MALAKISI TOBACCO VEINAL NECROSIS, A STRAIN OF POTATO VIRUS Y ISOLATED FROM *NICOTIANA TABACUM* L CULTIVAR 'SPEIGHT G.28' IN WESTERN KENYA.

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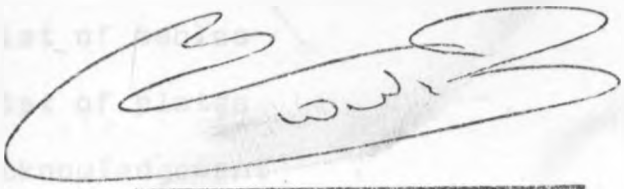
A THESIS SUBMITTED IN PARTIAL FULFILMENT OF REQUIREMENT FOR THE DEGREE OF MASTER OF SCIENCE IN PLANT PATHOLOGY OF THE UNIVERSITY OF NAIROBI.

APRIL, 1985

DECLARATION

I hereby declare that this thesis is my original work and has not been presented for degree in any other University.



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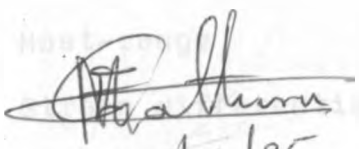

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LIST OF CONTENTS

	Page
- Declaration	ii
- List of tables	vi
- List of plates	viii
- Acknowledgement	xii
- ABSTRACT	xiv
- Chapter I : INTRODUCTION	1
- Chapter II : LITERATURE REVIEW	4
- Chapter III : MATERIALS AND METHODS	18
3:1 : Source of inoculum	18
3:2 : Inoculation procedure	18
3:3 : Source of plants	19
3:4 : Host-range	20
3:5 : Strain differentiation	23
3:6 : Transmission through seed of tobacco	24
3:7 : Transmission through insects	24
3:8 : Physical properties	26
- Dilution end point	26
- Thermal inactivation point	26
- Aging *in vitro*	27
3:9 : Preliminary clarification	28
3:10 : Purification procedure	30

3:11 : Electron microscopy 33

- Leaf dip preparation 33

- Crude sap preparation 33

- Partial purified preparation 34

3:12 : Estimation of the normal length 34

3:13 : Ultraviolet Absorption Spectrophotometry 34

3:14 : Source of antisera 36

3:15 : Serology 37

- Microprecipitin tests 37

- Chapter IV : RESULTS 38

4:1 : Field symptoms 38

4:2 : Host-range 38

4:3 : Strain differentiation 46

4:4 : Physical properties 49

4:5 : Transmission 50

- Transmission through seed of tobacco 50

- Transmission through insects 50

4:6 : Purification 51

4:7 : Electron microscopy 54

4:8 : Ultraviolet absorption Spectrophotometry 57

4:9 : Serological test 59

- Chapter V : DISCUSSION AND CONCLUSION 60
DISCUSSION 60
CONCLUSION 79
- Plate on host-range and Electron microscopy 82
- Chapter VI : LITERATURE CITED 96

LIST OF TABLES

NO.	TABLES	PAGES
1.	Flow chart of partial purification procedure of Malakisi Tobacco Vein Necrosis, Strain of PVY.	32
2.	Reactions of Nicotiana species and tobacco cultivars to inoculation with Malakisi Tobacco Vein Necrosis, Strain of PVY.	43
3.	Reactions of plant species and cultivars to inoculation with Malakisi Tobacco Vein Necrosis, Strain of PVY.	44
4.	Reactions of differential hosts to inoculation with Malakisi Tobacco Vein Necrosis, Strain of PVY.	45
5.	Analysis of variance on the effect of various clarifying agents on the retention of infectivity of Malakisi Tobacco Vein Necrosis Strain in clarified crude sap of *Nicotiana tabacum* cv 'White Burley' at 6,000 rpm and 15 minutes low speed centrifugation.	52

6. Separation of means (in Table 6) by the use of Duncan's new multiple range test. 52

7. Comparison of Malakisi Tobacco Vein Necrosis Strain with other reported filamentous viruses affecting tobacco in Literature. 67

8. Comparison of Malakisi Tobacco Vein Necrosis Strain physical properties with related potyviruses reported in literature. 70

9. Comparison of Malakisi Tobacco Vein Necrosis Strain particle length with literature data of other Potyviruses. 71

10. Comparison of responses of differential hosts to Malakisi Tobacco Necrosis Strain with literature data on other strains of PVY. 76

LIST OF PLATES

PLATE NUMBER	TITLE	PAGE
1.	Particle length distribution in a partially purified preparation of M-TVNS.	55
2.	Particle length distribution in leaf dip preparation of M-TVNS.	56
3.	Ultra-violet light absorption of a partially purified preparation of M-TVNS after two cycles of differential centrifugation.	58
4.	Non-inoculated leaves of *Nicotiana tabacum* cv 'Speight G.28' exhibiting veinal necrosis 7 days after inoculation.	81
5.	Non-inoculation leaves of *Nicotiana tabacum* cv 'White Burley' showing vein-clearing 14 days after inoculation.	82
6.	Non-inoculated upper leaves of *Nicotiana tabacum* cv 'Speight G.28' exhibiting vein-clearing 24 days after inoculation of lower leaves.	83
7.	Inoculated leaflet of Hybrid *Solanum tuberosum*. x. *S.demissium* 'Aquila.6' showing tiny necrotic local lessions 6 days after inoculation.	84

8. A photograph of Hybrid Solanum tuberosum. x. S.demissium, 'Aquila.6' exhibiting necrotic lesions on non-inoculated upper leaves 15 days after inoculation of lower leaves. 85

9. Chlorotic local lesions on inoculated leaf of Chenopodium amaranticolor Coste & Reyn 14 days after inoculation. 86

10. Inoculated leaf of C.quinoa Willd showing chlorotic local lesions 12 days after inoculation. 87

11. Non-inoculated upper leaf of Nicandra physaloides L showing vein-clearing and tiny necrotic lesions 20 days after inoculation of lower leaves. 88

12. Healthy (right) and inoculated (left) plants of Datura metel L showing mottling, leaf distortion and stunting 16 days after inoculation. 89

13. Electron-micrograph of a leaf dip preparation from Nicotiana tabacum L cv 'White Burley' showing the presence of flexous rod virus particles stained with 2% potassium phosphotungstate 90

14. Electron-micrograph of a crude sap preparation from Nicotiana tabacum L cv 'White Burley' showing the presence of aggregated flexous rod virus particles stained with 2% potassium phosphotungstate. 92

15. Electron-micrograph of a partially purified preparation from Nicotiana tabacum L cv 'White Burley' sap treated with ethyl ether as clarifying agent showing relatively less breakage of virus particles. 92

16. Electron-micrograph of a partially purified preparation from Nicotiana tabacum L cv 'White Burley' showing particles aggregation. 93

17. Electron-micrograph of a partially purified preparation from Nicotiana tabacum L cv 'White Burley' showing no particles aggregation. 94

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ABSTRACT

A tobacco virus disease, causing mainly mottling, vein-clearing and necrosis on leaves and stem was recently noticed throughout the tobacco growing areas in Western Kenya and seemed to have a serious effect on leaf losses, mainly on cultivar 'Speight G.28', which is the main cultivar grown in Western Kenya.

Field symptoms consist of green vein-banding, vein-clearing and mottling with pale yellow spots on a green background which in advanced stage changed into extreme white mottling. On 'Speight G.28', severe veinal and stem necrosis occured.

In this work, the new virus strain on tobacco has been isolated. characterized and identified. It has been reffered to as the Malakisi Tobacco veinal necrosis strain (M-TVNS).

Electron microscope examination showed that M-TVNS is a filamentous particles about 742 nm long. The results on symptomatology and host-range through mechanical inoculation, transmission through seed of Nicotiana tabacum cultivars and the aphid 'Myzus persicae' Sulz', ultra-violet absorption, and serological reactions suggest that the M-TVNS is strain of PVY.

Reactions on differential hosts used by de Bokx (1981) and Kahn and Monroe (1963) showed that M-TVNS is different from all the three groups of PVY strains as reported by de Bokx (1981). Its ability to induce systemic necrosis on leaves, veins and stem of root-knot resistant cultivar 'Speight G.28' identify it as a $M^{S}N^{R}$ strain described by Gooding and Tolin (1973).

CHAPTER I : INTRODUCTION.

INTRODUCTION

Tobacco, (*Nicotiana tabacum* L) is among the valued non-food crops. According to the U.S. Department of Agriculture report (1983), U.S. farmers gain more than one billion dollars on tobacco and the Government earns annually more than five and half billion dollars from tobacco taxes. The same report estimated the world production at 5.7 billion kg in 1973 from at least 87 countries in all continents (Lucas, 1975).

In Kenya, it was estimated that an area of three thousand ha was under tobacco in 1981 and a total yield of 3.7 million kg was obtained. This earned Kenya farmers an income of KShs.40 million and an amount of KShs.70 million went to the Government in taxes (B.A.T., 1981). As tobacco industry expands, the appearenace of new diseases or the spread of existing ones must be expected. In Kenya, the main diseases affecting tobacco are damping off, frog eye spot, brown leaf spot, powdery mildew and bacterial leaf spot caused by *Pythium debaryanum* Hesse *Cercospora nicotiana*, Ell & Eve, *Alternaria solani* (Ell & Eve) Masson, *Erysiphe cichoracearum* f. *nicotiana* D.C. and *Pseudomonas tabaci* (Wolf & Foster) respectively (Singh, 1975).

Tobacco is also affected by some viral diseases but few have been reported in Kenya. During a field survey conducted around Malakisi Leaf Center in June, 1983, most of the tobacco field had plants infected with tobacco mosaic virus (TMV), tobacco leaf curl virus (TLCV), tobacco ringspot virus (TRSV), rosette and a virus like disease causing mainly vein-clearing and necrosis on leaves. The last disease was recently noticed throughout the tobacco growing area in Western Kenya and seems to have a serious effect on leaf losses, mainly on 'Speight G.28', which is the main cultivar grown by Western Kenya farmers. The disease is characterized by vein-banding, vein-clearing and mottling of pale yellow spots on a green background and which in advance stage change into an extreme white mottling. On early infected plants of 'Speight G.28', severe veinal and stem necrosis occur, infected leaves collapsing on the stem followed by stem necrosis and plant death.

Other viruses have been reported either in the field or under experimental conditions as causing similar symptoms on tobacco. Among those are

potato virus Y (PVY), potato aucuba mosaic virus (PAMV), potato virus X (PVX), potato virus S (PVS), tobacco etch virus (TEV), and henbane mosaic (HMV) (Delgado-Sanchez, 1970; Berks, 1970; Welter, 1975; Shepherd, 1974; Govier, 1972).

In this work, the new virus strain on tobacco has been isolated, characterized and identified. It has been reffered to as the Malakisi Tobacco veinal necrosis strain (M-TVNS). The results on symptomatology and host-range through mechanical inoculation, transmission through seed of Nicotiana tabacum cultivars and the aphid 'Myzus persicae' Sulz, ultraviolet absorption, electron microscopy and serological reactions suggest that the M-TVNS is a strain of PVY. Reactions on differential hosts used by de Bokx (1981) and Kahn and Monroe (1963) showed that M-TVNS is different from all the three groups of PVY strains. Its ability to induce systemic necrosis on leaves, veins and stem of rootknot resistant cultivar 'Speight G.28' identify it as a $M^{S}N^{R}$ strain described by Gooding and Tolins (1973).

CHAPTER II : LITERATURE REVIEW.

LITERATURE REVIEW

The best known and most widely spread tobacco viruses throughout Africa are tobacco mosaic virus (TMV), tobacco ringspot virus (TRSV), tobacco leaf curl virus (TLCV), tobacco necrosis virus (TNV) and potato virus Y. Most of them are represented by different strains. Other worldwide viruses affecting tobacco are tobacco etch virus (TEV) and potato virus X (PVX). Potato virus S (PVS), potato virus A (PVA) and Henbane mosaic virus (HMV) were reported as causing more or less similar symptoms to those caused by M-TVNS on tobacco species in experimental conditions (Hopkins, 1956; Teakle, 1952; Kassanis, 1970; Zattlin and Israel, 1975; Damirdagh and Shepherd, 1967; Bartels, 1971; Govier, 1972).

TMV; a member of tobamovirus group; is an RNA containing virus with rigid tubular hellicaly symmetrical particles about 300 x 18 nm, mechanically transmitted but not by insects or other common vectors. Symptoms caused by TMV vary largely, depending on the strain. TMV can cause symptoms such as green mottling, vein-banding, yellow mosaic, necrotic spots, veinal necrosis, veinal or enation mosaic (Zattlin and Israel, 1975).

Nicotiana tabacum cvs cultivars develop vein-clearing, light green-dark mosaic, distortion and blistering. N.sylvestris reacts with mosaic or necrotic local lesion. Local lesions assay hosts for TMV are N.glutinosa, N.tabacum cvs 'Samsun NN' and 'Xanthii' nc', Phaseolus vulgaris cvs 'Pinto' and Chenopodium amaranticolor. They form necrotic local lesions at temperatures below 28°C (Holmes, 1946). TMV has shown to be very stable in crude sap. Preparation retains infectivity for decades, stand temperature up to 93°C and a dilution of 1:1,000,000. In 1975, Lucas reported that 90-100% of the plants may become infected by harvest time.

TLCV, a geminivirus, is also considered as one of the most destructive tobacco viruses in East Africa, Zimbabwe and South Africa (Lucas, 1975). The gemini particles measure 15-20 x 25-30 nm. They are transmitted by white fly, 'Bemissia tabaci', in a persistent manner but not manually with sap. The chief symptoms caused by TLCV are puckering or savoying of the leaves, accompanied by curling downward of the leaf margins. Leaves get crinkled and the veins on the underside become thickened (Osaki and Inouge, 1981).

Datura stramonium, Lycopersicon esculentum cultivars, Nicotiana tabacum cultivars and N.glutinosa have been reported as systemic diagnostic hosts for TLCV. Curling, vein-clearing, interveinal necrosis, stunting, spiral twisting of the leaves and yellowing are the main symptoms induced on these diagnostic hosts. There are no known local lesion host (Lucas, 1975; Osaki and Inouge, 1982).

In 1975, Lucas reported TRSV in South Africa. This is a nepovirus transmitted by a nematode 'Xiphinema americanum'(Bergson et al, 1964; Fulton, 1962, 1969). It is a multiparticles virus, containing the following three major classes of particles; empty protein shells without RNA, non-infectious nucleoprotein and an infectious nucleoprotein (Corbett and Robert, 1962; Davidson and Francky, 1969). TRSV has particles measuring 29 nm in diameter. The virus causes necrotic lesions that develop into ringspots or line patterns. TRSV has thermal inactivation point at 65^{o}C, dilution end point at 1:10,000 and preparation remains infective up to 6-10 days in crude sap at room temperature (Bergson et al, 1964).

TNV, belongs to a small virus group bearing the same name. The virus has polyhedral particles ranging from 25 to 30 nm in diameter.

It is one of the world wide distributed viruses affecting tobacco (Kassanis, 1970). The virus causes necrotic spots which often coalesce in the tissue along the midribs and veins on the lower leaves that have been infected through soil contact. Soilborne, it is transmitted by zoospores of the chytrid, Olpidium brassicae (Teakle, 1962). Phaseolus vulgaris cv 'Prince', Nicotiana tabacum and N.clevelandii are used as its diagnostic hosts. P.vulgaris and Chenopodium amaranticolor are reported as suitable local lesions assay hosts (Kassanis, 1970). The thermal inactivation point is between 85^{0} and 95^{0}C depending on the virus strain and its longevity in vitro is for many days (Babos and Kassanis, 1962).

Potato virus X, a member of potexvirus group, has been reported as causal agent of mottling and necrosis on Nicotiana species and other Solanaceous plants in the field (Shaw and Larson, 1962). A synergic action due to a combined infection by PVX and PVY causes a severe necrosis on tobacco cultivars in the field (Chagas et al, 1977, Corbett and Sisler, 1964; Kozard and Sheludko, 1969).

PVX is composed of one molecule of linear RNA. Its particles are rods, approximately 515 nm long and 13 nm wide. All potexviruses have particles with a modal length ranging from 470 to 580 nm. PVX is readily transmitted mechanically with sap. There are no known vectors (Mathews, 1981; Berks, 1970; Takashi and Knight, 1968). Cultivars of N.tabacum, Lycopersicon esculentum and Solanum tuberosum are reported as diagnostic systemic hosts, whereas Gomphrena globosa, Cassia occidentalis and Datura stramonium are local lesion assay hosts (Berks, 1970). On the main host, S.tuberosum, it induces an interveinal mosaic, sometimes scarcely visible, if not at all, depending on the strains, variety and environmental conditions. Virulent strains, cause rugosity of the leaves or even crinkling. Some hypersensitive varieties react with moderate or severe top necrosis, mainly to strains X1, X2 and X3, whereas X4 strains evoke only an interveinal mosaic in all varieties tested so far (de Berks 1970). PVX strains are reported as relatively stable in crude extract. Preparation loses infectivity between 68^{o} and $76^{o}C$, the dilution end points are between 10^{-5} and 10^{-6} and an aging in vitro of several weeks (Berks, 1970; Kahn and Monroe, 1963).

A carlavirus, PVS, has been reported as causal agent of vein-clearing, mottling and necrosis on Nicotiana debneyi. It has one molecule of a linear RNA. Particles are slightly flexuous rods 650 nm long and 12 nm wide (Welter, 1975). Carlavirus members have particle length ranging from 620 to 720 nm and a rather narrow host-range (mathews, 1981). Its local lecion assay hosts are Chenopodium amaranticolor, and C.album. On Solanum tuberosum, it induces roughness of the leaf surface and undulation of the leaf margin (Welter, 1975). Preparation shows rather low stability in crude sap. The thermal inactivation point is between 55^{0} and 60^{0} C. The dilution end point between 10^{-2} and 10^{-3} and a longevity in vitro of 3 to 4 days (Bagnall et al. 1955; Welter, 1975).

PVA, PVY, TEV and TVMV are members of potyvirus group and cause on tobacco more or less similar symptoms as M-TVNS in the field. HMV, of the same group, is reported as causing the same symptoms in tobacco in experimental conditions but has not been reported in the field.

Tobacco vein mottle and pepper vein mottle viruses were reported as showing almost the same experimental host-range as PVY strains. However they were able to induce systemic symptoms on Gomphrena globosa and Datura stramonium which are immune to PVY (Bartels, 1971; de Bokx, 1979; Damirdagh and Shepherd, 1967; 1970; Braunt, 1972; Govier, 1972).

The Potyvirus group members have flexuous filamentous particles with a modal length between 730 - 900 nm. They have one molecule RNA with a molecular weight of 35×10^6, representing 5% by weight of particles. They have relatively low stability in crude extract and most are considered as heat labile. Thermal inactivation point of potyvirus is mostly between 50-60^{0}C. However it was noticed that thermal inactivation points vary according to strain differences. Most have dilution end points within the range of 10^{-4}. But some strains of BCMV and PVY stand dilution up to 10^{-6}. they are described as highly susceptible to aging. Papaya ringspot virus does not survive beyond 8 hours and strains of PVY, BCMV and TEV retain their infectivity

up to a duration of 50 to 60 days (Brierly and Smith, 1962; Chagas et al, 1977; Kahn and Bartels, 1968; Matt, 1969; Simon, 1956; Yerkes and Graciano, 1960; Shepherd and Pound, 1960; Bos; 1971). Poty-viruses are strongly immunogenic and most produce relatively high titres. Serological relatedness exists between some members (Mathews, 1981; Purcifull et al 1970; Purcifull and Shepherd 1964). They are transmitted and spread mechanically with sap, through seed or in a non-persistent manner by aphid species (Pirone, 1964; Govier and Kassanis, 1974ab; Skotland and Burke, 1961). However, Bennet (1944) reported cases of transmission of TEV and CMV by Cuscuta californica.

TEV, a virus with flexuous filamentous particles of a normal length of 730 nm, affects tobacco and is considered of economic importance. It is transmitted mechanically through sap and by 10 species of aphids, notably by 'Myzus persicae' Sulz in a non-persistent manner. On tobacco cultivars, it causes local chlorotic spots, necrotic rings or arcs and systemic vein-clearing, mottling, necrotic spots and leaf distortion.

Occasionally systemic green vein-banding and interveinal mottling appears (Shepherd, 1974; Damirdagh *et al*; 1967, 1970; Holmes, 1946). It causes systemic symptoms on *Capsicum annum*, *Lycopersicon esculentum* and *Datura stramonium*. *Chenopodium amaranticolor*, *C.quinoa* and *C.album* are reported as suitable local lesions assay hosts and react with chlorotic local lesions. TEV is represented by two variants; the 'severe etch' induces more prominent stunting, chlorosis and necrosis rather than the 'mild etch' (Shepherd, 1974). TEV preparation looses infectivity above 55^{o}C, at a dilution of 10^{-4} and after a duration varying between 5 to 40 days (Shepherd and Shalla, 1969; Gooding and Bing, 1970; Hiebert *et al*, 1971; Matsui and Yamagushi, 1963; Purcifull, 1966; Purcifull *et al*, 1970).

PVA was reported as causing green vein-banding, vein-clearing and mild mottling on *Nicotiana tabacum* cultivars (Bertels, 1971). Particles of PVA are 730 nm long and 15 nm wide. It is transmitted by 7 species of aphids in the non-persistent manner. The more efficient are *Aphis frangulae*, *A.nastruitii* and *Myzus persicae*.

It is moderately immunogenic and antisera with titres of 1:512 are usually obtained. It has been shown to be serologically related to PVY, TEV, and HMV (Fribourg and Zoeten, 1970; de Bokx, 1975, et al, 1982ab). On the main host, Solanum tuberosum, PVA causes mild mosaic, roughness of the leaves surface and waviness of the leaf margin. Lycopersicon esculentum and Nicandra physoloides were reported as diagnostic systemic hosts. Aquila 'AG' hybrid is a useful local lesion assay host (Bartels, 1971). PVA has a very low stability in crude sap. It has a thermal inactivation point between 44° and 52° C, a dilution end point between 1:10 and 1:40 and a longevity in vitro of few days (Bartels, 1971; Fribourg and de Zoeten, 1970).

HMV is a flexuous filementous particle, 800 to 900 nm long and 12-13 nm wide. It was reported on Nicotiana tabacum cultivars as causing poorly defined chlorotic or necrotic lesions and vein-clearing followed by a green or yellow green systemic mosaic. However those symptoms were produced experimentally and have not been reported in the field.

Hyocyanamus niger, Nicotiana glutinosa, N.rustica and Chenopodium amaranticolor have been reported as diagnostic systemic hosts, when 'Aquila, 'A6' hybrid and N.sylvestris are considered as best local lesion assay hosts (Govier, 1972). HMV strains are transmitted by sap inoculation and readily by Myzus persicae, M.escalonicus, Aulocorthum circumflexum and Macrosiphum emphorbia. Serologically, HMV is related to PVY, Colombian datura virus and pokeweed mosaic virus (Kahn and Bartels, 1968). In tobacco or Datura stramonium sap, the thermal inactivation point is about 60°C, the dilution end point about 1:1,000,000 and most infectivity is lost after a few days at room temperature (Govier, 1972).

PVY is a virus with long flexuous particles 730 nm long and 11 nm wide. On the main host, Solanum tuberosum cultivars, PVY strains are reported as causing mild to severe mottle, streak or leaf drop streak with necrosis along the veins of the underside of the leaflet.

On Nicotiana tabacum cvs, all PVY strains, except Y^N strains induce vein-clearing, mottling, slight epinasty and whitish or brownish necrotic lesions (de Bokx et al 1982a; Delgado-Sanchez, 1970; Hiebert et al, 1971; Makkouk and Grumpk, 1974; 1975). N. tabacum cultivars, N.glutinosa, Solanum tuberosum cvs, Aquila 'A6 hybrid and Tinantia erecta are considered as systemic diagnostic hosts. Chenopodium amaranticolor, C.quinoa, Physalis floridana, S.tuberosum 'Duke of York' and 'Saco' are suitable local lesion assay hosts for some strains. However Aquila 'A6' hybrid produce local lesions for all PVY strains (Delgado-Sanchez and Grogan, 1966; Laird and Dickson, 1963; Chagas et al, 1977). All strains of PVY are readily transmitted by mechanical inoculation with sap, by core and stem grafting. Except PVY^C strain, other strains are readily transmitted by at least 25 species of aphid in a non-persistent manner. Myzus persicae is the most effecient vector. Other more or less efficient vectors are Aphis fabae, Macrosiphum euphorbia, M.certus, Phorodon humili and Rhopalosiphum insertum (Govier and Kassanis, 1974ab; Pirone, 1964; Stace-Smith and Tremaine, 1970). PVY strains are strongly immunogenic. Antisera with precipitin titres of 1:512 to 1:4096 can be obtained. PVY is serologically related to TEV, HMV, PVA, PeVM and BCMV.

Physical properties vary greatly with strain differences. In tobacco sap, the thermal inactivation point vary between 50^{0} and 65^{0}C, the dilution end point between 1:100 and 1:1,000,000 and a longevity in vitro varying from 2 to 50 days (Delgado-Sanchez, 1966; Gooding and Bing, 1970; Purcifull et al, 1970; Purcifull and Gooding, 1970). De Bokx and Huttinga (1981) and Kahn and Monroe (1963) had reported a classification of PVY strains into three groups; PVY^{O}, PVY^{N} and PVY^{C}. PVY^{O} group, the common strains, produces severe crinkle symptoms, rugosity or leaf-drop streak in potato, systemic necrosis in Physalis floridana and systemic mottling in tobacco cultivars. PVY^{N} group (Tobacco vein necrosis strains) induces severe systemic veinal necrosis in tobacco cultivars, systemic mottling in P.floridana and very mild mottling in almost all potato cultivars and are reported to be more destructive on tobacco than on potato. PVY^{C} group (Stripple streak strains including PVC); are not mostly transmitted by the aphid 'Myzus persicae'. Many potato cultivars are hypersensitive to strains of this group. Susceptible potato cultivars show systemic mosaic or stripple streak.

Symptoms induced in tobacco and Physalis floridana are similar to those induced by PVY^{O} strains. Gooding and Tolin (1973) has reported another delienation of PVY strains into three groups. This classification is based on the relation between the expression of the tobacco gene for resistance to rootknot nematode and biological activities of PVY strains in tobacco cultivars. The groups of strains are $M^{S}M^{R}$, $M^{S}N^{R}$ and $N^{S}N^{R}$. The first strain, $M^{S}M^{R}$, caused mottling and chlorosis on both root-knot resistant and susceptible cultivars. The second, $M^{S}N^{R}$, caused mottling and chlorosis on susceptible and necrosis on rootknot resistant cvs and the third, $N^{S}N^{R}$, induced necrosis on both susceptible and resistant cultivars. PVY^{O} decreses yield of tobacco by about 30% but PVY^{N} strains are very destructive and may cause a complete loss of the crop (de Bokx and Huttinga, 1981).

CHAPTER III : MATERIALS AND METHODS.

MATERIALS AND METHODS.

3:1 - Source of inoculum.

The virus was first reported on Nicotiana tabacum cv 'Speight G.28' around Malakisi area in Bungoma District. Diseased plant samples were brought to the laboratory of the Department of Crop Science of the University of Nairobi for study. The virus was multiplied in N.tabacum cv 'Samsun' ,'White Burley' and N.rustica inoculated by forefinger method.

3:2 - Inoculation procedure

Inoculations were done by carborandum fore-finger method (Noordam, 1973). Leaves of N.rustica L showing leaf malformation or those of N.tabacum cv 'White Burley' with vein-clearing and mottling were ground in a sterile mortar and sap diluted four fold in 0.5 M potassium phosphate buffer at pH 7.2. A pad saturated with the inoculum was rubbed on leaves previously dusted with 500 mesh carborandum. Plants were kept in the greenhouse and protected against external contamination by a systemic insecticide, Metasystox (Containing 25% Demeton-S-methylsupnoxid), sprayed weekly while plants were under observation for symptom development.

3:3 - Source of plants

Seeds of Nicotiana tabacum cvs 'White burley' and 'Samsun', N.debneyi L, N.rustica L, Chenopodium amaranticolor Costs & Reyn, C.quinoa Willd, C.album L, Physalis floridana Ryob, Nicandra physaloides L, Gomphrena globosa L, Datura stramonium L, D.metel L, Phaseolus vulgaris L and Solanum tuberosum L were supplied from the Department of Crop Science germ-Plasm bank, College of Agriculture and Veterinary Science, Kabete. Burley cultivars, 'Heavy Western' and 'Speight G.28' were obtained from B.A.T. Malakisi Leaf Centre. Seed of Solanum melongena L, Capsicum annuum L, Cucumis sativus L, C.melo L, Lycopersicon esculentum L and Zinnia elegans Jacq were obtained from Simlaw Seed East Africa Ltd. The hybrid Solanum demissium x S.tuberosum 'A6 and Nicotiana tabacum L cv 'Kentucky 35' were supplied by the Kenya Agriculture Research Institute, Quarantine Division, Muguga.

3:4 HOST-RANGE

Species and cultivars of Solanaceae, Leguminosae, Chenopodiaceae, Amaranthaceae, Cucurbitaceae and Compositeae were tested through mechanical inoculation. Seedling were grown in autoclaved Muguga Composition Soil mixture* in 15 cm diameter pots or plastic bags placed in a greenhouse with temperatures ranging between 20-27^{o}C.

According to Noordam's (1973) recommendation, test plants species were inoculated as follows:- Solanaceous at 2-4 leaves stage, Leguminosae at primary leaves, Amaranthaceae at two pairs of leaves, Cucurbitaceae on cotyledonary leaves and Chenopodiaceae at a clear mature stage. Reactions were observed between 7 to 28 days after which recovery tests were made on *Chenopodium amaranticolor* and *Nicotiana tabacum* cv 'Speight G.28' which served as assay hosts. The following species and cultivars were tested:-

N.tabacum cvs 'White Burley', 'Heavy Western', 'Samsun', 'Speight G.28' and 'Kentucky 35'.
N.rustica, *N.glutinosa* and *N.debneyi*.

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* Muguga composition soil mixture: Horse manure, Coffeehull, Forest soil and Ballast at a ratio of 1:1:2:1.

- Lycopersicon esculentum

  cvs 'Marglobe', 'Money maker', 'Rutgers'

- Solanum tuberosum .

  cvs 'Piratini', 'K.59', 'Desiree', 'Recent'
  'Eba', 'kers pink', 'Patrone', 'Multa'
  'Kenya Baraka',

- Hybrid S.tuberosum x S.demissium 'Aquila 6'

- S.melongena

  cvs 'Black beauty', 'Long purple'

- Capsicum annuum

  cvs 'Yolo wonder', 'Mercury', 'Long red Cayenne'

- Datura stramonium

- D.metel

- Physalis floridana

- Phaseolus vulgaris

  cvs 'Light brown NB* 507', 'White spherical'
  'NB 497'
  'Rose coco (small) NB 1122', 'GLP 2 NB 510',
  'Red haricot NB 86', 'Canadian wonder NB 26'
  'Mexican NB 1186', 'Cream oblong NB 1320',
  'Black haricot NB 16', 'Rose coco (spherical)
  NB. 2', 'Zebra NB 911', 'Rose coco (large)
  NB 1401', 'mwezi moja NM 518'.

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* National bean number

- Cassia occidentalis
- Chenopodium amaranticolor
- C.quinoa
- C.album
- Gomphrena globosa
- Zinnia elegans
- Cucumis sativus and C.melo

3:5 - Strain differentiation

Differentiation of M-TVNS was carried out by determining its biological activity on a set of differential hosts. The following differential hosts, used by Kahn and Monroe (1963), de Bokx (1981) and Delgado-Sanchez (1966) were tested with M-TVNS:

- Nicotiana tabacum cvs 'Samsun', 'White Burley' and 'Kentucky 35'.
- Datura stramonium
- D.metel.
- N.glutinosa.
- Physalis floridana
- Capsicum annuum cv 'Long red cayenne'
- Hybrid Solanum demissium x S.tuberosum 'A6'.

The virus was isolated by single lesion isolation method from Chenopodium amaranticolor and multiplied in 'White Burley'. Crude sap expressed in 0.05 M phosphate pH 7.2 was inoculated on differential hosts by forefinger method. Plants were then placed in a greenhouse and responses observed for 4 weeks. Observed symptoms were compared with literature data on PVY strains.

3:6 - Transmission through seed of tobacco.

Infected plants of Nicotiana rustica, N.tabacum cvs 'Samsun' and 'White Burley' were selected for seed production in the greenhouse. The seeds were sown and 25 seedlings of each species and cultivars were individually transplanted in 15 cm diameter polyethylene bags and kept in the greenhouse. They were weekly sprayed with metasystox and observed for 6 weeks. After 2, 3 and 4 weeks five plants from each species were taken at random and 2 g of leaves harvested from each of the selected plants were separately grounded and inoculum extracted. The sap was then inoculated on leaves of Chenopodium amaranticolor in vitro and five seedlings of N.tabacum, cv 'Speight G.28' in the greenhouse.

3:7 - Transmission through insects.

The main vectors known to transmit flexous filamentous viruses associated with tobacco virus diseases are aphid species. The green peach aphid, 'Myzus persicae' has been reported as vector of PVA, PVS, PAMV and TEV (Bartels, 1971; Welter, 1971; de Bokx, 1981; Kassanis, 1970; Shepherd, 1974). Other aphid species that are vectors of members of potyvirus group are Myzus ornatus, Macrosiphum euphorbia, Aleucorthum sp. Aphis nastrurtti and A.gossipi (de Bokx, 1981).

The green peach aphids '*Myzus persicae*' were collected from the field on Kale plants and freed of virus by rearing them on healthy chinese cabbage '*Brassicae Pekinensis* Rupr' in a cage. Plants of *N. tabacum* cv 'Speight G.28' and 'White Burley' were used as virus sources and cultivar 'Samsun' as the test plant. Aphids were starved for 30 to 60 minutes. Fifty apterous aphids in groups of 5 were allowed an acquisition feeding period of 15 minutes and immediately transfered on 10 healthy plants of cultivar 'Samsun' for an inoculation feeding period of 30 to 60 minutes. Plants were weekly sprayed with metasystox and observed for 5 weeks.

3:8 - Physical properties in crude sap

Two millilitres of each inoculum treatment was rubbed on six leaves taken at random from 12 plants of Chenopodium amaranticolor 30 days after transplanting. Local lesions were counted on four leaves showing the highest number for each treatment (Delgado-Sanchez and Grogan, 1966; Nordam 1973). The same pattern was followed for dilution end point, thermal inactivation point and longevity in vitro. All treatment were kept in humidified trays covered with polyethelene sheets and incubated at room temperature.

Dilution End Point (DEP)

Expressed crude sap was diluted from 10^{-1} to 10^{-7}. The undiluted sap and the seven dilutions were rubbed on leaves of C.amaranticolor previously dusted with carborandum.

Thermal Inactivation Point (TIP)

Thin walled specimen tubes were filled with 2 ml of crude sap. The tubes were brought to the following temperature levels: Unheated, 40, 45,

50, 55, 60, 65 and 70°C. Inocula were then rubbed on leaves of Chenopodium amaranticolor.

Aging in vitro (LIV).

Expressed crude sap was inoculated on detached leaves of C.amaranticolor after 0, 2, 4, 8, 16, 32, 48, 72, and 96 hr.

3:9 - Preliminary clarification

Alcohol (20%), ether (7, 7 and 10%), n-butanol (8.5%), chloroform (5%) and n-butanol-chloroform mixture (1:1) were compared as clarifying agents of M-TVNS. Heat at 40°C for 2 minutes was also used. Prior to addition of clarifying agents or heating, sodium diethyldithiocarbomate (DIECA) was sprinkled over the leaves at 5g/kg and crashed in a Vitamix blender. Extracted juice was then clarified by low speed centrifugation at 6,000 rpm for 15 minutes. The various treatments were made as follows:-

- Supernatant fluid was heated to 40°C for 2 minutes then centrifuged at 6,000 rpm for 20 minutes (Stace-Smith and Tremaine, 1970).
- Alcohol (95%) was added to the supernatant at 20 ml/100 ml sap, the mixture stirred for 5 minutes with a magnetic stirrer and clarified by low speed centrifugation at 6,000 rpm for 20 minutes and the supernatant collected (Stace-Smith and Tremaine, 1970).
- n-Butanol was mixed with the supernatant at 8.5 ml/100 ml sap, stirred for 5 minutes and clarified by low speed centrifugation at the same speed and time (Delgado-Sanchez et al 1966, 1970).

- Ether was progressively added to the supernatant fluid at 7, 7 and 10% of the sap volume and clarified by low speed centrifugation three times after each addition of ether (Nordam, 1973).
- Chloroform was mixed with supernatant at 5 ml/100 ml sap and the mixture stirred and centrifuged at 6,000 rpm for 20 min. (Shepherd and Gleen, 1960; Purciful et al, 1970, 1973).
- The mixture chloroform-butanol was added to the supernatant at equal ratio, stirred for 5 minutes and clarified by low speed centrifugation at 6,000 rpm for 20 minutes.

Four ml of each inoculum was rubbed on 10 leaves of Chenopodium amaranticolor previously dusted with carborandum. Local lesions were counted on 8 leaves of each treatment showing the highest number of lesions. Results were compared by the analysis of variance and separation of means by the Duncan's new multiple range test.

3: 10 - Purification procedure

A modified Noordam's procedure for purification of PVY was adopted as satisfactory for the purification of M-TVNS.

Sodium sulfite was sprinkled on leaves at a rate of 0.2 g per 100 g of leaves. Leaves were then ground in a Vitamix Blender and sap extracted by expressing through 2 layers of cheese cloth. To the homogenate, ethyl ether (7% of the sap volume) was added after shaking it with 0.2% sodium sulfite. The mixture was stirred with a magnetic stirrer for 5 minutes and left to stand for 30-60 minutes. Ethyl ether (7% of the sap volume) was once added to the homogenate and the mixture stirred again for 5 minutes. The homogenate was then clarified by low speed centrifugation at 5,000 rpm for 5 minutes in a superminor centrifuge. Pellets were discarded and the supernatant passed through a funnel with a wet pad to stop ether. Ethyl ether (10% of the sap volume) was added for the third and last time to the supernatant and the mixture stirred for 5 minutes and clarified once more at 5,000 rpm for 5 minutes. The

supernatant was adjusted to pH 7.6 with 0.2 M imidazole containing 7% ethyl ether and 0.2% sodium sulfite. The adjusted sap was once more run at 10,000 rpm for 10 minutes in a Beckman L5-50 ultracentrifuge set at 4-8^{o}C. The supernatant was given one or two differential centrifugation cycles and the obtained pellets were resuspended in 0.01 M potassium phosphate buffer containing 0.5 M urea.

Table 1 : Flow Chart of partial purification

Procedure of M-TVNS

Step and Fraction treated	Treatment	Fraction discard
1. Systemically infected leaves of 'White Burley' 21-25 days after inoculation.	Sprinkle with 0.2% $NaSO_3$ and macerate in a Vitamix blender Express sap through layers of cheesecloth.	Pulp
2. Expressed extract	Add 7% ethyl ether containing 0.2% $NaSO_3$, stir for 5 min. and leave to stand (30-60 min) Add more ether (10%), stir for 5 min. Clarify the homogenate at 5000 rpm for 5 min.	Pellets
3. Supernatant	Pour supernatant in a funnel through a wet pad. Add more ether (10% of the sap volume), stir for 5 min. Clarify once more by low speed centrifugation at 5000 rpm for 5 min.	Pellets
4. Supernatant	Filter supernatant as in point 3, adjust the sap to pH 7.6 with 0.2 M imidazole containing 7% ether and 0.2% $NaSO_3$. Centrifuge once more at 10,000 rpm for 10 min.	Pellets
5. Supernatant	Filter supernatant as in point 3. Centrifuge at 30,000 rpm for 90 min. using a Beckman L5-50 ultracentrifuge set at 4-8^{0}C	Supernatant
6. Pellets	Resuspend pellets in 0.01 M phosphate buffer pH 7.2 containing 0.5 M urea. Clarify at low speed (10,000 rpm for 10 min.)	Pellets
7.	Give one more cycle of	

3;11 - Electron microscopy

Leaf dip preparation

Negative staining with 2% potassium phosphotungstate at pH 6.5 (in water) as described by Hitchborn and Hill (1966) was applied. A drop of 2% potassium phosphotungstate was put by a hypodermic needle or pipette on a 300 mesh grid coated with formvar. A fresh cut of infected leaf of Nicotiana tabacum cv 'Speight G.28' was dipped in the stained grid for 30 seconds and the excess stain drained. The grid was then examined in the Carl Zeiss EM 9A electron microscope.

Crude sap preparation

Sap expressed from macerated infected tissue was clarified by low speed clarification at 6,000 rpm for 15 minutes. As undiluted sap was often too dense, the supernatant was diluted 1:10 to 1:100 with distilled water. A drop of undiluted sap and both dilutions were put on different grids coated with formvar and equal amounts of 2% potassium phosphotungstate at pH 6.5 added. The excess liquid was removed by a filter paper placed at the edge of the grid. The grids were then examined in the electron microscope.

Partially purified preparation

A partially purified preparation was placed on a clean surface of a glass slide. Grids coated with formvar were floated on the top for 3-5 minutes, after which they were removed and air dried. Then a drop of 2% potassium phosphotungstate pH 6.5 was placed on the grid. After removing the excess stain, the grids were examined in the electron microscope.

3:12- Estimation of the normal length of M-TVNS

Partially purified preparations and leaf dip preparations were stained with 2% potassium phosphotungstate at pH 6.5 (in water) prior to examination with electron microscope. Electron micrographs were made using a Carl Zeis EM 9A camera. Particles were compared to polystyrene particles (109 nm in diameter) as internal standard and measured after projection on wall and tracing them on paper.

3:13-Ultra-violet absorption spectrophotometry

Partially purified preparations were used for ultra-violet absorption spectrum in a Beckman M25 Spectrophotometer at slid width of 0.05 nm.

The reference standard contained 0.01 M phosphate buffer pH 7.2, in which the virus particles were suspended. Quartz cuvettes with an optical path length of the radiation of 1 cm were used and scanning done at wavelength ranging between 200 to 350 nm.

3:14 - Source of Antisera.

A partially purified virus preparation was injected in 6 kg New-Zealand White rabbit obtained from the Veterinary Department of the Kenya Agriculture Research Institute (KARI). Nine days prior to virus injection, blood for normal serum was collected by dripping. Two injections, one intramuscular, consisting of 1 ml of the virus mixed with an equal amount of Freud's incomplete adjuvant and another intravenous consisting of 0.5 ml of the virus with an equal amount of physiological saline (0.85% NaCl) were administered. Three similar intravenous injections were made 10 days after the first at 5 days intravals.

Ten days after the last injection, the rabbit was bled on the marginal vein of the ear by dripping. After being left to stand for two hours at room temperature, the clot was removed and left overnight at $4^{o}C$. The antiserum was decanted and centrifuged at 5,000 rpm for 30 minutes in the Superminor MSE Centrifuge and 0.01% sodium azide added as a preservative. The antiserum was then kept in a deep freezer.

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3:15 - Serological tests

Microprecipitin test

The titer of M-TVNS was determined by comparing antiserum serial dilutions against the partially purified virus (antigen), crude sap of diseased plants and healthy plants clarified by low speed centrifugation. Physiological saline (0.85%) was tested as a control. All serial dilutions were made with sterile 0.85% saline in 0.01 M trisoxymetnylaminomethane buffer at pH 7. Petri dishes coated with 0.2% formvar were used for micro-precipitin tests. Charts representing all dilutions were drawn and placed below petri-dishes. Crude sap from healthy and diseased plants was extracted from Nicotiana tabacum cultivar 'White Burley'. Drops of antigen-antisera and controls-antisera were placed in their respective positions as shown by the charts. They were then flooded with paraffin oil and petri dishes incubated for 2 hours at 37°C and examined by using a dissecting microscope at 40 or 60X magnification. Dishes were kept overnight at 4°C and re-examined the following day, Antisera of PVY^N, PVA, PVS and PVX supplied by Dr. de Bokx of Wageningen were compared to crude sap dilution of M-TVNS.

CHAPTER IV : RESULTS.

RESULTS

4:1 - Field symptoms

The main symptoms observed on Nicotiana tabacum cv 'Speight G.28', consisted of green vein-banding, vein-clearing and eventual vein necrosis. On earlier infected plants, veinal necrosis extended to the stem, followed by death of plants. On the recently introduced N.tabacum cv 'White Burley', vein-clearing and mottling were observed. Stunting was observed on a few plants.

4:2 - Host-range

Response of tested hosts was established by visual observation and confirmed by recovery tests on Chenopodium amaranticolor and N.tabacum cv 'Speight G.28' used as assay hosts. Nicotiana spp and other plant species were tested for host-range and symptomatology.

Most tobacco species and cultivars reacted by both local and systemic symptoms. Necrotic local lesions were observed on inoculated leaves of N.tabacum cv 'White Burley', 'Samsun' and 'Speight G.28' and N.rustica.

Systemic symptoms consisting of mottling, green vein-banding, vein-clearing and stunting were observed on all tobacco species and varieties. The cultivar 'Speight G.28' showed severe veinal and stem necrosis, followed by plant death. Few plants of Nicotiana rustica showed veinal and stem necrosis which did not lead to plant death. N.tabacum cv 'White Burley', 'Samsun', 'Heavy Western' and 'Speight G.28' showed positive reaction such as local lesions, vein-clearing, green vein-banding and mottling varying from green mottling to the extreme white mottling on old infected leaves. (Plates 4 and 5).

'White Burley' and 'Samsun' showed local lesions and necrotic patches on inoculated leaves and vein-banding, vein-clearing and mottling two weeks after inoculation.

In addition to vein-clearing and conspicuous mottling, veinal and stem necrosis were severe on the cultivar 'Speight G.28'. Among earlier infected plants of this cultivar death resulting in veinal and stem necrosis was observed. This variety showed the shortest incubation period and very high susceptibility (Plate 4).

Heavy western showed some tolerance, no local lesions were observed on inoculated leaves, whereas systemic vein-banding, vein-clearing and mild mottling were observed three weeks after inoculation but disappeared one month after their appearance However, although there was disappearance of symptoms, the virus was recovered on symptomless plants two months after inoculation.

N.glutinosa reacted with mottling twelve days after inoculation, whereas N.debneyi showed faint mottling after the same incubation period.

N.rustica showed a severe vein-clearing, leaf malformation and slight veinal necrosis but death was not observed as on N.tabacum cv 'Spieght G.28'. Symptoms were observed 10 days after inoculation.

Solanum tuberosum cultivars 'Piratini', 'K.59', 'Desiree', 'Recent', 'Eba', 'Kars pink', 'Multa', 'Patrone' and 'Kenya Baraka' reacted by mild mottling with an incubation period varying from two to four weeks.

Lycopersicon esculentun cvs 'Marglobe', 'Money maker' and 'Rutgers' showed mild mosaic three weeks after inoculation. Mild mottling was observed after two weeks on Capsicum annuum cvs 'Yolo wonder', 'Mercury' and 'Long Red Cayenne'.

The hybrid Solanum demission x S. tuberosum 'Aquila 6' showed a very short incubation period (6 days) and reacted with local and systemic necrotic lesions (Plate 7 and8).

Nicandra physaloides reacted after two weeks with local and systemic local lesions, vein-clearing and mottling (Plate 11), whereas mild mottling was observed two weeks after on Physalis floridana.

Chenopodium amaranticolor and C.quinoa reacted with chlorotic local lesions two weeks after inoculation (plate 9 and10). C.amaranticolor was used as local lesion assay host.

The following plant species and their cultivars were not susceptible to M-TVNS following mechanical inoculation and no virus was recovered on back inoculation:-

- S.melongena
- Phaseolus vulgaris
- C.album
- Gomphrena globosa
- Zinnia elegans
- Cassia occidentalis
- Datura stramonium

These results showed that M-TVNS has a narrow host-range, restricted to Solanaceae and Chenopodiaceae as most strains of PVY (de Bokx, 1981).

Table 2. Reaction of Nicotiana species and cultivars to inoculation with M-TVNS.

Species and cultivars	Response		Incubation period in days
	Local	Systemic	
Nicotiana tabacum L			
cultivars			
'White Burley 181'	NLL,NP	GVB, VC, S,Col,Mo	13
'White Burley 381'	NLL,NP	Mo, GVB, S, Col.	12
'Heavy Western'	0	M, Mo, VB, S	23
'Samsun'	NLL	Mo,GVB,VC,Col, NP	10
'Speight G28'	NLL, B, NP	SeVB,SeVC,SeVN,SN,D	7
Nicotiana rustica L	NLL	VC,D,VN,S N	10
Nicotiana glutinosa L	0	MMo	12
Nicotiana debneyi L	0	MMo	13

Table 3. Reactions of plant species and cultivars to inoculation with M-TVNS

Families, species, cultivars	Response		Incubation time (days)
	Local	Systemic	
Solanaceae.			
1. Solanum tuberosum L			
'Piratini'	0	MMo	20
'Eba'	0	MMo	17
'Desiree'	0	0	-
'Recent'	0	MMo	19
'Patrone'	0	0	-
'Kars pink'	0	0	-
'Multa'	0	MMo	15
'Kenya Baraka'	0	MMo	18
'K.59	0	MMo	14
2. S.tuberosum x S.demissium	NLL	NLL	7
3. Capsicum annuum L			
'Yolo wonder'	0	MMo	22
'Mercury'	0	MMo	20
4. S.melongena L			
'Black beauty'	0	0	-
'Long purple'	0	0	-
5. Lycopersicum esculentum L			
'Rutgers'	0	MMo	20
'Money maker'	0	MMo	21
'Marglobe'	0	MM	19
6. Physalis floridana Rydb	0	MMO	13
7. Datura stramonium	0	0	-
8. Nicandra physaloides L	0	0	-
B. Leguminosae			
1. Phaseolus vulgaris L			
'White spherical'	0	0	-
'Rose coco (small)'	0	0	-
'GLP 2'	0	0	-
'Red haricot'	0	0	-
'Canadian wonder'	0	0	-
'Mexican'	0	0	-
'Clean oblong'	0	0	-
'Black haricot'	0	0	-
'Rose coco (large)'	0	0	-
'Zebra'	0	0	-
'Rose coco (spherical)'	0	0	-
'Mwenzi moja	0	0	-
2. Cassia occidentalis L	0	0	-
C. Chenopodiaceae			
1. Chenopodium amaranticolor Coste & Reyn	CLL	0	14
2. C.quinoa L	CLL	0	12
3. C.album L	0	0	-
D. Amarathaceae Gomphrena globosa L	0	0	-
E. Cucurbitaceae			
1. Cucumis sativus L			
'Poinsett'		0	-
'Ashrey'	0	0	-

Abbreviations used in Table 2 and 3

NLL	:	Necrotic local lesions
NP	:	Necrotic patches
B	:	Blight
Mo	:	Mottling
MMo	:	Mild mottling
GVB	:	Green vein-banding
VC	:	Vein-clearing
S	:	Stunting
SeVB	:	Severe vein-banding
SeVC	:	Severe vein-clearing
SeVN	:	Severe veinal necrosis
SN	:	Stem necrosis
D	:	Death
L	:	Leaf distortion
Col	:	Extreme chlorosis on all leaves
CLL	:	Chlorotic local lesions
MM	:	Mild mosaic
O	:	Immune
VN	:	Veinal necrosis
M	:	Mosaic

4:3 - Strain differentiation

The set of differential hosts used by de Bokx (1981), Kahn and Monroe (1963) and Delgado-Sanchez (1966) were inoculated with M-TVNS. Table 8 shows the reaction observed.

Datura stramonium and Gomphrena globosa were immune to M-TVNS. This fact exclude any PVY-PVX association in M-TVNS tobacco interaction. On D. metel, M-TVNS induced green vein-banding, chlorosis, mottling and stunting (Plate12), chlorotic mottle on Capsicum annuum. 'Long red Cayenne' and mild mottling on Physalis floridana.

On tobacco cv 'Speight G.28' systemic vein clearing, veinal and stem necrosis were induced but 'White Burley' and 'Samsun' showed green vein banding, vein clearing and chlorosis without systemic necrosis although mild necrosis were observed on inoculated leaves (Plate 3 & 4).

Table 4 : Reactions of differential hosts to inoculation with M-TVNS

Species or cultivar	Responses		Inocubation period (days)
	Local	Systemic	
Nicotiana tabacum			
cvs 'Samsun'	NeLL	VC, MMo	10
'White Burley'	NeLL	VC,GVB,Mo	9
'Kentucky 35'	-	GVB,VC, Mo	11
Datura stramonium	-	-	-
D.metel	-	GVB,D, Mo	7
N.glutinosa	-	GVB, MMo	12
Gomphrena globosa	-	-	-
Capsicum annuum			
cv 'Long Red Cayenne'	-	Mo	7
Physalis floridana	-	Mo	9

Abbreviation used in table 4

NeLL	:	necrotic local lesions
D	:	leaf distortion
MMo	:	mild mottling
Mo	:	mottling
GVB	:	green vein-banding
VC	:	vein- clearing

4:4 Physical properties.

Results showed that M-TVNS in *Nicotiana tabacum* cv 'White Burley' sap has a thermal inactivation point between 55°C and 60°C. Its dilution end point stood between 1:100 and 1:1,000. The longevity *in vitro* at room temperature was of 2 days. Those characteristics suggested low stability of M-TVNS as reported for most potyviruses (Mathews, 1981).

4:5 - Transmission

Transmission through tobacco seed

None of the 75 seedlings from seed of infected *Nicotiana tabacum* cvs 'White Burley' and 'Samsun' and *N.rustica* showed symptoms after six weeks of observation and the virus was not recovered. Those results suggested that M-TVNS is not transmissible through seed of tobacco.

Transmission through insects

M-TVNS was successfully transmitted by green peach aphid, *Myzus persicae* from *N.tabacum* cv 'Speight G.28' to cultivar 'Samsun'. Out of 10 tested Samsun plants, six were positively aphid inoculated and showed symptoms three weeks after inoculation. The virus was recovered from all aphid infected plants four weeks after inoculation.

4:6 - Purification

Five chemicals and heat at 40°C were compared as clarifying agents. Extracts clarified with chloroform induced the highest number of lesions on Chenopodium amaranticolor (438) and the Duncan's new multiple range test showed that chloroform as treatment was significantly different from all other treatments. The remaining treatments did not show any statistical differences (Table 6). However when chloroform was used in purification, separately or in combination with n-butanol, results showed a high rate of breakage of particles. The use of alcohol in a modified method of Stace-Smith and Tremaine (1966), resulted in a strongly aggregated preparation and considerable losses during clarification by low speed centrifugation following the first differential centrifugation cycle. Ether was added progressively at rates of 7, 7 and 10ml/100ml of extract between two speed centrifugation as suggested by Noordam (1973). This method gave acceptable partially purified preparation without considerable breakage and loss of particles by low speed centrifugation but this method did not stop particle aggregation (Plate 16). However, when 0.5 M urea was added to this preparation, considerable reduction of virus particle aggregation was achieved (Plate 17).

Table 5 : Analysis of variance on the effect of various clarifying agents on the retention of infectivity of M-TVNS in clarified crude sap of Nicotiana tabacum cv 'White Burley' at 6,000 rpm and 15 minutes low speed centrifugation.

Clarifying agent and treatment	Number of local lesions on Chenopodium amaranticolor.								
r:	1	2	3	4	5	6	7	8	Tot.
Heat	16	7	12	3	17	56	32	18	160
Alcohol	13	19	10	58	18	4	26	6	154
n-Butanol	23	26	53	47	24	13	24	12	222
x But-Chlor	15	2	12	1	22	14	11	13	90
Ether	32	14	17	35	16	49	24	55	242
Chloroform	93	48	43	13	66	62	79	34	438
Total	192	116	147	157	163	198	195	134	1306

x: Butanol-chloroform

Anova table

Source	df	SS	MS	F
Total	48	56806.00		
Level	1	35534.08		
"Total"	47	21271.92		
Blocks	7	1035.92	147.99	
Treatment	5	9104.42	1820.88	5.73
Error	35	1131.58	318.05	

F cal : 5.73 $F^{5}_{35}(0.05)$: 2.49

There were significant differences among treatments

Table 6 : Separation of means by the use of Duncan's new multiple range test.

$LSD_{0.05}$=18.11

Means	54.75^{C}	30.25^{E}	27.275^{B}	20^{H}	19.25^{A}	11.25^{B-C}
11.25	43.5**	19.00**	16.03	8.75	8.00	-
19.25	35.50**	11.00	8.03	0.75	-	
20.00	34.75**	10.25	7.275	-		
27.275	27.48**	2.98	-			
30.25	24.50**	-				
54.27	-					

Abbreviations used:

C = chloroform

E = ether

B = n-butanol

H = heat

A = alcohol

B-C = n-butanol and chloroform mixture.

** -= Significant differences.

4:7 - Electron icroscopy.

The M-TVNS particles, in a leaf dip preparation stained with 2% potassium phosphotungstate pH 6.5 (in water) were flexous particles. Their length varied from about 200 to 1000/nm in partially purified preparation and 600 - 1500 nm in leaf dip preparation. The normal length was estimated from the frequences in 13 classes taken 6 below and 6 above the highest frequency class. A value of 685 nm was obtained from 96 particles representing 74% of particle population in a partially purified preparation (Plate 1). This was 7.77% shorter than the normal length of 742.75 nm obtained from 74 particles of leaf dip preparation (Plate 2). This difference is due to a significant amount of breakage which occured during partial purification process and appeared onto formvar coated grids during electron microscopy observation. The normal lengths of purified potato Y and turnip mosaic viruses reported by Delgado-Sanchez and Grogan (1966) and Shepherd and Pound (1960) were also 6.3 and 9% shorter than the normal length of particles obtained from leaf dip preparation.

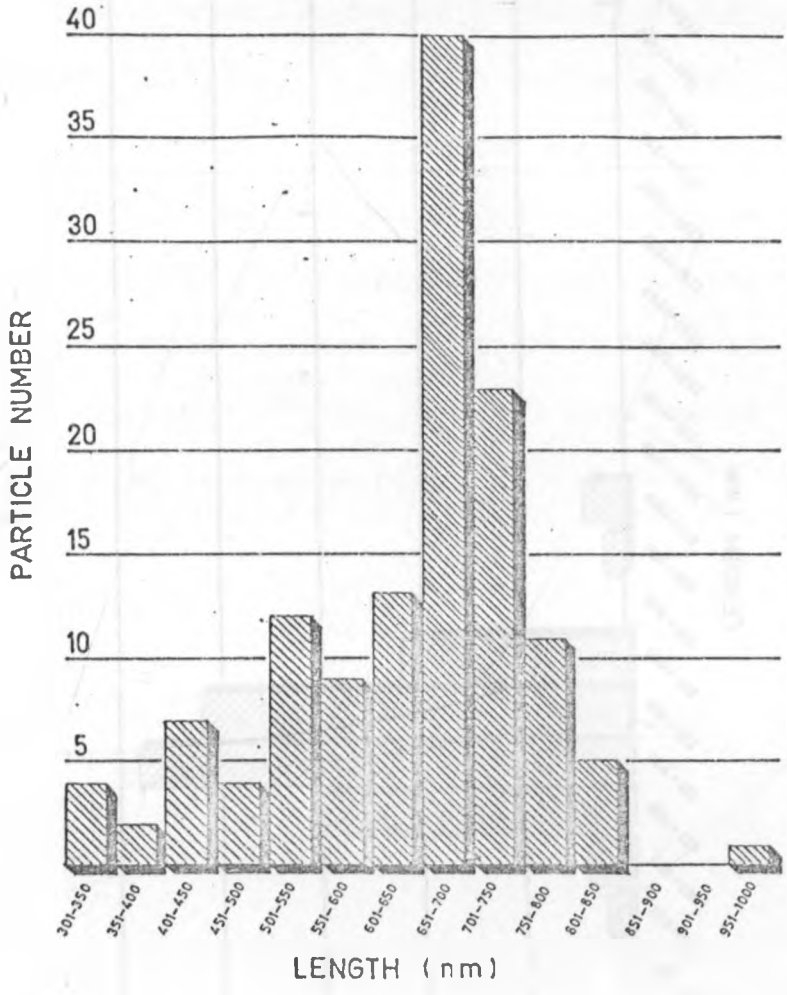


Plate 1 : Particle length distribution in a partially purified preparation of M-TVNS.

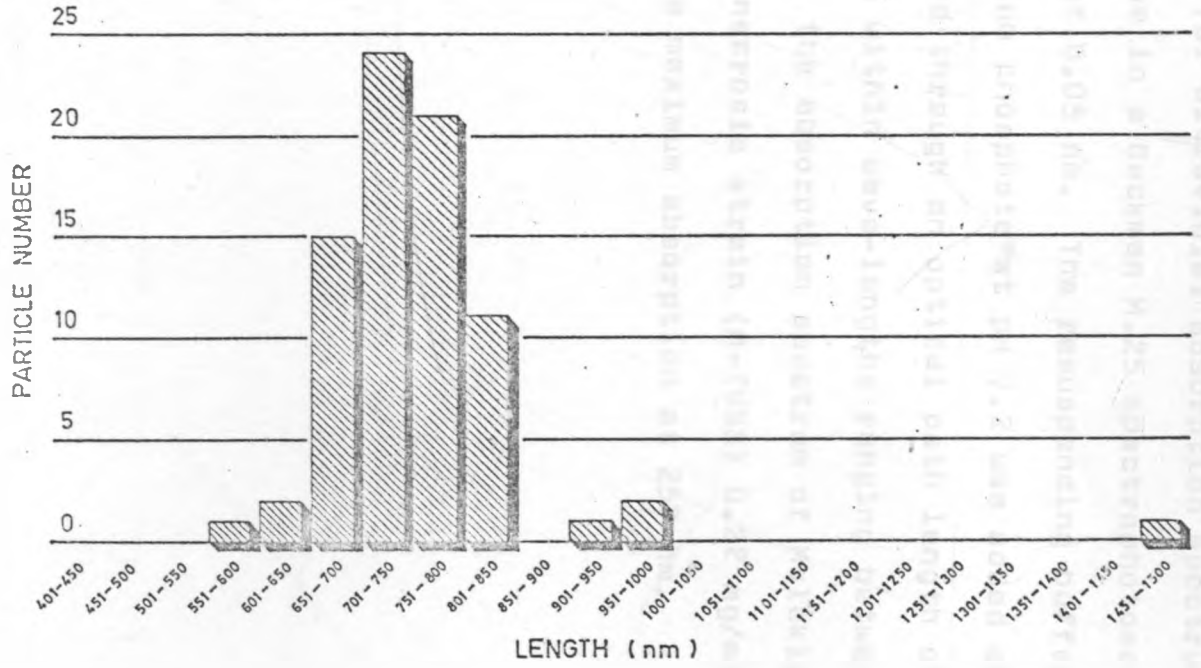


Plate 2 : Particles length distribution in leaf dip preparation of M-TVNS.

4:8 - Ultraviolet absorption spectophotometry.

A partially purified preparation of M-TVNS was tested for ultraviolet absorption spectrum. The test was done in a Beckman M.25 spectrophotometer at slit width of 0.05 nm. The resuspending buffer, 0.01 M potassium phosphate at pH 7.2 was added as a reference standard through an optical path length of 1 cm. When scanned within wave-lengths ranging between 200 to 300 nm, the absorption spectrum of Malakisi tobacco veinal necrosis strain (M-TVNS). 0.22 mg/ml buffer showed a maximum absorption at 257 nm.

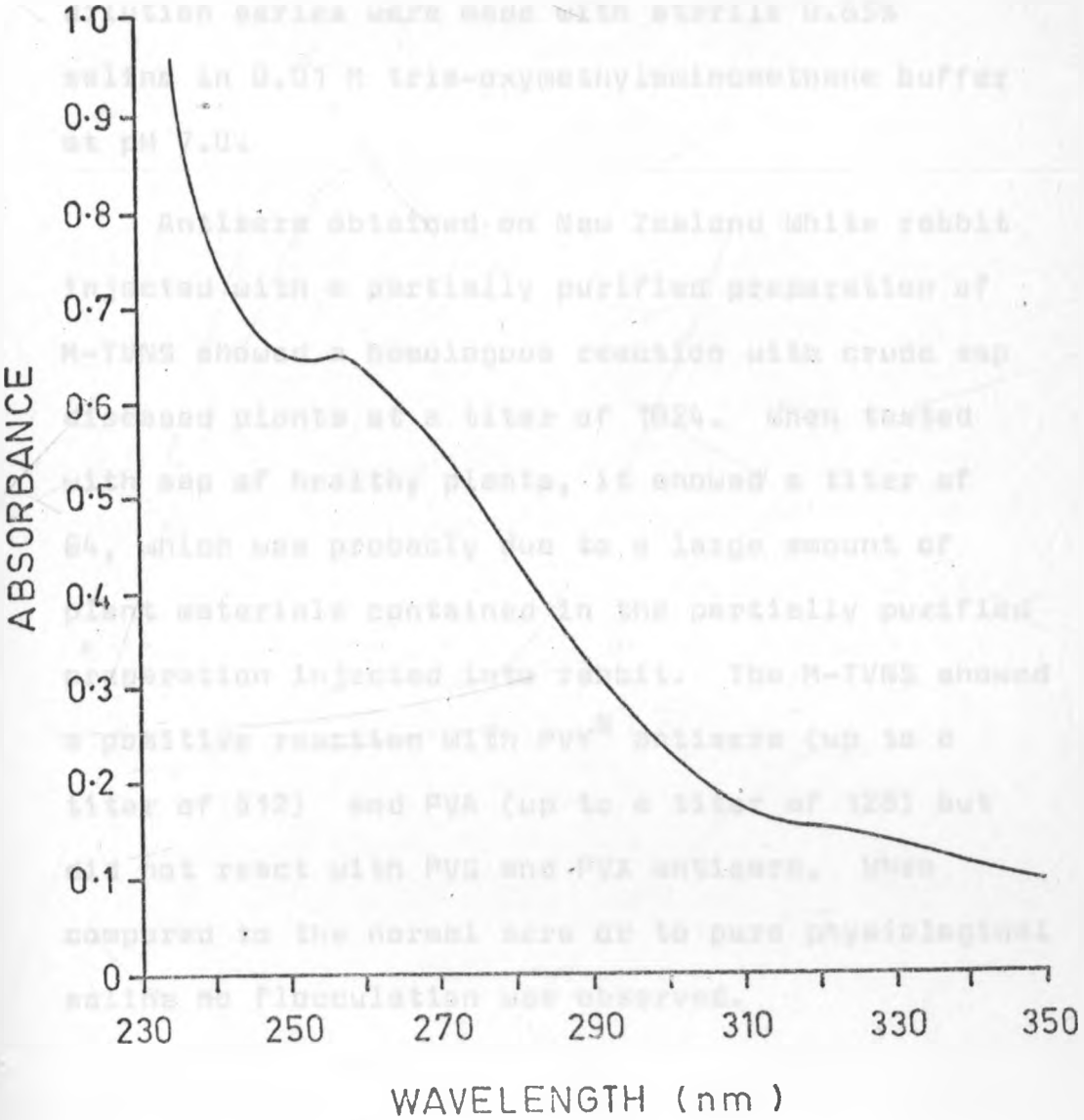


Plate 3 : Ultra-violet light absorption spectrum of a partially purified preparation of M-TVNS after two cycles of differential centrifugation..

4:9 - Serological tests.

All serological tests and comparisons were carried out by using van Slogteren microprecipitin tests in petri dishes coated with formvar and all dilution series were made with sterile 0.85% saline in 0.01 M tris-oxymethylaminomethane buffer at pH 7.0.

Antisera obtained on New Zealand White rabbit injected with a partially purified preparation of M-TVNS showed a homologous reaction with crude sap diseased plants at a titer of 1024. When tested with sap of healthy plants, it showed a titer of 64, which was probably due to a large amount of plant materials contained in the partially purified preparation injected into rabbit. The M-TVNS showed a positive reaction with PVY^N antisera (up to a titer of 512) and PVA (up to a titer of 128) but did not react with PVS and PVX antisera. When compared to the normal sera or to pure physiological saline no flocculation was observed.

CHAPTER V : DISCUSSION AND CONCLUSION

DISCUSSION AND CONCLUSION

5:1- Discussion

Identification of plant viruses is based firstly on virus classification in broad group and secondly on the differentiation between related virus within a group.

According to Matthews (1981), the more useful criteria to place plant viruses in a broad group are:

a - amount and kind of nucleic acid

b - particle morphology

c - type of vectors

those allowing a more narrow classification are properties such as:

a - symptomatology and host-range

b - amino-acid composition of the protein coat

c - resistance to chemical and physical agents

d - serological relationships which serve both to limit broad groups and differentiate related plant viruses within a group.

In plant diseases, the production of symptoms demonstrates biological activities of the virus particles (Gibbs and Harrison, 1976; Matthews, 1981; Corbett and Sisler, 1966). Applied on a large number of hosts and confirmed by recovery test on assay hosts, symptoms reflect activities of a particular protein, coded by the virus RNA (Matthews, 1981) and serve as criteria of virus identification.

Tobacco ringspot and tobacco necrosis viruses have been reported as causal agents of systemic necrosis on tobacco cultivars (Babos and Kassanis, 1962, Fulton, 1962). However, M-TVNS was differentiated from both TRSV and TNV. These viruses have polyhedral particles.

Rod shaped flexous viruses such as PVA (Bartels, 1971), PVS (Welter, 1975), PVX (Berks, 1970), TEV (Shepherd, 1974), HMV (Govier, 1972) and PVY (Delgado-Sanchez, 1966 a and b, 1970; de Bokx, 1981; Kahn and Monroe, 1963) were reported on tobacco and cause partially more or less similar symptoms to M-TVNS. The comparisons of M-TVNS host-range, physical properties, serology and size with literature data reported on those viruses (Table 7, 8, and 9 are

discussed below:-

Among the listed viruses, PVX and PVS belong to Potexvirus and Carlavirus groups respectively. PVA, PVY, TEV and HMV are classified as Potyviruses (Matthews, 1981).

Potexvirus and potyvirus groups are differentiated by the modal length of their particles, stability in crude sap, transmission methods and serological reactions. Members of potexvirus group have a normal length of 470-580 nm and are mechanically transmitted. They are not however transmitted by common vectors as aphids, Whiteflies, etc (Matthews, 1981). Potyviruses have 700 to 900 nm as range of normal length. This is considerably longer than that of potexviruses. In addition they are transmitted by aphid species in a non-persistant manner. They also give a relatively low stability in crude sap compared to potexviruses which can remain infective for weeks or years (Table 10). PVY has been reported as the causal agent of local lesions on Gomphrena globosa, Cassia occidentalis and chlorotic and mosaic mottle on Datura stramonium which were immune when mechanically inoculated with M-TVNS (Berks, 1970). Serologically M-TVNS was not related to PVX but did so with PVY and PVA of Potyvirus group. Therefore, it should be

concluded that by its modal length, host-range and serological relatedness that M-TVNS is not a potexvirus but a potyvirus.

M-TVNS induced various symptoms on Nicotiana tabacum cultivars such as vein-clearing, green vein-banding, mottling and veinal and stem necrosis which were not reported on the carlavirus PVS. M-TVNS did not infect Chenopodium album which was reported as assay host for PVS. Symptoms such as roughness of the leaf surface and undulation of the leaf margin caused by PVS on Solanum tuberosum cultivars were not induced mechanically by M-TVNS. Serologically M-TVNS was not related to PVS and its modal length of 742 nm is far longer than carlaviruses which do not exceed 700 nm (Welter, 1970, Matthews,1980).

Within Potyvirus group, TEV, PVY, PVA, and HMV are transmitted by aphids and affect tobacco (Delgado-Sanchez, 1970; de Bokx, 1981; Shepherd, 1974, Govier, 1972). However, HMV has never been reported affecting tobacco in the field and has a longer modal length of 800 to 900 nm which differentiate it from M-TVNS with a modal length of 742 nm in leaf dip preparations (Govier, 1972).

Both M-TVNS and PVA induced green vein-banding, vein-clearing and mottling on white burley cultivars but M-TVNS had been shown to be pathogenic on Nicotiana rustica, N. debnevi, Chenopodium amaranticolor and C.quinoa which have not been reported as hosts of PVA (Bartels, 1971). PVA had been associated with necrosis and death on Lycopersicon esculentum and mosaic and top necrosis on Solanum tuberosum cultivars which symptoms were not observed after mechanical inoculation with M-TVNS (Bartels, 1971). M-TVNS have shown a relatively higher stability in crude extract than PVA which was reported to exhibit a TIP between 44-52^{o}C and DEP standing between 1:10 and 1:40 (Table 7). M-TVNS and PVA have been serologically related with a heterogenous titer of 1:128 but they differ significantly in their host-range, and physical properties.

TEV has been reported as causal agent of necrotic etching, chlorotic mottle, distortion and green vein banding on tobacco varieties. It induces also mottling, vein-banding and leaf distortions on Datura stramonium and chlorotic local lesions on C.album, However, C.album and D.stramonium were immune when mechanically inoculated with M-TVNS (Holmes, 1946; Shepherd, 1974). TEV also induces leaf distortion, root necrosis, wilt and death on Capsicum annuum,

which although sensitive to M-TVNS did not exhibit such symptoms. M-TVNS had shown a relatively higher TIP and lower DEP compared to TEV (Table 7). These comparisons suggest that M-TVNS and TEV differ mainly by their biological activities in Chenopodium album, Capsicum annuum and Datura stramonium and physical properties.

When mechanically inoculated on Nicotiana tabacum cultivars, M-TVNS has induced symptoms such as vein-clearing, green vein-banding, mottling and necrosis as reported on PVY strains. Reaction of C.amaranticolor and C.quinoa to M-TVNS was similar to that reported on PVY-Chenopodium sp association (de Bokx, 1981; Delgado-Sanchez, 1966). As PVY strains, M-TVNS caused mild mottling on Physalis floridana, Solanum tuberosum cultivars and was unable to infect Gomphrena globosa, Zinnia elegans, Cassia occidentalis, D. stramonium, S.melongena, Cucumis sativus and C.melo (Delgado-Sanchez and Grogan, 1966). PVY strains have been reported to have a TIP of 50-62^{o}C, a DEP ranging from 10^{-2} to 10^{-6} and LIV of 2 to 50 days (de Bokx, 1981). M-TVNS had appeared to be a strain of relatively low stability in crude sap but still with physical properties within the limits reported for PVY strains.

M-TVNS had showed a modal length of 742 nm, which is within the range reported for PVY strains. It was more serologically related to PVY^N with a titer of 1:512 in a microprecipitin test. These similarities in host-range and symptomatology, physical properties, particle length and serological relatedness suggest that M-TVNS is a strain of PVY.

Table 9 Comparison of Malakisi Tobacco vein necrosis strain with other reported flexous filamentous viruses affecting tobacco in Literature.

	Response to VIRUSES					
HOST	M-T V N S	PVA	PVS	PVX	PVY	TEV
Nicotiana tabacum. L Cv. 'Barley'	Mo, VC, VB NP, St	GVB, VC MiMo	O	NR, Mo	Mo, VN, VB, VC VN	VC, NE, CM, CS Dt, VB, NR
Nicotiana tabacum. L 'Speight G.28'	Mo, CV, VB, VB	O	O	NR, Mo	Mo, VN, VB, SmN	VC, NE, CM Dt, VB
Nicotiana glutinosa. L	Mo	O	O	-	Mo	
Nicotiana rustica. L	VB, VC,	O	O	-	VC, Mo	O
Nicotiana debneyi. L	Mo	O	VC, Mo,	-	Mo	O
Solanum tuberosum. L	Mo, NL	M, TN, Rg	L, Rg Ul	M.	Mo, Lds, VN NR	O
Capsicum annuum. L	Mo,	O	O		Mo	CM, D, RN, WD.
Lycopersicum esculentum. L	M	N, Dth	O	M,	Mo	MD, DiMo
Phaseolis vulgaris. L	O	O	O	O	S	O
Chenopodium amaranticolor, Coste & Reyn	CLL	O	CLL	O	CLL	CLL
C. album. L	O	O	CLL	O	CLL	CLL
C.quinoa	CLL	O	O	O	CLL	CLL
Physalis floridana.Rydb	Mo,	O	O	O	Mo, NLL	O
Nicandra physaloides. L	Mo, NLL	VC, Mo, St	O	O	Mo, NLL	O
Gomphrena globosa. L	O	O	O	LL	O	O
Zinnia elegans. Jacq	O	O	O	O	O	O
Cassia occidentalis. L	O	O	O	CLL	O	O
Datura stramonium. L	O	O	O	CR, MMo	O	Mo, VB, Lds

Abbreviations used in table 7.

MMo	=	Mild mottling
Mo	=	Mottling
VC	=	Vein-clearing
NP	=	Necrotic patches
St	=	Stunting
VB	=	Green vein-banding
GVB	=	Dark green vein-banding
VN	=	Veinal necrosis
SmN	=	Stem necrosis
NL	=	Necrotic lesion
CLL	=	Chlorotic local lesion
NLL	=	Necrotic local lesion
M	=	Mosaic
TN	=	Top necrosis
Rg	=	Roughness of leaf surface
N	=	Necrosis
D	=	Death
L	=	Latent symptoms
SiC	=	Slight chlorosis
UL	=	Undulation of the leaf margin
NR	=	Necrotic ring
CR	=	Chlorotic ring

Continued: (Table 7)

MMo = Mosaic mottle

Lds = Leaf distortion

NE = Necrotic etch

RN = Root necrosis

WDt = Wilt and death

DMo = Diffuse mottling

Table 8 Comparison of M-TVNS physical properties of M-TVNS with those of related potyvirus reported in literature.

VIRUSES	Physical properties			
	TI P in °C	D E P	LIV	AUTHOR
M-TVNS	55-60	1:100-1:1,000	2-3 days	
PVA	44-52	1:10-1:40	days	Bartel, 1971
PVY	50-62	1:100-1:1,000,000	2-7 (50) days	Bokx & Huttinga, 1981
BCMV	50-65	1:1,000-1:1,0000	1-4 days	Bos, 1971
TEV	55	1:10,000	5-40 days	Shepherd, 1974
HMV	60	1:1,000,000	Few days	Govier, 1972
CVMV	50-65	1:100-100,000	2-10 days	Hollings, 1971
CoAMV	57-60	1:1,000-10,000	1-3 days	Bokx, 1974
PRSV	54-56	1:1,000	8 hours	Purcifull, 1977
PeVMV	55-60	1:1,000-1:10,000	7-8 days	Braunt, 1972
SNMV	55-60	1:1,000-1:1,000,000	2-4 days	Bos, 1972
TuMV	62	1:1,000-10,000	3-4 days	Tomlison, 1970

Table 9. Comparison of M-TVNS particles length with leterature data of other Potyviruses.

VIRUS	SIZE (nm)		
	LENGTH	WIDTH	AUTHOR
- M-TVNS	742		
- Potato Virus Y	730	11	Bokx 1981
- Potato Virus A	730	15	Bartel 1971
- Bean Common Mosaic Virus	750	15	Bos 1971
- Tobacco Etch Virus	730	13	Shepherd 1974
- Henbane Mosaic Virus	900	-	Govier 1972
- Carnation Vein Mottle Virus	790	12	Hollings 1971
- Cowpea Aphid Sorna Mosaic Virus	750	-	Bock 1974
- Papaya Ring Spot Virus	800	12	Purcifull 1972
- Pepper Veinal Mottle Virus	770	12	Braunt 1972
- Soybean Mosaic Virus	750	-	Bos 1972
- Turnip Mosaic Virus	722	-	Hill, 1972

Abbreviations used in table 8 and 9

- MTVNS = Malakisi Tobacco Veinal Necrosis Strain.
- PVA = Potato Virus A.
- PVY = Potato Virus Y.
- BCMV = Bean Common Mosaic Virus.
- TEV = Tobacco Etch Virus.
- CVMV = Carnation Vein Mottle Virus.
- CoAMV = Cowpea Aphid Borne Mosaic Virus.
- PRSV = Papaya Ring Spot Virus.
- PeVMS = Pepper Vein Mottle Virus.
- SBMV = Soybean Mosaic Virus
- TuMV = Turnip Mosaic Virus.

De Bokx (1981) and Delgado-Sanchez (1970) based PVY strain differentiation on reactions observed on Nicotiana tabacum cv 'White Burley' and 'Samsun', Physalis floridana, Solanum tuberosum cv 'Duke of York' and other potato cultivars. The above species and cultivars allow the classification of PVY isolates into three groups of strains. PVY^{O}, the common strains are characterized by mild mottling, veinal clearing and banding and chlorosis on N.tabacum cultivars. PVY^{O} produces also mottling and necrosis on potato cultivars, necrosis on P. floridana and it is reported as being more destructive on potato cultivars than on tobacco (de Bokx, 1981; Delgado-Sanchez, 1966, 1970). By comparison they described the necrotic strains group PVY^{N} as the cause of mild mottling on potato cultivars and P.floridana and severe veinal and stem necrosis on tobacco cultivars and are known to be of more economic importance on tobacco than on potatoes. The third group, PVY^{C} strains behave as PVY^{O} strains but are not transmitted by aphids and potato cultivars are hypersensitive to this group.

Although using the same differential hosts as de Bokx (1981) and Delgado-Sanchez (1970), Kahn and Monroe (1963) introduced the use of Datura stramonium and D.metel, Gomphrena globosa, Capsicum annuum cv 'Long Red Cayenne' and Nicotiana tabacum cv 'kentucky 35' as differential hosts. Both D.stramonium and G.globosa are immune to all PVY strains and are mainly used to detect a possible association of PVY and PVX. D.metel reacts by mottling and chlorosis to all PVY strains. Capsicum annuum cv 'Long Red Cayenne' reacts by mottling to PVY^{O} and PVY^{C} but is resistant to PVY^{N} (Kahn and Monroe, 1963).

Gooding and Tolin (1972) based differenciation of PVY strains in relation to the resistance gene to root-knot nematode in tobacco cultivars. The first strain, $M^{S}M^{R}$ caused mottling and chlorosis on both root-knot resistant and susceptible cultivars. The second, $M^{S}N^{R}$ caused mottling and chlorosis on susceptible and necrosis on root-knot resistant cultivars and the third $N^{S}N^{R}$ induced necrosis on both root-knot susceptible and resistant cultivars.

The comparison (Table 10) of reactions of differential hosts inoculated with M-TVNS with reported data on the three group of strains of PVY suggest the following observations:-

M-TVNS caused necrotic spots on inoculated leaves of White Burley, Samsun and Heavy western but caused severe systemic necrosis on leaves, vein and stems of a tobacco cv 'Speight G.28'. This reaction differenciated M-TVNS from PVY^{O} strains which are not able to induce systemic necrotic symptoms on any Nicotiana tabacum cultivars. M-TVNS has induced mild mottling on Physalis floridana but not necrotic lesions as do PVY^{O} strains. M-TVNS is different from PVY^{N} strains by its inability to cause systemic necrosis on other N.tabacum cvs such as 'White Burley', 'Heavy Western', 'Samsun' and 'Kentucky 35'. It differs from PVY^{N} by causing chlorotic mottling on 'Long Red Cayenne' which is known to be resistant to PVY^{N} strains (Kahn and Monroe, 1963). By comparison to PVY^{C} strains, which are only mechanically inoculated, M-TVNS was readily transmitted by Myzus persicae. M-TVNS caused systemic necrosis on leaves, veins and stems of root-knot resistant tobacco 'Speight G.28' but not on susceptible cvs such 'White Burley', 'Samsun' and 'Heavy Western'. This suggests a similarity with the $M^{S}N^{R}$ strain reported by Gooding and Tolin (1973).

Table 10 : Comparision of the Malakisi Tobacco Veinal Necrosis Strain on Tobacco and other indicator plants with Literature data of three strains of Potato Virus Y (PVY)

Differential host'	M-TVNS	PVY^N	PVY^C	PVY^O
Nicotiana tabacum. L 'Samsun'.	VC MiMo, NeLL	VB, SeVN, LDt	MiMo, NeLL	MiMo, NeLL
N.tabacum. L 'White Burley'	VC MiMo, NeLL	VB, SeVN, LDt	MiMo, NeLLi	MiMo, NeLL
N.tabacum. L 'Kentucky. 35'	VB, MoVC	VB, SeN	MiMo, VB	MiMo VB
Datura stramonium L	Im	Im	Im	Im
D.metel. L.	Mo, VB	Mo VB	MoVB	MoVB
N.glutinosa. L	VB, MiMo	SeVN	MiMo	MiMo

Table 10 : CONTINUED

Differential Hosts'	M-TVNS	PVY^N	PVY^C	PVY^O
Gomphrena globosa. L	VB, MiMo	SeVN	MiMo	MiMo
Capsicum annum. L 'Long Red Cayenne'	Mo	Im	Mo	Mo
Solanum demissium x S. tuberosum 'A6'	NeLL	NeLL	NeLL	Nell

Authors: de Bokx et Al 1981; Kahn et Al 1963; Delgado-Sanchez, 1966, 1970;.

Abbreviations used in Table 10

VC	:	Vein-clearing
VB	:	Vein-banding
Mo	:	Mottling
MMo	:	Mild mottling
NL	:	Necrotic lesions
SeVN	:	Severe veinal necrosis
LDt	:	Defoliation
SeN	:	Severe necrosis
Ne	:	Necrosis
NeLL	:	Necrotic local lesions

5:2 CONCLUSIONS

M-TVNS, a strain of PVY, was noticed on tobacco during the 1982 tobacco growing season in Bungoma District, Western Kenya. The virus was isolated from Nicotiana tabacum cultivar 'Speight G.28', on which it induced vein-clearing, vein-banding, mottling and veinal and stem necrosis.

Characterization and identification of the virus was carried out through symptomatology and host-range, transmission through seed of N.tabacum cultivars and aphid 'Myzus Persicae', ultra-violet absorption, electron microscopy and sero-logical reactions.

When the results were compared to other tobacco mottle and necrosis inducing viruses, the following conclusion were reached:-

1. M-TVNS is a flexous filamentous particles and thus different from TNV and TRSV which have poly-hedral particles.

2. By its modal length of 742 nm, its transmission by 'Myzus persicae' and inability to infect Datura stramonium, Gomphrena globosa and Cassia occidenta-lis and serological unrelatedness, M-TVNS was found to be different from PVX.

3. M-TVNS modal length, serological reaction and its lack of virulence on Chenopodium album differentiated it from PVS.

4. M-TVNS showed a narrow host-range, being limited to the families Solanaceae and Chenopodiaceae. It induced mottling, vein-clearing, green vein-banding and stunting on Nicotiana tabacum cvs 'White Burley', 'Samsun' and 'Heavy Western'. Necrotic lesions were observed on inoculated leaves of these cultivars. The root-knot resistant tobacco cultivar 'Speight G.28' reacted by a severe veinal and stem necrosis and cultivar 'Kentucky 35' reacted by green vein banding, vein-clearing and mottling. Leaf malformation, chlorosis, mild stem necrosis were induced on N.rustica L. These reactions suggested similarity with PVY. They also differentiated M-TVNS from PVA. PVA induces dark green vein-banding to cultivar 'Burley' but does not cause systemic necrosis on tobacco cultivars (Bartels, 1977). M-TVNS was differentiated from TEV which causes necrotic etching, chlorotic mottle, distortion and green vein-banding on tobacco varieties (Shepherd, 1974).

5. M-TVNS caused mottling and slight mosaic on Lycopersicon esculentum cultivars 'Marglobe', 'Money maker' and 'Rutger' as do PVY but did not cause necrosis and plant death induced by PVA.

PVA induces mosaic and top necrosis on potato cultivars (Bartels, 1971). These symptoms were not observed when potato cultivars were mechanically inoculated with M-TVNS.

6. In contrast to M-TVNS specificity, which causes chlorotic local lesions on Chenopodium amaranticolor and C.quinoa and necrotic lesions on Nicandra physaloides, PVA does not cause such symptoms on these hosts as reported by Bartels (1971).

7. Serological M-TVNS gave an antiserum titer of 1:1024 in microprecipitin tests. According to the considered criteria, although not exhaustive, M-TVNS was shown to be a strain of PVY.

8. By its inability to produce necrosis on all tobacco cultivars tested but only on the root-knot resistant cultivars 'Speight G.28', its ability to induce chlorotic mottle on Capsicum annuum cultivar 'Long Red Cayenne' and transmission by Myzus persicae M-TVNS was shown to be different from PVY^{O}, PVY^{C} and PVY^{N} but similar to $M^{S}N^{R}$ as causing veinal and stem necrosis only on Nicotiana tabacum cultivar bearing the gene of resistance to the root-knot nematode Meloidogyne sp and mottling on root-knot susceptible cultivars.

Plate. 4 : Non-inoculated leaves of Nicotiana tabacum cv 'Speight G.28' exhibiting veinal necrosis 7 days after inoculation.

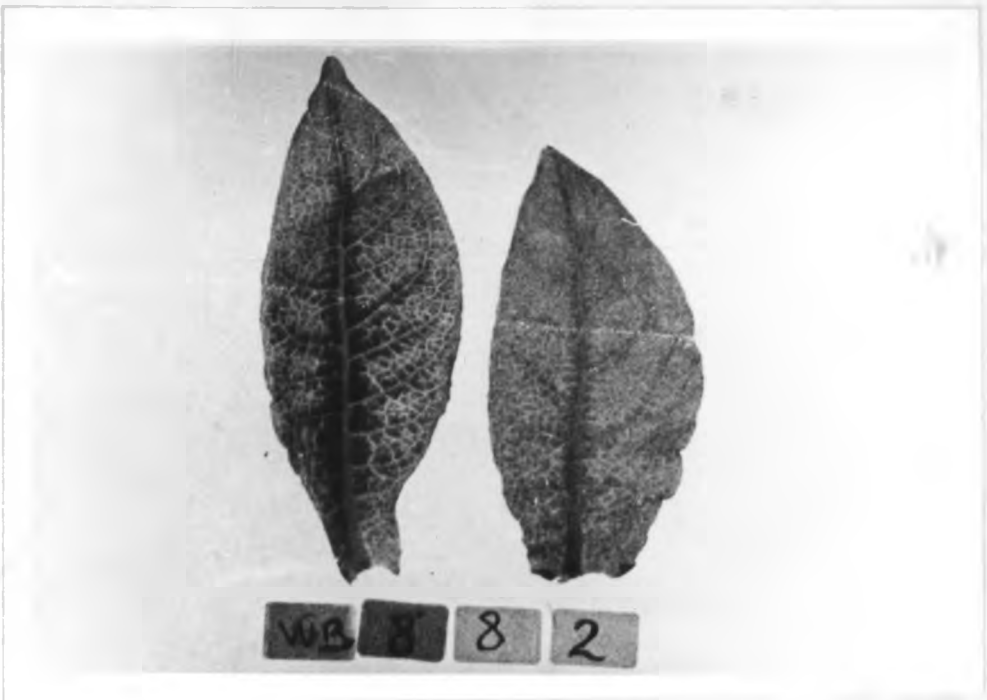


Plate.5 : Non-inoculated leaves of Nicotiana tabacum L. cv 'White Burley' showing vein-clearing 14 days after inoculation.

Plate.6 : Non-inoculated upper leaves of Nicotiana tabacum cv 'Speight G.28' exhibiting vein-clearing 24 days after inoculation of lower leaves.

Plate. 7 : Inoculated leaflet of Hybrid Solanum tuberosum.x.S.demissium, 'Aquila.6' showing tiny necrotic local lesions 6 days after inoculation.

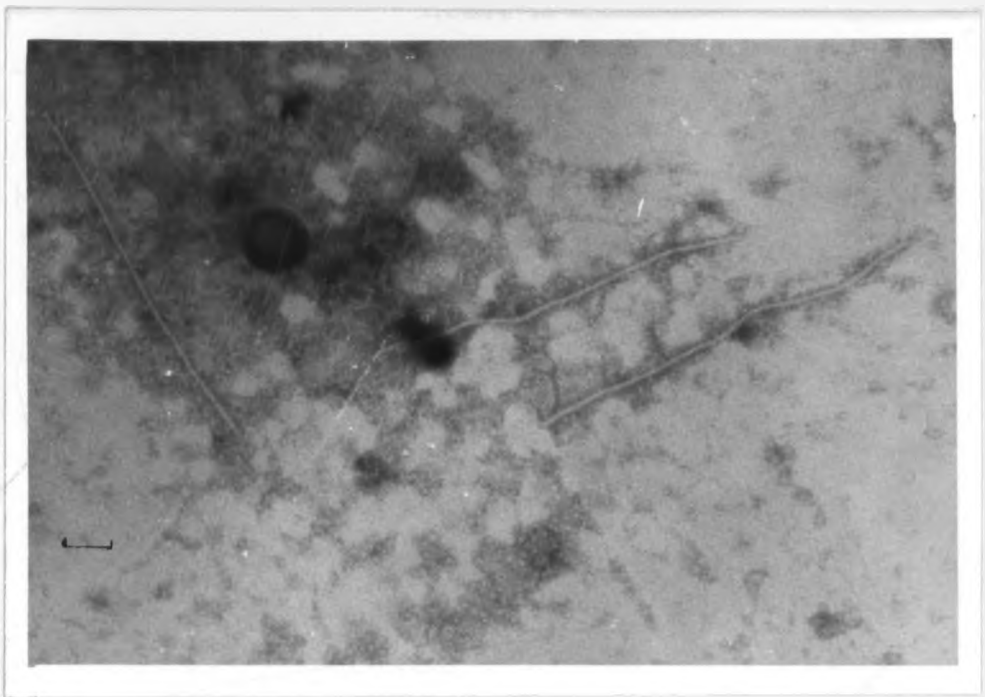
Plate. 8 : A photograph of Hybrid Solanum tuberosum.x.S.demissium, 'Aquila.6' exhibiting necrotic lesions on non-inoculated upper leaves 15 days after inoculation of lower leaves.

Plate. 9: Chlorotic local lesions on inoculated leaf of Chenopodium amaranticolor Coste and Reyn 14 days after inoculation.

Plate. 10: Inoculated leaf of Chenopodium quinoa Willd showing chlorotic local lesions 12 days after inoculation.

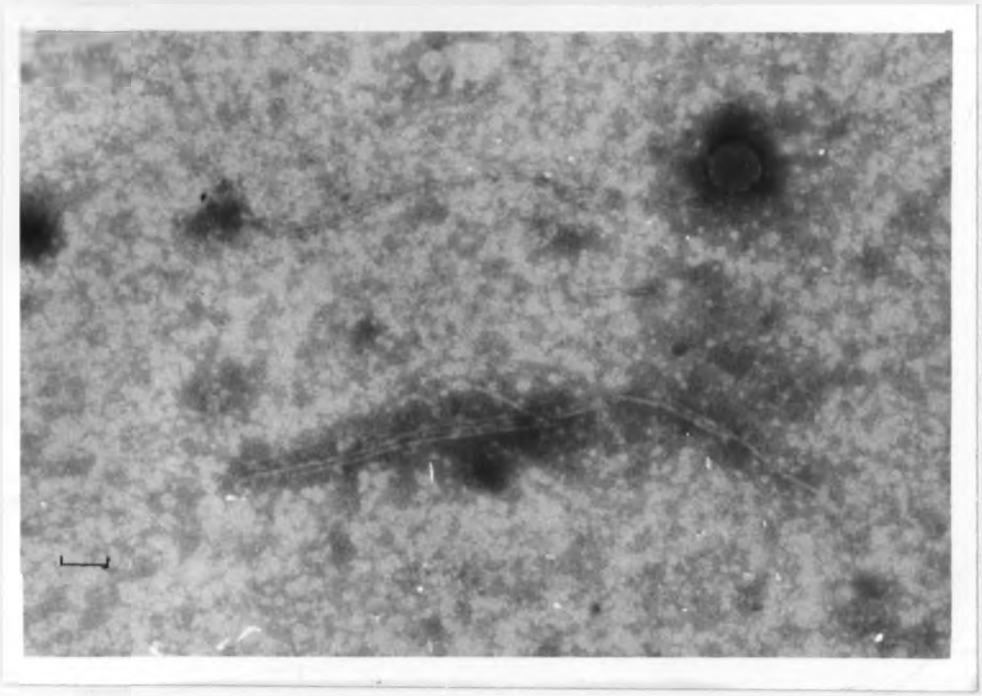
Plate. 11 : Non-inoculated upper leaf of Nicandra physaloides L showing vein-clearing and tiny necrotic lesions 20 days after inoculation of lower leaves.

Plated. 12 : Healthy (right) and inoculated (left) plants of Datura metel L showing mottling, leaf distortion and stunting 16 days after inoculation.



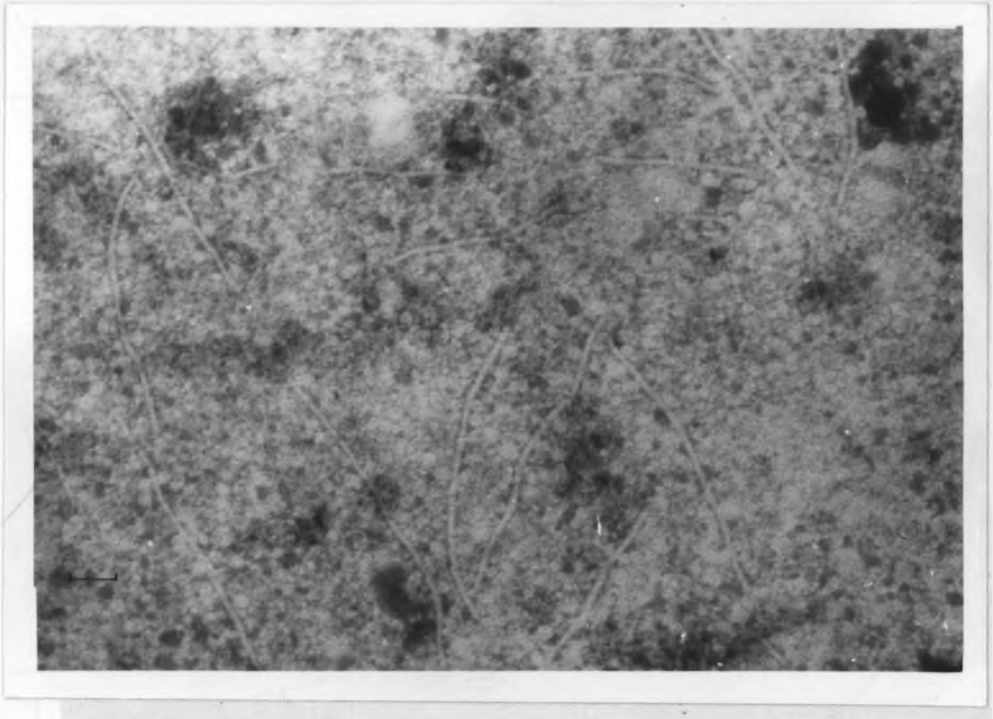
109 nm

Plate. 13 : Electron-micrograph of a leaf dip preparation from Nicotiana tabacum L cv 'White Burley' showing the presence of flexous rod virus particles stained with 2% potassium phosphotungstate (pH 6.5 at 55,000.x Magnification).



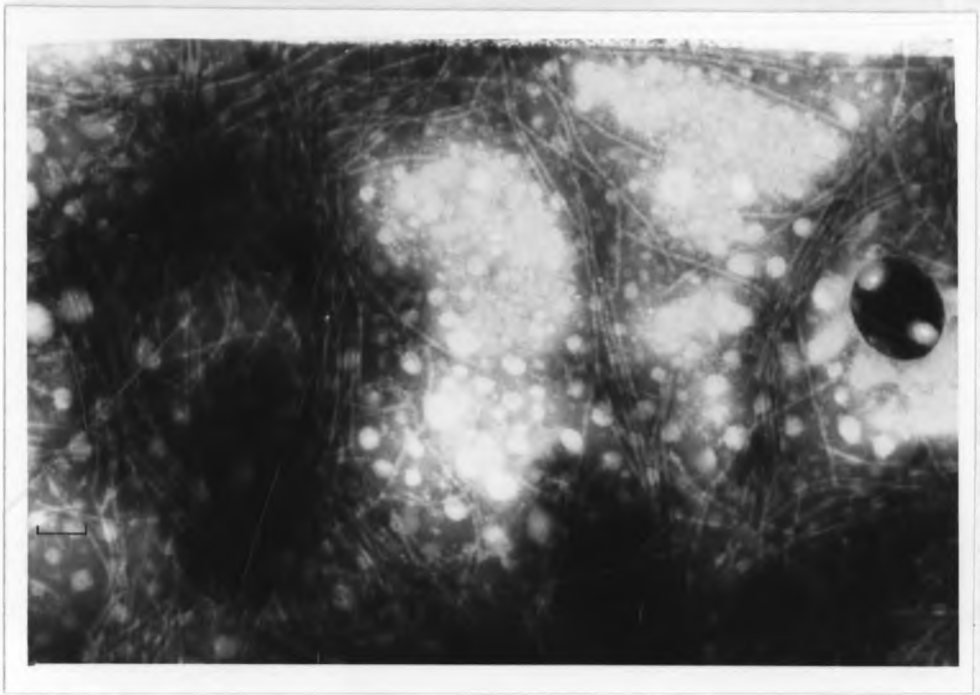
109 nm

Plate.14 : Electron-micrograph of a crude sap preparation from Nicotiana tabacum L cv 'White Burley' showing the presence of aggregated flexous rod virus particles stained with 2% potassium phosphotungstate (pH 6.5 Magnification 55,000.x).



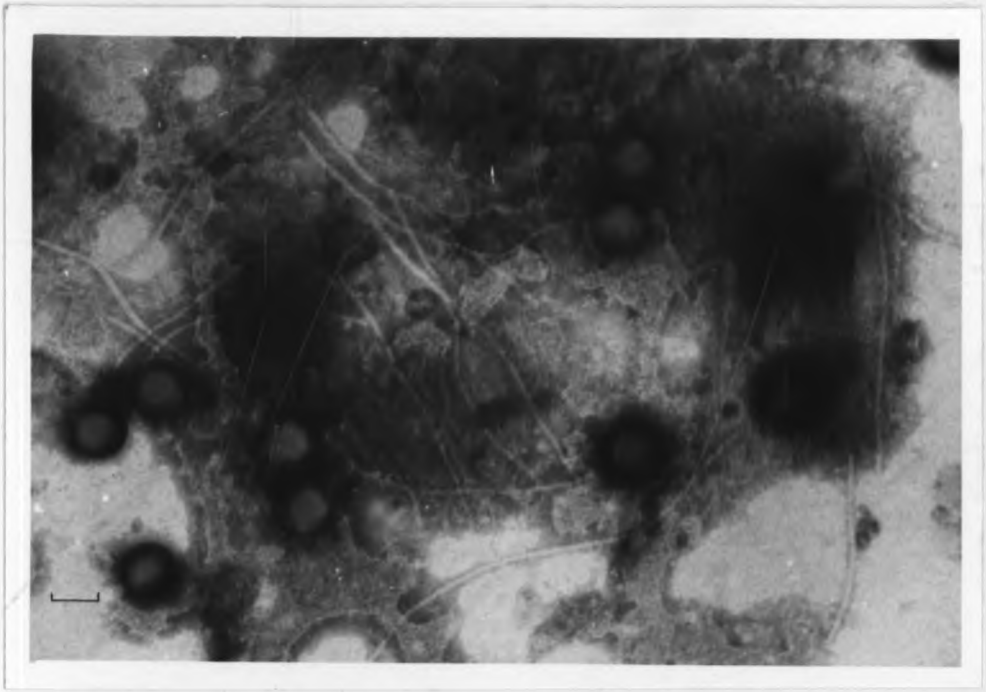
109 nm

Plated. 15 : Electron-micrograph of a partially purified preparation from Nicotiana tabacum L cv 'White Burley' sap treated with ethyl ether as clarifying agent showing relatively less breakage of virus particules. (Stained with 2% Potassium phosphotungstate at 6.5 Magnification 55,000.x).



109 nm

Plate. 16 : Electron-micrograph of a partially purified preparation from Nicotiana tabacum L cv 'White Burley' purified according to Noordam procedure. Note particle aggregation as 0.5 M urea was not added to the resuspending buffer. (Stained with 2% potassium phosphotungstate pH 6.5, Magnification 55,000 x).



109 nm

Plate. 17 : Electron-micrograph of a partially purified preparation from Nicotiana tabacum L cv 'White Burley' purified according to Noordam procedure. Note particle aggregation absent as 0.5 M urea was added to the resuspending buffer. (Stained with potassium phosphotungstate (2%) pH 6.5, Magnification 55,000.x).

CHAPITER VI : LITERATURE CITED

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