosa	0	Mo, Vy, St	0	Vc, Mo, Ly	0	Mo, Lc
Nicotiana rustica	0	YM	0	Ivc, Mo	Cl	Ivc,Mo,Lc
Nicotiana tabacum cv. Xanthi-nc	0	Mo	0	Mo	0	Mo
cv. Samsun	0	M	Cs	Mo, Vc	0	M
cv. KM10	0	0	0	Mo	Nl	M, Ln
Pisum sativum cv. Green east	0	0	0	0	0	0
Phaseolus vulgaris cv. Top crop	0	Ivc, Vb	0	Mo, Vb,Ivc	Nl	Vb, Mo
Physalis floridana	0	M, Ly, Lp,Sn	Nl	M, Ly	Ns	M, Ly, Ln
Solanum melongena cv. Black beauty	0	0	Nl	M	Nl,Cs	M,
Vigna radiata	0		0	Ivc, Mo	0	Mo, Cb
Vigna unguiculata cv. Bubebe (IT-328-16)	o	Mo	0	Vb, Mo, Lc	0	Ivc, Mo

Key :-

0	No symptoms	Ln	Leaf narrowing	Sn	Stem necrosis
Cb	Chlorotic blotch	Lne	Leaf necrosis	St	Stunting
Cl	Chlorotic lesion	Lp	Leaf puckering	Vb	Vein banding
cM	Chlorotic mottle	Ly	Leaf yellowing	Vc	Vein clearing
Cs	Chlorotic spots	M	Mosaic	Vy	Vein yellowing
Ivc	Interveinal chlorosis	Mo	Mottling	yM	Yellow mosaic
Lc	Leaf curling	Nl	Necrotic lesions		
Ld	Leaf deformation	Ns	Necrotic spots		

Fig. 2a. Flue-cured tobacco plant (cv. K35) showing mosaic, irregular chlorotic and dark green vein banding caused by TMV

Fig. 2b. Flue-cured tobacco plant (cv. K35) infected by TMV as t approaches maturity

Fig. 2b

Fig. 3a

Fig. 3a. Tobacco plant (cv. K35) showing yellowing and mottling due to TMV.

Fig. 3b. Tobacco plant showing interveinal chlorosis, leaf puckering, stunting and reduced leaf size due to mixed infection of TMV and PVY.

Fig. 3b

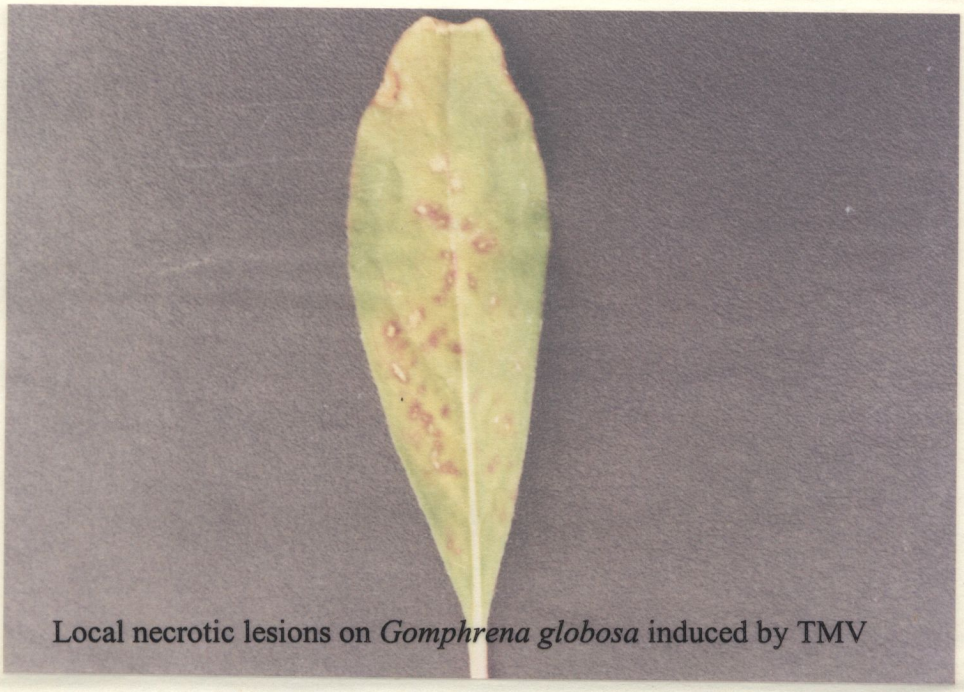

Fig. 3c

Fig. 3c. Local necrotic lesions on *Gomphrena globosa* induced by TMV

Fig. 4a. Leaf malformation, yellowing and dwarfing on young leaves of tobacco cv. NC346 caused by PVY.

Fig. 4a

Fig. 4b. Systemic mottling and vein banding caused by PVY on mechanically inoculated *Capsicum annuum* cv. California wonder.

Fig. 4b

2.0 LUSAKA PROVINCE.

Two large scale farms (Kasondi ranch and Bonanza) and one small scale (Lukali) farm were surveyed at the end of February 1997.

4.2.1. Kasondi Ranch

(a) Field Symptoms

A survey was conducted on 8 hectares of burley tobacco cv. BRK102 transplanted in mid December at Kasondi ranch. Cultivar BRK102 was under experimental suitability and it was planted in a farm in which virginia tobacco was grown in the previous year. Infected plants showed necrotic spots, vein clearing, mosaic, mottling, leaf puckering, leaf deformation, yellowing and stunted growth. Chlorotic rings, ringspots, vein banding and severe mosaic were observed in some infected plants (Fig. 5a,b). Chlorosis appeared around the midrib and then spread to small veins (Fig. 5c). The average disease incidence recorded in the field was 30% of the plants examined (Table 3). Pigweeds (*Amaranthus* spp.) and *Solanum nigrum,* showing distinct mosaic and leaf deformation, were observed in and around the tobacco fields.

(b) Host range reactions

The virus inoculum from mixed infected leaves induced local reactions followed by systemic symptoms on *Cucumis sativus* cv. Pepino; *Datura stramonium, Gomphrena globosa, Nicotiana clevelandii, N. rustica, N. tabacum* cvs Xanthi-nc, samsun; *Phaseolus vulgaris* cv. Top crop, *Physalis floridana, Solanum melongena* cv. Black beauty, *Vigna radiata* and *Vigna unguiculata* cv. Bubebe (IT-328-16). On *Physalis floridana* symptoms became severe (12 days after inoculation) which subsequently resulted into death of the plant (Fig. 5c). However, *Chenopodium quinoa* and *C. amaranticolor* developed only local necrotic spots and chlorotic

lesions respectively. *Capsicum annuum* cvs California wonder, Yoloy; *C. frutescens* cv. Tabasco; *Lycopersicon esculentum* cv. Heinz and *Nicotiana glutinosa* developed systemic symptoms only (Table 5). Typical mosaic with prominent vein banding was induced clearly on *N. tabacum* cv. Xanthi-nc (Fig.5d)

(c) Serology and Electron microscopy

The results on serological test by DAS ELISA indicated that both, field samples and infected indicator plants, contained PVY and TRSV. They reacted positively with PVY and TRSV antisera and negatively with TSWV, TMV, AMV and CMV antisera. Few flexuos rod-shaped particles were detected in electron microscope examination. Estimated particle lengths were between 560-980nm.

4.2.2. Lukali Farm.

(a) Field Symptoms

A flue-cured tobacco field Placed with cultivar RK3 in a 4 hectares small scale farm in Kabile tobacco scheme area, located in Lusaka west, was inspected for viral symptoms and sample collection. Seeds for cultivar RK3 were imported from Zimbabwe. Field symptoms on infected plants, from which the leaf samples were collected, consisted of systemic vein clearing, veinal necrosis, yellowing and reduced leaf size, the latter being especially noticeable on the top leaves (Fig. 6a). Numerous small buff colored necrotic lesions and vein banding were frequently observed on infected plants (Fig. 6b). Some weeds in the same field, such as *Bidens pilosa* and *Amaranthus spp.* were found to exhibit the same symptoms as on tobacco plants. Aphids were also observed in the tobacco field. Lukali farm exhibited the highest virus disease incidence of 40% (Table 3).

(b) Host range reactions

Test plants mechanically inoculated with the inoculum from Lukali farm showed varying reactions which are summarized in Table 5. Severe symptoms were observed on *Nicotiana rustica* which started as chlorotic lesions on the inoculated leaves followed by systemic chlorotic and necrotic lesions. The necrosis extended into the midribs and eventually into veins. The veins turned dark brown to black. The young leaves were puckered and reduced in size (Fig. 6c). However, no symptoms were observed on *Chenopodium amaranticolor,* while *Nicotiana tabacum* cv.Samsum developed severe mosaic followed by leaf necrosis and stunted growth. Leaf curling on *Capsicum annuum* cvs California wonder and Yoloy appeared on the top leaves . The interveinal chlorosis and leaf yellowing were more pronounced on the lower leaves of *Nicotiana glutinosa* (Table 5).

(c) Serology and Electron Microscopy

Sap extract from both field sample and infected test plants indicated the presence of TMV, AMV and PVY by DAS ELISA test. However the sap reacted negatively with antisera against TSWV, TRSV and CMV.

Observations of the electron microscopy revealed that the sample contained multiple-infections of sligthly flexous and rigid-rods. Estimated particle lengths were 448-784 and 306nm respectively.

4.2.3. Bonanza Farm

(a) Field Symptoms

Large Scale farm at Ngwerere, Lusaka east containing 30 hectares of the flue-cured tobacco (cultivar K35) was investigated for virus disease occurrence. A section of 8 hectares (section iv) tobacco field was selected for close symptoms observation and sample collection. The

infected tobacco plants were slightly stunted. The leaves had mottling or mosaic, yellowing, interveinal chlorosis, chlorotic spots, puckered appearance and were distorted. Necrotic spots concentrated along the main veins were observed on severely affected plants (Fig. 7a). The incidence of the disease was 20% (Table 3).

(b) Host range reactions

Mechanical inoculation with freshly expressed sap from Bonanza tobacco field leaves produced local chlorotic spots on *Physalis floridana* 6 days after inoculation followed by mosaic, leaf curl, stunted growth and eventually leaf and stem necrosis and death of the plant (12 days after inoculation) (Fig. 7b). *Capsicum annuum* cvs California wonder, Yoloy; *C. frutescens* cv. Tabasco; *Nicotiana glutinosa, N. rustica and Solanum melongena* cv. Black beauty developed mixed symptoms. The infected plants were stunted as compared to healthy uninoculated plants. Mosaic or mottling was common in almost all the inoculated test plants except *Chenopodium quinoa, Pisum sativum* cv. Green east and *Vigna radiata* which reacted with chlorotic blotch, interveinal chlorosis, leaf yellowing, chlorotic spots and leaf curling (Table 5). Symptoms on inoculated *N. tabacum* cv. Samsun started as mottling followed by interveinal chlorosis which subsequently resulted into prominent green vein banding (Fig. 7c).

(c) Serology and Electron Microscopy

Antigens in the sap extracted from both infected field sample and indicator plant leaves gave reactions of PVY and TMV when tested against their corresponding antiserum by DAS-ELISA. There was no reaction with the antisera of CMV, AMV, TSWV and TRSV. Results from electron microscope indicated the presence of rigid-rod shaped particles with estimated lengths of 308-336nm and a fair amount of flexous rods with estimated length 728nm.

Table 5. Host range symptoms induced by samples from Lusaka Province.

Indicator plant species	Lukali Tobacco Farm		Bonanza Tobacco Farm		Kasondi Ranch	
	Symptoms		Symptoms		Symptoms	
	Local	Systemic	Local	Systemic	Local	Systemic
Capsicum annuum cv. California wonder	0	Ivc, M, Lc	0	M, Vb, Cb, Lc	0	M, Ivc, Lc
Capsicum annuum cv. Yoloy	0	Mo,Ivc, Lc	0	M, Vb, Cb,Lc	0	M
Capsicum frutescence cv. Tabasco	Nl	Mo,Lne, St	0	Ivc, Lc, Mo	0	Mo, Cb, Vb,Ln
Chenopodium amaranticolor	0	0	Cs	Mo	Cl	0
Chenopodium quinoa	Nl	Lne	Nl	Cb, Lc, Ly	Ns	0
Cucumis sativus cv. Pepino	0	Ns, M	Nl	M	Cl	Mo, Ivc, yM
Datura stramonium	0	Ivc,yMo, Ns	Nl	Ivc,Mo	Ns	Ivc, Mo, Cl
Glycine max cv. Kaleya	Nl	Cs, Mo	Nl	Cs, Mo	0	M
Gomphrena globosa	0	Ivc, Cb, M	Nl	Mo	Nl	Ivc, Lne
Lycopersicon esculentum cv. Heinz	0	Ivc, Mo, St	Nl	Ivc, Mo, Ly	0	Cb, M, Rs
Nicotiana clevelandii	Cl	Ivc, Mo, St	Cl	Mo	Nl	Vm,Ld
Nicotiana glutinosa	Nl	Ivc, Vy,Ly	Cl,Ns	Ivc, M,Vy		Mo, Cs, Vc
Nicotiana rustica	Cl, Nl	Mo, Cl,Nl,Lne,St	Vm,Lc	Vm,Lc, Mo, Ly,Cs	Vm	Vm
Nicotiana tabacum cv. Xanthi-nc	0	M, Vy	0	Mo	Ly	Ivc, Mo,Vb, Cb,St
cv. Samsun	Ns	M^s, Lne St	Ns	Mo, Ivc,Vb	M	Mo, M
cv. KM10	0	Mo	Cl	Mo	0	Ly, Mo
Pisum sativum cv. Green east	Nl	yMo, Ns, Lne	Nl	Cs, Ly	0	Ly, Crs
Phaseolus vulgaris cv. Top crop	Vne	Mo, Lp, Lne	0	Mo, Vb	Nl	vb, Ivc
Physalis floridana	Nl	Ns, Lc, Mo, St	Cs	Mo, Lc,St, Lne,Sne	Nl	Cs, Cl, Lne, Sne
Solanum melongena cv. Black beauty	Nl	Mo, Lc, St	Nl	Mo, Lc, Lne, St	Nl	Mo
Vigna radiata	0	Ivc, Mo	0	Ivc, Lc	Nl	Lp, Lc, M
Vigna unguiculata cv. Bubebe (IT-328-16)	0	Cs, Vm	0	Ivc, Cs, Mo	Nl	Cs,Mo

Key:-

0	No symptoms	Lne	Leaf necrosis	St	Stunting
Cb	Chlorotic blotch	Lp	Leaf puckering	Vb	Vein banding
Cl	Chlorotic lesion	Ly	Leaf yellowing	vM	Vein mottling
Crs	Chlorotic ringspots	M	Mosaic	Vne	Veinal necrosis
Cs	Chlorotic spots	Mo	Mottling	Vy	Vein yellowing
Ivc	Interveinal chlorosis	Nl	Necrotic lesions	yM	Yellow mosaic/ mottling
Lc	Leaf curling	Ns	Necrotic spots		
Ld	Leaf deformation	Rs	Ringspots		
Ln	Leaf narrowing	Sn	Stem necrosis		

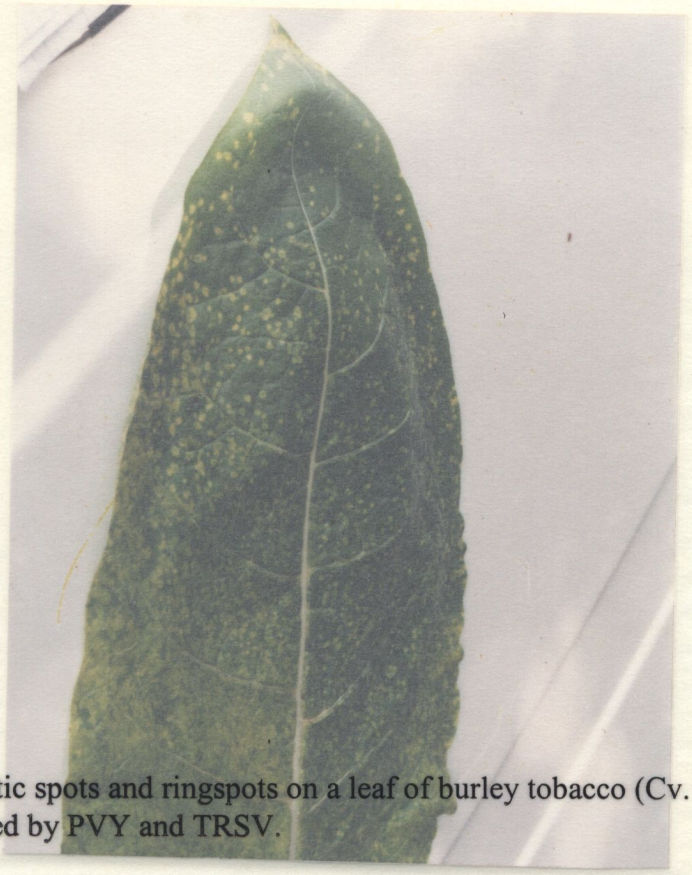

Fig. 5a. Necrotic spots and ringspots on a leaf of burley tobacco (Cv. BRK102) infected by PVY and TRSV.

Fig. 5b. Tobacco leaf (cv. BRK102) showing chloritic spots, vein banding and severe mosaic due to PVY infection.

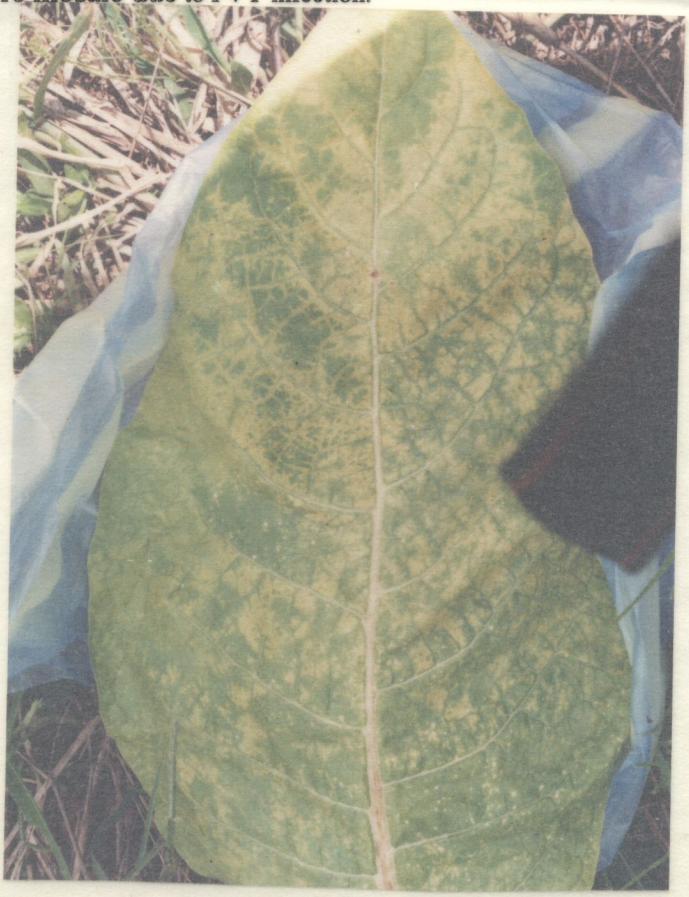

Fig. 5a

Fig. 5b

Fig. 5c

Fig. 5c. Uninoculated and inoculated *Physalis floridana* with PVY and TRSV. The inoculated plant developed leaf and stem necrosis followed by death of the entire plant.

Fig.5d. Vein banding and mild mosaic on *Nicotiana tabacum* cv. Xanthi-nc due to PVY

Fig. 5d

Fig. 6a

Fig. 6a. Vein banding, leaf deformation and reduced leaf size on top leaves of tobacco (RK3) caused by TMV and PVY.

Fig. 6b. Numerous small buff coloured necrotic lesion, vein necrosis, leaf curling and reduced leaf size on tobacco (c.v. RK3) due to mixed infection of TMV, PVY and AMV.

Fig. 6b

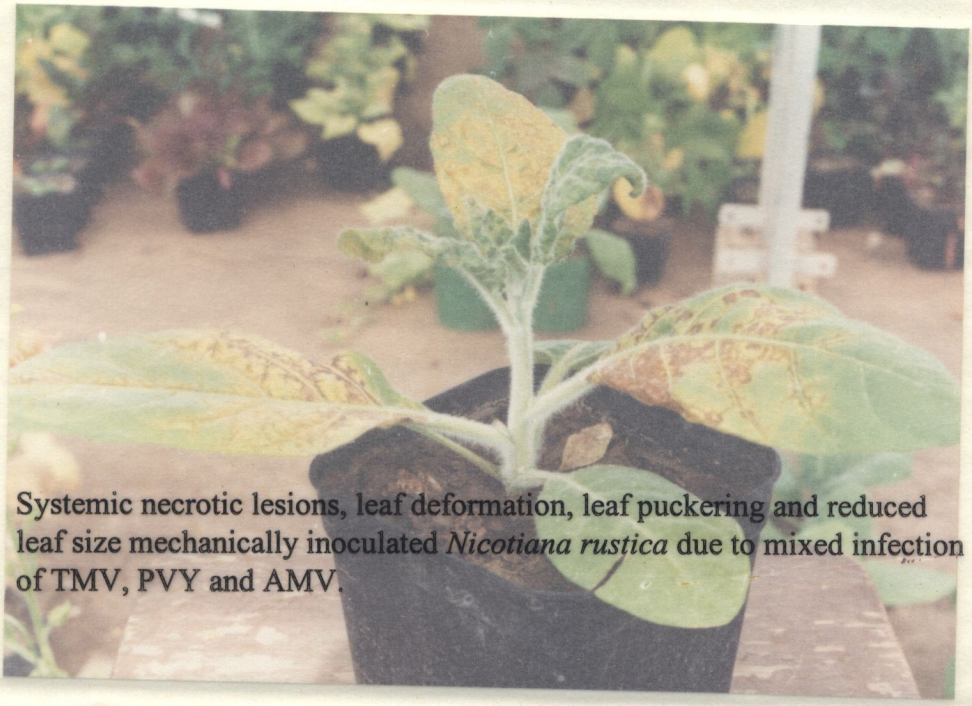

Fig. 6c. Systemic necrotic lesions, leaf deformation, leaf puckering and reduced leaf size mechanically inoculated *Nicotiana rustica* due to mixed infection of TMV, PVY and AMV.

Fig. 6c

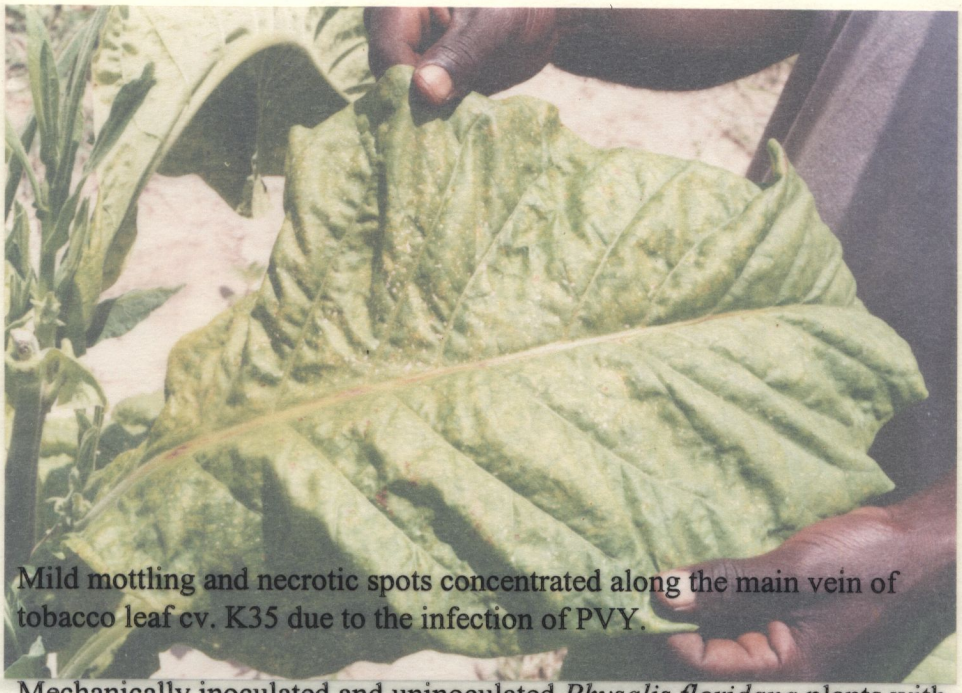

Fig. 7a

Fig. 7a. Mild mottling and necrotic spots concentrated along the main vein of tobacco leaf cv. K35 due to the infection of PVY.

Fig. 7b. Mechanically inoculated and uninoculated *Physalis floridana* plants with PVY and TMV.

Fig. 7b

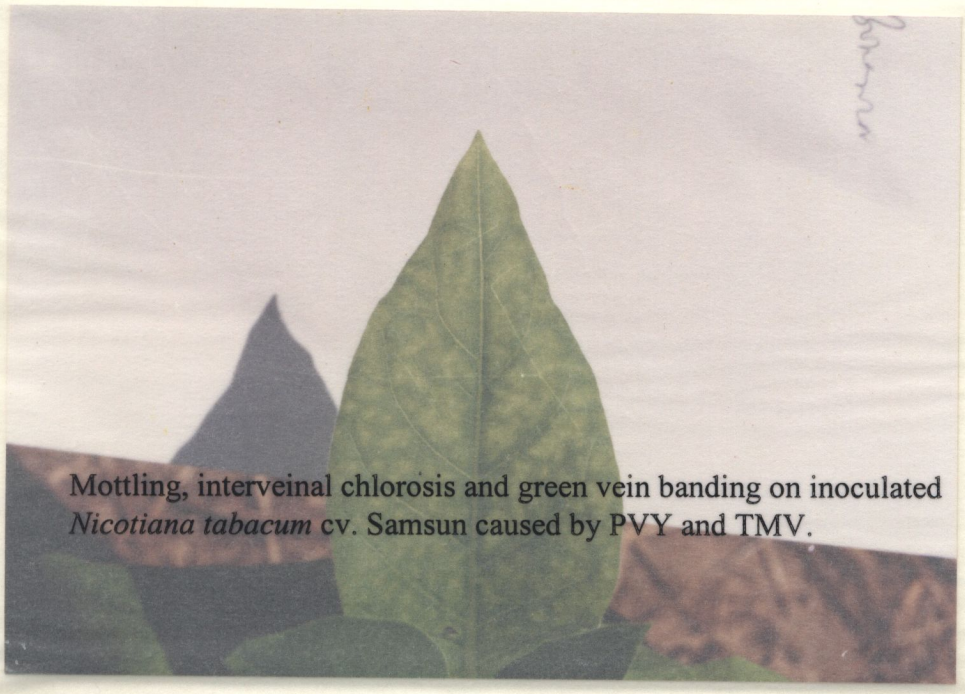

Fig. 7c. Mottling, interveinal chlorosis and green vein banding on inoculated *Nicotiana tabacum* cv. Samsun caused by PVY and TMV.

Fig. 7c

3.0. CENTRAL PROVINCE

Field survey for virus symptoms, sample collection and determination of disease incidence was conducted in mid February, 1997 in three tobacco fields of which one field was under large scale farming and the rest were under medium scale farming.

4.3.1. Mkushi Coffee Estate

(a) Field Symptoms

Mkushi coffee estate is a large scale farm located in Mkushi farming block, about 50 km east of Mkushi town. Fifty two (52) hectares were under flue-cured tobacco cv. NC 346. Tobacco fields (8 hactares) surveyed showed 31% incidence of disease infection but symptoms in infected plants were more severe. Symptoms observed in diseased plants included severe mosaic, inteveinal chlorosis, abnormal growth/stunted growth and leaf curling (Fig. 8a). Some plants showed loss of apical dominance, reduced leaf size and yellowing concentrated mainly along the margin from the leaf apex. Vein clearing and necrotic lesions, which turned into brown colour, were also seen in the foliar part of some tobacco plants.

(b) Host range reactions

When extracted sap was inoculated to test plants; *Chenopodium quinoa, C. amaranticolor, Glycine max* cv. Kaleya, *Gomphrena globosa, Nicotiana clevelandii* and *Pisum sativum* cv. Green east reacted by producing necrotic local lesions. Systemic chlorotic blotch were more pronounced in lower leaves of *Chenopodium quinoa*. The inoculum induced vein necrosis on inoculated leaves of *Phaseolus vulgaris* cv. Top crop and *Lycopersicon esculentum* cv. Heinz. *Nicotiana rustica* developed severe systemic necrotic infection (Fig. 8b). Systemic symptoms appeared in all indicator plant species inoculated. (Table 6).

(c) Serology and Electron microscopy

The antisera to AMV, TMV and TRSV reacted positively with the sap extracted from both field sample and symptomatic test plants. However, antisera to CMV, TSWV and PVY gave negative results in DAS-ELISA test. Negatively stained preparations of crude extract viewed in the electron microscope confirmed the presence of rigid rod-shaped particles with estimated lengths between 280-306nm. However neither spherical nor baciliform virus particles were observed.

4.3.2. Zanje Estate

(a) Field Symptoms

Field survey on a medium scale (8 hectares) burley tobacco cv. Banket A1 in Kasavasa area, South east of Kabwe town revealed the following symptoms on the affected plants; chlorotic spots, necrotic lesions, interveinal chlorosis, mosaic and stunted growth in some plants with severe symptoms (Fig. 9a). Leaf curling, puckering, rugosis and distortion with the upper leaves being narrow appeared on some tobacco plants.

The plants were also heavily infested with aphids which appeared to be concentrated on the lower sides of the leaves. Some weeds (*Nicandra spp*) in the field appeared to possess similar symptoms as the ones observed on tobacco plants. The disease infection was 25%.(Table 3)

(b) Host range reactions

Screenhouse inoculated tests demonstrated that leaves inoculated with sap from Zanje Estate sample developed necrotic lesions on *Chenopodiun amaranticolor, C. quinoa, Nicotiana clevelandii, N. glutinosa, N. tabacum* cv. Xanthi-nc, *Phaseolus vulgaris* cv. Top crop and

Vigna unguiculata cv. Bubebe (IT- 328-16). Systemic symptoms on *Capsicum frutescens* cv. Tabasco started as mild mottling followed by interveinal chlorosis, leaf curling and chlorotic spots which changed to necrotic spots. Systemic chlorotic spots preceded by indistinct mottling were recorded from *Datura stramomium* which later developed leaf necrosis. Other systemic symptoms recorded from host range species included necrotic ringspots, leaf yellowing, chlorotic blotch, leaf distortion, stunted growth, leaf narrowing and vein clearing. Interveinal chlorosis and mild mottling were recorded on *Vigna radiata*. However, *Solanum melongena* cv. Black beauty remained symptomless (Table 6).

(c) Serology and Electron microscopy

Crude sap extracted from the original field sample and indicator plants showing viral symptoms tested positive to TMV and TRSV antisera but negative to TSWV, AMV, PVY and CMV antisera. Electron microscopic examination revealed rod-shaped particles typical of tobamoviruses in the crude sap of infected plants. The estimated length of virus particles was 306nm.

4.3.3. Green Leaf Farm

(a) Field Symptoms

Field symptoms of viral infection and sample collections were also done at Green leaf farm situated in Chibwe farming block in Kapiri Mposhi district. An area of 16 hectares was under flue-cured tobacco production where only cultivar K35 was grown. Half of the field was investigated for the presence of virus disease. Many plants showed severe leaf deformity with mosaic, chlorotic mottle symptoms (Fig. 10a). Few plants displayed dark green blistering, leaf blotch on lower sides of the leaves, leaf puckering, distortion, chlorotic and necrotic lesions

However in this area the crop was severely destroyed by hail stones. The disease incidence was 17% (Table 3).

(b) Host range reactions

Results of host range study are shown in Table 6. All cultivars of *Capsicum annuum* when inoculated with virus inoculum from Green leaf farm produced systemic interveinal chlorosis followed by either leaf curl and chlorotic blotch or mosaic and stunting at 7-20 days postinoculation. However systemic mottling or mosaic appeared in all indicator plants species. The inoculum incited vein yellowing followed by mottling on *Nicotiana glutinosa* (Fig. 10b). Both *Chenopodium amaranticolor* and *C. quinoa* developed local necrotic lesions, systemic interveinal chlorosis and local chlorotic lesions followed by mottling and leaf yellowing respectively. In *Pisum sativum* cv. Green east, the symptoms started as necrotic lesions and inoculated leaves were followed by leaf curl, marginal leaf chlorosis and indistinct mottling.

(c) Serology and Electron microscopy

DAS-ELISA test confirmed the presence of PVY and TMV in the sample while the sap showed negative reaction to antisera to TSWV, AMV, CMV and TRSV. Negatively stained virus particles obtained from infected tobacco leaves contained flexous and rigid rods approximately 728nm and 308nm long respectively.

Table 6. Host range symptoms induced by samples from Central Province.

Indicator plant species	Mkushi Coffee Estate		Zanje Estate		Green Leaf Farm	
	Symptoms		Symptoms		Symptoms	
	Local	Systemic	Local	Systemic	Local	Systemic
Capsicum annuum cv. California wonder	Cl	Ivc, Mo, Lc	0	Ivc, Mo, Lc	0	Ivc, Lc, Cb
Capsicum annuum cv. Yoloy	0	Ivc, Cs	0	Cs, Nrs	0	Mo, Ivc, Lc,Cb
Capsicum frutescens cv. Tabasco	Cs	Mo, Vc	0	Mo, Ivc, Lc, Cs, Ns	Nl	Ivc, Mo, St
Chenopodium amaranticolor	Nl	M	NL	0	Cl	Ivc
Chenopodium quinoa	Nl	Cs, Cb, Ly	Nl	Crs, Ly	Nl	Mo, Ly
Cucumis sativus cv. Pepino	0	M, Ns, Ly	Cl	M, Crs	Nl	Mo, M
Datura stramonium	Rs	Rs, Ivc, yMo, Cb	Ln	M, Cs, Lne	0	Mo
Glycine max cv. Kaleya	Nl, Vne	Ivc,Cb, Vb, M, Vy	Cl	Cs, Cb	Nl, Lc	Mo
Gomphrena globosa	Nl, Cb	Mo	Nl	Mo	Nl	Mo
Lycopersicon esculentum cv.Heinz	Vne	M, Lne	Ns	Mo	Ns	Ivc, Lc, Mo
Nicotiana clevelandii	Nl	Mo	Nl	M, Ld	Nl	Cl, Mo
Nicotiana glutinosa	0	Mo	Nl	0	0	Vy, Mo
Nicotiana rustica	Nl	M, Nl	0	M, Ln, St	Cl	Ivc, Mo
Nicotiana tabacum cv. Xanthi-nc	0	Mo	Nl	Vc, M	0	Mo, M
cv. samsun	Cs	Mo, M	Ns	Mo	Ns	Mo,Ivc
cv. KM10	0	Mo	0	Mo	0	M, Ly
Pisum sativum cv. Green east	Nl	yMo, Ns, Lne	0	Cs	Nl	Cs, mC,Mo
Phaseolus vulgaris cv. Top crop	Vne	Mo, Lp, Vb, Lne	Nl	Cs, Mo	0	Ivc, Mo
Physalis floridana	Ns	M, Ly	Cl	Ns, Ly, Lne	0	Ns, Mo, Ly, Vm, St
Solanum melongena cv.blackbeauty	0	M, St	0	0	Nl	Mo, St
Vigna radiata	0	Ivc, M, Lc	0	Ivc, Mo, Lc, Ns	0	Mo, M, Ns
Vigna unguiculata cv. Bubebe (IT-88-16)	0	Mo	Nl	Ivc	0	Ivc, Mo, Ly

Key:-

0	No symptoms	Lne	Leaf necrosis	Rs	Ringspots
Cb	Chlorotic blotch	Lp	Leaf puckering	Sn	Stem necrosis
Cl	Chlorotic lesion	Ly	Leaf yellowing	St	Stunting
Crs	Chlorotic ringspot	M	Mosaic	Vb	Vein banding
Cs	Chlorotic spots	Mo	Mottling	Vc	Vein clearing
Ivc	Interveinal chlorosis	mC	Marginal chlorosis	Vne	Veinal necrosis
Lc	Leaf curling	Nl	Necrotic lesions	Vy	Vein yellowing
Ld	Leaf deformation	Ns	Necrotic spots	yMo	yellow mottling
Ln	Leaf narrowing	Nrs	Necrotic ringspots		

Fig. 8a

Fig. 8a. Abnormal growth or stunted growth and leaf culing on tobacco cv. NC346 caused by mixed infection of AMV, TMV and TRSV.

Fig. 8b. Severe systemic necrotic infection due to TRSV on *Nicotiana rustica*.

Fig. 8b

Fig. 9a

Fig. 9a. Leaf curling, puckering and distortion on burley tobacco cv. Banket due to TMV infection.

Fig.9b. Burley tobacco infection by TRSV.

Fig. 9b

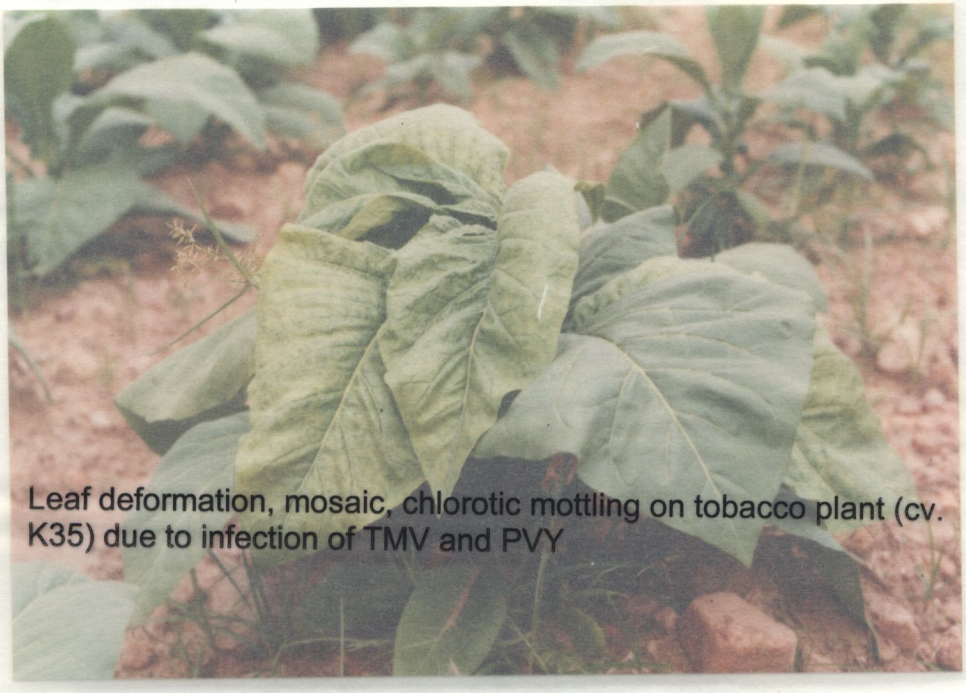

Fig. 10a
Fig. 10a
Leaf deformation, mosaic, chlorotic mottling on tobacco plant (cv. K35) due to infection of TMV and PVY

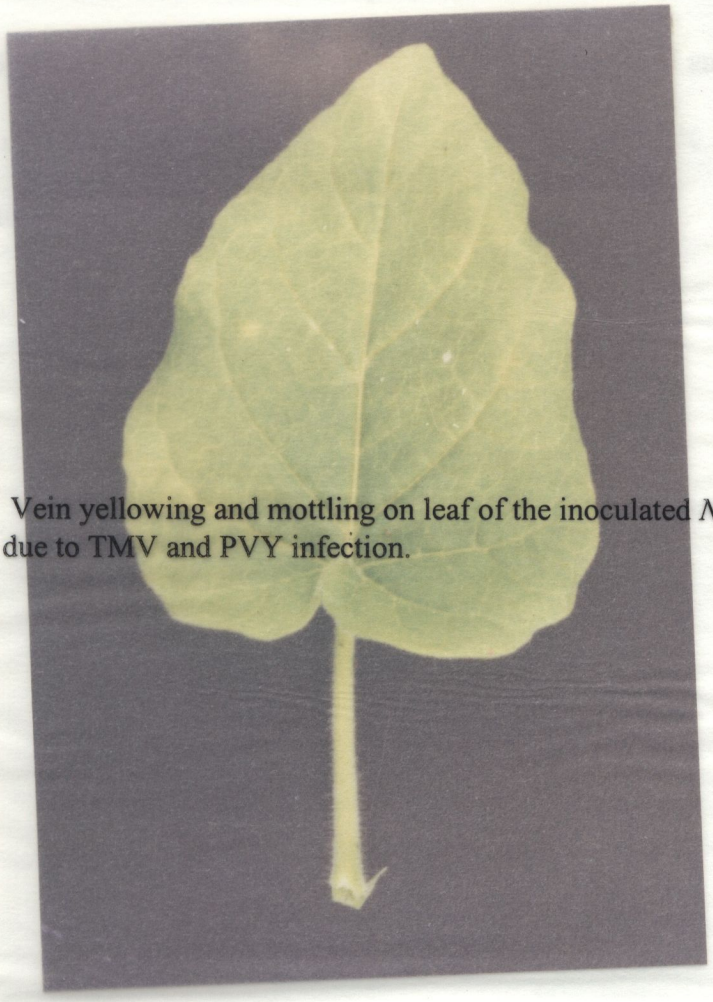

Fig. 10c Vein yellowing and mottling on leaf of the inoculated *Nicotiana glutinosa* due to TMV and PVY infection.

Fig. 10b

4.4. Prevalence

The results of the present study on Field symptoms, host range, serology and electron microscopy show that TMV was more prevalent (77.7%) followed by PVY which was recovered in 66.6% of the tobacco fields surveyed. However, AMV and TRSV were relatively less predominant and were recovered only in 33.3% and 22.2% of the surveyed fields respectively (Fig. 11).

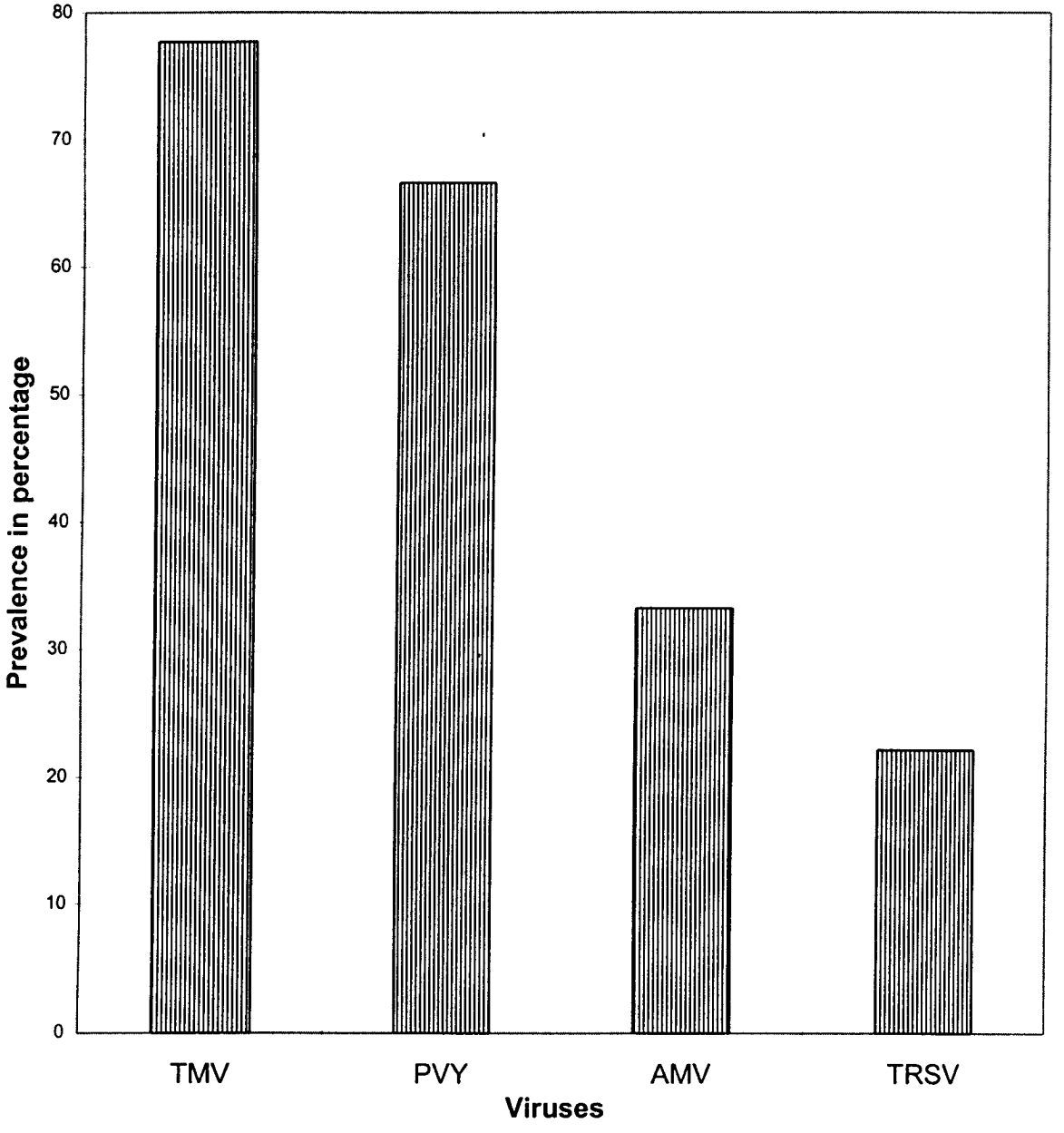

Fig 11. Prevalence of viruses in tobacco fields in Central, Southern and Lusaka of Zambia during 1997

5.0. DISCUSSION

The present investigation focused on the identification of viruses on the basis of field symptoms, reactions of indicator host species, serological tests (DAS-ELISA) and electron microscopy. The importance of these techniques in the identification of tobacco viruses has been proved by Ford and Tolin (1983); Tedford and Nielsen (1990) and Swanepoel and Nel (1995). In the present study TMV, PVY, AMV and TRSV have been identified.

Characteristic symptoms of PVY in tobacco have been described as mild mottling, vein clearing, necrotic lesions on veins, midribs or stems; leaf distortion and stunting (Lockhart and Fischer, 1976; Latorre, 1983). Similar symptoms were noticed in tobacco plants at Petergreen PRC tobacco farm and the infective agent appears to be PVY.

Potato virus Y was also recovered in samples from Bonanza, Green Leaf and Charles Itamoya tobacco fields in combination with TMV. Combined effects of PVY and TMV have been previously demonstrated by Sievert (1973) in tobacco and Sherwood *et al.* (1988) in pepper. The observed symptoms at Bonanza and Charles Itamoya tobacco fields were similar to those observed by Sievert (1973) and Sherwood *et al.* (1988). The simultaneous presence of PVY and TMV in tobacco plants caused severe stunting, chlorosis and necrosis and could be responsible for reduced tobacco yield. Results indicate that symptoms were more pronounced at the periphery of the fields, adjacent to focus line of weeds. This demonstrated that weeds play an important role in the epidemiology of virus diseases. Weeds may act as virus reservoirs and / or overwintering sites for aphid vectors. Similar results have been reported for PVY in tobacco (Latorre, 1983).

DAS-ELISA technique confirmed the presence of single infection at Petergreen PRC tobacco farm, since it reacted positive to the antiserum of PVY but did not produce any reaction to AMV, TMV, TSWV, TRSV and CMV. DAS-ELISA also confirmed the mixed infection of TMV and PVY in Bonanza, Green Leaf and Charles Itamoya tobacco samples.

The flexous rod-shaped particles with lengths indicative of the presence of PVY were observed in samples from Green Leaf farm (728nm), Kasondi ranch (560-980nm), Bonanza (728nm) and Petergreen farm (728-784nm). Particle shape and length of potyvirus PVY have been reported as flexous rods with estimated length between 680-900nm (Hollings and Brunt, 1981). Turpen (1986) assessed the purity of PVY by electron microscopy and particles were determined to be 734 ± 4 nm in length. However, Dossar and Mungur (1982) revealed the existence of elongated virus particles estimated to be 750nm viewed in electron microscopy which were subsequently confirmed to be PVY. Agranovsky (1993) reported filamentous particles 700-750nm in length present in the sap extract from pepper plants which were confirmed to be PVY. Therefore the findings of the present study for the particle lengths of PVY are within the ranges reported in the previous studies. However, the present study does not preclude the presence of other virus particles with similar shape and particle length.

The symptoms observed in tobacco fields infected by TMV alone (Popota College Farm) as dark green vein banding, mottling, blotching, mosaic and stunted growth were likewise generally characteristic of previously described symptoms for the virus disease on tobacco (Lucas, 1975; Diallo and Mulchi, 1981; Diallo *et al.*, 1981; Reilly 1983). Since TMV is transmitted mechanically it eventually comes in contact with the injured cells of a host plant. The uniformity of TMV incidence (25%) in the field presumably originated from

contaminated workers' hands or equipment that came in contact with health plants (Johnson *et al.*, 1983).

The sample from Popota tobacco college farm in which only TMV was detected induced similar symptoms on *Capsicum annuum* cv. California wonder; *Gomphrena globosa* and *Nicotiana glutinosa* to those of TMV reported by Ford and Tolin (1983) (Table 4). However, Ford and Tolin (1983) observed no symptoms on *Phaseolus vulgaris* cv. Bountiful, which is contrary to the present findings in which *P. Vulgaris* cv. Top crop developed systemic interveinal chlorosis followed by vein banding. Probably this variation could be explained on the basis of cultivar difference. Since TMV occurred in combination with AMV and PVY, the symptoms induced on *Datura stramonium* possibly were due to the presence of TMV. *Datura stramonium* has been reported to be insusceptible to both PVY and AMV (Brunt *et al*.,1990)

DAS-ELISA test revealed the single infection of TMV at Popota tobacco college Farm. Double infections of TMV-PVY and TMV-TRSV were indexed from Charles Itamoya and Green Leaf samples respectively. However, Mkushi Coffee Estate and Lukali tobacco samples tested positive to TMV, AMV, TRSV and TMV, PVY, AMV respectively. The presence of TMV in samples was indicated by the presence of rigid rod shaped virus particles with lengths of 308-336nm (Bonanza sample), 306-364nm (Lukali sample), 280-306nm (Mkushi sample), 156-308nm (Popota sample), 306nm (Zanje sample) and 308nm (Green leaf sample). The particle lengths obtained in this study are in agreement with the reports of Noordam (1973) and Van Regenmortel (1981). However, the particle length observed in Popota sample was much shorter because some of the particles appeared broken. This could be due to sample preparations for electron microscopic examination.

The present results indicate that TMV is widespread in all the three surveyed provinces. Because TMV is readily transmitted (Zaitlin, 1975; Fulton and Scott, 1977; Mercer *et al.*, 1982), the use of TMV-resistant cultivars might be a practical and inexpensive method of controlling tobacco diseases caused by TMV. Tobacco resistant cultivars to TMV strains have been identified by Johnson and Main (1983). Genetic engineering technique which involves cell manipulation that include somatic hybriditization through protoplast fusion and transformation by insertion of foreign DNA into the cell genome have been used as a control measure TMV on tobacco (Register *et al.*, 1989).

Primary symptoms of alfalfa mosaic virus in tobacco usually occur in the form of chlorotic spots or blotches in the leaves. New systemically infected leaves exhibit vein clearing and then white rings, arcs and coalescing line patterns of necrotic tissue. Bud leaves may be distorted and sometimes there is a bright mosaic pattern (Akehurst, 1981). The results of the present study show that AMV occurred in combination with other viruses. It was detected at Lukali farm in combination with TMV and PVY, while at Mkushi coffee Estates, AMV was identified together with TRSV and TMV. These mixed infections have been reported to cause wide variation in symptoms or increased severity in tobacco (Lucas, 1975). Similar findings were observed in the current study.

The incidence of the mixed infection of TMV, PVY and AMV under field condition at Lukali (40%) was significantly higher than either combination detected (Table 3). The presence of weeds and aphids in and around Lukali tobacco field could be associated with the high

incidence of the disease. Aphids have been reported to play an important role in the transmission of AMV from one host to another, given the extent of infestation in tobacco (Froshier, 1974; Kaiser and Hannan, 1983). Susceptible weed species represent significant source of virus inoculum. In addition, the growing of susceptible cultivar (RK3) to TMV may be responsible for the highest virus incidence at Lukali farm. The lowest virus disease incidence at Petergreen PRC farm (7%) could be due to good disease management practices which were observed such as control of weed and pest by use of chemicals.

Reactions of test plants to AMV infection showed that there was some variation in symptoms as observed by Brunt *et al.*(1990). Alfalfa mosaic virus (AMV) is known to induce mild yellow mottle, chlorotic rings or spots and occasional elongated reddish lesions (on veins and veinlets) on *Phaseolus vulgaris* cv. Top crop (Silber and Heggestad, 1965). In the present experiment *Phaseolus vulgaris* cv. Top crop developed local vein necrosis followed by systemic mottling, leaf puckering and subsequent leaf necrosis. Yellow mottling was on *Datura stramonium* and *Pisum sativum* cv. Green east. Similar symptoms have been recorded on the same host range species in the previous studies (Zaumeyer and Patino, 1960; Silber and Heggestad, 1965; Brunt *et al.*, 1990). However, many symptoms seen under field conditions were also reproduced in the screenhouse experiment (Tables 5, 6). The yellow mottling observed in this study was mixed with other symptoms which were not previously reported. This suggests the possibility of other viruses being present which were subsequently detected by DAS-ELISA. Identification of AMV by electron microscopy has been found to be difficult since the AMV particles degrade easily in the electron microscope preparations (Zaitlin, M. - Personal communication). This could be the reason for the failure of the electron microscopy to resolve the particle morphology of AMV in the present study.

Although AMV has not been recognized previously as a pathogen of field tobacco in Zambia, the virus has the potential of becoming quite destructive. Ndunguru and Kapooria (1997) have reported the presence of AMV in field grown pepper and also in other plant species in Lusaka province. The effects of various strains of AMV in different tobacco cultivars have been demonstrated by Tedford and Nielsen (1990).

Tobacco ringspot virus was recovered at Mkushi Coffee Estate, Zanje Estate and Kasondi Ranch tobacco fields in combination with TMV, AMV and PVY. Typical field symptoms such as severe mosaic, interveinal, chlorosis, veinal necrosis, yellowing, stunted growth and reduced leaf size were noticed in all three tobacco fields. Reilly (1983) demonstrated double infection of TRSV and TMV under field conditions. However, field symptoms observed at Zanje Estate tobacco due to TRSV and TMV were different from those reported previously in flue-cured tobacco by Reilly (1983). This variation could be attributed to the difference in tobacco varieties grown. The combined infection of TRSV and TMV was detected in burley (air-cured) tobacco. The mixed infections observed at Mkushi Coffee Estate and Kasondi Ranch tobacco fields were either TRSV, AMV and TMV or TRSV, PVY and TMV respectively.

Correct virus diagnosis by symptom expression is usually straight forward in singly virus-infected plants. Occasionally, double infected plants can be diagnosed by symptoms alone. However, complex symptoms result in doubly and triply infected plants. Thus, laboratory techniques must be used accurately to identify viruses in mixed infections (Reilly, 1983). The

presence of weeds in and near tobacco fields contribute to the knowledge of epidemiology of the ringspot disease of tobacco. Pigweed (*Amaranthus spp.*) has been reported to be susceptible to TRSV (Brunt *et al.*, 1990). As tobacco is seldom grown in succeeding years on the same land, it would appear that these virus infected weeds serve as a continuing source of the virus inoculum. The transmission of the virus by nematodes from weeds has been reported by Akehurst (1981) and from this it can be concluded that weed epidemiology of ringspot virus of tobacco (TRSV) as well as other crop plants is very important in disease outbreaks.

Sap extract from Zanje Estate tobacco incited chlorotic spots or ringspots on *Chenopodium quinoa, Datura stramonium, Glycine max* cv. Kaleya, *Cucumis sativus* cv. Pepino, *Pisum sativum* cv. Green east and necrotic ringspots on *Capsicum annuum* cv. Yoloy, and *C. frutescens* cv. Tabasco. These symptoms were in line with those reported by Ramsdell (1978) and Brunt *et al.* (1990). However, Reilly (1983) cited that TRSV reduced the mottling effect of TMV in simultaneously inoculated plant. The virus suspension from Mkushi Coffee Estate developed chlorotic ringspots on *Datura stramonium* and chlorotic / necrotic spots on *Capsicum annuum* cv. Yoloy, *C. frutescens* cv. Tabasco, *Cucumis sativus* cv. Pepino, *Nicotiana tabacum* cv. Samsun, *Pisum sativum* cv. Green east and *Physalis floridana.* However, these symptoms were not satisfactory indicators for the diagnosis of TRSV. Presence of TRSV in Zanje Estate, Mkushi Coffee Estate and Kasondi Ranch tobacco was confirmed by DAS-ELISA as demonstrated by Shiel and Castello (1985).

6.0 CONCLUSION.

The prevalence of TMV (77.7%) and PVY (66.6%) in all the three provinces indicate that these virus diseases are potentially a limiting factor in tobacco production in Zambia. Therefore for farmers to produce high yield and high quality tobacco there is a need to understand virus interactions, virus reservoirs and efficient vector transmission under tropical conditions. When compared to TMV and PVY, AMV occurred at lower frequencies (33.3%) followed by TRSV (22.2%). However, this does not mean that these viruses would not become serious in the foreseeable future.

Increase in the incidence of TMV, PVY, AMV and TRSV in tobacco in the future years will represent potentially new and serious problems in tobacco production in Zambia. The extent to which these viruses will eventually occur in other tobacco growing areas remains unknown. The combination of the wide host range and the severe effects of these viruses on many economically important crop plants indicate that TMV, PVY, AMV and TRSV have the potential to become most important and destructive pathogens in Zambia, if no efforts are made to check them. It is therefore imperative that all available virus disease management practices such as avoidance and elimination of virus sources, control of vectors and use of resistant cultivars should be deployed to minimize losses occurring from virus disease in Zambia.

Visual observations on field symptoms in the present study were made in an effort to develop descriptions of viruses that could be useful to farmers and extension personnel in recognizing tobacco with multiple or single virus infection under Zambian field conditions. However, the

diversity of symptoms displayed by doubly and triply infected plants was so great that positive diagnosis of each virus was not easy. Thus, serological assay, electron microscopy, molecular hybridization and Polymerase chain reaction (PCR) should be considered as reliable and effective proof in virus diagnosis.

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Appendix (i). Tobacco Production by type, Dry weight (tonnes) in Zambia.

YEAR	FLUE-CURED (VIRGINIA)	AIR-CURED (BURLEY)	TOTAL
1985	2196	566	2762
1986	3057	619	3676
1987	2956	651	3607
1988	3738	610	4348
1989	3249	1016	4265
1990	2831	1534	4365
1991	4077	1979	6056
1992	3662	2312	5974
1993	3516	3224	6740
1994	1723	1083	2806
1995*	2240	1560	3800
1996*	1949.7	1892	3841

Source: Quarterly Digest of Statistics. Third and fourth Quarters, 1996. Central Statistical Zambia.

- Agricultural statistics bulletin 1995/96, ministry of agriculture, food and Fisheries; Republic of Zambia.

Appendix ii. Analysis of variance for virus disease incidence in three provinces surveyed

Source of variation	Degree of freedom	Sum of squares	Mean sum of squares	F-value
Farms	8	5792.00	724.00	43.606*
Error	63	1046.00	16.603	
Total	6838.00			
CV	16.30%			

*Significantly different at 0.01 probability level.