Potato virus y (PVY) in irish potatoes *(Solanum tuberosum)* and tree tomato *(Cyphomandra betaceae)* and the influence of potato susceptibility to the virus and the aphid virus-vector on the spread of PVY Mosaic in Kenya

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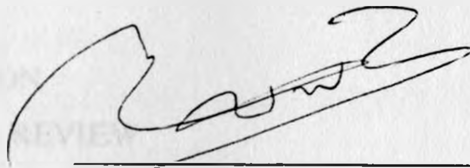

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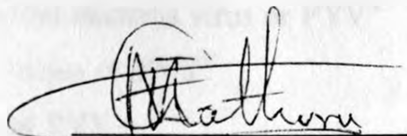

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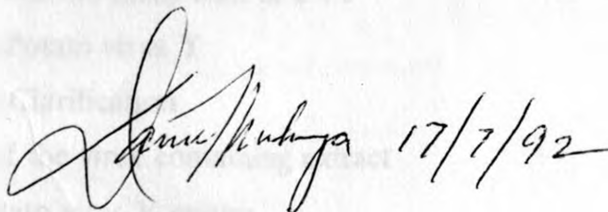

Prof. D.M. MUKUNYA

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[2]AAFA: Association of African Faculties of Agriculture

ABSTRACT

Potato virus Y (PVY) occurs on some important crops, weeds and shrubs all over the world. The most common known hosts of PVY strains are *Solanum tuberosum*, *Capsicum annuum*, *Nicotianna tabacum*, *Lycopersicon esculentum* among crops, and *Solanum atropurpureum* and *Datura metel* among weeds. PVY has been also reported as a potent pathogen of *Cyphomandra betaceae*, a fruit shrub commonly known as Tree-tomato, largely distributed in small scale farm plots all over East Africa, with its habitat stretching into Kenya, Tanzania, Uganda, Rwanda, Burundi and eastern highland of Zaire.

The natural habitat of this fruit shrub is mostly restricted to highlands of these countries where the irish potato is one of the most important staple food and cash crop for the majority of farmers. However, tree-tomato fruit is mostly consumed by the household and rarely considered as a source of income. In all these areas there is not a single tree without a virus disease like symptoms. As PVY has been reported elsewhere as being virulent to this fruit shrub, the first part of this work was aimed at ascertaining the identity of the causal agent of the endemic virus disease like symptoms observed in eastern Africa. The hypothesis was that if the causal agent is of a viral nature, then the most probable agent may be one the three viruses that have been reported on tree-tomato, namely Cucumber mosaic virus (CMV), Potato virus X (PVX) or PVY.

The second part of this study dealt with the form of occurrence of PVY strains on potato variety in two selected areas in Kenya, where the irish potato is considered as one of the most important crops.

The third and last part of this study examined the influence of the susceptibility of mostly grown potato varieties to aphid and to PVY on the spread of PVY infection in the field.

The causal agent of tree-tomato diseases was identified through host range, physical properties, electron microscopy and transmission tests.

The host range showed that only Solanaceae species were susceptible. Other families such as Chenopodiaceae, Leguminosae, Cucurbitaceae, Amaranthaceae and Compositae were not susceptible.

The fact that none of the Cucurbitaceae plant species was susceptible confirms that the virus prevailing in tree-tomato in Kenya is not a CMV strain. In addition, electron microscopic observation showed that the virus had flexuous filamentous particles and not polyhedral particles as CMV.

Observation of tested indicator plants, the physical properties, the modal length of 738 nm in a purified preparation, and the ELISA have proved that this virus is a strain of PVY readily transmissible to potato and pepper crops.

The form of occurrence of PVY strains in the two selected areas of potato production was carried out by the observation of symptoms induced by various isolates on a set of indicator plant species and varieties and by ELISA using antisera against PVY, PVX, PVS and PVA. The various symptoms observed on differential hosts showed clear deviation from PVY strains. The ELISA showed that PVY occurs rarely as single infection but mostly within a complex of two, three or four viruses. The results of ELISA tests showed that when collecting infected potato plants samples in the field, around 85.7% of the samples contain potato virus Y. However, only 16.7% of the sample were pure PVY infection. These results show that PVY can be found at the rate of 69% in the form of mixed infection with one, two or three other viruses. Association with one virus (X or A) was estimated at 25.5%, that with two different viruses at 32.5%, and that with all three other viruses at 9.5%.

Results showed that among 13 PVY isolates could be separated into two groups. The common strains PVY°, not causing veinal necrosis on tobacco varieties and the necrotic strains PVY^N, causing necrotic symptoms on *Nicotiana tabacum* var 'White Burley', 'Samsun' and 'Kentucky'. In addition, within each group, strains showed differences in their ability to infect pepper cv 'Long Red Cayenne'. The study showed an equal proportion of common strains PVY^O (7 strains) and necrotic strains PVY^N (6 strains). It equally showed that most tested strains are virulent on pepper (9/13). Thus, when not grown for commercial purposes, pepper should be considered as potential reservoir host for most Kenya PVY strains.

The susceptibility level to PVY infection also showed a wide variation among varieties (ratio of 7:1 for the 1st crop and 3:1 in the second crop). Such variation should mainly be attributed to several factors, among which the genetic resistance to the virus itself, the resistance to vectors, the initial infection rate in potato tuber seeds of each variety, and the transmission potential of the most predominant vector.

The mechanism of resistance or susceptibility to PVY has been described by several authors. However, the comparison of data on PVY rate and aphid infestation rate showed that there is a strong correlation between the two parameters. The first crop shows that there is a weak correlation (r2 = 0.25). The same fact was observed in the second trial with a more significant correlation (r2 = 0.72). However, the behaviour of 'Kenya Baraka' (lowest aphid population and highest rate of PVY) suggests that a higher susceptibility to PVY cannot be corrected by the inclusion of aphid-resistant gene into it. This means a variety should have a combination of both types of resistance to give satisfactory performance.

The other important parameter to consider is the effect of PVY infection on tuber yield. It appears that the rule that higher virus infection means a reduction

on yield does not apply with a uniform intensity on a set of varieties. This means that two varieties may have the same rate of virus infection but be affected differently on their yield. Another parameter to consider here is the genetic production potential of a variety. Among the eight varieties tested, some are classified as high, medium or low-yielding. From that assumption, a mere comparison of yields cannot be a sufficient criteria to assess the pressure of virus infection on the variety yield. Among the eight varieties, 'Annet', 'Kenya Baraka', 'Feldeslohn', 'Dutch Robjin' and 'Bvumbe' are considered as high producers and can yield over 40 tonnes per ha, while 'Kerr's Pink', 'Pimpernell' and 'B.53' are considered as medium and yield between 20 to 40 tonnes per hectare. The obtained production shows that varieties with the highest PVY rate during the second crop, namely 'Kenya Baraka', 'Dutch Robjin' and 'Feldeslohn' have recorded the highest yield reduction varying from 47.8 to 52.5%, while varieties with the lower PVY rate, notably 'Pimpernell' and 'Annet' have a lower yield reduction varying between 22.3 - 23.9 %. The intermediate level shows the same trend, this group includes 'Bvumbe' and 'B.53' with a reduction of 33.8 to 35.7%, while the variety 'Kerr's Pink' is in a group of its own where we find the highest PVY rate (52%) corresponding to a comparatively low reduction (29.6%) in yield as compared to the highest and the average group.The conclusion is that reduction should be considered as a more reliable parameter to assess than the yield itself.

INTRODUCTION

Among crops grown in Kenya, maize (*Zea mays* L.), beans (*Phaseolus vulgaris* L.) and potatoes (*Solanum tuberosum* L.) are the most important food crops. Potato occupies the third position after maize and beans in both acreage and production (Annon, 1984).

Potatoes are grown in the Kenya highlands, between 1,500 to 2,700 m. The most favourable areas for potato production in the country are Meru and Embu in Eastern province, the Western part of Abedares, Ol-Kalou and Kinangop in Central Province and Molo and Mau Narok in Nakuru district in the Rift Valley province. The less favourable areas, where it is grown as a subsistence crop include Uasin Gishu, Trans Nzoia, Nandi, Kericho, Machakos and Taita Taveta districts (Durr and Lorenzyl, 1980).

In 1980, potato crop was estimated to occupy about 55,700 ha. Crop forecast surveys of 1985 estimated potato production at around 590,000 metric tonnes (Annon, 1985). All of it was consumed locally. The above figures are mere estimates and do not translate the real situation in the field. In Kenya, the average production of 5 tonnes per hectare is common in most potato growing areas, except in Meru, where yields reach 9.4 tonnes per hectare. In Britain, Holland and the United States, a well managed potato crop, gave an average yield of 26.4 - 40.3 tonnes per hectare (Hide and Lapwood, 1978). Thus, there is potential for export but this cannot be met due to low yield per unit area of land.

Several factors have been identified and associated with low potato yields. The major ones are poor farming system, lack or non use of inputs like fertilizers, pesticides and certified seed, insufficient rainfall, pests and diseases. Among those, pests and diseases are the most important. Among the diseases, late blight, bacterial wilt and potato scab caused by *Phytophthora infestans, Pseudomonas solanacearum* and *Streptomyces scabies* respectively, are the most important (Annon, 1974). However, most farmers are vaguely if not unaware of such

diseases and attribute such blight, wilt or other crop calamity to frost or lack of sufficient rainfall. Among large scale farmers, a few use chemicals to control these diseases.

Thus, to minimise losses caused by fungi and bacteria on crops, governments of developing countries spend great sums of scarce foreign exchange to import various pesticides. For instance, between 1978 and 1984, the Kenya Government imported pesticides worth more than 1.4 billion Kenya Shillings (Annon, 1985). Unfortunately, fungicides do not assure total protection of crops against diseases. Experience has shown that when emerging strains of a pathogen such as *Phytophthora infestans* develop resistance to fungicides, it therefore becomes difficult to control them. While chemicals assure only partial protection against fungi, they do not control plant viruses at all.

Bock (1976), showed that in a potato field free from pests, fungi and bacteria, virus diseases were equally significant in yield reduction. However due to limited information about viruses and the diseases they cause, control of viruses has been overlooked. Furthermore most farmers are not aware of viral diseases and their effect on yield reduction. This is due to the fact that most virus diseases of potatoes are often symptomless or latent.

Viruses pathogenic to potato crop in the country are: Potato leaf roll virus (PLRV), Potato virus Y (PVY), Potato virus (PVX), Potato virus S (PVS), Potato virus A (PVA) and alfalfa mosaic virus (AlfMV) (Annon, 1974). Among these viruses, PLRV and PVY cause great yield reduction. For instance PLRV has been reported to cause yield losses between 50 and 68.5 per cent (Annon, 1974). Strains of PVY have been reported as causing yield losses of between 30 and 90 percent (de Bokx and Huttinga, 1981).

Apart from studies carried out by Bock and Robertson (1976) and Bondole *et al* (1986), no systematic work has been done to determine the range of variation of

PVY in the country and the implications of such variability to breeding PVY resistant potato varieties. This study will offer a positive impact on potato breeding in the country.

PVY strains are aphid transmitted and tuberborne. Their main vector, the green peach aphid (*Myzus persicae*. Sulz) and bean aphid (*Aphis fabae*. L) are widely distributed in most potato growing areas and multiply throughout the year (Eastop, 1957, Le Pelley 1959). This double way of transmission and favourable conditions of aphid population build up have contributed to the spread of PVY and other aphid-borne potato viruses in the country. However the endemic state of this virus in Kenya is mainly due to the use of infected seed potato stocks by small scale farmers.

The use of infected tubers by unaware farmers, year after year will increase the incidence of infected seed-potatoes and brings the danger of a degeneration of potato crop in the country. Furthermore, viruses can not be controlled by any known pesticides but rather through evasive measures designed to reduce source of inoculum inside and outside the crop, to limit spread by vector and thus minimize the effect of infection on yield. All those measures form the most important part of a certification programme in a country.

The most reliable and effective way to control plant viruses in the use of resistant varieties. Resistance to PVY in potato is often of the hypersensitive type and strain specific. A variety resistant to a PVY strain may be susceptible to others. Thus whenever a resistant variety is cultivated widely, a new strain of the virus will arise to attack it. Thus, it is of great importance to study the range of variation of PVY isolates existing in the country and to test all local and exotic potato varieties to all defined or well differentiated strains. This study will provide valuable information on potato variety resistance to PVY strains.

The second way to control potato viruses is by the use of virus free seed potato stocks. This is achieved by the production and distribution of high quality certified seed-potatoes. The first step in the production of such stocks is the choice of a location, with unfavourable conditions to aphid population build up. Although such a location may exist in Kenya, like the Kibirichia area, a low incidence of PLRV and PVY was detected in those areas (Annon, 1974). Tubers from such an area, with low incidence of infection (5%), are still a potential threat when given and planted in an area where aphid population is higher. Thus the best way is to have the ability to detect all infected plants and if a field or plot reaches a critical virus incidence level (2%), it should be discarded as source of certified seed.

In Kenya, potato tubers carry PLRV, PVA, PVS, PVX and PVY. Detection of such viruses by mere visual observation of symptoms is not very dependable. Often potyvirus infection tends to remain latent at the early stage of growth and can remain so for the whole plant growth cycle. Therefore to locate diseased plants in the field or in a bulk of seed-potatoes requires the use of sufficient sensitive techniques. These methods are designed for testing leaves and tubers. The most used are based on the reactions of differential plant species, various serological tests and electron microscopy.

Given the economic importance of viral diseases on potatoes in the country, lack of concise studies on the variation of major viruses, methods of screening potatoes for resistance and proper methods for the detection of these viruses, this study intended to achieve the following objectives:

1. To identify the virus strain associated with Tree-tomato (*Cyphomandra betaceae*) and determine its importance on potato crops.
2. To determine the range of variation of PVY in selected areas of Kenya.
3. To determine in which forms PVY strains occur in the field, their relative importance compared to other viruses infecting potatoes.

4. To assess the susceptibility level of commercially grown potato varieties to PVY and the aphid virus-vector in the field.
5. To establish the effect of aphid infestation level on the rate of PVY infection under field conditions.
6. To assess the correlation between PVY infection rate and the tuber yield of commercial potato varieties in Kenya.
7. To determine suitable varieties to be considered for further improvement such as interspecific cross-breeding with one of the wild potato species with a higher resistance to aphids.

Such approach is the most appropriate for developing countries, which in most cases do not have adequate facilities for the production of pesticides and certified seed.

LITERATURE REVIEW

General properties of Potyviruses

Members of this group of viruses are flexuous rods with single stranded RNA. They are grouped on the basis of the most common length of their particles. All potyviruses have a modal length between 720 and 900 Serologically, potyviruses are related (Brandes and Wetter, 1959, Brandes and Berks, 1965, Edwardson 1974).

They have aphid species as vectors and are mostly transmitted in non-persistent manner. They are also transmitted through seed, tuber, pollen grain, the ovule and the flowers of infected plants. Few potyviruses are transmitted by dodder (Lambers, 1972; de Bokx and Huttinga, 1981).

Potyviruses have a relatively low stability in crude sap and are generally considered as thermolabile. However thermal inactivation points of potyviruses vary according to strain differences. For instance Bean common mosaic virus (BCMV) strains differ markedly in their thermal inactivation point (Bos, 1971, Buruchara, 1979).

Potyviruses have a dilution end point close to 10^{-4}. However for few members, such as PVY infectivity is lost at 1:40 dilution and few others such as BCMV and PVY strains remain infective up to a dilution of 10^{-6} (Bartels, 1971, Fribourgh and de Zoeten, 1971, de Bokx *et al*, 1982).

They are described as highly susceptible to aging. Papaya ringspot virus (PRSV) does not survive beyond 8 hr, whereas few strains of BCMV, PVY and TEV retain their infectivity up to a duration of 50 to 60 days (Shepherd and Gleen, 1960; Yerkes and Graciano, 1960; Brierly and Smith 1962; Chagas *et al*, 1977).

Except for few members such as BCMV, virus strains of this group can not be differentiated on the basis of physical properties alone.

Nomenclature, Distribution and Economic importance of PVY

Johnson (1930) reported a virus on tobacco, which he named 'Tobacco vein-banding virus'. This was the first reported strain of PVY in tobacco and it was named according to the morbid anatomy. This was a strain of PVY^0 group (de Bokx and Huttinga, 1981).

Holmes (1939), when subdividing viruses into those which infect bacteria, higher plants and animal and by using binomial system, called PVY '*Marmor epsilon*'. Smith (1937), preferred to identify the virus by using the generic name of the crop followed by a number. As the virus was predominant on potato, he thus referred to the virus as 'Solanum virus 2' (Smith, 1937).

Holmes (1939) felt that knowledge on viruses was still inadequate for a proper identification or nomenclature. He suggested that the method of nomenclature should be somewhat based on abnormal or morbid anatomy and pathophysiology. This suggestion brought up first names of plant viruses that are still being used to day. According to symptoms induced on differential potato varieties, the virus was named 'potato acropetal mosaic virus'. Bawden and Kassanis in 1947, isolated for the first time a necrotic strain from tobacco and designated it as Tobacco veinal necrosis virus or PVY^N. This strain was later considered as the strain type for the group PVY^N (Beemster and Rozendal, 1972). Simons (1956) reported another PVY strain from pepper, which was named as pepper vein-banding virus (PVBV-PVY).

PVY^O Strains occur worldwide. However PVY^N strains occur largely in Europe, Africa and South America. PVY^C, including PVC, occur in Australia, India, parts of the United Kingdom, continental Europe and South America (Barghava and Joshi, 1959; de Bokx and Huttinga, 1981, Annon, 1981).

PVY strains are of economic importance on potato and tobacco crops and in combination with other viruses e.g. Tobacco mosaic virus' (TMV). PVY causes

significant yield losses on pepper and tomato. On potatoes and tobacco, and depending on the strain and variety, yield losses may vary from 30 to 90 percent (Simons, 1950; de Bokx and Huttinga, 1981).

Diseases caused by PVY strains

PVY strains are causal agents of vein-clearing, mottling, mosaic, green vein-banding and necrosis of veins, leaves and stem, stunting, leaf drop, streak and death of *Solanum tuberosum, Nicotiana tabacum, Capsicum annuun, Lycopersicon esculentum* and *Cyphomandra betaceae*.

Diseases caused by PVY strains on potato varieties

Symptoms in potato vary widely with strain and variety. They range in severity from latent or mild symptoms, to severe necrosis or to the death of infected plants.

Strains of PVY^O group are the most important on potato varieties and evoke much more severe symptoms than PVY^N. Strains of PVY^0 group cause stunting, severe mosaic and rugosity on the leaflet surface, vein streak or leaf drop streak with necrosis along the veins on the underside of the leaflet. By their severity on potato varieties, these strains are easily detected by visual observation. However they have a relatively slow translocation from leaves to tubers. Potato varieties often show mature-plant resistance to PVY strains and their spread can easily be reduced by roguing plants with conspicuous symptoms (Bock and Robertson, 1976).

Strains of PVY^N cause rather very mild symptoms. They can remain latent in the host and symptoms only show late in the growing season. However, PVY^N strains spread more rapidly in the field than PVY^O strains. The rapid spread is due to several factors, the most important being the overlooking of infected plants during roguing because of vague symptoms, rapid translocation from leaves to tubers and less marked mature-plant resistance toward PVY^N. They multiply

better at relatively high temperatures (22°C - 26°C) than low temperatures (15-18°C) (Beemster and Rozendal, 1972).

Strains of PVY^C induce almost the same symptoms as PVY^0 strains but evoke stipple streak symptoms. Most potato varieties are hypersensitive to these strains. Aphid species transmit some strains of this group with great difficulty if at all (Weirsema, 1972).

Tobacco diseases

Only strains of PVY^0 and PVY^N have so far been reported as causing tobacco diseases in the field (Kahn and Monroe, 1963). Most tobacco varieties react to PVY^N with vein-clearing, mild to extreme mottling, green vein-banding and leaf narrowing. Lower leaves show an extreme white mottling. These symptoms are common on varieties 'White Burley', 'Kentucky', 'Samsun' and 'Turkish' (Lucas, 1975; Delgado-Sanchez, 1966). The diseases caused by this group of strains is also called tobacco vein-banding disease.

PVY^N strains cause vein-clearing which change into veinal necrosis. This necrosis extends through the midrib to the stem and causes death of the plant. Some strains of this group cause necrosis only on root-knot nematodes resistant varieties such as 'Speight G. 28' and just mottling on root-knot susceptible varieties. The strains are often referred to as 'Tobacco veinal necrosis virus (TVN-PVY) (Kahn and Monroe 1963; Bondole *et al*, 1986).

Disease of Tree-tomato

Although PVY was isolated from this crop in India by Bhargava and Joshi in 1956, no authors have specified to which PVY strain group this isolate belongs. A similar isolate was found in Uganda by Bock and Robertson (1974) and is to day widely distributed in Kenya (Annon, 1979). Symptoms induced in tree-tomato consist of dark green or brown vein-banding, mosaic, blistering, malformation

and distortion of leaves. Severe stunting and plant death has been noticed as well.

Tomato disease

On tomato, PVY strains cause mild mottling and have less effect on yield reduction. But when PVY is associated with other viruses such as TMV, losses on yield are rather high (Simons, 1956).

Pepper Disease

The effect of PVY on pepper varies greatly with strain and variety. General symptoms consist of mottling and green vein-banding. However most strains of PVY^N group are avirulent on pepper (Annon, 1981). Bock and Robertson (1976) also found that Kenya PVY isolated from pepper could not be aphid transmitted from pepper to potato varieties. The pepper isolates of PVY are often referred to as pepper vein banding virus (PVBV-PVY).

Variation of PVY and differential hosts

PVY shows a wide range of variation. It comprises many strains, classified into three distinct groups. These groups are differentiated on the basis of symptoms induced mainly on potato and tobacco varieties. Besides potato and tobacco, other standard differential plant species are *Nicotiana glutinosa, Solanum demissium* Y (SDY), *Solanum demissium* A (SDA), The Hybrid *Solanum chacoense* (TE_1), *Physalis floridana* L., *Capsicum annum* L. cv 'Long Red Cayenne, *Datura stramonium* and *Datura metel*. Of those, *Datura stramonium* and *Solanum demissium* A, are immune to all PVY strains. *Datura stramonium* is used to detect the presence of PVX and *Solanum demissium* A, to check a double infection with PVA. Cases of such double infections (PVY-PVX or PVY-PVA) are common in potato fields (Kahn and Monroe, 1963; Delgado-Sanchez, 1966; de Bokx and Huttinga, 1981).

The three groups of strains of PVY strains are: the common strains PVY^0, the necrotic strains PVY^N, known also as 'Tobacco veinal necrosis virus' and the non-aphid transmitted group known as PVY^C. PVY^C group includes also PVC (de Bokx and Huttinga, 1981).

The Common Strains or PVY^0 group

Differential hosts react as follows to these strains:

Solanum tuberosum L: Most varieties are severely affected by the strains of this group. Symptoms exhibited are stunting severe mosaic and rugosity of the leaflets. Sensitive varieties react with necrosis which may affect only some veins on the lower surface of the leaves or may form severe necrosis on leaves and stems. Such severe necrosis may ultimately cause older leaves to collapse and either drop from the plant (called leaf drop streak) or remain hanging (called palm tree type) (Beemster and Rozendal, 1972).

Nicotiana tabacum. L: cvs 'Samsun', 'White Burley','Kentucky' and 'Heavy Western' react with mild mottling, vein-banding and sometimes with necrotic local lesions on inoculated leaves. However, all tobacco varieties do not develop systemic necrosis or plant death.

Nicotiana glutinosa. L: react by mild mottling, does not develop stunting or leaf narrowing. Leaf production is not affected. Thus *Nicotiana glutinosa* is often used as multiplication host for PVY strains (Delgado-Sanchez and Grogan, 1966).

Capsicum annum L. cv 'Long Red Cayenne'. This variety reacts to PVY strains by mild to conspicuous mottling and often by green vein banding (Kahn and Monroe, 1963).

Physalis floridana. L: develops local and systemic necrosis and mottling.

'Aquila 6' reacts by necrotic local lesions.

Solanum chacoense (TE1). reacts also with necrotic local lesions.

Solanum demissium Y. develops necrotic lesions on mature detached leaves.

The tobacco veinal necrosis virus or PVY^N -

Strains of the PVY^N group cause very different symptoms on potato, tobacco, pepper and *Physalis floridana*. PVY^N strains can be detected by the following reactions of differential hosts:

- *Solanum tuberosum* L: Infection by strains of this group remains latent at the early stage of potato growth and appears late in the season with mild or vague symptoms (Beemster and Rozendal, 1972).

- *Nicotiana tabacum* L: Tobacco varieties react with systemic necrosis on veins and stems, leading to plant death.
- *Nicotiana glutinosa*. L: reacts with similar symptoms to PVY^N as to PVY^0 strains.
- *Capsicum annum* L. cv Long Red Cayenne'. This variety and other differential peppers are immune to strains of this group.
- *Physalis floridana:* contrary to reactions induced by PVY^0 strains, *Physalis floridana* reacts to PVY^N strains with mottling.
- 'Aquila 6'; *Solanum chacoense* (TE1) and *Solanum demissium* Y react with necrotic local lesions to PVY^N.
- *Datura metel* L: reacts to PVY^N with mottling and green vein-banding.

Stipple streak strains or PVY^C

On *Solanum tuberosum.* L. varieties, PVY^C strains, including PVC, cause similar symptoms as PVY^0 strains. However most commercial potato varieties are hypersensitive to PVY^C strains. On all other differential hosts, PVY strains evoke the same strains as PVY^0 group.

Other variants of PVY

Gooding and Tolin (1973) found that strains of PVY isolated from tobacco in the Southwestern U.S. could be differentiated into three strains. The first strain (M^SM^R) caused mottling and chlorosis on both root-knot resistant and susceptible varieties. The second (M^SN^R) caused necrosis on root-knot resistant varieties but not on root-knot susceptible varieties; and the third (N^SN^R) caused necrosis on

both root-knot resistant and susceptible varieties. However potato varieties or other differential host reactions to these three strains were not investigated.

Bondole *et al* (1986) isolated a necrotic strain from *Nicotiana tabacum* cv 'Speight G. 28' and White Burley' in Western Kenya. The isolate induced systemic necrosis on root-knot resistant variety 'Speight G. 28' but not on susceptible variety 'White Burley'. This strain was different from PVY^N strains, which induce systemic necrosis on all tobacco(Kahn and Monroe,1963). It also infected capsicum annum cv 'Long Red Cayenne' which is immune to most PVY^N strains. This strain was also pathogenic on potato varieties, on which it induced late mild mottling like other strains of PVY(n) group. The isolate was designated as Malakisi tobacco veinal necrosis virus - (Ma-TVNV-PVY).

A new potyvirus, code named UF, was isolated and identified by CIP scientist (Annon, 1981). This strain does not produce local lesions on leaves of 'Aquila 6' but is systemic producing vein-necrosis. It produces local lesions in *Solanum demissium* Y and *Solanum chacoense* as do other PVY strains. It was aphid transmitted from *Nicotiana occidentalis* to potato cultivars. Potato varieties 'Kennebec', 'Radosa' and Wauseon' develop partial vein necrosis and low leaves chlorosis with green areas. Varieties 'Clavela', 'Maria tropical' and 'Arran Pilot' react with systemic chlorotic spots, mosaic and deformation of leaf margins. The strain UF did not react serologically against PVY^0 and PVY^N. However it did give a positive reaction against an antisera of PVY^C from Netherlands. UF and PVY^C behave similarly when grafted to potato and on other indicator hosts. The main difference is that the UF strain is aphid transmitted while most PVY(c) are not (Annon 1981).

PVY strains causing pepper vein-banding have been reported as inducing mottling in *Zinnia elagans* L, which is usually immune to other PVY strains (Simons, 1956).

Multiplication and bio-assay host of PVY

Nicotiana tabacum cvs 'Samsum', 'Xanthii', Turkish' and 'White Burley' have been reported as suitable multiplication hosts for PVY (Huttinga, 1973, de Bokx and Huttinga, 1981). However some tobacco varieties are severely affected by the necrotic strains of PVY^N group. This is the case of 'White Burley., 'Samsun', and 'Speight G.28' which react with severe reduction of leaf area and thus produce an insufficient quantity of infected leaves for purification (Kahn and Monroe 1963, Bondole *et al.* 1986).

Nicotiana glutinosa is known to react to most PVY strains with moderate mottling and less reduction of leaf mass (Delgado-Sanchez, 1966). Other varieties of *Nicotiana tabacum* such as 'Kentucky' and 'Heavy western' behave as *Nicotiana glutinosa* and were successfully used as multiplication host (Bondole, *et al*, 1986).

Reported local lesion assay hosts for PVY are *Chenopodium amaranticolor, Chenopodium quinoa, Solanum demissium* Y, *Solanum chacoense* and the hybrid 'Aquila 6' (Delgado-Sanchez, 1966, de Bokx, 1972, Annon, 1981). *Chenopodium* spp are raised from true seed and are easy to maintain permanently in great numbers in the greenhouse for virus detection routine tests. The hybrid 'Aquila 6' reacts to most PVY strains except to the newly identified strain U.F. (PVY). *Solanum demissium* Y and *Solanum chacoense* react to all PVY strains differentiated so far.

Purification of Potato virus Y

More recent works in the ultrathin sectioning and biochemical studies of plant cell constituents have shown a variety of ways in which the particles of plant viruses differ from the normal constituents of their host cells (Gibbs and Harrison, 1976).

Thus, the aim of purification is to produce fully active virus preparation as free as possible from host material. Purification aims also to obtain as little as

possible distortion of shape and size of virus particles (Huttinga, 1973). A purified preparation with less plant materials and less distortion of virus particles is mostly preferred for antiserum production and assessment of the virus particle diameter and length. Purification of plant viruses follows the following steps: extraction of crude sap, stabilisation of the sap, clarification and fractionating of the virus containing extract from other host materials and concentration (Noordam, 1973).

Extraction and Clarification

Infected leaves are usually crushed mechanically using, for example, a pestle and mortar, or an electric blender to liberate their sap. This sap is usually filtered through muslin to remove cells and fibre etc. (Matthews, 1981).

However, sap from plant species is slightly or very acidic and contains various polyphenol oxidases and tannins produced from oxidized phenolic compounds (Matthews, 1981). It had been noticed that most plant viruses precipitate irreversibly when sap acidity reaches their isoelectric point, which for most potyviruses is around pH 4 (Damirdagh and Shepherd, 1970). Some plant viruses, including potyviruses, are inactivated by intermediate products of polyphenol or agglutinated by tannin (Gibbs and Harrison, 1976)

The effect of sap pH and these inactivating or precipitating systems may be minimized in various ways. For potyviruses, the crude sap is stabilized at pH 7.2 - 9, by grinding leaves in higher molar buffer containing one of the various reducing agents such as sodium sulphite, thioglycollic acid, mercaptanol, etc., and chelating agents such as diethyl dithiocarbamate (DIECA), ethylene diamine tetra-acetate (Gibbs and Harrison, 1976).

Potyviruses are best extracted in phosphate buffer pH 7.2 or borate buffer at pH 9, containing one of the reducing agents (Huttinga, 1973). By extracting and clarifying BYMV, PMV and PYV^N in these two buffers, Huttinga (1973)

obtained a purified preparation with homogenous particle sizes and without aggregation. However, this method should not be considered as standard for other PVY strains.

Fractionating of the virus containing extract

Virus containing extract can be separated from the other host components in sap and concentrated by moderate or higher centrifugation force or by addition of higher molecular weight compounds such as polyethylene glycol (PEG) (Gibbs and Harrison, 1976). However potyviruses are very unstable and prone to breakages, thus, do not stand chemical treatment and higher centrifugation force (Huttinga, 1973). Thus moderate centrifugation at 26,500 g is preferred for fractionation of PVY isolates from host materials.

Serology of Potato virus Y strains

Antiserum of PVY and its relationships to other potyviruses

PVY strains are strongly immunogenic. Antisera with homologous precipitin titer of 1:512 to 1:4096 have been produced in rabbits injected with purified preparation emulsified with Freud's incomplete adjuvant (de Bokx and Huttinga, 1981). Apart from the virus concentration and the purity of the suspension, the injection route and the interval between injection are very important and change with the virus for immunization (Noordam, 1973).

The titer of anti-PVY varies with the applied test. It shows a titer of 1:2048 in precipitin test, 1:4096 in ELISA and less or just 1:128 in the immuno-diffusion test (Van Slogteren, 1972, Sampson and Taylor, 1968). However the relationship of PVY to other potyviruses is mainly assessed through agar immuno-diffusion test (Purcifull and Shepherd, 1964). Due to their modal length preventing potyviruses to diffuse in agar gel, they require to be degraded by various substances. The most successfully used for PVY degradation being Sodium dodecyl sulphate (SDS) (Purcifull and Shepherd, 1964).

Strains of PVY, although closely serologically related, exhibit considerable serological variability. PVY is serologically distantly related to tobacco etch virus, henbane mosaic and potato A viruses (de Bokx and Huttinga, 1981, Purcifull and Gooding, 1970).

Resistance to PVY strains in the potatoes

According to several tests conducted by CIP scientists, effective resistance to PVY strains occur in some potato varieties (Annon, 1981).

Hypersensitivity to PVA, PVX^B, PVY^C and PVX is determined by the genes Na, Nb, Nc and Nx respectively. In the case of PVY, Nc gene controls only the unimportant strain PVY^C. The gene Nc, determining top necrosis response in potatoes to PVY^C is found in about half of the cultivated varieties. Most of those varieties are however not hypersensitive to the more important PVY^0 and PVY^N strains (Cockerham, 1959; de Bokx, 1972, Wiersema, 1972).

A few varieties possess all four genes; Na, Nb, Nc and Nx for hypersensitivity to all potato viruses; e.g. 'Ambassadeur', 'Commandor', 'Craigs', 'Defience', 'Graigs', 'Snow-white' and 'Ulser Knight' (Wiersema, 1972). Such varieties are immune to PVA, PLRV, PVX and PVY. Unfortunately, apart from a few varieties, most possess only one of the four genes. The consequence of this type of resistance in cultivated varieties is that resistance to PVY is strain specific. Since no gene for hypersensitivity to PVY^N and PVY^0 are available and this type of resistance is only strain specific, breeding for extreme resistance or immunity is preferred by most breeders nowadays. Such extreme resistance or immunity was found to be controlled by either two of the four genes. This type of resistance is found in some clones of *Solanum tuberosum* spp *andigenum, Solanum chacoense* and *Solanum stoloniferum* (Cockerham, 1959, Wiersema, 1972, Annon, 1981).

Potato resistance to aphids and its implication on the spread of virus diseases in the field.

Until the end of the seventies, there was little information about the effect of potato resistance to aphids on the spread of virus diseases. This is still due to lack of collaboration between entomologists, breeders and virologists. An integrated approach combining potato resistance to virus and to aphids could be of great contribution to the control of field spread of non-persistently transmitted potato viruses, mainly in the less developed countries, where the major producer (The small scale farmers) cannot afford costly certified seed potatoes.

The difficulties encountered in the control of non-persistently transmitted potato viruses such as PVY, PVA and PVS, lies in their very mode of transmission. Kennedy *et al* (1962), remarked that the very rapid acquisition of non-persistently transmitted viruses and the frequently repeated alighting, probing and take off which is characteristic of alates, forms an ideal combination for the spread of virus diseases. For this reason, breeding potato for resistance to aphids as means to control virus diseases has been overlooked for many years. However, in the forties, various workers in Europe and America noticed varietal differences to aphid infestations (Adams 1975, Gibson 1971a). Glandular hairs on potato stem and leaves were described as a possible means of limiting aphid damage to potato crops (Gibson 1971b). They showed that three glandular pubescent species, *Solanum polyadenum Grenm*, *Solanum berthaultii* and *Solanum tarijense Hawkes* have high densities of 4-lobes (Type A) and simple glandular trichomes (Type B) on leaves, stems and petioles. Upon contact by an insect, a viscous exudate is released and accumulated on the insect's body. For the green peach aphid, *Myzus persicae* (Sulzer) and potato aphid, *Macrosiphum euphorbia* (Thomas), the exudate typically accumulates on tarsi impending the insect's movements or trapping the insects on the leaf (Gibson and Turner, 1977). The trichome exudate also accumulates on the insect's mouth parts, preventing feeding and leading to considerable mortality. These exudate were found to be

polyphenol compounds (Gibson and Turner, 1977). The ability of a potato variety to produce these exudate was referred to as the polyphenol oxidase activities (PPO) of its glandular hairs (Raman, 1985). However, if a high density of trichome glandular hairs is a measure of resistance to aphid, no correlation has ever been found between hair density and the intensity of PPO activities. No study has been done on all Kenya commercially grown potato varieties on the relation between their susceptibility to aphid and to PVY infection or any other aphid transmitted virus. A study carried out by Nderitu (1983) on eight potato varieties showed no significant correlation between vegetative factors (growth rate, leaf areas, leaf shape, colour of stem and tuber) to the rate of aphid infestation on those varieties, while it revealed a strong correlation between the glandular hair density and the rate of aphid population on each variety. Unfortunately as an entomologist the author did not consider the effect of the glandular hair density and the rate of aphid population on the susceptibility of the local varieties to PVY in the field under Kenya conditions. The resistance to aphids has been shown to depend more on the nature of the chemical secreted by glandular trichomes than on their density. This fact explains why most cultivated potato varieties (*Solanum tuberosum*) do not show great variations in their PPO activity, while the PPO activity is higher on the leaves of Colombian wild potato species (Ryan *et al*, 1983). Among the latter, the most used as source of resistance to aphids are *Solanum berthaultii* and *Solanum polyadenum*. The current trend at CIP and most potato growing countries is to assess their local varieties on their susceptibly to aphids and to determine the most suitable to be used as one of the parents in the crossing with one of the wild aphid resistant potato species.

Field and Laboratory detection of potato viruses

A batch of potato tubers is qualified suitable for seed, if it can produce strong, healthy plants with a high yield. For this reason, tuber must be almost free from tuber transmitted pathogens. Fungi, bacteria and viruses must be absent.

Thus the aim of disease inspection in the field and tuber indexing in the laboratory is to assess the incidence rate of diseases caused by tuber borne pathogens and when this incidence is found beyond a critical level, the whole crop is discarded as seed potato source.

In general, all inspected fields are checked for tuber transmitted pathogenic fungi, bacteria, viruses and nematodes. The important fungi are *Phytophthora infestans* and *Rhizoctonia solani*. Among bacteria, *Pseudomonas solanacearum*, *Erwinia atroseptica* and *Streptomyces scabies* are the most common. Viruses transmitted through tuber include PLRV, PVA, PVM, PVS, PVX and PVY (Hiddema, 1972). Of these viruses, PLRV and PVY are the most important in Kenya (Annon, 1974).

For most fungal and few viral diseases, with conspicuous symptoms, field inspection is carried out by visual observation for the detection of diseased plants. This is the case for Phytophthora late blight and potato leaf roll virus. For viruses with mild symptoms such as PVX and PVY, leaf samples are collected during potato growth and checked in the laboratory or in the greenhouse through various methods. For PVY, the commonly used methods are: the use of bio-assay host 'Aquila 6', microprecipitin test, enzyme linked immunosorbent assay (ELISA) and electron microscopy (de Bokx, 1979).

Methods used for such tests vary greatly with the virus nature. A test method suitable for PVM may become inefficient for the detection of PLRV. The time of collecting leaf sample affects greatly the sensitivity of any particular method. This is due to the following factors: the variation of virus concentration is subjected to the virus strain and the environmental conditions, different viruses have different translocation pattern in the plant tissues and potato varieties react differently to different strains. It has been shown that PVY^N translocates faster from leaves to tubers than PVY^0 strain and that PVY^0 multiplies equally at low temperature (15° - 18°C) and high temperature (22° - 26°C), whereas PVY^N does not multiply

at low temperature but does so at 22° - 26°C (de Bokx and Piron, 1977). Thus any test carried out to detect PVY when temperatures are low is bound to miss PVY^N infected potato plants. Each country has set a critical incidence rate or maximum index, above which a potato field should be discarded as source for basic seed or certified seed. Such critical index has not been set for any African country. Critical index set and followed by European Economic Community (EEC) members are presented in the following table.

Table 1. Maximum index for seed potato certification scheme in the EEC countries

Tuber grade*	Maximum index(%)
S, SE	2
E	3
A	4
B	8
C	12

S, SE, E = Seed-potato quality grade in descending order for basic seed.
A, B C = Seed-potato quality grades in descending order for certified seed

Thus the index determines which grade should be attributed to seed-potato lot. If the maximum index exceeds 12, the crop is rejected.

Despite the care given to field inspection and foliage testing in the laboratory, this gives no guarantee for health and figure obtained is not often realistic and quite often judgement from field inspection can misfire. This fact is due to symptomless undetected plants and less appropriate test used for testing leaves (Hollings, 1975). Thus field inspection should be followed by collection of tuber samples from the field. The tubers are treated with Rindite or other chemicals to

break the dormancy and planted in pots in the greenhouse. Growing plantlets are checked for virus 6 weeks after planting.

For an effective seed potato certification programme, each potato growing area in a country should establish for each virus the following aspects: the most sensitive method, and the right sequence for the three required field inspections.

Transmission and Epidemiology

PVY strains are readily transmitted by inoculation with sap, stem and core grafting (Delgado-Sanchez, 1966). The disease is passed to next growing season mainly through contaminated tubers. Plants raised from contaminated tubers do not usually show symptoms at the first generation (de Bokx and Cuperus, 1978). In the field, PVY, except PVY^C strains are efficiently transmitted by the green peach aphid *Myzus persicae*. Other vectors are *Myzus certus*, *Aphis fabae*, *Macrosiphum euphorbiae*, *Phorodon humilii* and *Rhopalosiphum insertum* (Kennedy *et al*, 1962). In Kenya, it was confirmed that *Myzus persicae* and *Macrosiphum euphorbia* are important vectors of PVY, whereas *Aphis solani* was not (Annon, 1974).

The importance of transmitting PVY through tubers from one generation to another is related to the period between the time of large flights of aphids and crop lifting date. Thus lifting dates are never fixed earlier than about 10 days after large flights of aphids have been monitored (Hiddema, 1972). The virus is transmitted in a non-persistent manner. Plants infected with PVY contain a 'helper component', which is a labile proteinaceous material of M.Wt 1 to 2 x 10^5. The 'helper component' mediates transmission of the virus by aphids (Govier and Kassanis, 1974). This fact could explain the failure to transmit PVY from pepper to potato plants by Bock and Robertson (1976). It can be speculated that the 'helper component' may be both aphid and host specific

MATERIALS AND METHODS

1. Source of inoculum

The virus extracted from *Cyphomandra betaceae.* is most apparent during cold weather and infected plants were located at Thika and Kabete. Leaves showing mosaic, vein clearing and blisters were harvested from naturally infected tree tomato plants at Thika and Kabete and brought to the laboratory of Crop Science Department of the Faculty of Agriculture for study. Virus isolates used in the study of PVY variations and forms of occurrence were from diseased potato plants located at Kabete and Molo in Kiambu and Rift Valley district respectively.

2. Inoculation procedure

All reported viruses infecting tree tomato are readily transmitted by mechanical inoculation with sap. Cucumber mosaic virus (CMV) was found to be inhibited by a plant subsistence. In this work, the virus proved to be extremely difficult to transmit through sap by manual inoculation. When old infected leaves were used as inoculum source very few plants of *Nicotiana tabacum* cv 'White Burley' were infected. Leaves picked at different age levels showed that the use of young top leaves could give over 90% infectivity in susceptible host plant species and varieties. The inclusions of 0.1% thioglycollic acid into 0.05 M phosphate buffer pH 7.5 improved significantly the rate of infection. Thus infected top youngest leaves were macerated in the buffer at a ratio of 1:1 (W:V). The extract was clarified by low speed centrifugation at 6000 rpm for 5 min.. The clarified fraction was used to inoculate leaves of indicator plants dusted with carborundum (500 mesh) by the forefinger method.

3. Source of differential plant species

Seeds of differential host plants as listed in table 2. were provided by the following institutions: the Department of Crop Science of the University of Nairobi, the Kenya Agricultural Research Institute (KARI), National Seed Quality Control Service (N.S.Q.C.S.) and the Nairobi regional centre of the

International Potato Centre (CIP). Seeds of a few differential hosts were purchased from Simlaw East Africa Ltd., Nairobi.

4. Host-range

This study was carried out to confirm the relationship between the tree-tomato strain and other strains of PVY on the basis of symptomatology. The isolate was tested by mechanical inoculation of species and varieties from the Solanaceae, Leguminosae, Chenopodiaceae, Amaranthaceae, Cucurbitaceae and Compositae (Table 2). For this evaluation, seedlings were grown in sterilized Muguga soil mixture[3] in 15 cm diameter pots or plastic bags placed in green house with temperature ranging between 20°-28°C. Test plants were inoculated at various stages: Solanaceae at 2-4 leaf stage, Leguminosae at primary leaves, Amaranthaceae at two leaves, Cucurbitaceae on cotyledonary leaves and Chenopodiaceae at a leaf mature stage. Such plants were sprayed weakly with metasystox (25% Demeton-s-methyl sulphonoxid) in order to control insects such as aphids that are known to be active vectors of PVY in the field and in the greenhouses. The plants were observed for symptoms expression at varying period depending on the species and variety. Recovery test was done only from plants without obvious disease symptoms. Detached mature leaves of Hybrid 'Aquila 6' were used for this bio-assay.

5. Physical properties

The resistance of virus particles to chemical and physical agents can be used as a tool in the differentiation of both groups of viruses and viruses within

[3]Muguga soil mixture composition: Horse manure, coffee husk, forest soil and ballast t a ratio of 1:1:2:1

Table 2.Indicator plant species used in the host-range study for the tree-tomato strain of PVY

Family	Species	Variety
Solanaceae	*Nicotiana tabacum*	Samsun
		White burley
		Kentucky
	Nicotiana glutinosa	
	Nicotiana debneyi	
	Nicotiana rustica	
	Nicotiana benthamiani	
	Lycopersicon esculentum	Roma
		Rutger
		Money maker
	Capsicum annum	Long red cayenne
	Physalis floridana	
	Nicandra physaloïdes	
	Datura metel	
	Datura stramonium	
	Solanum tuberosum	Kerr's pink
		Piratini
		Atzimba
		Cruza
		Serrena

Table 2: (cont..)

	Solanum tuberosum	Anita
		I-853
		I-1062
		Norland
		Aracy
Chenopodiaceae	*Beta vulgaris*	
	Chenopodium album	
	Chenopodium amaranticolor	
	Chenopodium quinoa	
	Spinaceae olearaceae	
Amaranthaceae	*Gomphrena globosa*	
	Amaranthus caudatus	
Cucurbitaceae	*Cucumis melo*	
	Cucumis sativus	Ashrey
		Poinsett
Leguminosae	*Phaseolus vulgaris*	Canadian wonder
		Rose Coco
		Mwezi moja
Compositae	*Zinnia elengans*	

each group (Mathews, 1981). Thus, it was observed that Potexviruses are relatively more stable than potyviruses and that within Potyviruses, some strains of BCMV are more resistant to heat than other members of the same group (Buruchara, 1979). The mostly used physical properties in the identification of plant viruses are the thermal inactivation point, the dilution end point and the longevity *in vitro*. The three parameters were assessed in crude sap without any prior dilution in distilled water or addition of any agent.

The sap was slightly clarified by slow speed centrifugation at 5,000 rpm for 5 min., submitted to three different treatments (heat, dilution and aging) and bio-assayed on detached leaflets of Aquila 6.

Thermal inactivation point.

One ml of aliquot of the clarified sap were put in thin walled test tubes and heated in water bath at temperature ranging from 40 C to 80 C for 10 min. with an increasing interval of 5 C between treatment levels. The thin walled test tubes were cooled and the containing sap inoculated on detached leaflets of Aquila 6. The leaflets were kept on moist tissue paper in trays covered with polythene sheet to perserve the moisture. The moisture was continuously maintained and symptoms observed for 6 days.

Dilution end point.

Serial dilutions from 1:100 to 1:100,000 were made by diluting 1 ml of the sap in distilled water. Saps of each dilution level were immediately inoculated on detached leaflets of Aquila 6. The inoculated leaflets were kept and symptoms observation carried out as described above.

Longevity in vitro.

The crude sap was distributed in equal aliqouts of 2 ml, kept at room temperature and inoculated on detached leaflets of Aquila 6 at 12 hrs interval.

These leaves were kept on moist trays covered with polythene sheets, observed daily and the aging in vitro point determined six days after inoculation.

6. *Transmission*

6.1 Transmission by aphid species

Initial populations of *Myzus persicae* and *Macrosiphum* sp were harvested from potato and tree tomato respectively. The two populations were freed from any virus by multiplying them on two separate cages of healthy chinese cabbage (*Brassica chinensis*). They were later transferred to two new sets of healthy plants of the same species. The two species were tested for their ability to transmit the virus from *Cyphomandra betaceae* and *Nicotiana tabacum cv. 'Kentucky'* to *Nicotiana glutinosa, Physalis floridana* and *Cyphomandra betaceae*.

In the first test, aphid populations were collected from infected tree tomato plants in the field and introduced directly to the test plants. A second lot of aphids were freed from virus by rearing them on chinese cabbage "*Brassica Chinensis*". Tests with this second lot of aphids were carried out by following a non persistent pattern. A lot of *Myzus persicae* were starved for 60 min. and given a virus acquisition feeding period of 30 to 120 min. After that they were immediately transferred onto leaves of healthy test plants. Inoculation feeding periods varied from 60 min. to 2 hr. and then plants were sprayed with metasystox (containing 25% Demeton-s- methylsulphonoxid). Plants were kept in the green house sprayed weekly and observed for 8 weeks. Recovery tests were done at the end of this period by extracting sap from symptomless plants and inoculating detached mature leaves of *Solanum demissium* Y (SDY) and Aquila 6

6.2 Transmission through seed of tree-tomato

Fruits were picked from ten infected tree tomato plants. Five plants were of the red pulp type and five the yellow pulp type. Seeds were used to raise seedlings in the greenhouse. Seeds from 2 fruits of each tree were extracted, washed with tap water and air dried separately. Five seedlings of each tree source were labelled separately as T1 T2 .... T5, raised in 15 cm polythene bags and sprayed weekly with either Metasystox or Rogor E to prevent aphid and white fly infestation. Plants were observed, symptoms noted and recovery tests done after 4 months through manual inoculation with extracted sap on Aquila 6 and SDY respectively.

6.3 Transmission through potato tubers

Tubers were harvested from nine manually infected varieties of potatoes, namely Piratini, Atzimba, Cruza, Serrena, Anita, I-853, I-1062 and Norland. These plants were confirmed to be infected through the bioassay recovery test on Aquila 6. Seven tubers were sampled from each variety and kept up to the sprouting time. These tubers were planted in plastic pots, 15 cm in diameter. After emergence, plants were weekly sprayed with either Metasystox or Rogor E. After 6 weeks, plants showing symptoms were counted and recovery tests done on detached leaves of Aquila 6 for all the plants including the symptomless ones.

6.4 Dissemination in a tree tomato field

Healthy seedlings of tree tomato were raised in the greenhouse and transplanted in a plot measuring 25.5 m x 12 m. Spacing between rows and within rows were 4 m and 1.5 m respectively. The healthy seedlings were transplanted in April 1985 at the beginning of the long rains. A second lot of two month old plants and infected by mechanical inoculation were brought in the field two months later and interplanted in rows of healthy plants in a ratio of 1 to 6. Spraying of the plants with insecticides or fungicides was avoided in this field. Aphids were collected in the field and symptoms observation carried out for seven months. Other damage by insects other than aphids were also noted. Plants showing PVY symptoms were counted monthly.

7.0 Purification of tree tomato PVY strain

Purification of the isolate from tree tomato was done by differential centrifugation, using low and moderate centrifugation force (10,000 g and 26,500 g respectively), according to Huttinga's method (1979) (Fig 1).

A 100 g of infected leaves of *Nicotiana tabacum* cv 'Kentucky' was homogenized in a waring blender with 150 ml. of 0.1M thioglycollic acid buffer (pH 9), 40 ml of Carbon tetrachloride and 40 ml of chloroform. The homogenate was clarified by centrifuging at 10,000 g for 10 min. to separate the water phase containing the virus. Then the viral suspension was concentrated by applying moderate centrifugation force at 26,500 g for 1.5 hr. The resulting pellet was resuspended in 0.1 M tris-HCl buffer, pH 9. (1/100 v/v of the initial sap volume). This virus suspension was re-clarified at low-centrifugation force at 8,000 g for 10 min. This partially purified preparation was used for ELISA and electron microscopy.

8. Electron microscopy

The electron microscopic examination for the tree tomato isolate was carried out on the following preparations: leaf-dip preparation, clarified leaf extract, and ultra-thin sections of healthy and infected plant tissues.

8.1 Leaf-dip preparation

Leaf-dip preparations were negatively stained according to Hitchborn and Hill (1965). In this procedure a drop of 2% potassium phosphostungstate (K-PTA) at pH 6.5 was put on a formvar coated 300 mesh copper grid with a needle of a hypodermic syringe or a pasteur pipette. A freshly cut triangular piece of infected leaf of potato was used to touch a drop of stain (K-PTA) for approximately 3 seconds. The excess stain was drained off with a piece of filter paper placed at the edge of the grid. The grid was then air dried and examined in a Carl Zeiss EM9 A electron microscope.

8.2 Electron microscopy of clarified leaf extract

This test was carried out as a routine virus recovery check on inoculated test plants without clear visible symptoms. Virus particles observed were measured and compared to the average length of the purified preparation. Plant sap was expressed through two layers of nylon cloth or muslin. To the homogenate 0.1% thioglycollic acid (TGA) was added at a ratio of 1:1. The mixture was clarified by slow centrifugation at 6000 rpm for 10 min. and the supernatant collected for application onto the grids. A sample of ten grids was prepared for each level (lower, middle and upper leaves) of three test plants. Negative staining was done according to Hitchborn and Hill (1965). A drop of clarified extract, undiluted and diluted (1:100), was put on different grids and an equal amount of 2% K-PTA pH 6.5 added. The excess liquid was drained with a filter, the grids air dried and examined under the electron microscope.

8.3 Electron microscopy of partially purified preparation

Examination of purified preparations is a more convenient way to assay the concentration and purity of a virus suspension after purification and more important it gives electron microscope micrographs with numerous virus particles needed in the measurement of the virus modal length. In this study, examination was carried out to determine the modal length and subsequently the group to which the virus under study belongs.

Grid specimens were prepared by placing a drop of purified virus preparation on a parafilm. A formvar coated copper grid was floated on the top of the drop for 3 to 6 min. after which it was removed and air dried. A drop of 2% K-PTA, pH 6.5 containing polystyrene particles (109 nm in diameter) was placed on the virus containing grid for 8 min. and the excess stain removed by placing a filter paper at the grid edge. The grid was air dried and examined under the electron microscope.

8.4 Electron microscopy of Ultra-thin sections of leaf tissues

The purpose of this study was to assess the diagnostic value of inclusion bodies induced by PVY. This was done by a comparative examination of inclusion bodies induced by two different strains of PVY on differential test plants. The tree tomato isolate was compared to an isolate from field infected potato variety 'Kerr's pink'.

Ultra-thin sections of leaf tissues infected by PVY were made from *Nicotiana tabacum* varieties 'White Burley', 'Kentucky' and 'Samsun', *Nicotiana rustica, Physalis floridana, Datura metel* and *Cyphomandra betaceae.* Sections of infected and healthy leaves were fixed, dehydrated and embedded according to a modified method of Morgan and Rose (1967).

Leaf sections of 1 mm^2 from mottled and green (control) areas were fixed in 1% glutaraldehyde for 1 hr. and washed 10 times in Sorenson's buffer at 7 min interval. Post fixation was done in 1% osmium tetraoxide for 30 min. and washed three times in the same buffer. Dehydration was executed in increasing concentration of acetone. The dehydrating reagent was washed off in tree solutions made of durcapan I and acetone at various ratio (1:3, 2:2, and 3:1) and in pure durcapan I (propylene oxide) and tissue embedded in Durcapan II (Epoxyresin). It was left overnight at 40°C and in the oven at 60°C for 48 to 72 hours. Sections were cut with glass knives on an Austria Reichert ultra-microtome and stained with uranyl acetate and lead citrate. Fig. 2 shows the flow chart followed during the preparation of ultra-thin sections.

Fig. 1. Flow chart for the purification of PVY according to Huttinga(1979)

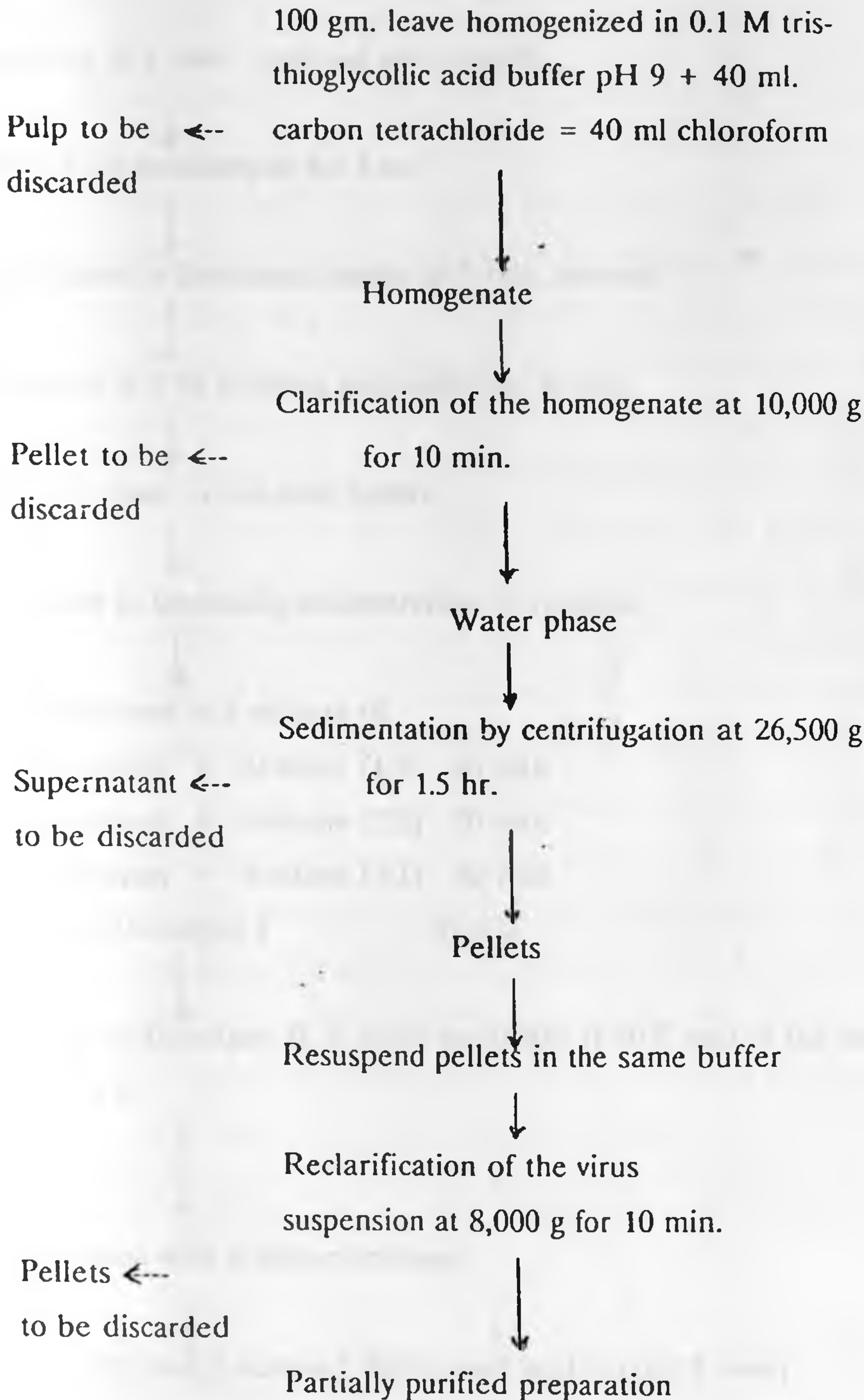
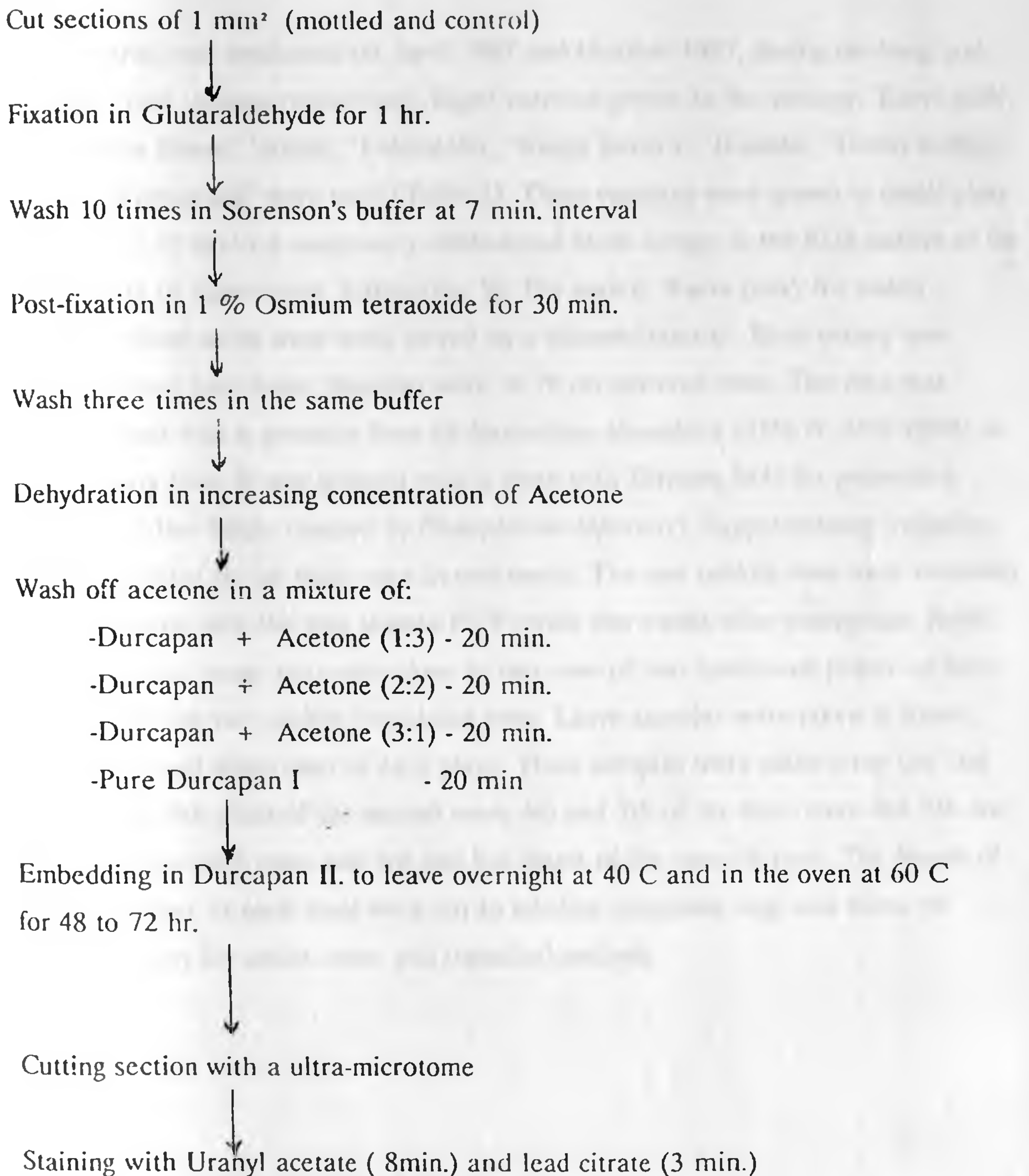


Fig.2. Flow chart for the preparation of ultrathin section for microscopic examination of histological inclusions induced by the strain of PVY extracted from *Cyphomandra betaceae*



9. The influence of PVY aphid-vectors to the incidence and occurrence of PVY in potato varieties

9.1 Assessment of potato varieties to aphid infestation

The trial was conducted on April 1987 and October 1987, during the long and short rain seasons respectively. Eight varieties grown in the country, 'Kerrs pink', 'Rosyln Eburu', 'Annet', 'Feldeslohn', 'Kenya Baraka', 'Bvumbe', 'Dutch Robjin', and 'Pimpernell' were used (Table 3). These varieties were grown in small plots (6.5 x 5.25 m) in a completely randomized block design at the field station of the Faculty of Agriculture, Kabete(fig. 3). The variety 'Kerrs pink', for which uncertified seeds were seed, served as a diseased control. Each variety was replicated four times. Spacings were of 75 cm between rows. The crop was fertilized with a granular form of diamonium phosphate (15% N; 45% P205) at planting time. It was sprayed once a week with Dithane M45 for protection against late blight (caused by *Phytophthota infestans*). Supplementary irrigation was applied to the trials once in two weeks. The two middle rows were manually inoculated with the tree tomato PVY strain two weeks after emergence. Aphid counts and assay tests were done in two rows of non inoculated plants on both sides of the two middle inoculated rows. Leave samples were taken at lower, middle and upper part of each plant. These samples were taken from the 2nd and the 9th plant of the second rows, 4th and 7th of the third rows and 5th and 6th of the sixth rows and 3rd and 8th plants of the seventh rows. The leaves of each plant, at each level were put in labelled polythene bags and taken to laboratory for aphid count and statistical analysis.

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to aphids and PVY infection pressure in the field.

Cultivar	Maturity	Drought resistance	Disease resistance	Disease sus ceptibility	Yield
Annet	early	moderate	Late blight PVX,PLRV	Bact.wilt RKN	high
Feldeslohn	medium late	moderate	PLRV RKN	Bact.wilt Late blight	high
Kerr's pink	early	resistant		Bact.wilt Late blight RKN,PVX,PLRV	medium
Kenya Baraka	late	moderate	Late blight RKN,PVX,PVY	Bact.wilt PLRV	High
Robjin	late	highly susceptible		Bact.wilt Late blight RKN,PVX,PLRV, PVY	high
Roslyn Eburu(B53)	medium, late	highly susceptible	Late blight	Bact.wilt PVX,PLRV,PVY	medium, high
Bvumbe	early, medium	fair	Late blight PLRV	PVX,PVY, Bact.wilt	high
Pimpernel	medium	fair	PLRV	Late blight PVX,PVY,RKN	medium

Yield(potential in Kenya):-High:>40 tons
-medium: 20-40 tons

abreviations: RKN= root knot nematode
Bact.wilt= bacterial wilt

Maturity: early: 3-3.5 months
medium-late: 3.5-4 months
late: 4-5 months

9.2 Assays for polyphenol oxidase activities (PPO) in potato leaves

The PPO activities of the eight varieties tested for PVY were assessed by using a procedure described by Ryan *et al* (1983) and modified by Ave et al (1986). In each plot, 3 plants were selected at random from the inner rows except the 2 edge rows. For each plant, 3 small leaflets (10 cm^2 total area) were removed from the terminal foliage and placed in a test tube. Within 20 min. of excision, 3 ml of buffer containing 0.7% sodium phosphate, 1% Triton X-100 and 0.075% p-phenylene diamine (Free from base) were added to each tube. The p-phenylene diamine was added to stabilise and enhance the colour of the reaction product. After vigorous agitation for 30 sec. on a vortex mixer to maximize discharge of trichome glands, the tubes were held at 27°C for 20 min. in an incubator and placed in a 37°C water bath for 15 min. during which a violet colour developed. Colour intensity was determined by measuring percentage transmittance (%T) at a wavelength of 470 nm with a spectronic 20R spectrophotometer. In this assessment low %T values indicated high enzymatic activities and vice versa.

9.3 Yield assessment on field infected potato varieties

The assessment of tuber yield was carried out on the eight tested potato varieties during the long rainy season between April and July, 1987 and the following short rainy season between October, 1987 and January, 1988. The objective was to assess the effect of PVY infection on the yield of potato varieties as the resistance of a variety to a disease should be rated more by the way its yield is affected than by the mere expression of the disease intensity. One week after most potato plant haulms were dried, tubers were harvested separately from nine potato plants selected at random in each plot after exclusion of the two edge rows on each side of the plot. The tuber weight of each plant was taken separately and the data analyzed statistically.

9.4 ELISA

ELISA test were carried out for the following objectives:

- To determine in which state of purity or association PVY isolates were found in potato fields.
- To assess the rate of PVY infection in tubers. Samples of tubers from Agriculture Development Corporation (ADC) and those of the control 'Kerrs pink' from rural markets were tested before the first field experiments as well as tubers harvested from the two experiments in the field.

8- To assess the rate of PVY infection in the field during two seasons (short rains 1987 - long rains 1988).

The ELISA procedure used was that set up by the International Potato Centre (CIP). The first kit was obtained from the [4]N.S.Q.C.S. and the second from the regional office for East Africa of CIP.

The ELISA were carried out through the following steps:

1. Eight drops of coating antibodies* were poured in each well of the microtitration plate. The plates were put inside plastic bags, sealed with an adhesive tape and incubated at 37°C for 3-4 hr.
2. Leave samples were selected either when showing conspicuous symptoms or by taking one leaflet from the top, middle and bottom of the plant for every sample. For tubers, sprouts and a small section of the heel end were used. Each sample was put in a small polythene bag and labelled.
3. Each sample was ground in the extraction [5]buffer to a ratio of 1:3.
4. After step (1), microtitration plates were washed three times with PBS + Tween buffer and 6 drops of each sample were poured into each well. The plates were again sealed and incubated in a domestic refrigerator overnight.

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[4]N.S.Q.C.S: National Seed Quarantine and Certification Service

[5]Buffers descriptions are presented in annex 35

5. After step (4), microtitration plates were washed three times or more with PBS** Tween buffer and 6 drops of conjugate buffer* added into each well. Plates were then incubated for 3-4 hr at 37°C.
6. The plates were left for 30 to 60 min. after which time, positive (yellow colour) and negative (colourless) reactions were noted.

10. Variations of PVY strains

The tree tomato isolate was collected from the experimental plot previously planted at the field station (Kabete) for the study of the evolution of PVY rate in a tree tomato field. Fifty four other isolates were collected from two locations: Kabete in Kiambu District and Molo in the Rift Valley District. In the first location, all isolates were collected from mixed stands of potatoes with either beans, maize or peas.

In Molo, isolates were collected from pure stands of seed potatoes production plots of Agricultural Development Corporation (ADC). In Kabete location, collection of isolates was simplified by the fact that most plants showed conspicuous symptoms of leaf drop and veinal and stem necrotic streaks, characteristic of PVY infection on Kerr's pink . However collection of samples, at the ADC plots were complicated due to continuous roguing practice and thus sole faint mottling or slight leaf tapering were indicators of potential infection by PVY.

Leaf samples of different varieties and from different plots of those two locations were collected, put in moisturized polythene bags, labelled and taken to the Crop Science Department at Kabete, and inoculated manually on a set of differential hosts. Successfull inoculation was achivied only on 36 samples out the 54 collected from the field. The differential hosts showing symptoms were subjected to ELISA. Isolates found to be pure PVY and or isolated from PVY - PVX mixture were reinoculated on a second set of differential hosts and their

reactions compared after 6 weeks. According to the primary sources (location and variety), the 36 isolates were labelled in the following ways:(Table 4)

WKP-(Wangige-Kerr's pink): Isolates collected in Wangige and extracted from potato variety Kerr's pink;
UKKP-(Upper Kabete-Kerr's pink): isolates collected in upper Kabete and extracted from potato variety Bvumbe;
MBV-(Molo-Bvumbe): isolates collected in Molo and extracted from potato variety Bvumbe;
MRT-(Molo-Rosylin Tana): isolates collected in Molo and extracted from Rosyln Tana;
MKB-(Molo-Kenya Baraka): isolates collected in Molo and extracted from Kenya Baraka;
MAN-(Molo-Annet): isolates collected in Molo and extracted from annet; and
MDR-(Molo-Dutch Robjin): isolates collected in Molo and extracted from potato variety Dutch Robjin.

All differential hosts were kept in an aphid-free greenhouse, watered daily and observed for 6 weeks. Symptoms were recorded as they appeared and the incubation time noted

Table 4: Grouping of 36 recovered virus isolates according to location and variety

Group	Location	Source	Isolate (IS.)
WKP	Wangige	Kerr's pink	IS.01;IS.02;IS.03;IS.11; IS;12;IS.15;IS.25; IS.26;IS.29;IS.33;IS.34; IS.35;IS.36
UKKP	Upper kabete	Kerr's pink	IS.05;IS.09;IS.16;IS.22; IS.31;IS.32
MBV	Molo	Bvumbe	IS.04;IS.17
MRT	Molo	Rosylin Tana	IS.06;IS.23
MKB	Molo	Kenya Baraka	IS.07;IS.08;IS.10;IS.18; IS.19;IS.20;IS.21
MAN	Molo	Annet	IS.13;IS.14
MDR	Molo	Dutch Robjin	IS.24;IS.27;IS.28;IS.30

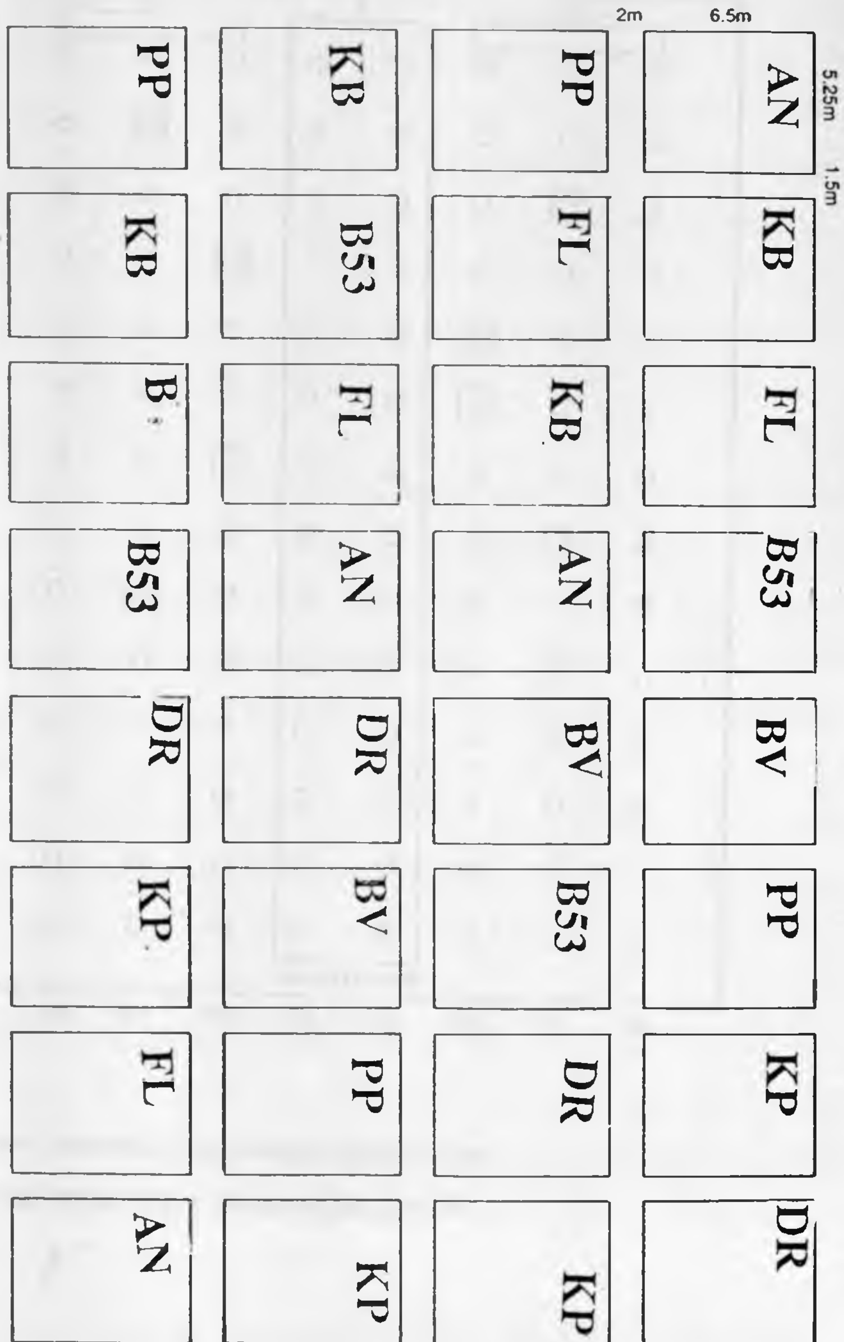
11. Overall experimental plan

The experiment was conducted at Kabete, Kenya (Latitude 0°15'S, Longitude 36°44'E, altitude 1953 m). The experiments in the field were conducted during the long rainy season between April and July 1987 and the following short rainy season between September and December 1987.

Eight potato varieties, namely 'Dutch Roslyn', 'Bvumbe,' 'Feldeslohn,' 'Kenya-Baraka, 'Roslyn Eburu (B.53), 'Kerrs pink', Pimpernell and 'Annet,' were tested in this experiment.

Fig. 3 and 4 shows a schematic representation of the experimental layout in the field. Leaf samples to be tested were collected from the 8 potato varieties distributed randomly in 4 blocks. Each variety (v) was represented once in each block. Each plot (p), including only one variety, had 8 rows of 14 plants each. During the first growing season two rows of each plot were artificially inoculated with PVY inoculation with the aid of a sprayer gun 7 days after emergence whereas the other rows were left to natural infection by aphid species. Each plot was bordered by two potato rows on all sides. Blocks were separated by a corridor of 2 m. For the study of PVY variation in Kenya, 7 plants of each differential host species were included in the experiment in the greenhouse. Of those 5 plants were inoculated and two kept as healthy controls.

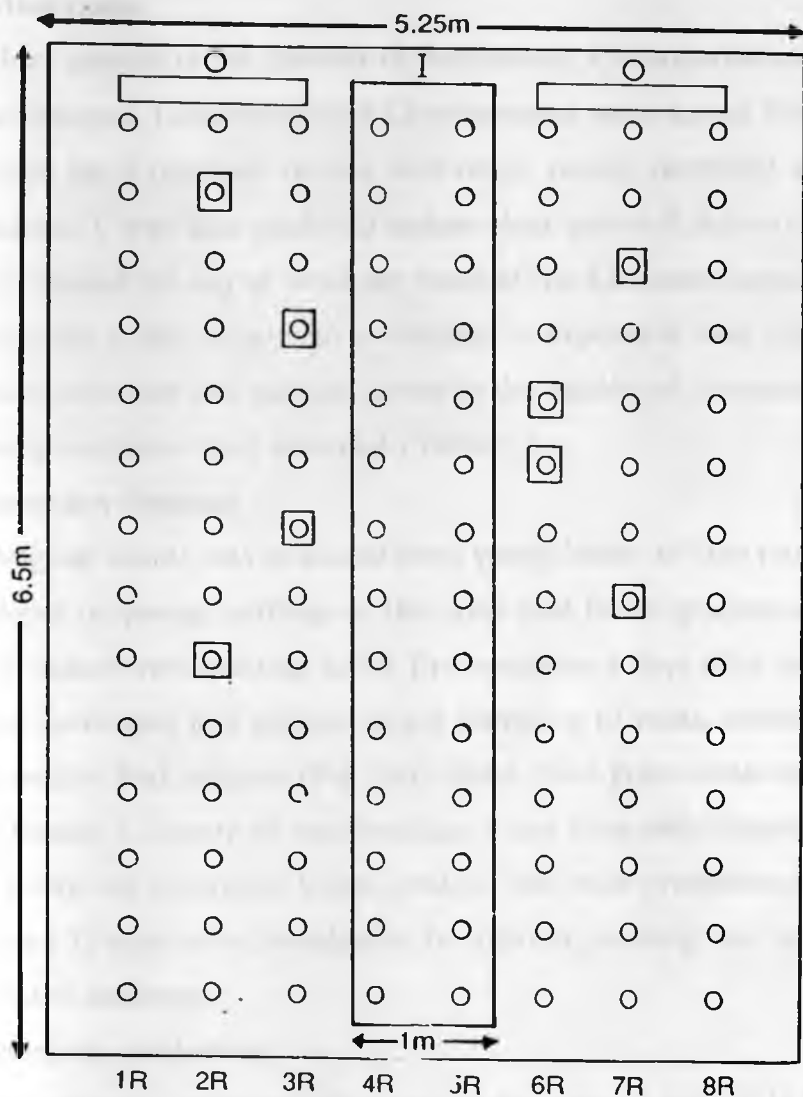
Fig.3. General experimental layout for the field trials on potato varieties



Abbreviation

AN= Annet; KB= Kenya baraka; FL= feldeslohm; B53= Rosylin Eburu; BV=Bvumbe; PP= Pimpernell; KP= Kerr's pink; DR= Dutch_Robjin

Fig.4. Disposition within a single experimental plot



Legend: I: two manually inoculated middle rows

O: lateral rows under observation for PVY.

RESULTS

3.1. TREE TOMATO STRAIN OF PVY

3.1.1 Host-range

Plant host species in the families of Solanaceae, Chenopodiaceae, Leguminosae, Amaranthaceae, Compositae and Cucurbitaceae were tested. Results showed that this strain has a relatively narrow host-range, mainly restricted to the family of Solanaceae. It was also unable to induce clear and well defined local chlorotic or necrotic lesions on any of bio-assay hosts of the Chenopodiaceae family. However the strain appears to be virulent to important food crops, mainly potatoes, tomatoes and pepper, grown in the vicinity of tree-tomato. The following reactions were recorded (Table 5.):

Cyphomandra betaceae

The original isolate was extracted from young leaves of tree tomato. When inoculated on young seedlings of the same host in the greenhouse, the isolate was able to induce vein clearing as the first symptom 6 days after inoculation. This disease developed into mosaic, severe distortion of veins, veinlets and midribs and irregular leaf margins (Fig. 5ab). Later, dark green areas associated with the veins formed a pattern of vein-banding. Apart from vein clearing that developed after 6 days on inoculated leaves, most of the other symptoms were systemic and appeared 12 days after inoculation. In addition, stunting was very pronounced on inoculated seedlings.

Lycopersicon esculentum

Tomato varieties commercially grown in the country reacted to the virus by producing symptoms varying from vein-clearing, to mottling, necrosis and stunting. Stunting was severe on "Roma" while necrotic blotches were observed on "Money maker". "Rutgers" and "Marglobe" reacted with diffuse to severe mottling but no stunting was observed.

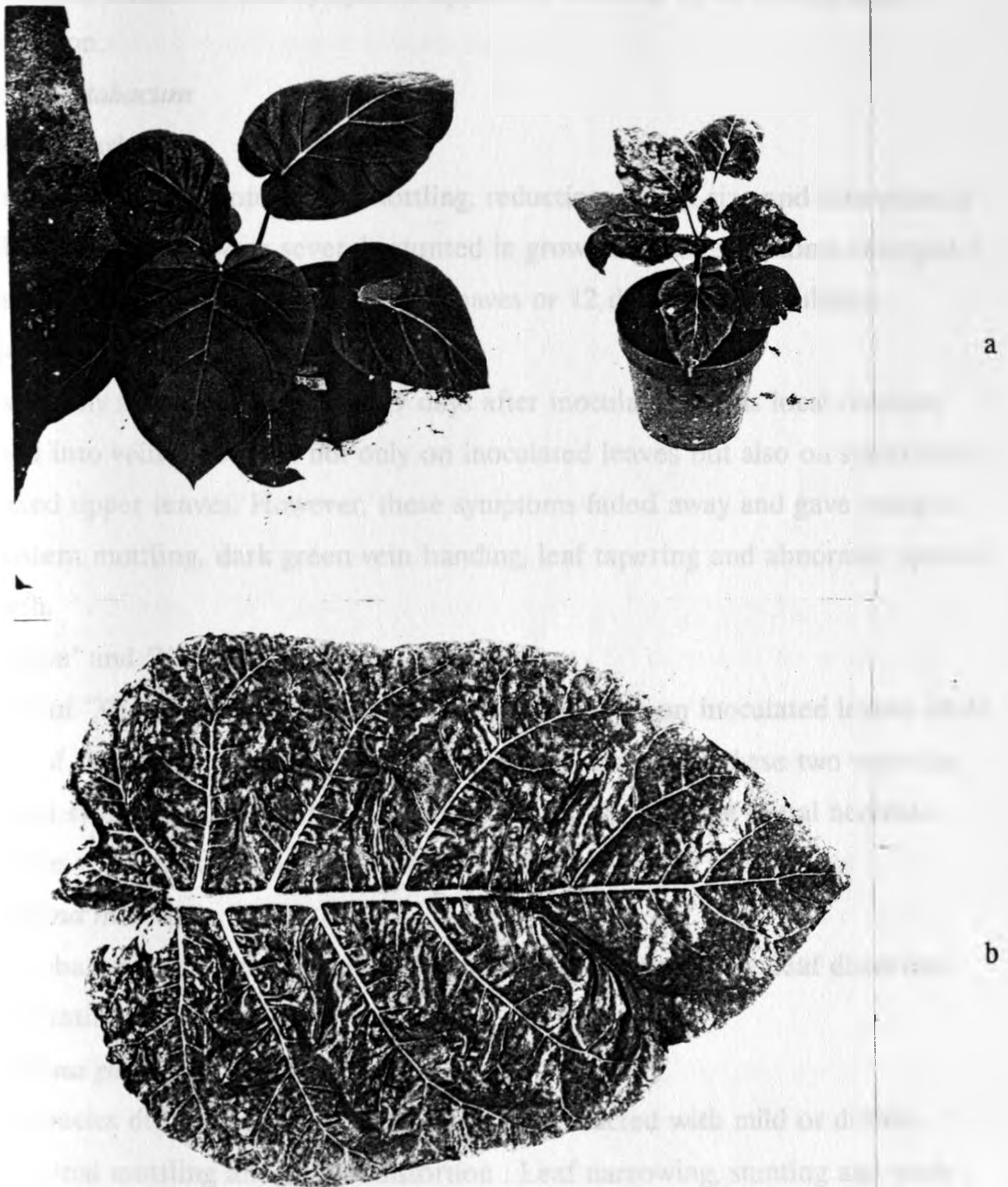


Fig.5. Reactions of tree tomato seedling (a) manually inoculated with the tree tomato strain of PVY(right) and the control(left) four weeks after inoculation and a close up (b) of an infected tomato leaf showing mottling, green vein banding and leaf surface and margin distortions

Capsicum annuum

The varieties "Yolo wonder" and "Long red cayenne" were inoculated and showed mottling or mosaic. These symptoms appeared between 12 to 18 days after inoculation.

Nicotiana tabacum

- 'White Burley'

This variety showed interveinal mottling, reduction of leaf size and distortion of leaflets. The plants were severely stunted in growth. These symptoms emerged 6 days after vein clearing on inoculated leaves or 12 days after inoculation.

- 'Kentucky'

This variety showed vein clearing 9 days after inoculation. This local reaction turned into veinal necrosis not only on inoculated leaves but also on systemically infected upper leaves. However, these symptoms faded away and gave place to persistent mottling, dark green vein banding, leaf tapering and abnormal upward growth.

'Samsun' and 'Xanthii'

Plants of 'Xanthii' variety reacted with veinal necrosis on inoculated leaves while those of 'Samsun' did not show any local lesions. However, these two varieties showed symptoms characterized by diffuse mottling, systemic veinal necrosis, extreme white mottling on lower leaves, leaf tapering and stunting.

Nicotiana rustica

This tobacco species reacted with diffuse mottling, conspicuous leaf distortion and stunting 7 days after inoculation.

Nicotiana glutinosa

This species did not show any local lesions but reacted with mild or diffuse interveinal mottling and veinlet distortion . Leaf narrowing, stunting and early senescence were also observed. However, stunting was observed on less than 30% of inoculated plants, while most had normal growth and larger leaves.

Nicotiana debneyi

Blotchy mottling was observed on leaves. The mottling was mostly of pale green to whitish colour than yellowish. Leaf tapering and reduction of abnormal upward growth were conspicuous. Symptoms appeared 10 to 12 days after inoculation.

Physalis floridana

Reacted with necrotic lesions on inoculated leaves. Systemic symptoms consisted of interveinal mottling, necrotic blotches, irregular leaf margin and severe flower abortion. Subsequently, this host produced very few seeds. Symptoms were observed 10 days after inoculation.

Solanum tuberosum

The following reactions were observed:

- cv. 'Piratini (CIP 7202108)' reacted with mottling, reduction of leaf size, puckering and rolling downward of leaflet.
- cv. 'Atzimba (CIP 720045)' exhibited stunting, leaf narrowing and mosaic.
- cv. 'Anita (CIP 720047)', and the following CIP varieties: 'Serrena (CIP 720087)', 'Cruza (CIP 676064)', and 'Aracy (CIP 720111)' reacted with similar symptoms and within the same incubation time. The symptoms consisted of severe stunting, mottling, veinal necrotic streak, visible on the back of the leaflet. This necrosis progressed toward the midribs, and the petiole and later caused leaf death of most haulms. Most plants of these varieties had etiolated stems by the time they were mature. They produced less than 5 tubers each and of small size (>4 cm in 0).
- cv. 'I-853 (CIP 575001)' and 'I-1062 (CIP 575010)', reacted with the shortest incubation period and showed severe yellowing of leaves, roughness of leaf surface and upward leaf rolling. Necrotic streaking was also severe on these varieties. These symptoms appeared more or less similar to those caused by potato leaf roll virus (PLRV). Recovery test on *Chenopodium amaranticolor* and 'Aquila 6' showed PVY-like symptoms and Electron microscopic examination of leaf dip preparation from these plants showed rather flexuous particles. This was not sufficient ground to

discard the effect of PLRV on the appearance of the above symptoms as PLRV is not mechanically transmitted and as probably PVY concentration could have been higher compared to PLRV, hence it could not be detected by electron microscopy.

- 'Norland (CIP 800089)'. This variety did not show any mottling or mosaic but showed severe roughness of leaf surface and cracking. Leaf puckering, rolling downward, and severe necrosis were also observed. However, all these symptoms were on the inoculated leaves and no virus was recovered during recovery test.

Nicandra physaloïdes

Reacted with necrotic blotches on inoculated leaves and severe mottling and stunting(Fig. 6).

Datura metel

Datura metel showed vein clearing as first symptoms on inoculated leaves and later mottling and green vein banding.

Chenopodium spp.

All three Chenopodium species, namely: *Chenopodium amaranticolor, C. album* and *C. quinoa* were not susceptible to the tree tomato strain of PVY.

Aquila 6

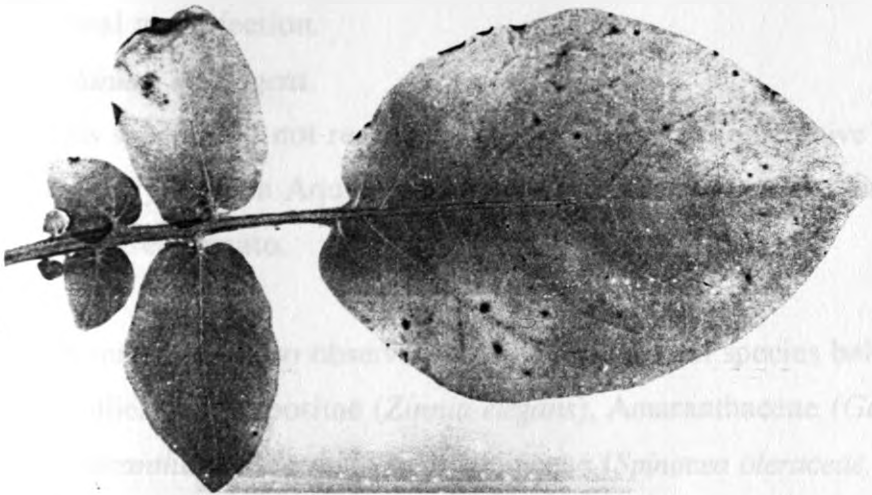
'Aquila 6' reacted with necrotic local lesions, with regular margin and 1 to 2 mm in diameter. However, such symptoms were obtained only on mature detached leaves but not on inoculated undetached leaves (Fig. 6).

Solanum demissium Y (SDY)

Detached mature leaves of SDY reacted as 'Aquila 6'. However, leaves of inoculated plants showed severe mosaic, very large necrotic blotches, which coalesced and severe stunting (Fig. 6).

Solanum demissium A (SDA)

This species was immune to the virus. This reaction indicates that the virus can not be a strain of PVA, which causes symptoms similar to PVY on tobacco varieties such as 'White Burley'.



a

b

g.6. Assay host *Aquila* 6 showing necrotic local lesions (a) and *Chenopodium amaranticolor* (b) with not well defined local lesions

Datura stramonium

It is considered as one of the most important indicator species in the differentiation of elongated viruses of potyvirus and potexvirus groups. It reacts positively to PVX and to PVA but not to PVY(Berks, 1970; de Bokx and Huttinga, 1981). However in this experiment , none of the inoculated plants showed any symptoms and repeated recovery assay tests on Aquila 6 did not reveal any infection.

Solanum melongera

This species did not react after inoculation and the negative results of bioassay recovery tests on Aquila 6. proved it to be immune to the virus strain isolated from tree tomato.

Immunity was also observed on differential plant species belonging to the families of Compositae (*Zinnia elegans*), Amaranthaceae (*Gomphrena globosa, Amaranthus caudatus*), Chenopodiaceae (*Spinacea oleraceae, Beta vulgaris*), Leguminosae (*Phaseolus vulgaris*) and Cucurbitaceae (*Cucumis melo, C. sativa*). A summary of reactions by indicator plant species and varieties to PVY strain from tree tomato is presented in Table 4.

Table 5. Responses of indicators plant species and varieties to PVY stain extracted from *Cyphomandra betaceae*

Indicator plant species/ variety	Reactions	
	Local symptoms	Systemic symptom
Cyphomandra betaceae	*VC*	*Mo,LD,GVB,St*
Nicotiana tabacum		
cv 'Xanthii'	*VC,VNe*	*M,VNe,Lt,St*
'White Burley'	*VC,VNe*	*M,Mo,St,Rl*
'Samsun'	*VC,*	*VC,M,VNe,St,Lt*
'Kentucky'	*VC,VNe*	*VC,M,VNe,St,GVB*
Lycopersicon esculentum		
cv 'Roma'	*VC*	*M,St*
'Rutger'		*M*
'Money maker'	*VC*	*Ne,M,*
'Marglobe'		*M*
Capsicum annuum		
cv 'Long Red Cayenne		*M,Mo*
Physalis floridana	*Ne*	*Mo,St*
Nicandra physaloides	*Ne*	*M,St*
Hybrid 'Aquila 6'	*NLL*	*No*
Solanum tuberosum		
cv 'Piratini(CIP 7202108)'	*No*	*M,LP,St,RL*
'Atzimba (CIP 720045)'	*No*	*Mo,St,Rl*
'Cruza (CIP 676064)'	*No*	*M,VNS,Et*
'Serrena (CIP 720087)'	*No*	*M.VNS,Et*
'Anita (CIP 720047)'	*NLL*	*M,VNS,Et*
'I-853 (CIP 575001)'	*No*	*M,VNS,Ne,RLS*
'I-1062 (CIP 57010)'	*No*	*RLS,VNS, LD*
'Norland (CIP 800085)'	*Ne*	
'Aracy (CIP 720111)'	*No*	*St,M,VNS,Et*
Chenopodium quinoa	*No*	*No*
C. amaranticolor	*No*	*No*
C.album	*No*	*No*
	No	*No*
Datura stramonium	*No*	*No*

Abbreviations used in Table 4.

Et = Etiolation or abnormal upward growth

GVB = green vein banding

LD = Leaf distortion

LP = leaf puckering

Lt = leaf tapering

M = mottling

Mo = mosaic

Ne = necrosis

NLL = necrotic leaf spot

No = no reaction

Rl = reduction of leaf size

RLS = roughness of the leaf surface

St = stunting

VC = vein clearing

VNe = vein vecrosis

VNS = veinal necrotic streaks

3.1.2 Transmission of tree tomato strain of PVY

Transmission through aphids

When using *Myzus persicae* population reared in the greenhouse, the rate of transmission of virus strain from *Cyphomandra betaceae* was assessed on seedlings of tobacco species, *Physalis floridana* and tree tomato. Results showed that *Myzus persicae* acquired and transmitted the virus successfully from leaves of *Nicotiana tabacum* cv 'Kentucky' to *Nicotiana glutinosa*, *Physalis floridana* and tree-tomato plants at 58.3, 64.2 and 27.2 % respectively. The average transmission rate was 49.3%. However, when *Macrosiphum* sp. population was collected from infected tree-tomato plants and transferred immediately on healthy test plants, the transmission rate obtained was (41.6, 14.4 and 26.2 %) on *Nicotiana glutinosa, Physalis floridana* and tree-tomato respectively (Table 6). The results confirmed that *Myzus persicae* is an important vector of PVY strains. However the role of other vectors such *Macrosiphum sp, Aphis gossypii*, and *Aphis fabae*, commonly found on potato crops planted in both monocropping and mixed cropping systems should not be overlooked. For each ecosystem, the pattern of the distribution of aphid species populations over the year should be determined as each species reaches its peak during a particular month, and such pattern studied in each major potato growing area in Kenya could be of great importance in the choice of planting and harvesting date.

Table 6. Rate of transmission of PVY by aphids from *Nicotiana tabacum* cv 'Kentucky and *Cyphomandra betaceae*

Aphid species	Plant source	Tested plant	Number of infected plant	percentage
Myzus persicae	*Nicotiana tabacum* cv'*Kentucky*	*N.glutinosa*	7/12	58.3
		P.floridana	9/14	64.2
		C.betaceae	3/11	27.2
		total	19/37	
		Average	6.3/12.3	49.3
Macrosiphum spp	*Cyphomandra betaceae*	*N.glutinosa*	5/12	41.6
		P.floridana	2/14	14.4
		C.betaceae	4/15	26.6
		Total	11/41	
		Average	3.6/13.6	27.5

Transmission through seeds of tree tomato

Results of virus transmission through seed of tree tomato presented in Table 7 show that the transmission rate among tested seed sources(trees) varied from 0 to 100% and the tree tomato with red pulp fruit had a much lower transmission rate (12%) than the tree tomato with yellow pulp fruit (52%). However such a difference can not be considered a sign of genetic differences in the two tree tomato types nor an indication that the red pulp type poses less danger as a source of infection to neighbouring crops susceptible to PVY, PVX and CMV which multiply easily on tree tomato. The major emerging issue on both tree tomato and potato crops is the manifestation of a high transmission rate of PVY through tree tomato seeds and its implication in the choice of a safer propagation procedure.

Table 7. Transmission rate of PVY through seeds of *Cyphomandra betaceae*

Yellow type			Red type		
Source of inoculum	No. of infected seedling	Percentage	Source of inoculum	No. of infected seedling	Percentage
Tree1	3/5	60	Tree1	1/5	20
Tree2	1/5	20	Tree2	0/5	0
Tree3	4/5	80	Tree3	0/5	0
Tree4	5/5	100	Tree4	2/5	40
Tree5	0/5	0	Tree5	0/5	0
Total	13/25		Total	3/25	
Average	2.6/5	52	Average	0.6/5	12

Transmission of tree tomato strain of PVY through tubers of potato varieties

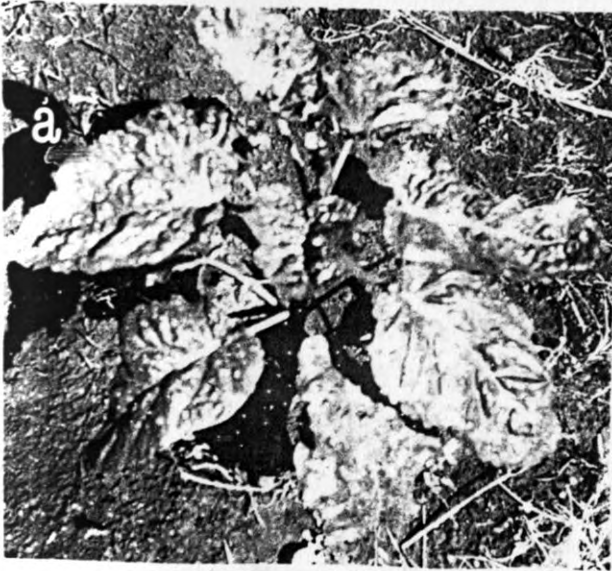
This test confirmed that the tree tomato strain of PVY is transmitted through tubers. The transmission rates among varieties varied from 0 to 42%. This is an indication of varietal differences to support of PVY and can be considered as one of the criteria to consider in the selection of virus resistant varieties. Tubers from 4 varieties (Serrena, Anita, I-1062 and Norland) did not have the virus. This negative result may be due to the small size of the samples used because mechanical and insect inoculation showed that these varieties are susceptible to this strain of PVY. The highest rates were recorded on Aracy (42.8%) , Atzimba and Piratini (28.5%)(Table 8).

Table 8. Transmission of PVY through tubers of *Solanum tuberosum* varieties

Variety	No. infected plant	percentage of infection
Atzimba	2/7	28.5
Cruza	1/7	14.2
Aracy	3/7	42.8
Serrena	0/7	0
Anita	0/7	0
I-853	1/7	14.2
I-1062	0/7	0
Piratini	2/7	28.5
Norland	0/7	0
Total	9/63	
Average	1.22/7	14.25

Field dissemination of tree tomato strain of PVY through aphid species

When diseased and healthy tree-tomato seedlings were interplanted in the ratio of 1 to 6, data on showed that the virus spread to 100% of the tree-tomato plants in the experimental plots(fig 7ab), within seven months with an initial inoculum of 14.28%(annex 36). Spacing of 4 m between lines and 1.5 m within tree rows(fig. 7c) showed that the virus could not be transmitted by contact of the tree foliage but mainly by alate aphids and apterous aphids. The average increase of PVY infection for every two weeks was 7.93 %. The highiest increases were recorded from the 15th of august 1986 to the 1st of october 1986 or from 45 to 90 days after transplantation of tree seedling(fig.8). Such high rate in the increase of infection may be explained by the fact that younger seedlings are more susceptible to diseases and by a higher aphid populations as demonstrated on the potato trials a year later. During this period of seven months, two experimental plants died.



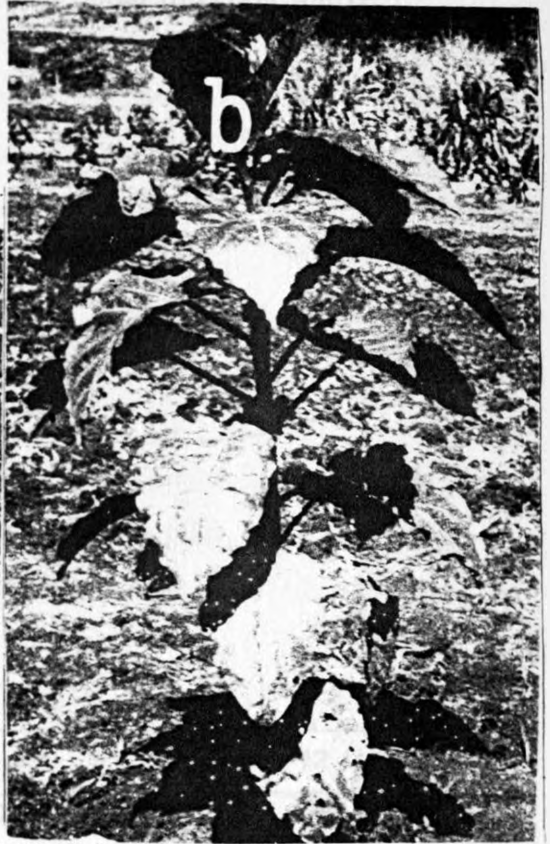
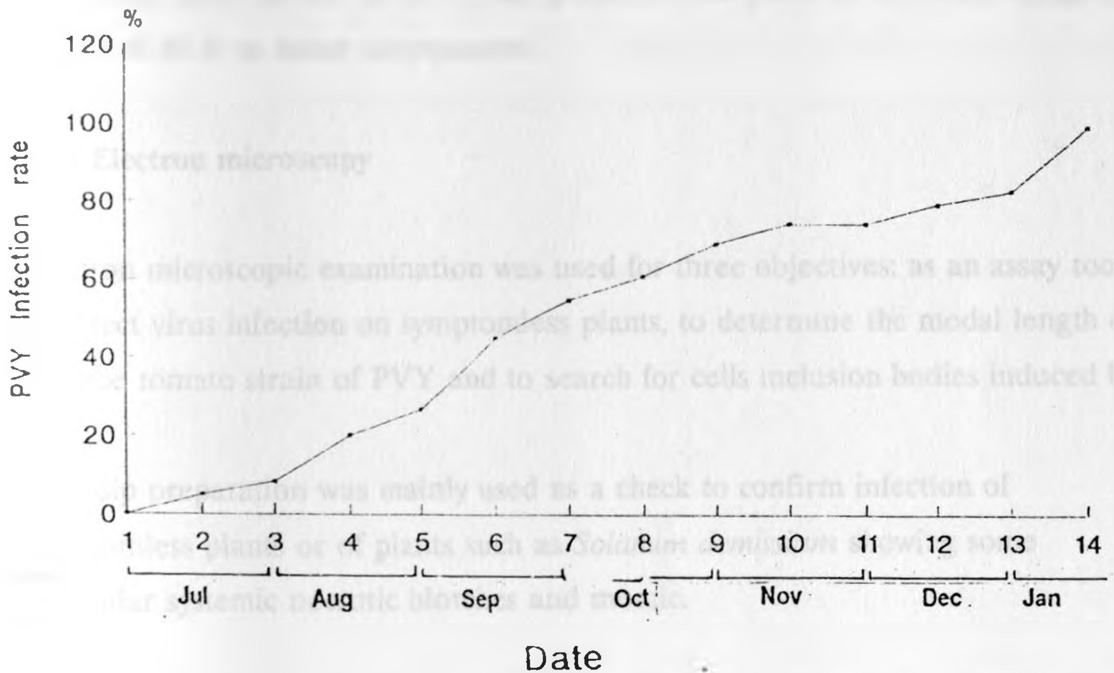


Fig.7. Diseased plants in the natural aphid transmission experimental plot showing severe leaf distortion on upper leaf and ant damage on lower leaves and the view of the plot.

Fig. 8. Evolution of the spread for PVY infection in a field of *Cyphomandra betaceae* submitted to an initial infection source



3.1.3 Physical properties

The virus strain isolated from *Cyphomandra betaceae* showed relatively low stability in crude sap of infected *Nicotiana tabacum* cv. 'Kentucky'. The virus was inactivated after 10 min. at 65°C, had a dilution end point of 10^{-3} and a longevity *in vitro* of 66 hr at room temperature.

3.1.4 Electron microscopy

Electron microscopic examination was used for three objectives: as an assay tool to detect virus infection on symptomless plants, to determine the modal length of the tree tomato strain of PVY and to search for cells inclusion bodies induced by virus.
Leaf dip preparation was mainly used as a check to confirm infection of symptomless piants or of plants such as *Solanum demissium* showing some particular systemic necrotic blotches and mosaic.

Examination of a purified preparation was specially used to determine the modal length of the virus extracted from tree-tomato. The virus particles isolated from *Cyphomandra betaceae,* stained with 2% potassium phosphotungstate pH, 6.5 (in water) appeared to be flexuous(fig. 9a). Measurements of 229 particles on electron micrographs showed that particle length varied from 330 nm to 920 nm(fig. 9b). However, the average of 224 particles representing 97.7% of measured particles was 738 nm. This modal length is still within the range 648-740 nm, reported for PVY and its strains (de Bokx and Huttinga, 1981).

Observations were carried out on ultra-thin sections made from healthy and diseased leaf tissue samples of *Cyphomandra betaceae*, *Nicotiana tabacum* var. 'White Burley and 'Samsun' in order to identify inclusions such as pinwheels and other amorphous characteristic structures that have identification value. The most

consistent features observed (fig. 10) were the destruction and the reduction of both chloroplast size and number particularly in the mesophyll cells. The content of nucleus were disintegrated and replaced by more or less elongated intranuclear inclusions and lipid globule. In same cases the chloroplast appeared to have been destroyed and the granna released in the cytoplasm. It also appeared that bundles made of virus particles have replaced most of the cytoplasmic content. However structures as pinwheels, one of the mostly used identification features for Potyviruses (Mathews, 1981) were not observed.

Fig. 9. Electron micrograph showing particles of PVY obtained from partially purified preparation

300 nm

a

b

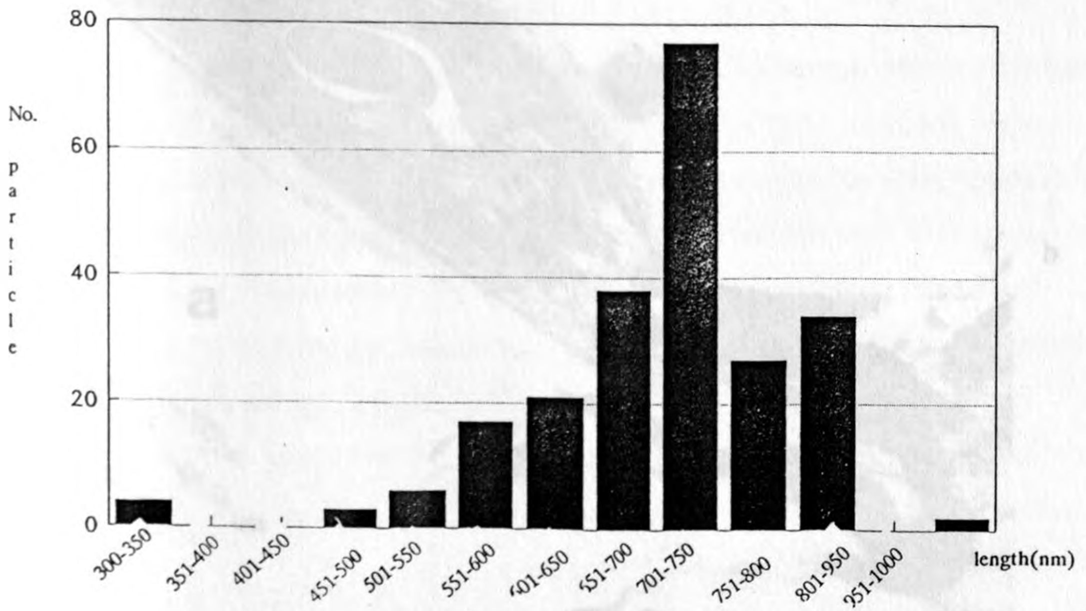
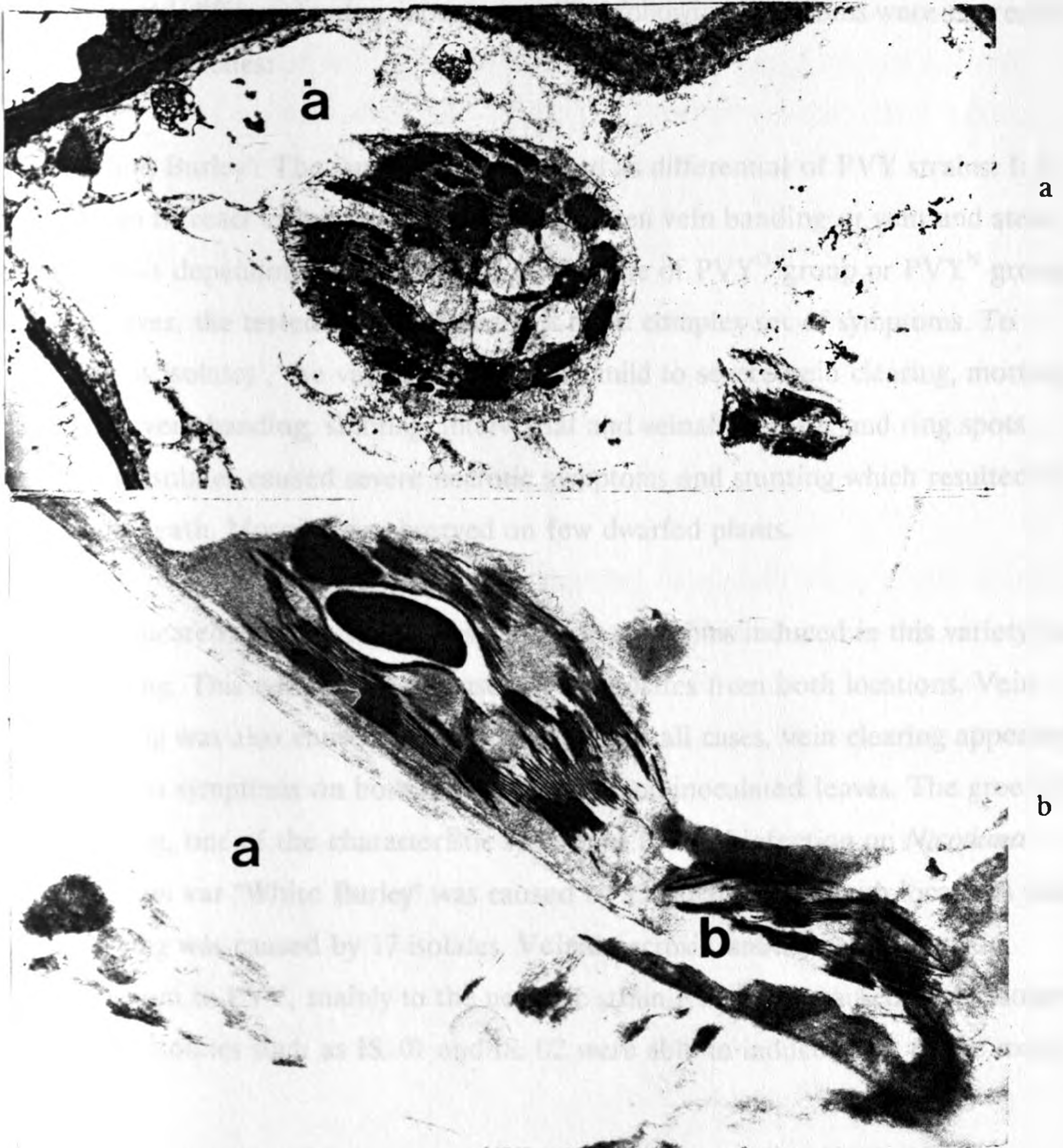


Fig. 10. Electron microscopy of Ultra-thin sections prepared from mottled leaf tissue of *Cyphomandra betaceae* showing elongated particles in the nucleus, lipid globules and size and number reduction of chloroplast.



3.2 COMPARISON OF PVY ISOLATES FROM POTATOES, *Solanum tuberosum*

3.2.1 Reactions of differential hosts to virus isolates

The comparison was carried out among 36 isolates collected from two different locations. . The following symptoms were induced by one group or another of virus isolates.

Nicotiana tabacum: Tobacco varieties of this species exhibited symptoms which could be attributed to more than one reported potato viruses. Symptoms appeared differently after inoculation. The following symptoms were expressed by various varieties:

-'White Burley': The variety is widely used as differential of PVY strains. It is known to react either by mere mottling, green vein banding or vein and stem necrosis depending on whether the strains are of PVY^O group or PVY^N group. However, the tested isolates induced a more complex set of symptoms. To various isolates , the variety reacted with mild to severe vein clearing, mottling, green vein banding, stunting, interveinal and veinal necrosis, and ring spots. Some isolates caused severe necrotic symptoms and stunting which resulted in plant death. Mosaic was observed on few dwarfed plants.

As indicated in table 9, the most common symptoms induced in this variety was mottling. This symptom was caused by 20 isolates from both locations. Vein clearing was also caused by the 20 isolates. In all cases, vein clearing appeared as the first symptoms on both inoculated and non-inoculated leaves. The green vein banding, one of the characteristic symptoms to PVY infection on *Nicotiana tabacum* var "White Burley" was caused by 13 isolates from both locations while stunting was caused by 17 isolates. Veinal necrosis, another characteristic symptom to PVY, mainly to the necrotic strain PVY^N was caused by 14 isolates. Some isolates such as IS. 01 and IS. 02 were able to induce three of the main

symptoms observed on 'White Burley'. They were able to induce vein clearing, mottling and stunting but failed to cause green vein banding. In the overall , all isolates, regardless of their origin and source (location and variety), except IS. 34, caused symptoms which could be related to PVY. The isolate IS. 34 caused characteristic ring spots which have never been related to any PVY strain. This was an indication that another virus was present in the infective complex. Such a trend of symptoms distribution was also observed on other *Nicotiana tabacum* mainly cv. Kentucky, Xanthii and Samsun

-'Kentucky': All symptoms described on 'White Burley' were observed on this variety. In addition, this variety showed a marked trend of developing leaf tapering coupled with a severe stunting and well-marked veinal necrosis, turning into greyish colour. Basal old leaves developed an extreme white mottling. However, as shown in Table 9, symptoms in brackets mean that the particular isolate did not induce these symptoms on all plants of an indicator species.

-'Samsun': Except for more conspicuous local lesions on inoculated leaves, this variety exhibited all symptoms induced in 'White Burley' and 'Kentucky'. However, most plants reacted with relatively mild symptoms but with a common stunting characteristic.

Nicotiana rustica: The following symptoms were observed as caused concurrently by one isolate or by separate isolates: Inoculated leaves showed necrotic local lesions of different sizes. Indicator plant species reacted with clearing to inoculation with IS.1, IS.3, IS.4, IS.6, IS.19, IS.22, IS.32 AND IS.36. Chlorotic and necrotic ring spots were caused by IS.5. Systemic yellow spots were caused by five isolates (IS.6, IS.21, IS,28, IS.30 and IS.35). However, other common symptoms of PVY such as mottling, vein-clearing , mosaic and stunting were induced by a large number of isolates (IS.1, IS.2, IS.3, IS.4, IS.5, IS.6, IS.7, IS.9, IS.12, IS.13, IS.15, IS.16, IS.19, IS.20, IS.22, IS.23, IS.25, IS.26, IS.27, IS.IS.29, IS.32, IS.33, IS.36). Necrotic local lesions were caused by four isolates (IS.21, IS.22, IS.24 and

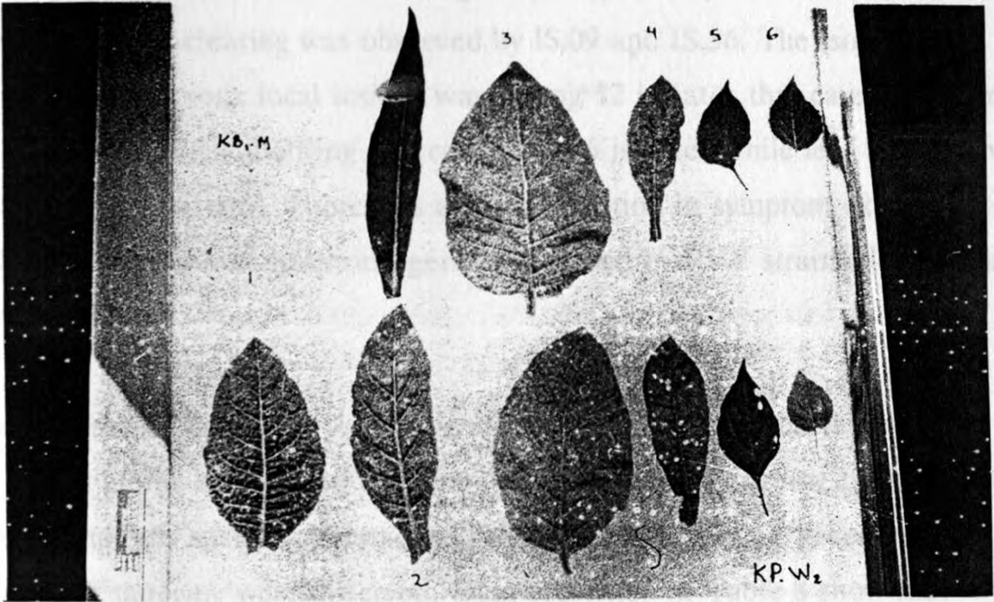
IS.28). Vein clearing appeared after 10 days which was the shortest incubation period and was caused by 12 isolates. Mosaic and stunting were caused by 8 and 4 isolates respectively. Chlorotic ring spots , which in most cases were caused by IS. 05 and IS.34 reflected the presence of mixed infection as such symptom could not be associated to PVY strains.

Nicotiana debneyi: The distribution of symptoms and their causal agents shows that this species reacted to most isolates by local or systemic necrotic lesions. The lesions were either scattered or coalescing along the midrib. Necrotic local lesions were caused by 10 isolates (IS.01; IS.02; IS.03; IS.04; IS.05; IS.06; IS.07; IS.21; IS.22; and IS.310). Systemic necrosis in form of necrotic blotches, veinal necrosis or veinal necrosis reaching the midrib were caused by 16 isolates (IS.01; IS.02: IS;03; IS.04; IS.05; IS.05; IS.07; IS.08; IS.11; IS.20; IS.24; IS.25; IS.26; IS.28; IS.29; IS.32, IS.34. Other symptoms observed on this species included mottling which was caused by 9 isolates (IS.09; IS.10; IS.11; IS.14; IS.21; IS.22; IS.25; IS.30 AND IS.36). Green vein banding was caused by 4 isolates (IS.06; IS.21; IS.23 AND IS.36), stunting by 6 isolates (IS.11; IS.19; IS.20; IS.23; IS.31 and IS.34) and abnormal upward growth by 2 isolates(IS.19 and IS.34).

Nicotiana glutinosa: Symptoms presented in Table 9 show that among the seven groups of isolates, characteristic scattered yellow or olive-like spots, small or big, were caused by five isolates. Apart from these symptoms, most plants of this differential species reacted to 22 isolates by a mild mottling and to 19 isolates by stunting without any other symptoms. Vein clearing induced as first symptom on other tobacco species and varieties was caused by three isolates only. Because of these reactions, *Nicotiana glutinosa*, although recommended as a good multiplication host for PVY and other viruses, could not help much in differentiating or separating different strains.

Physalis floridana: A remarkable fact with this differential host was that only three isolates (IS.20; IS.23 and IS.34) caused necrotic local lesions and 18

Fig. 11. Close up of differential host leaf infected by two isolates showing different symptoms



isolates caused indistinctive yellowing, though at different incubation times. This resembled to an early senescence of leaves. Eight isolates caused mild interveinal necrosis or necrotic blight, Stunting was caused by 13 isolates. *P. floridana* was one of the differentials which reacted with symptoms close to those caused by PVY strains as reported by de Bokx and Huttinga(1981).

Nicandra physaloïdes: Necrotic and irregularly shaped local lesions were caused by 7 isolates. Vein-clearing was observed by IS.09 and IS.36. The isolate IS.28 associated with necrotic local lesions was among 12 isolates that caused systemic blight. Mild to obvious mottling was caused by 16 isolates while leaf tapering was associated with 8 isolates. There was a great deviation in symptom expression from known or reported symptoms generally caused by PVY strains(de Bokx and Huttinga,1981).

Capsicum annuum cv. "Long Red Cayenne": This differential reacted with a range of symptoms as reported on various PVY strains. According to the tested isolates, symptoms such as systemic necrosis, mottling, stunting, green vein banding, leaf tapering were observed. Data presented in Table 8 show that systemic necrosis was initiated by 13 isolates while mottling was caused by 14 isolates. Among those 6 isolates (IS.08, IS.14, IS.21, IS.23, IS.25 and IS.29) were able to cause both symptoms. Isolates IS.08 and IS.14 also caused stunting of the plants.

'Hybrid Aquila 6': Reactions shown by this differential were the determining factor which showed that most of the isolates tested were rather a mixture of viruses. Detached 'Aquila 6' leaves are reported as responding only by local lesions to all PVY strains(de Bokx and Huttinga,1981). Fourteen isolates were able to induce such symptoms. However, to 7 isolates, some plants reacted with systematic mottling, with severe veinal necrosis and irregular blotches followed by plant death. Isolates IS.30 and IS.31 caused mottling.

Gomphrena globosa: Some deviations from PVY was also observed here. To 15 isolates, plants reacted with necrotic local lesions. It is known that *Gomphrena globosa* is immune to PVY strains and such necrotic lesions are induced by other viruses such as PVX and PVA (Berks, 1970; Bartels, 1971; de Bokx and Huttinga, 1981).

Datura stramonium: This species reacted with mottling or mosaic to 15 isolates (IS.01; IS.02; IS.03; IS.06; IS.13; IS.15; IS.17; IS.20; IS.21; IS.23; IS.26; IS.28; IS.33; IS.36).Ring spots were induced by 8 isolates (IS.01; IS.20; IS.21; IS.23; IS.25; IS.28; IS.29 and IS.34). This species is also known to be immune to PVY strains and symptoms such as ring spots are caused by PVX (Berks, 1970; Bartels, 1971; de Bokx and Huttinga, 1981). Such reactions were a clear indication of a complex infection, most probably with PVX being one of the viruses.

Datura metel: Nine isolates caused mostly green vein banding. This symptom was associated with severe or mild mottling (7 isolates). Ten isolates caused a characteristic systemic necrotic lesion along the veins and mid-ribs or just systemic interveinal necrosis. Mosaic was caused by 5 isolates and stunting by 4 isolates.

Chenopodium amaranticolor: Almost half the isolates or 16 isolates failed to induce symptoms on this assay host. Those were the IS.01; IS.02; IS.05; IS.07; IS.09; IS.10; IS.11; IS.12; IS.13; IS.14; IS.15; IS.16; IS.19; IS.24; IS.33 and IS.34. This observation may lead to speculation that there were some negative factors which prevented the expression of infection on this assay host, known to have a wide host range. In addition these isolates which failed to induce local lesions on *C. amaranticolor* were able to infect other tested differential plant hosts with symptoms common to PVY, PVA or PVX, and these viruses are known as able of inducing chlorotic or necrotic lesions on this assay hosts (Berks, 1970; Bartels, 1971; de Bokx and Huttinga, 1981). Apart from any adverse abiotic factor in the greenhouse, the most possible explanation may be the mutual inhibiting effect of

complex infection. The remaining isolates were able to induce chlorotic local lesions. However, such lesions showed a great variation in size, shape and degree of coalescence.

Chenopodium quinoa: As for *C. amaranticolor*, chlorotic local lesions were observed in most sets of plants. However, some isolates failed to cause local lesions of any kind (IS.2, IS.9, IS.12, IS.13, IS.17, IS 18, IS.21, IS.24, IS.33, IS.34 and IS.35)
Chenopodium album: Although mostly used as an assay host for a number of viruses, this differential is immune to PVY strains(de Bokx and Huttinga,1981),. However, The isolates IS.10 and IS.12 reacted with chlorotic local lesions. Apart from ring spots on *Nicotiana* spp and local lesions on *Gomphrena globosa* , this was another proof that there was a mixture of potato viruses.

Symptoms observed after the first manual inoculation of differential hosts showed a great deviation from symptoms reported as caused by PVY strains. This deviation was reflected by the fact that some differential plant host that usually immune to PVY strains reacted by various symptoms. These reactions were observed on *Gomphrena globosa, Datura stramonium and Chenopodium album.(fig 11)*

Table 9. Reactions induced by virus isolates extracted from *Solanum tuberosum* varieties in Kiambu and Rift Valley District

Differential	Isolates & Reactions							
	IS1 (WKP)	IS2 (WKP)	IS3 (WKP)	IS4 (BVM)	IS5 (UK KP)	IS6 (MRT)	IS07 (MKB)	IS08 (MKB)
Nicotiana Tabacum cv								
- White Burley	M,VC,St.	M,VC,St.	SeNe,VC,St.	M,St.	VC,M,GVB	VC,GVB,M,blw	Mo,GVB	St.
- Kentucky	M,VC	M,VC,VN	VC,M	M	VC,M,LT	No	GVB,St,Lt	VC,GVB
- Samsun	RS,St,blw	RS,St,blw	VC,SeVNe,M,St,blw	RS,M,St,blw	VC,M,St.	VC,M,GVB,Blw	M,blw,St.	No
Nicotiana rustica	VC	M	VC	VC,NLL,St.	M,CRS	VC,NLL,M,SYS	M,St.	VC -- No
Nicotiana debneyi	NLL,VC,VN	NeLL,VC,VN	NLL,VC,VN	NLL,MrN,VC	M,VNrb,NLL	NLL,No,GVB	VC,NLL,VNrb	IVN,MVrb
Nicotiana glutinosa	VC,M	No	VC,M,VN,St,Blw	VC,Ep	M,St.	-	M	M,St.
Nicandra physaloides	IVN,St.	M	M,St.,Lt.	IVN,M,Mo,St.	VN,Rb.	No	IVN	M
Physalis floridana	M,IVN,St.	M	M	M	VC,Mo	VC,GVB	IVN	M--No
Datura stramonium	Mo,RS	Mo	M	-	No	VC,Mo,Lt,St.	No	Mo
Datura metel	-	-	-	VC,LD,St.,GVB	-	-	-	Mo
Capsicum annum L.R.C.	Ne	Mo,Lt,St.	M	Mo	No	No	No	M,Ne,St.
quila 6	-	-	-	NLL	-	-	NLL	SeNe
Chenopodium quinoa	CLL(vi)	No	CLL	CLL(I)	CLL(I)	CLL	CLL(I)	CLL
Chenopodium amaranticolor	No	No	CLL	CLL(i)	No	CLL	No	CLL
Chenopodium album	No	No	No	No	No	No	No	No
Gomphrena globosa	NeLL	NeLL	NeLL	NeLL	No	CLL (Ne)	No	NLL

Table 9. Reactions induced by virus isolates extracted from *Solanum tuberosum* varieties in Kiambu and Rift Valley District (cont..)

	IS9 (UKKP)	IS010 (MKB)	IS11 (WKB)	IS12 (WKP)	IS13 (MAN)	IS14 (MAN)	IS15 (WKP)	IS16 (UKKP)
Nicotiana Tabacum cv	VC,M,St.	M,No,GVB,St.	No	M,IVN,St.	M,GVB,St.	M,No,LD	M,GVB,St.	GVB,No,St.
- White Burley	VC,M,Lt,St.	VC,M	No	No	No,GVB,LT	M,VN,Lt	M,GVB,Lt,St	M,GVB,Nbi
- Kentucky	VC,M,Lt,St.	-	IVN,St.	No	-	-	-	-
- Samsun	M,St.	VC	M	IVN	-	-	-	-
Nicotiana rustica	M,IVN,St,NLL	IVN,VNrb	IVN,VNrb	NLL,VNrb,VC	M,GVB,LD,St.	VNrb	M,GVB,LD,St	No
Nicotiana debneyi	M	M	IVN,M,SYS,St.	No	-	M	-	-
Nicotiana glutinosa	VC,M,IVN,St.	M	-	No	NLL	M	No	M,Nbl
Nicandra physaloïdes	VC,M	INV,St.	IVN,VN,St.	M	No	M,St	M,Nbl	M
hysalis floridana	No	M	-	-	No	M,Nbl	No	No
Datura stramonium	-	-	-	-	M,GVB,St.	Nbl	M,No,GVB,LD, St	Nbl
Datura metel	No	IVN	IVN	Nbl	VC,M	M,NL,St.	M,LD	M,LD,St.
Capsicum annum L.R.C.	NLL	NLL	-	-	-	-	-	-
Aquila 6	CLL(i)	Nbl	NLL	No	-	-	-	-
Chenopodium quinoa	No	NLL	CLL	No	-	-	CLL	CLL
Chenopodium amaranticolor	No	No	No	No	-	-	-	-
Chenopodium album	No	NLL	-	NeLL	-	-	-	-
Gomphrena globosa	-	-	No	NLL	NLL	NLL	NLL	No

Table 9. Reactions induced by virus isolates extracted from *Solanum tuberosum* varieties in Kiambu and Rift Valley District (cont..)

Differential	Isolates & Reactions							
	IS17 (MBV)	IS18(M-KB)	IS19	IS20	IS21	IS22 (UKKP)	IS23 (M-RT)	IS24 (M-DR)
Nicotiana tabacum cv								
- White Burley	VNrb	M,GVB,blw	VC,VN,M,GVB	VC,M,VN	VC,M,GVB,blw	M,VC,Nbl	NLL,VN	No,VN,Nbl
- Kentucky	M,Lt,up	-	VC,GVB,Lt	VC,VN,Lt,St, blw	GVB,M,St.,blw	GVB,M,St,blw	VC,VN,blw,Lt	M,GVB,blw
- Samsun	-	-	M,blw,St.	M,St.	VN,blw	M	M	M,St.
Nicotiana rustica	-	-	VC,No,St.	M,Nbl,St	VC,NLL,Sys	VC,NLL,M	M,Nbl,St.	NLL,VNrb,Dt
Nicotiana debneyi	-	-	St,Lt	VN,VNrb,St.	NLL,M,GVB, VNrb	NLL,M	No,GVB,St.	Nbl,VNrb,Dt
Nicotiana glutinosa			M	M,Nb,RS	M	M,St	No,St	No
Nicandra physaloides	M,Y,Nbl		M,Lt	NLL,M,Nb	VP,Rb,St.	Rb	NLL,M,Lt	Nbl,M,St
Physalis floridana	M	Y	M	NLL,M	VC,GVB,St	No	NeLL,M,St	M,St.
Datura stramonium	VC,M,Nvl		No	M,No,RS	No,RS,NLL	CLL	M,RS	No
Datura metel	No,GVB	VC---> No	No,GVB	M, Nb	Sns	Sns	Sns	GVB,M
_psicum annum L.R.C.	M,St,Nb	Y	No	M,No,St	Nbl,M	M	M,Nbl	No
Aquila 6	NLL		NLL	Nbl,St,NLL	NLL(I)	NLL	NLL,M	NLL
Chenopodium quinoa			NLL(I)	NLL(I)	No	CLL	CLL(I)	No
Chenopodium amaranticolor	No	No	No	No	No	No	NLL(I)	No
Chenopodium album								
Gomphrena globosa	-	-	No	NLL	NLL	NLL	NLL	No

Table 9. Reactions induced by virus isolates extracted from *Solanum tuberosum* varieties in Kiambu and Rift Valley District (cont..)

Differential	Isolates & Reactions							
	IS25 (WKP)	IS26 (W-KP)	IS27 (N-DR)	IS28 (N-DR)	IS29 (W-KP)	IS30 (N-DR)	IS31 (UK-KP)	IS32 (UK-KP)
Nicotiana tabacum cv								
- White Burley	N,Nbl,VN,St	VC,VN,N,St	VC,N	VC,VN,N,blv	VC,VN,St	VC,GVB,Nbl	VC,N,GVB	No,GVB,Lt
- Kentucky	VC,GVB,blv	GVB,blv	GVB,Lt	GVB,Lt	N,GVB	N,GVB	N,Lt,St	N,Lt
- Samsun	St,blv	N,St	N,St	N,St	Wlb,N,St	N,St	N,St,blv	N
Nicotiana rustica	VN,No,Nbl	Nbl,Vn,No	N,blv	NLL,Sys	N	CLL,VC,Sys	No	VC,N
icotiana debneyi	Nbl,Dt,N	Rb,VNrb	No	Nbl,VNrb,Dt	Nb,VNrb,Dt	VC,N	NLL,VC,St	VNrb,blv
Nicotiana glutinosa	N,St	N,St	N,Sys	Sys,St	Sys	N	N	N
Nicandra physaloides	NLL,Lt,Rb	N,NLL,It	Nbl,N	CLL(1),Nbl	Rb	NLL,No,St	Nbl	N,St,It
Physalis floridana	N,Nbl,St.	Not	N	Y,St	No	VC,GVB	No	Nbl
Datura stramonium	No,RS	No,RS	No	No	CRS	N,No	No	No
Datura metel	Sns	Sys,Nbl	GVB,N,LD	Sns	Sns	VC,No,Sys	Sns	No
Capsicum annum L.R.C.	N,IVN	NoLd	NoLD	No,BLt	N,VN	No	No	N
Aquila 6	NLL	Nbl	NLL	NLL,Nbl	Nbl -	NRS,No	NeLL,N,St	No
Chenopodium quinoa	CLL	CLL	CLL	CLL(I)	NLL	CLL	CLL	CLL
Chenopodium amaranticolor	CLL	CLL(-)	CLL	CLL(I)	CLL	CRS	CLL	CLL
Chenopodium album				-			-	-
Gomphrena globosa	NLL	No	NLL(g-r)	NLL(g-r)	NLL	NLL	No	No

Table 9. Reactions induced by virus isolates extracted from *Solanum tuberosum* varieties in Kiambu and Rift Valley District (cont..)

Differential	Isolates & Reaction			
	IS33 (W-KP)	IS34 (W-KP)	IS35 (W-KP)	IS36 (W-KP)
Nicotiana Tabacum cv				
- White Burley	VC,Nb!,Dt	N,RS,St	SeVC,NC,St,En	VC,VN,Nbl,St,Dt
- Kentucky	VC,GVB	VC,Rb,N	N,GVB,blw	N,GVB,Lt
- Samsun	N	VC,VNrb	St,blw	N
Nicotiana rustica	VN,Nbl,St,Dt	N,RS	Sys	VC,VN,Nbl,St
Nicotiana debneyi	No	VN,op,St	No(+)	VC,N,GVB
Nicotiana glutinosa	No(+)	N	K	Sys
Nicandra physaloïdes	N,Nbl,At	N,lt	Lt,op	VC,N,Nbl
Physalis floridana	Y,St	NLL,Nbl,N	Y,St	St,N
Datura stramonium	No,St	No,RS	-> No	N,St
Datura metel	Sns	GVB,N	GVB,Sys,N	N,St
Capsicum annum L.R.C.	No,VN,IVN,St	No,Nbl,VN	No,Blt	No,Nbl
Aquila 6	Nbl(br-g)		NLL	
henopodium quinoa	No	No	No	CLL(I)
Chenopodium amaranticolor	No	No	CLL(I)	Nbl
Chenopodium album			No	
Gomphrena globosa	NLL	NLL(g-r)	No	NLL(g-r)

Abbreviations used in Table 9

( )-- No = symptoms not consistent or persistent

AN = Annet

blw = extreme mottling on the lower leaves

Bv = Bvumbe

CLL = chlorotic local lesion

CLL (Ne) = chlorotic local lesion turning necrotic lesions

CLL(i/I) = chlorotic local lesions with irregular margins or not well defined chlorotic lesions

CRS = Chlorotic ring spot

DR = Dutch Robjin

Dt = plant death

GVB = Green vein-banding

IS = isolate

IVN = Interveinal necrosis

KB = Kenya Baraka

KP = Kerr's Pink

LD = leaf distortion

LP = leaf puckering

Lt = leaf tapering

M* = Molo

M = mottling

Mo = Mosaic

Nbl = blight

Nbl(br-g) = necrotic blotch turning grey at later stage

NLL(g-r) = necrotic local lesions with reddish outer margins or frog eye type

Ne = Necrosis

Nll = Necrotic local lesion

NeLL(I) = necrotic local lesions with irregular margins

No = no symptoms

No(+) = no symptoms but virus recovered

Rb = roughening of leaf surface

RS = ring spots

RT = Roslyn Tana

Sevn = Severe veinal necrosis

Sm = Severe mottling

SMo = Severe mosaic

Sns = Systemic necrotic spot

St = Stunting

Svne = Severe veinal necrosis

Sys/SYS = systemic regular yellow spots

up = upward abnormal growth or etiolation

VB = vein banding

VC = vein-clearing

VN = Veinal necrosis

VNrb = Veinal necrosis reaching the midrib and stem

VNS = Veinal necrotic streak

W = Wangige

Y = Yellowing

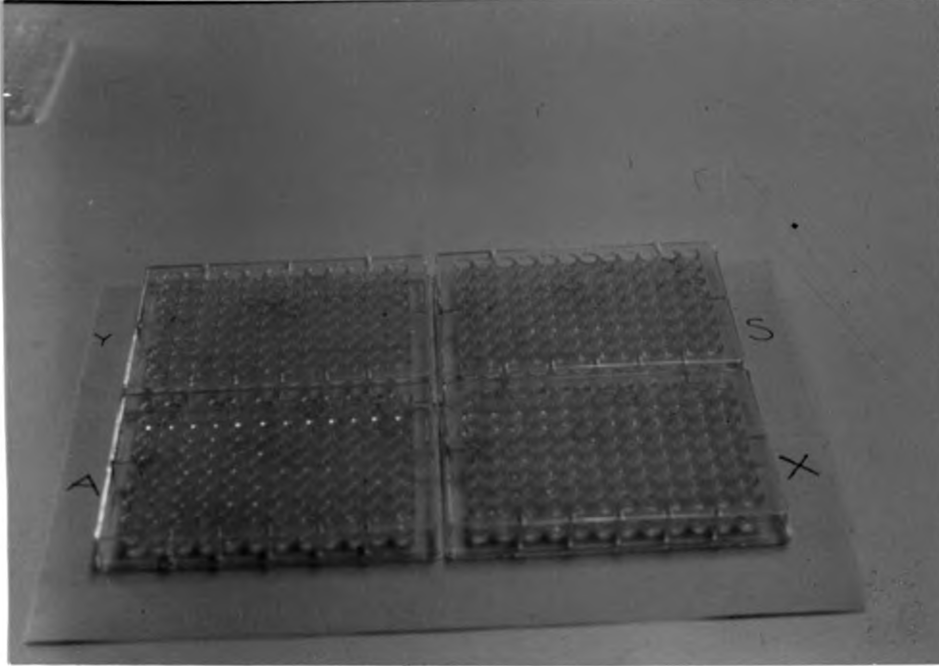
3.2.2 Forms and rates of occurrence of PVY strains in Potato crops

As demonstrated by reactions of differential hosts, PVY strains were rarely found on single infections on potato crops in the field. Thus, any studies on the extent of variation of PVY need, as a pre-requisite condition, to determine in which form the PVY occurs. The collection of isolates on the basis of symptoms on potato plants could not help much in assessing both the variation and the rate of occurrence of potato virus Y in potato crops. To determine in which form and at which rate potato virus Y strains occurred in the field, ELISA tests were conducted on all differential hosts, which reacted positively (fig. 12). The results of ELISA (Table 10) tests showed that when collecting infected potato plants samples in the field, 85.7% of the samples will contain potato virus Y. However, only 16.7% of the sample will be made of pure PVY infection. These results show that PVY can be found at the rate of 69% in the form of mixed infection with one, two or three other viruses. Associations with one either PVX or PVA was estimated at 26.2%, that with two different viruses at 33.3% (PVY-X-A; PVY-X-S and PVY-S-A), and that with all three other viruses at 9.5%(PVY-X-S-A).

Table 10. Forms and rates of occurrence of PVY strains in potato crops.

Type of virus	Rate of infection	
	Elisa indexed samples	Percentage of occurrence
PVY	7	16.7
PVX	6	14.3
PVY-A	4	9.5
PVY-X	7	16.7
PVY-S	0	0
PVY-X-A	5	11.9
PVY-X-S	4	9.5
PVY-S-A	5	11.9
PVY-X-S-A	4	9.5

Fig. 12. Detection of mixed infections by the use of ELISA test showing the addition of PVA, PVS and PVX to PVY infection.



3.2.3 Strain of PVY separated by differentials hosts and ELISA

Thirteen virus isolates separated by differential hosts and confirmed through ELISA test to be PVY were submitted to bio-assay for their differentiation into PVY strains. The isolates included one extracted from tree-tomato, seven from pure or single infection of PVY and five from a double infection with PVX that were separated after two serial inoculation on variety Annet which is immune to PVX. Table 11 shows a summary of reactions given by various differential hosts. The details of these reactions are also dealt with individually to indicate the isolates and their respective pathogenicity as presented below:

Nicotiana tabacum

cv. 'Samsun':

The following various reactions were observed:

Vein-clearing was caused by isolates TT, IS.15, and IS.27,

Veinal necrosis which followed as a characteristic symptom of PVY was only induced by the isolate TT.

Mottling was cause by IS.02, IS.04, IS.08, IS.14, IS.15, IS.19, IS.24, and IS.27, respectively. This symptom is associated with PVY as reported by other workers (de Bokx and Huttinga, 1981)

IS.14 induced mosaic in this variety.

Extreme mottling of the basal leaves was induced by 8 isolates, namely: IS.01, IS.02, IS.04, IS.15, IS.19, IS.27, IS.35, while stunting occured in plants inoculated with IS.01, IS.02, IS.04, IS.08, IS.15, IS.24, IS.27 and IS.35.

cv. 'White Burley'

Vein-clearing was caused by isolates IS.01, IS.02, IS.14, IS.19, IS.24, IS.35, and TT; and veinal necrosis by IS.02, IS.04, IS.14, IS.19, IS.24, and TT.

Green vein banding is generally caused by both strains of PVY° and PVY^{N} but mostly by PVY° strains (Delgado-Sanchez and Grogan, 1970; de Bokx and Huttinga, 1981). The reaction was observed on differential sets inoculated with IS.15, IS.18 and IS.19. The IS.19 also caused veinal necrosis on the inoculated leaves.

Mottling was induced by isolates IS.01, IS.04, IS.08, IS.15, IS.17, IS.18, IS.24, IS.27, and TT.

Mosaic which is rare on PVY-infected plants was induced by IS.14, IS.17, and IS.24.

'White Burley' stunting was as common as mottling. It was caused by IS.02, IS.04, IS.15, IS.17, IS.35, and TT.

cv. 'Kentucky'

Vein clearing which is often associated with veinal necrosis which follows a few days later on this variety was induced by IS.01, IS.02, IS.04, IS.08 , IS.19, IS.24, IS.27, and TT. Except for IS.01, IS.08 and IS.27, the remaining isolates caused veinal necrosis and/or necrotic blotches.

Veinal necrosis was induced by IS.01, IS.02, IS.04, IS.08, IS.15, IS.18, IS.19, IS.24, and IS.35,.

Observations on symptoms induced by all tested isolates showed that symptoms such as mottling, green vein banding and stunting could not help in differentiating PVY strains. However, vein-clearing was shown to be more predominant causing isolates to be able to induce subsequent veinal necrosis and/or necrotic blotches. Necrotic symptoms such as veinal necrosis, midrib necrosis, necrotic blotches were caused in a consistent way on 'White Burley' and 'Kentucky' but not on 'Samsun', on which only the isolate TT from tree-tomato was able to induce necrosis. It should be concluded that the variety 'Samsun', does not always react by necrosis to strains of PVY^{N} group.

Nicotiana glutinosa

In most cases, this differential reacted with mild to more or less severe mottling without plants showing any stress on vegetative growth. However stunting was induced by IS.08, IS.18 and IS.24. Etiolation or an abnormal upward growth was caused by IS.04 and TT while vein clearing was induced by IS.01 and TT. IS.02 did not induce any symptom but was recovered in bio-assay test on Aquila 6. The isolate IS.27 caused systemic yellow spots on the leaf blades without affecting growth, and the isolate TT caused veinal distortion and leaf tapering. Thus, this tobacco species could not help much in differentiating PVY strains.

Capsicum annum cv. 'Long Red Cayenne'

Three isolates failed to induce symptoms on pepper. Among those, two of those inducing necrotic symptoms on tobacco varieties, namely isolates IS.19 and IS.24. Ten isolates induced various symptoms on this species. The isolates IS.02, IS.04, and TT, which caused necrosis on tobacco varieties and species and considered as of PVY^N group caused mosaic and leaf distortion, whereas IS.01, IS.08, IS.35 and IS.17 of PVY° group caused mottling and necrotic blotches. Such distribution shows that in all PVY^N strains affecting pepper, only IS.14 could not induce necrosis on pepper and of all PVY° strain, only IS.15 could not cause necrotic symptoms on pepper.

Considering norms of differentiations reported by de Bokx and Huttinga (1981), the isolates, on the basis of their reaction on these three tobacco varieties, can be separated as follows (Table 12):

PVY^N group strains: IS.02, IS.04, IS.014, IS.019, IS.024 and TT.

PVY° group strains: IS.01, IS.08, IS.15, IS.18, IS.17, IS.35, IS.27.

Apparently the isolate TT, from tree-tomato may be classified as the necrotic strain of PVY but this finding does not exclude the fact the PVY° group of strains may also be common in tree-tomato

Table 11: Reaction of differential hosts inoculated with 13 PVY isolates from Solanum tuberosum and Cyphomandra betaceae.

Diffential host	Isolates												
	IS.15	IS.17	IS.18	IS.19	TT	IS.4	IS.8	IS.1	IS.14	IS.24	IS.27	IS.35	IS.2
Nicotiana tabacum cv.Samsun	M,St,VC, Blw	No+	No	M,blw,St	VC,VNe, St	M,St, Blw	M,St,Blw	M,St,Blw	M,Mo	M,St,	VC,M, St,Blw	St,Blw	St,Blw
cv. White burley	M,GVB,St	Mo,St	M,GVB,Blw	VC,M,GVB, VNe	VC,M,LD, St,VNe	M,St, VNe	St	VC,M,St	VC,M,Mo, LD, VNrb,Dt	VC,Mo, VNe,Nebl	VC,M	VC,St,Et	VC,VNe St,Dth
cv. Kentucky	M,GVB,Lt, St	No+	M,GVB,Lt	VC,GVB,Lt	VC,VNe, GVB,Lt	VC,VNe, M,Lt	VC,M,GVB	VC,M	Mo,GVB,Lt	VC,VNe,M ,GVB,Blw	VC,GVB,Lt	M,GVB, Blw	VC,VNe ,M,Lt
Datura Stramonium	No	No	No	No	No	No	No	No	No	No	No	No	No
D. metel	M,Mo,GVB, LD,St	Mo,GVB	VC(-)	Mo,GVB	VC,Mo, GVB	VC,GVB, LD,St	Mo	No(+)	Nebl	M,GVB	VC,M,GVB, LD	GVB	No
N.glutinosa	M	M	M,St	M	VC,M,Lt	VC,Et	Mo,St	VC,M	M	M,St	M,Sys	M	No(+)
Capsicum annuum	M,LD	M,St, Nebl	No	No	M,Mo	Mo	Mo,Nebl, St	Nebl,Mo	Ne,M,ST	No	Mo,LD	Mo,Blt	Mo,Lt, St
Physalis floridana	M,Nebl,St	No	M	M	Nebl,M	M	M(-)	M,IVNe,St	M	M,St	Nebl,M	M,St	M
Aquila 6	NLL	NLL	NLL	NLL	NLL	NLL	NLL	NLL	NLL	NLL	NLL	NLL	NLL
C. amaranticolor	CLL	No	No	No	No	CLL	CLL	No	No	No	CLL	CLL	No
C.quinoa	No	No	CLL	CLL	CLL	CLL	CLL	CLL	No	No	CLL	No	No

Descriptions for abreviations

Blt = blight

Blw = extreme mottling on the lower leaves

CLL= chlorotic local lesions

Dth = plant death

Et = Etiolation

GVB = green vein banding

IVNe = interveinal necrosis

LD = leaf distortion

Lt = leaf tapering

M = mottling

Mo = mosaic

M(-) = no persistant mottling

Nebl = blight

Ne = necrosis

NLL = necrotic local lesions

No = no reaction

No(+) = reaction disappears but virus is recovered

St = stunting

VC = vein clearing

VNe = vein necrosis

VNrb = veinal necrosis reaching the midrib and stem

Table 12. Distribution of 13 PVY strains into PVY^o and PVY^N groups

Isolate	Rate of occurence			
	PVY(o)	PVY(n)	Pepper(+)	Pepper(-)
IS.1	*		*	
IS.2		*	*	
IS.4		*	*	
IS.8	*		*	
IS.14		*	*	
IS.15	*		*	
IS.17	*		*	
IS.18	*			*
IS.19		*		*
IS.24		*		*
IS.27	*		*	
IS.35	*		*	
IS. TT		*	*	
	7/13	6/13	10/13	3/13

Table summary: PVY^o - 7 strains PVY^N - 6 strains

PVY^o virulent to pepper - 6 strains

PVY^N virulent to pepper - 4 strains

3. SUSCEPTIBILITY OF POTATO VARIETIES TO APHIDS AND THE EFFECTS ON THE PVY INCIDENCE

3.1 Susceptibility of potato varieties to aphids

The fluctuation of aphid populations on eight potato varieties was assessed in a completely randomized block design trial. The first trial was conducted during the long rain season from april to july 1987, and the second from october 1987 to january 1988. Each variety was replicated four times. Although study on aphid species distribution was not carried out, it was obvious to notice by visual assessment the predominance of the potato aphid *Macrosiphum euphorbia* and the green peach aphid *Myzus persicae* and a less important presence of the bean aphid *Aphis fabae* due probably to the presence of several bean experimental plots in the area. Other aphid species were also spotted on potato leaves (Fig. 20).

Aphids were collected on four rows of each plot. As shown in Fig. 4, aphids were not harvested on plants of the two middle rows, but from the second and third rows and the sixth and seventh rows located on both sides of the two middle rows. Aphid collection and counts were carried out every week from the fifth week after plantation to the 14th week. Before statistical analysis, aphid count (N) was transformed into ($\sqrt{N}$).

As shown in Fig. 13, 14, 15 and 16 and row data in annexes 1 and 3, the total number of aphids collected on eight varieties, for a period of nine weeks, showed a great variation both in time and between varieties. A ratio of 3,5:1 was found between 'Kerr's Pink', the control, with the highest aphid infestation (N = 1035) and 'Kenya Baraka' with the lowest infestation (N = 294). Four varieties, 'Kenya Baraka' (N = 294), 'B.53' (N = 404), 'Bvumbe' (N = 516) and 'Pimpernell' (N = 553), showed infestation level lower than the average (656.1).

Apparently, the other three varieties 'Dutch Robjin' (N = 849), 'Feldeslohn' (N = 943) and 'Annet' (N = 767) and the control 'Kerr's Pink' showed a higher infestation

rate. Generally, the curve in Fig. 15, shows that there is a strong fluctuation of aphid population during the nine weeks of observation. The curve reflecting the evolution of an average aphid population shows a ratio of 4:1 between the sixth and the eleventh week. The curve also shows that there are two peaks of infestations. A less important maximum reached at the 8th week and a stronger peak reached on the eleventh week. The same pattern of aphid population was shown by five of the eight compared varieties, namely 'Kerr's Pink', 'Dutch Robjin', 'Feldeslohn', 'Annet', and 'Bvumbe'. The curve evolution of an average population showed that aphid infestation became relatively significant on the 8th week, when it reaches an average of 2.7 aphids per plant. However, the highest peak is reached on the 11th week with an average of 4.3 aphids per plant. When considering the general average of aphid number per week and per plant, it can be concluded that aphid population was relatively low during the long rain season. This state does not mean that the aphid population is generally low during this season but they are mostly washed off the leaves by rains and wind.

When dealing with different sets of a population, it is considered that those are proportions and therefore analyses of variance and comparison of means are not carried out on raw data but on transformed values (Steel and Torries, 1960). The transformed values were obtained using ($\sqrt{N}$)(annex 2).

The analysis of variance were done and means compared both according to Duncan and Dunnet test (annexes 4, 5, 6). The first was used as an appropriate test to compare several means at various ranges and the second is indicated as suitable for comparing several treatment means to a single control ('Kerr's Pink') by using a single *t* (Dennet) values (Steel and Torries, 1960).

Means represented in annex 5. show that there is a ratio of 2:1 between the highest means (KP = 4.88 and the lowest KB = 2.89). The analysis of variance (annex 4) shows a highly significant *F* value. The comparisons of means by using Duncan test (p

= .05%) shows there is a significant difference between 'Kerr's Pink' (highest rate) and 'Kenya Baraka', 'Bvumbe', 'B.53' and 'Pimpernell', respectively.

There was no significant difference between 'Kerr's Pink' and 'Dutch Robjin' (second highest), 'Feldeslohn' (the third highest rate) and 'Annet' (fourth highest). However, the same test at p = .01% revealed less significant differences between means. Such differences were only observed between 'Kerr's Pink', 'Kenya Baraka' and 'Bvumbe', respectively. Duncan test (p= .05%) also revealed a significant difference between 'Dutch Robjin' (the second highest) and 'Kenya Baraka' (lowest rate). However, the same 'Dutch Robjin' showed differences only to 'Kenya Baraka'at p= .01%. Another emerging difference was between 'Feldeslohn' and 'Kenya Baraka' at p=.05 and p=0.1% respectively.

The comparison of all treatments means to the control 'Kerr's Pink' mean by using Dunnet test revealed that there was a significant difference between 'Kerr's Pink' and four varieties, namely 'Kenya Baraka', 'Bvumbe', 'B.53' and 'Pimpernell'. However, there were no differences between the control and means of 'Dutch Robjin', 'Feldeslohn' and 'Annet'. All significant differences are summarised in annex 5.

The second field experiment on the assessment of the field resistance of potato varieties to aphid and PVY was carried out during the following short rains season between November 1987 and January 1988 at an neighbouring field on the same site. Tubers obtained from the previous experiment were sorted and those having 4.5 - 5.0 cm in diameter and showing no disease symptoms were selected and kept separately in meshed polythene bags. The bags containing seed tubers were kept under natural alternation of light and darkness and protected against aphid infestation by a layer of aldrin powder. After sprouting, a sample of 12 tubers was removed at random from each variety and each replicate subjected to ELISA test. The remaining tubers were planted in the field. As in the first experiment, varieties were tested following a completely randomized blocks design with four replicates and given the same treatments (spacing, fertilizer, weeding) and more irrigation (once every two weeks)

due to scarcity of rains during that cropping season. The collection and counts of aphid were done weekly following a systematic pattern as during the previous experiment. Before analysis and comparison of means, aphid count (N) was transformed into $\sqrt{N+1}$.

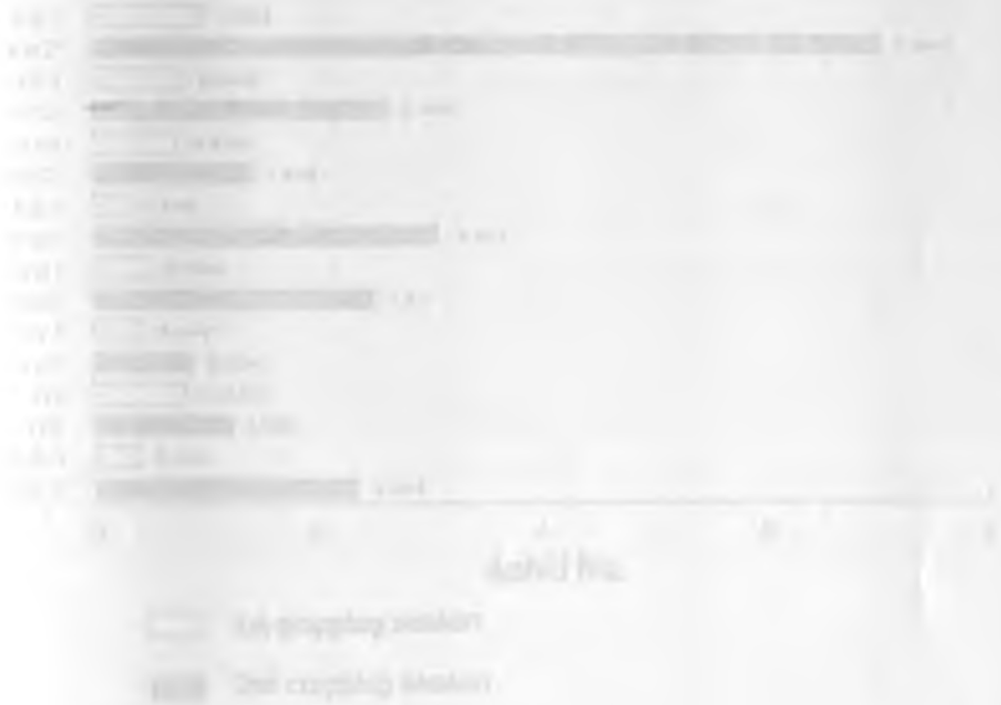
Comparison of total aphid population collected on each variety (annexes 7 and 9, Fig. 15 and 16) for a period of 9 weeks, showed a wide variation. A ratio of 7:1 was found between the control 'Kerr's Pink' showing the highest aphid count (N = 7044) and 'Bvumbe' (N = 884). Five varieties, 'B.53', 'Annet', 'Feldeslohn', 'Bvumbe' and 'Pimpernell' showed an infestation level lower than the average (X = 2659.25). Varieties with a higher than the average rate were 'Dutch Robjin' (N = 2661) 'Kerr's Pink' (N = 7044), 'Kenya Baraka' (N = 3131).

The observation of the weekly total number of aphid also showed strong fluctuations (Fig. 16). The curve of an average aphid population showed that aphid starts infesting potato critically after 6 or 7 weeks (more than 3.4 aphid/plant) and the infestation reaches its pick between the 9th and the 12th week, with an average infestation per plant varying from 12 to 17.9 aphids per plant. The second experiment also showed that aphid infestation is higher during the short rains season. The average population observed this season was four times higher than the average population observed during the previous experiment (Fig. 16).

The Anova table of transformed values ($\sqrt{N+1}$) showed a highly significant F value (11.2) among varieties, while there was no difference between blocks (annexes 8 and 10).

The comparison of means by the Duncan new multiple range test (annex 11) showed that there were differences (p=.05 and p=.01 level) only between the control 'Kerr's Pink' and all other tested varieties. The variety 'Kenya Baraka', which showed a rather low infestation rate during the previous season showed the second highest total population (N = 3131) and an average per plant of (X = 7.19). However it did not

show any significant difference. Nevertheless such attraction of aphid during the second season showed that the low value observed during the first season was mainly due to a low total population of aphid, generally observed during long rains season. The control Kerr's pink appeared to be different to all other varieties when compared by using the single t (Dunnet) value at both p= .05 and p=.01 levels (Annex 12).



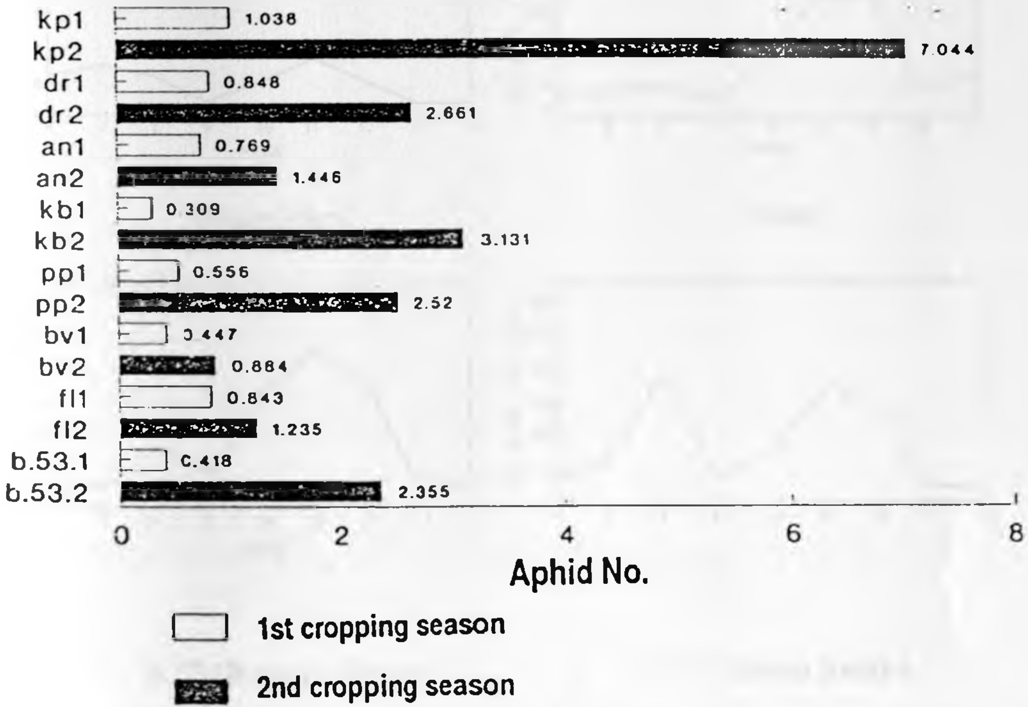


Fig. 13. Total counts of aphid population collected on *Solanum tuberosum* varieties during the long and short rains season. 1987/88.

Fig. 14. Evolution of aphid population levels on *Solanum tuberosum* during the long rains season 1987

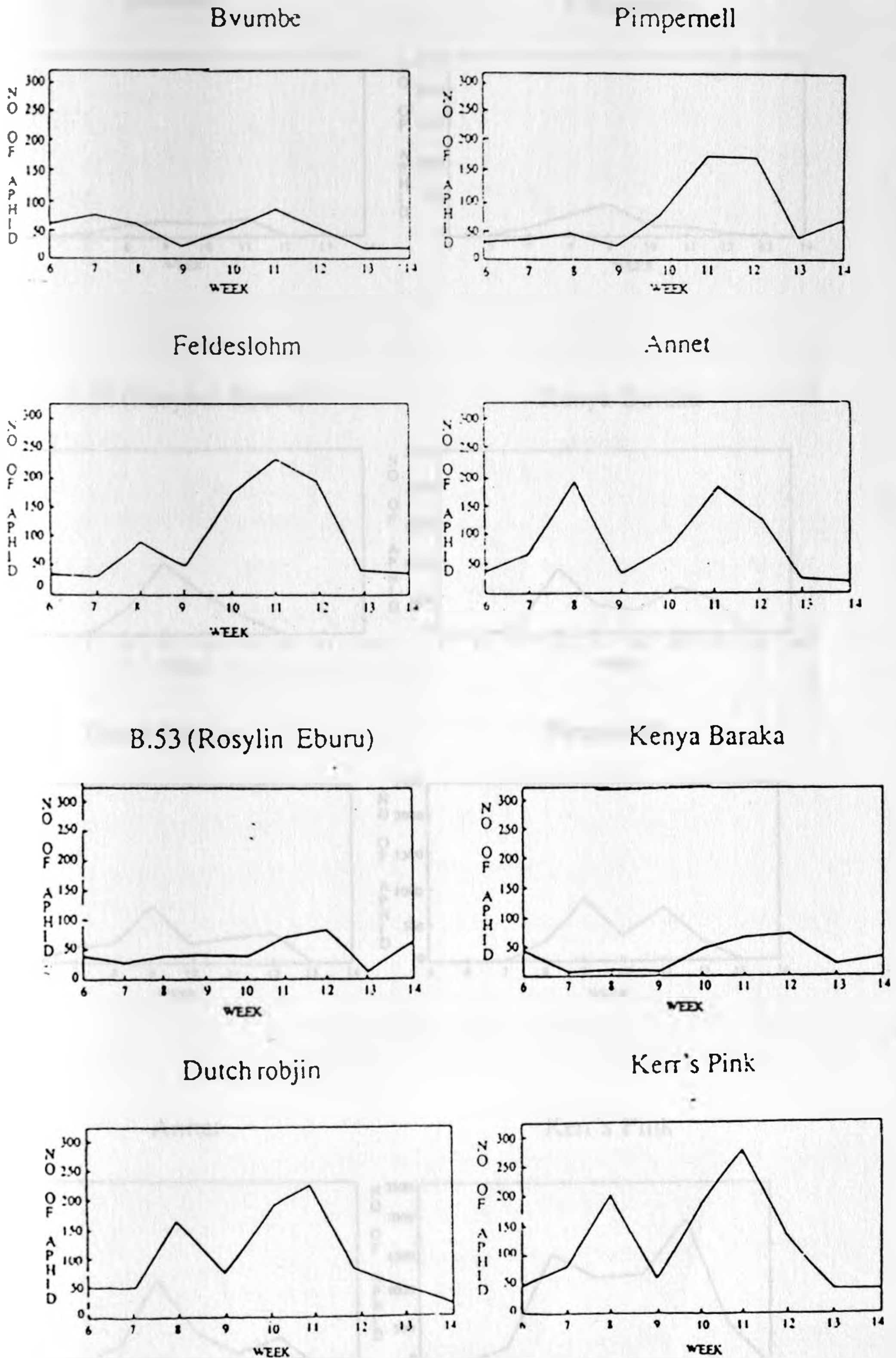
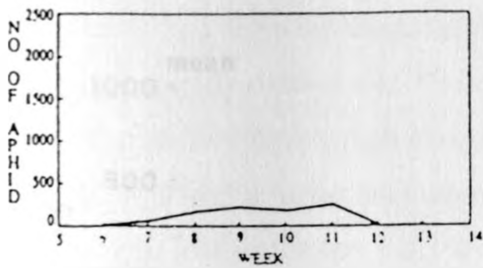
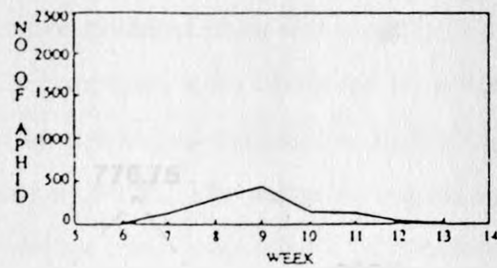


Fig. 15. Evolution of aphid populations levels *Solanum tuberosum* varieties during the short rains season 1987/88.

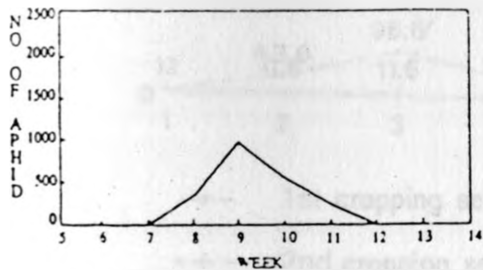
Bvumbe



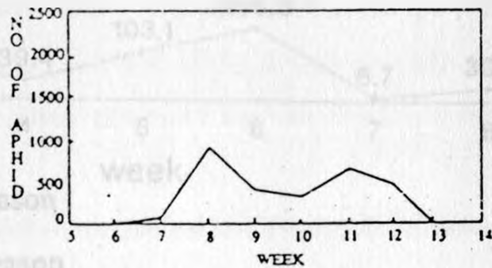
Feldeslohm



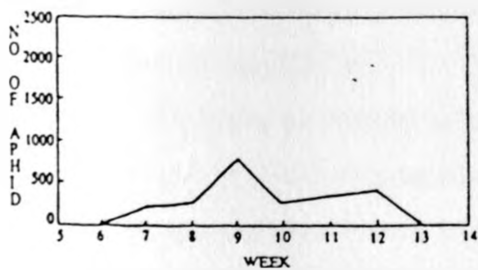
B.53 (Rosylin Eburu)



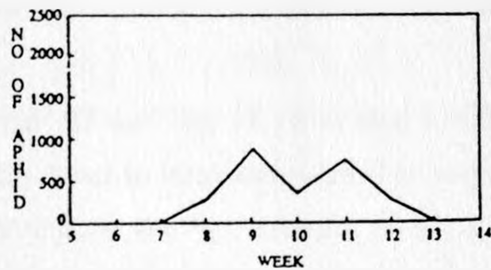
Kenya Baraka



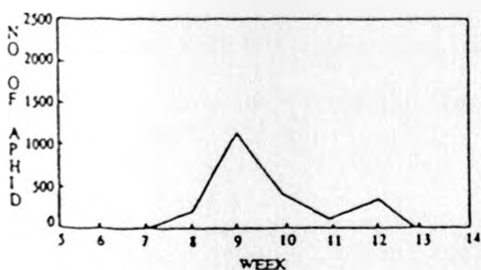
Dutch Robjin



Pimpernell



Annet



Kerr's Pink

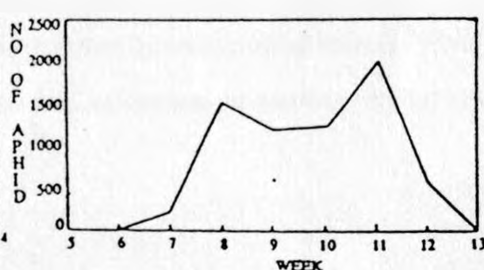
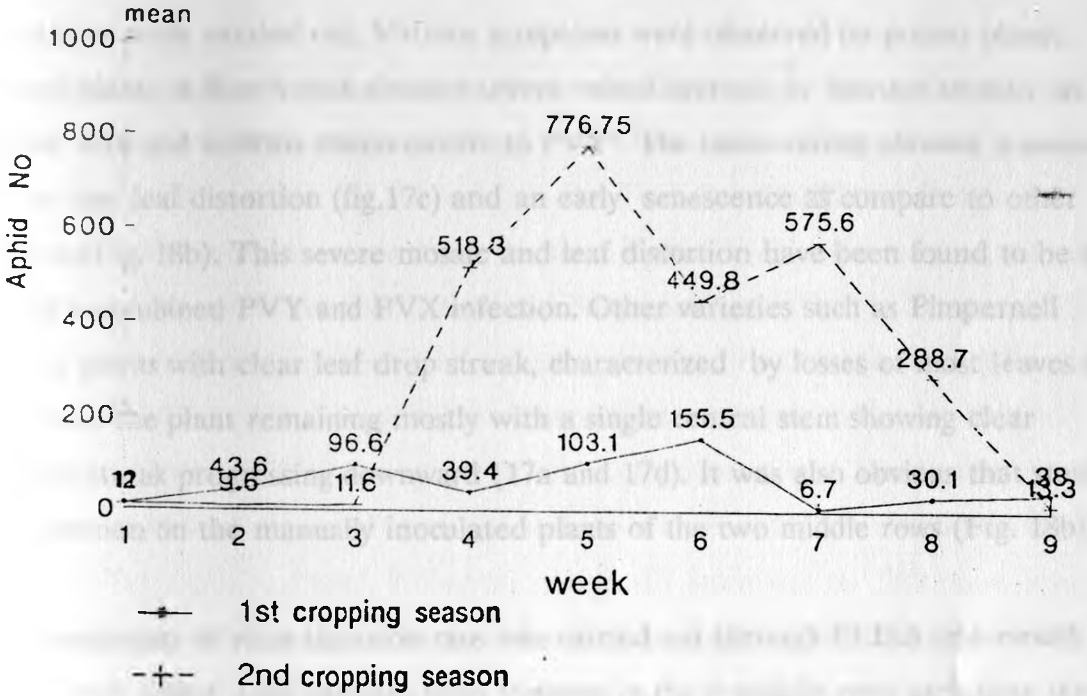


Fig. 16. Evolution of the weekly aphid population counts per variety during the long and short rains seasons



3.2 Assessment of virus infection rate

The assessment of the relationships between susceptibility to aphid infestation and the PVY infection rate was one of the major objectives of this study. The aphid infestation and virus infection rates were assessed from the same field and the correlation study carried out. Various symptoms were observed on potato plants. Several plants of Kerr's pink showed severe veinal necrosis or necrotic streaks on the veinlet, vein and midribs characteristic to PVY^{o}. The same variety showed a severe mosaic and leaf distortion (fig.17c) and an early senescence as compare to other varieties(Fig. 18b). This severe mosaic and leaf distortion have been found to be the sign of a combined PVY and PVX infection. Other varieties such as Pimpernell showed plants with clear leaf drop streak, characterized by losses of most leaves and stems and the plant remaining mostly with a single central stem showing clear necrotic streak progressing downward (17a and 17d). It was also obvious that stunting was common on the manually inoculated plants of the two middle rows (Fig. 18b).

The assessment of virus infection rate was carried out through ELISA one month before crop lifting. Leaf samples from 9 plants in the 4 middle rows excluding the two manually inoculated inner rows, were harvested at random from each plot, put in separate polythene bags. The sap was extracted mechanically using an electrical press and subjected to ELISA .

Results of ELISA, presented in annex 13 and Fig. 19, show that a wide variation existed between variety susceptibility level to virus infection. The ratio between the most infected variety ('Kenya Baraka') and the less infected variety ('Pimpernell') stood at 7:1, with the coefficient of variation standing at 13.3 for a general mean of 22.2%.

Considering that each plot was submitted to a strong initial inoculum (25% of infected plants) making up the two manually inoculated middle rows, it can be concluded that such variation show the existence of genetic variation among the 8 varieties.

However differences between means of tested varieties and the control 'Kerr's Pink' could only be determined through statistical analysis. As these data are percentages, it is recommended that they are transformed into Arc sin ($\sqrt{X}$) (Steel & Torries, 1960). The analysis of variance and the comparison of means through both Duncan and Dunnet tests (Annexes 14, 15, 16) revealed the following observations.

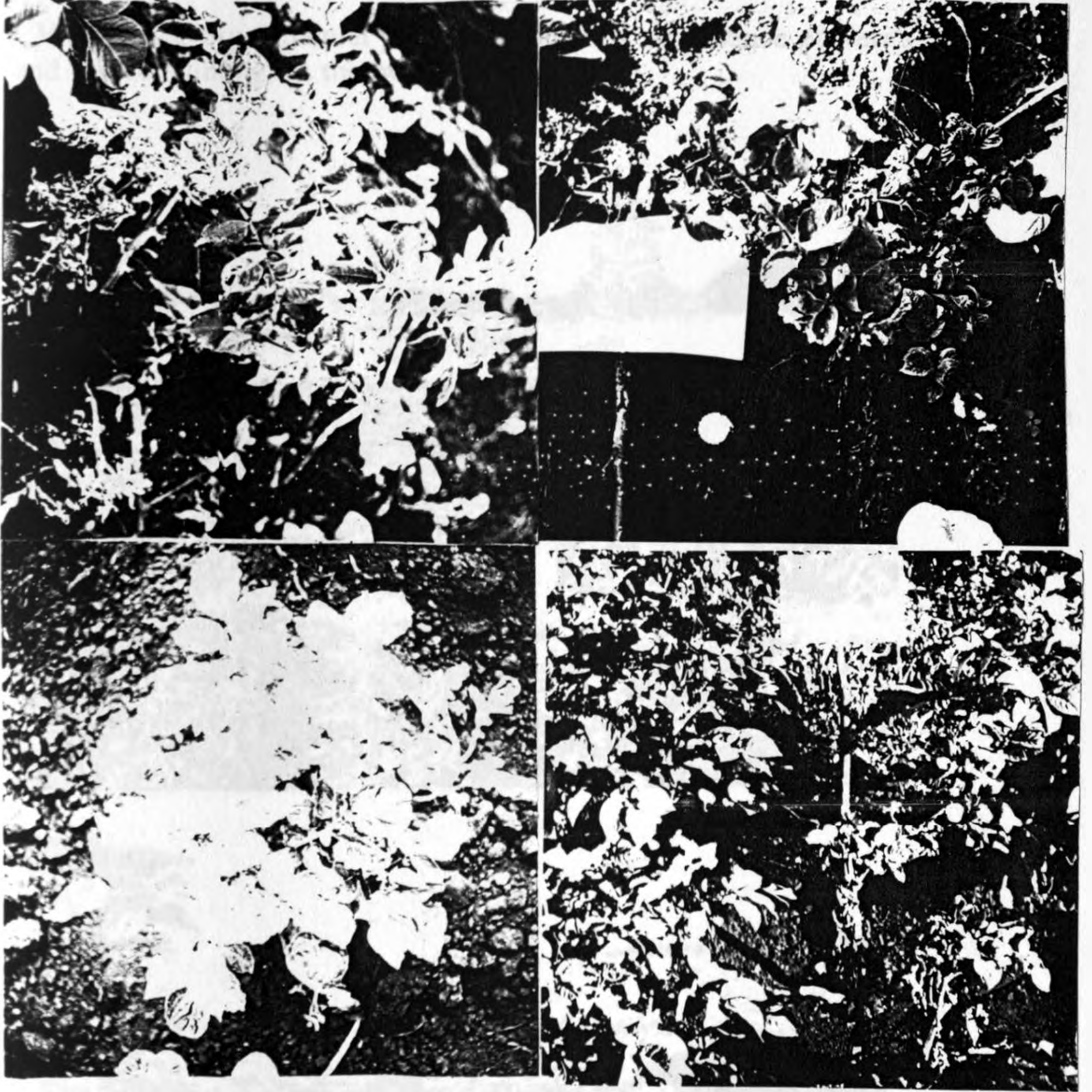
A multiple range comparison, using Duncan multiple range test showed that there were differences between 'Kenya Baraka', the most infected variety, and 'Pimpernell' (p=.05), 'B.53' (p=.05) and 'Annet' (p=.05). Two varieties, 'Dutch Robjin' and 'Feldeslohn', having the second and the third highest PVY infection rate, also showed differences to two of the three varieties ('Pimpernell' and 'B.53'). The control 'Kerr's Pink', for which non certified seed was used, behaved better than 'Kenya Baraka', 'Dutch Robjin' and 'Feldeslohn' but showed difference only to the less infected variety ('Pimpernell'). The most important observation is that 'Kerr's pink', for which non certified seed was used appeared to have a lower infection rate than varieties planted from certified seed. However, statistically there was no difference between 'Kerr's Pink' and those varieties. In addition, the comparison between the control 'Kerr's Pink' and all other varieties means by using the Dunnet's test revealed no difference.

The assessment of PVY infection rate was also carried during the following season to determine the extent of secondary infection on potato varieties. One month before crop lifting, potato leaves were harvested randomly on the four middle rows excluding the two inner rows as during the first trial. The major differences were that the two inner rows were not manually inoculated, as the aim was to assess the increase in PVY infection rate after the second crop. Another difference was that the infection rates were calculated on 12 plants instead of 9 plants. The PVY infection after two season showed a relatively important variation from the highest PVY infection rate ('Kerr's Pink'= 52%) and the lowest infection rate ('Pimpernell'= 14.5%) meaning a ratio of 4:1. The average infection rate was 29.85, and a coefficient of variation of 39.3%. (annexes 17, 18, 19 and 20).

The analysis of variance and the comparison of means by the Duncan new multiple range showed significant differences between 'Kerr's Pink' and 'Pimpernell' (p=.05%), 'Bvumbe' and B. 53 (p=.01%). The Dunnet test showed differences only between the control 'Kerr's Pink' and 'Pimpernell' (p=.05 and p=.01 respectively) and 'Bvumbe' (p=.01%).

Fig.17. Various field symptoms of PVY infection on potato varieties tested for aphid and PVY resistance; leaf drop necrotic streaks, excessive leaf formation on single stem potato plant and mosaic and leaf distortions.

Fig.17. Various field symptoms of PVY infection on potato varieties tested for aphid and PVY resistance; leaf drop necrotic streaks, excessive leaf formation on single stem potato plant and mosaic and leaf distortions.

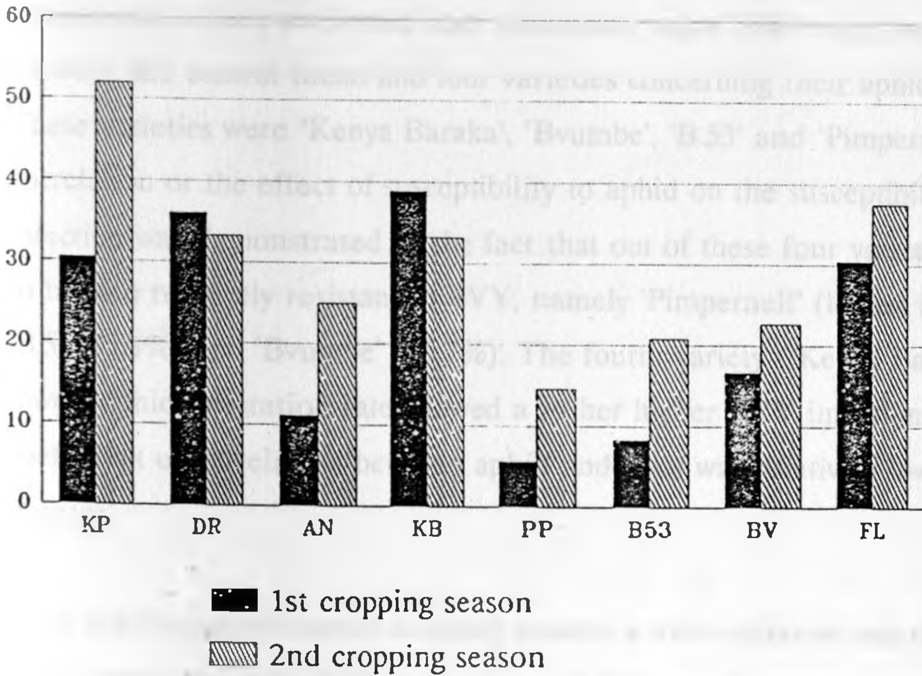


g. 18. View of the experimental plots showing early senescence of Kerr's pink and severe stunting on the two middle rows

a

b

Fig. 19. PVY infection means recorded through ELISA on *Solanum tuberosum* varieties during the long and short rains seasons



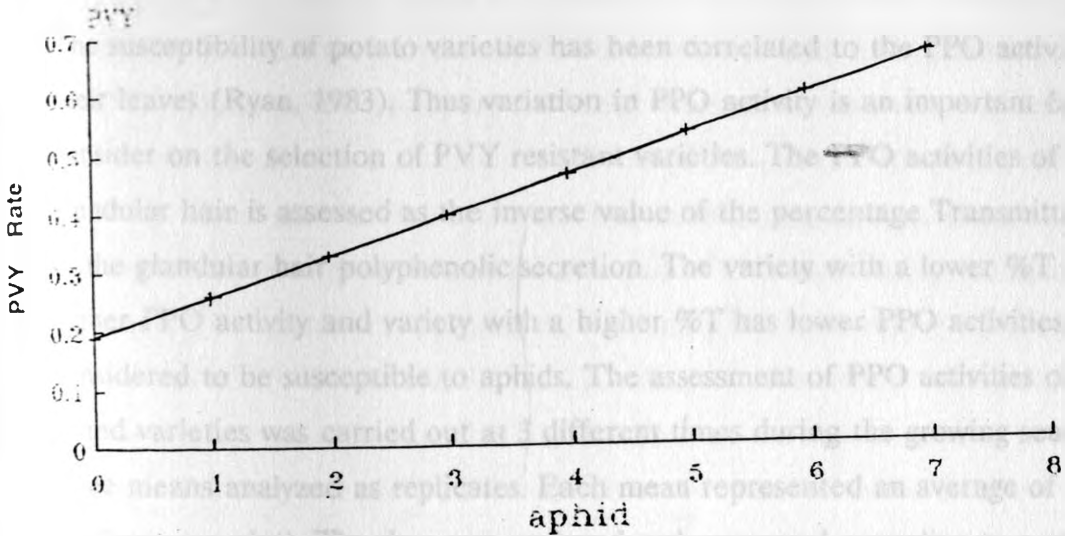
abreviation: KP= Kerr's pink; DR= Dutch Robjin; AN= Annet; KB= Kenya Baraka; B53= Rosylin Eburu; PP= Pimpernell; FL= Feldeslohm and BV= Bvumbe.

3.3 Correlation between aphid infestation and PVY infection rate in the field

The observation of raw data recorded during the first cropping season on both aphid population and PVY infection rate reveal a wide range of variation. This is a sign of variation in genetic resistance set up among the eight cultivars or between the control Kerr's pink and the 7 tested varieties. Although the analysis of variance of transformed values confirmed such variations, major differences were found only between the control mean and four varieties concerning their aphid susceptibility. These varieties were 'Kenya Baraka', 'Bvumbe', 'B.53' and 'Pimpernell'. The correlation or the effect of susceptibility to aphid on the susceptibility to PVY infection was demonstrated by the fact that out of these four varieties, three seemed to be also relatively resistant to PVY, namely 'Pimpernell' (lowest infection rate 5%), 'B.53' (8.3%) and 'Bvumbe' (16.7%). The fourth variety ('Kenya Baraka') with the lowest aphid infestation rate showed a rather higher PVY infection rate. The coefficient of correlation between aphid and virus was relatively low ($r2 = 0.25$) but positive .

After the first experimental cropping season, a wide variation was observed on both aphid population and PVY rate at the end the second cropping season. The analysis of variation on both cases showed some major differences. On the rate of aphid infestation, the comparison of means showed differences between the control 'Kerr's Pink' and all tested varieties, while no differences was observed among all seven varieties. The comparison of the PVY infection means showed differences between the control 'Kerr's Pink' and three varieties, namely Pimpernell, 'Bvumbe' and 'B.53'. The same three varieties were shown to be better and significantly different to the control 'Kerr's Pink' and other varieties. The study of correlation showed a coefficient of correlation $r2 = 0.72$ and $r2 = 0.74$ for raw data and transformed data respectively. The regression curve of PVY infection rate in relation to aphid population is defined by the equation $y = 18.5 + 0.15.x$ and $y = 0.19 + 0.068.x$ for raw and transformed data respectively (Fig. 20).

Fig. 20. The regression curve reflecting the influence of aphid population infestation level on the intensity of PVY infection at the end of the short rains season



3.4 Assessment of Polyphenol Oxidase Activity (PPO)

The analysis of variance of variety susceptibility level to aphid and to PVY has shown that there is variation between varieties and correlation between the two factor (r2 = 0.26) meaning that the susceptibility of a potato variety to PVY is influenced greatly by its susceptibility level to aphids.

The susceptibility of potato varieties has been correlated to the PPO activities of their leaves (Ryan, 1983). Thus variation in PPO activity is an important factor to consider on the selection of PVY resistant varieties. The PPO activities of potato glandular hair is assessed as the inverse value of the percentage Transmittance (%T) of the glandular hair polyphenolic secretion. The variety with a lower %T has a higher PPO activity and variety with a higher %T has lower PPO activities and is considered to be susceptible to aphids. The assessment of PPO activities of the eight tested varieties was carried out at 3 different times during the growing season and the three means analyzed as replicates. Each mean represented an average of 12 plants (3 plants per plot). The data was analyzed and compared according to a completely randomised block design and means compared by using the Duncan new multiple range test and the Dunnet test(annexes 21, 22). The observation of raw data showed a very narrow range of variation (61.3 - 77.7%T). This fact was also revealed by a low standard deviation of 4.6%T and coefficient variation of 6.6%. The transmittance value were transformed into Arc Sin (√%T) and analyzed. The analysis of variation and comparison of means by Duncan test showed that there was no difference among varieties.

The Dunnet test did not reveal any differences between the control Kerr's pink and all tested varieties. The control even showed the lowest %T, meaning a relatively higher PPO activities than other varieties. Another observation from both raw data and transformed data is that all tested Kenya commercially grown potato varieties have a uniform PPO activity level (except 'Bvumbe'). The analysis also showed that although relatively uniform, the %T of 'Kenya Baraka' was relatively higher

compared to others. This fact may be the explanation of its higher susceptibility to PVY, although it showed the lowest aphid infestation level.

The correlation study of transformed data on aphid and PPO activities showed a relatively important correlation between the two factors ($r = -0.35$). The understanding of such negative correlation coefficient and the related curve of regression is that the increase of %T means lower enzymatic activities and therefore a higher susceptibility to aphid and PVY. Analysis of data collected on the first trial showed that there was a correlation between the increase in aphid population rate on potato leaves and its PPO activities. The same parameter was observed, analyzed and compared to the aphid infestation rate and to PVY rate during the second cropping season. The assessment was also carried out three times during the second growing season, on 30th December, 15 and 27th January respectively; and the three means analyzed as replicates (annexes 23, 24). Each means represented an average of 12 plants (3 plants per plot). PPO activities was measured as the inverse values of the percentage of transmittance (%T) according to Ryan *et al* procedure (1986). The obtained data was analyzed according to a completely randomized block design and means compared by using the Duncan new multiple range test and the Dunnet test.

The observation of raw data in annex 24 , shows a margin of 16.4%T, a standard deviation of 5.8, and a coefficient of variation of 8.4% for an average of 69.8% T. However, the histogram of means (Fig. 21) shows a relatively uniform trend. Compared to the first experimental crop, the analysis of variance and comparison of means showed more differences. The control 'Kerr's Pink' showed a significant difference to all varieties except 'Kenya Baraka'. The latter was also significantly different to the remaining six varieties (Feldeslohn, 'Dutch Robjin', 'Annet', 'Pimpernell', 'B.53' and 'Bvumbe'). The variety 'Bvumbe', with the lowest %T (%T = .90) appeared significantly different to all varieties except to 'B.53', the second lowest. According to this comparison of means we can differentiate two groups, a group with a lower %T, made of 'Bvumbe', 'B.53', 'Pimpernell' and 'Annet'; and the other group with higher %T, made of Kerrs Pink, Kenya Baraka,

Feldeslohn and Dutch Robjin. These results are more consistent to the rate of aphid infestation than the first experimental season. This is mainly due to the relatively high population of aphid as compared to the long rains season.

The correlation study showed a strong relationship between the PPO activity of varieties and the aphid infestation rate (r2 = 0.71) and between PPO activities and the rate of PVY infection (r2 = 0.79). The regression curve of aphid population on potato variety leaf PPO activities was defined by the equation y = 24.7x - 18

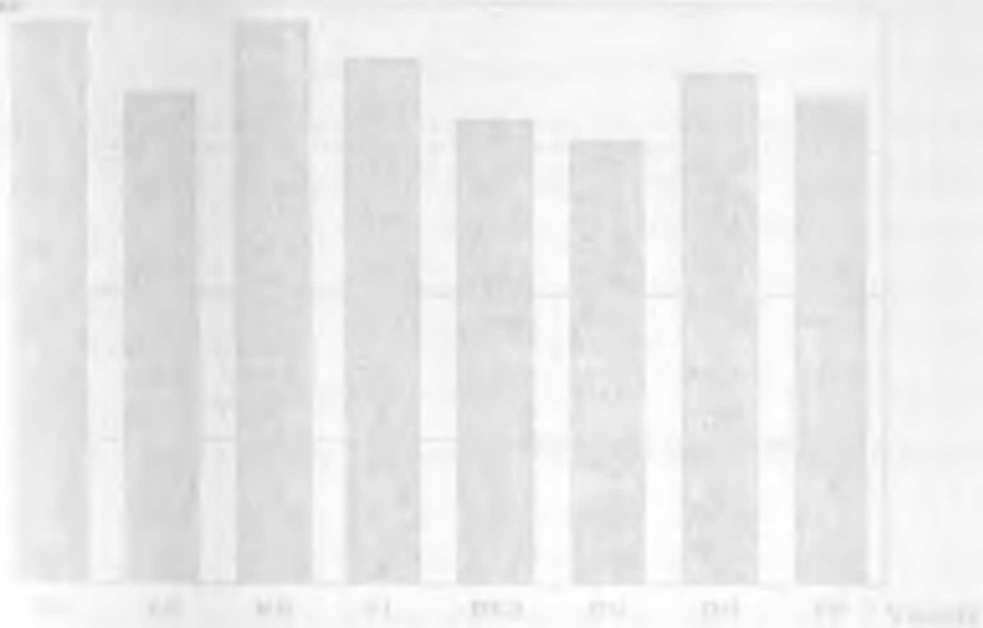
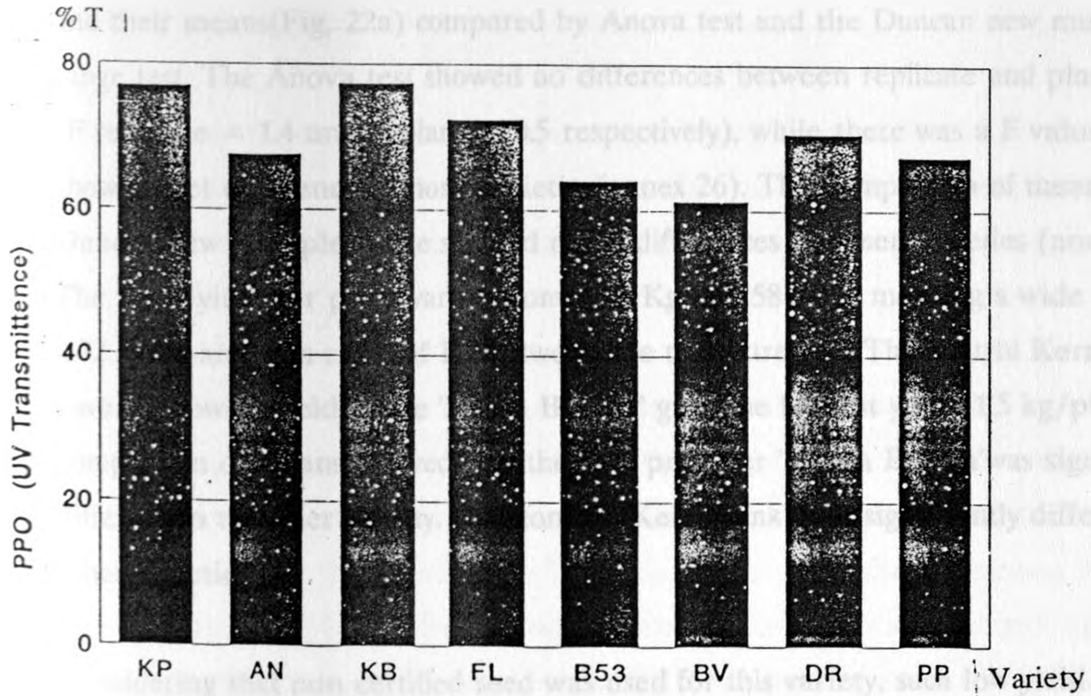


Fig. 21. Average levels of polyphenoloxidase activities in potato varieties after the short rains season. 1987/88



abreviation: KP = Kerr's pink; DR = Dutch Robjin; AN = Annet; KB = Kenya Baraka; B53 = Rosylin Eburu; PP = Pimpernell; FL = Feldeslohm and BV = Bvumbe

3.5.Effect of PVY infection on potato tuber

The ability of a variety to maintain a high and uniform yield under infection pressure is the ultimate aim of plant breeding for resistance to diseases or pests. Therefore the resistance or susceptibility of a variety cannot only be measured by the infection rate but also by the way the crop yield is affected. The average tuber weight per plant were compared. Nine potato plants were selected at random, the fresh weight of their tuber measured separately (Annex 25). A total of 36 plants per variety were weighed and their means(Fig. 22a) compared by Anova test and the Duncan new multiple range test. The Anova test showed no differences between replicate and plant means (F replicate = 1.4 and F plant = 0.5 respectively), while there was a F value (16.8) showing net differences among varieties(annex 26). The comparison of means by the Duncan new multiple range showed many differences between varieties (annex 27). The tuber yield per plant varied from 0.86 Kg to 1.58 Kg, , meaning a wide margin of 0.72 kg or almost a ratio of 1:2 between the two extremes. The control Kerr's pink gave the lowest yield, while 'Kenya Baraka' gave the highest yield (1.5 kg/plant). The comparison of means showed that the best producer 'Kenya Baraka'was significantly different to all other variety. The control 'Kerr's Pink' was significantly different to all other varieties.

Considering that non certified seed was used for this variety, such low yield should be expected and does not mean that the yield of this variety is the most affected by the virus infection. The second highest yielding variety, 'Bvumbe' showed a significant difference with all other varieties. The two varieties with the lowest yield did not show any difference. According to the comparison of means by the Duncan new multiple range test and the major differences emerging from it, the eight potato varieties can be grouped into three classes:

The highest producers 'Kenya Baraka' and 'Bvumbe', with an average yield of 1.54 - 1.58 kg per plant or an estimated yield of 44 tonnes per hectare. The two varieties did not show differences in their yield.

The middle class is made of 'Dutch Robjin', 'Annet', 'Feldeslohn' and 'Pimpernell', with an average yield varying from 1.17 to 1.36 kg per plant or an expected yield of 34.3 - 39.9 tonnes per hectare. Except for a significant difference observed between 'Pimpernell' (1.17 kg/plant) and 'Dutch Robjin' (1.36 kg/plant), there were no other differences among those varieties.

The lowest producer class included 'B.53' and 'Kerr's Pink' with an average yield lower than 1 kg/plant.

However, all these differences do not reflect the ability of different varieties to sustain high yield under PVY or any virus infection. The yield of varieties controlled by various factors; the most important being the genetic set up, the cropping system, soil, climate (precipitations, light intensity and temperature) and resistance to pests and diseases. However, the most important of all is the genetic set up. Among the eight tested varieties, some are classified as high yielding and others as medium or low yielding. Therefore, the fact that the variety 'Kenya Baraka' shows the highest yield (1.5 kg per plant or 44 tonnes per hectare) does not mean that this variety is the most resistant. This variety has both the highest yield and the highest PVY infection rate (1.5 kg and 38.6% respectively). Such inconsistency means that there is not a significant correlation between the PVY infection rate and the yield of these varieties, based on one cropping season.

It is common for researchers or plant pathologists to assess a crop resistance by comparison of the yield per unit area (acre or hectare). Such approach is meaningful only when comparing the average yield of different populations of the same variety. Meaning that these populations not only are compared under the same cropping conditions, but also have the same genetic set up. In addition, such comparisons are really meaningful for vegetatively propagated crops, as within such population, the absence of crossing assure a negligible variation of yield among plants. Such variation appears when different populations of a variety are submitted to different levels of positive or negative external factor such as cropping systems or diseases. Thus the

best way to compare the eight varieties submitted to this experiment was to use the CV observed within each variety (Fig. 23). The analysis of variance and comparison of CV of the eight varieties (annex 29) showed several significant differences. The control 'Kerr's Pink' had the highest CV and was significantly different to all other varieties, closely followed by 'Bvumbe' and 'Kenya Baraka'. The lowest CV was observed on 'Pimpernell'. The latter showed the lowest PVY infection rate and a relatively low aphid infestation level. The correlation study also showed that there is a net relationship between the PVY infection rate and the tuber weight CV within the same variety. Such CV is supposed to be extremely low for vegetatively propagated crop as potato. The increase of the tuber weight CV among plants of the same variety can be considered as a suitable parameter to consider in this case.

Sampling for the assessment of tuber weight per plant was done as during the first trial. Nine potato plants were selected at random and the fresh weight of their tuber measured separately (annex 30, Fig. 22b). A total of 36 plant tuber lots per variety were weighed and their means analyzed and compared by Anova test and the Duncan new multiple range test. The Anova test (annex 31) did not show differences among plants (F = 0.6), but there were significant F values for blocks and varieties (6.6 and 7.4 respectively). The average tuber weights per plant were significantly lower than during the first season and varied from 0.61 kg/plant ('Kerr's Pink') to 1.02 kg/plant ('Bvumbe'). As after the first trial, the control 'Kerr's Pink' gave the lowest yield per plant, while 'Bvumbe' gave the highest average. The best producer showed significant differences to the other six varieties, namely 'Kerr's Pink', 'B.53', 'Feldeslohn', 'Dutch Robjin', 'Kenya Baraka', and 'Pimpernell' but not against 'Annet', which was the second best yielding variety (0.89 kg/plant) (annex 32). After the second crop, which was planted from the second generation tuber, the eight varieties could be classified as follows:

- The fist group including 'Bvumbe', 'Annet' and 'Pimpernell', relatively showed the best average (0.89-1.02 kg/plant) and differences among them
- The second group including the remaining varieties showed no differences among them and gave a comparatively lower yield.

The suggestion emerging from the first cropping season on tuber weight per plant was that the effect of PVY infection rate could be detected by assessing the coefficient of correlation between the rate of PVY infection and the tuber weight per plant. Such correlation was also examined on the second crop. The study showed a significant correlation ($r = 0.58$) and the tuber weight was affected according to a linear regression curve ($Y = 0.98 - 7.10^{-3} *x$). Another fact observed during the first crop was that the PVY infection rate tends to increase the CV of tuber weight per plant within variety. The same study (Fig. 21b) on the second crop showed a relatively lower coefficient of correlation between the two parameters ($r = 0.05$). However, the regression curve model ($y = 0.68 + 5.10^{-4} *x$) gives a significant slope.

The observation of yield reduction on all varieties (Table 13) shows that varieties with the highest PVY rate during the second crop, namely 'Kenya Baraka', 'Dutch Robjin' and 'Feldeslohn' have recorded the highest yield reduction varying from 47.8 to 52.5%, while varieties with the lower PVY rate, notably 'Pimpernel' and 'Annet' have a lower yield reduction varying between 22.3 - 23.9. The intermediate level shows the same trend, this group includes 'Bvumbe' and 'B.53' with a reduction of 33.8 to 35.7. The variety 'Kerr's Pink' is in a group of its own where we find the highest PVY rate (52%) corresponding to a comparatively low reduction (29.6%) in yield as compared to the highest and the average group.

Table 13. PVY infection and Yield reduction recorded on potato varieties after two cropping seasons

Variety	1st crop		2nd crop		
	PVY(%)	Yield (Ton/ha)	PVY (%)	Yield (Ton/ha)	YR
Kenya baraka	38.8	47.4	33.2	22.5	52.5
Bvumbe	16.7	46.2	22.7	30.6	33.8
Dutch Robjin	36.1	40.8	33.3	21.1	47.8
Feldeslohn	30.5	36.6	37.5	18.9	48.4
Annet	11.1	36.2	25	26.7	22.3
Pimpernell	5.6	35.1	17.5	26.7	23.9
B.53	8.3	29.4	20.8	18.9	35.7
Kerr's pink	30.6	25.8	20.8	18.3	29.06
General mean	22.2	37.18	26.35	32.53	36.3

Fig. 22. The average tuber yield per plant (kg) recorded on *Solanum tuberosum* varieties during long rains season(a) and short rains(b)

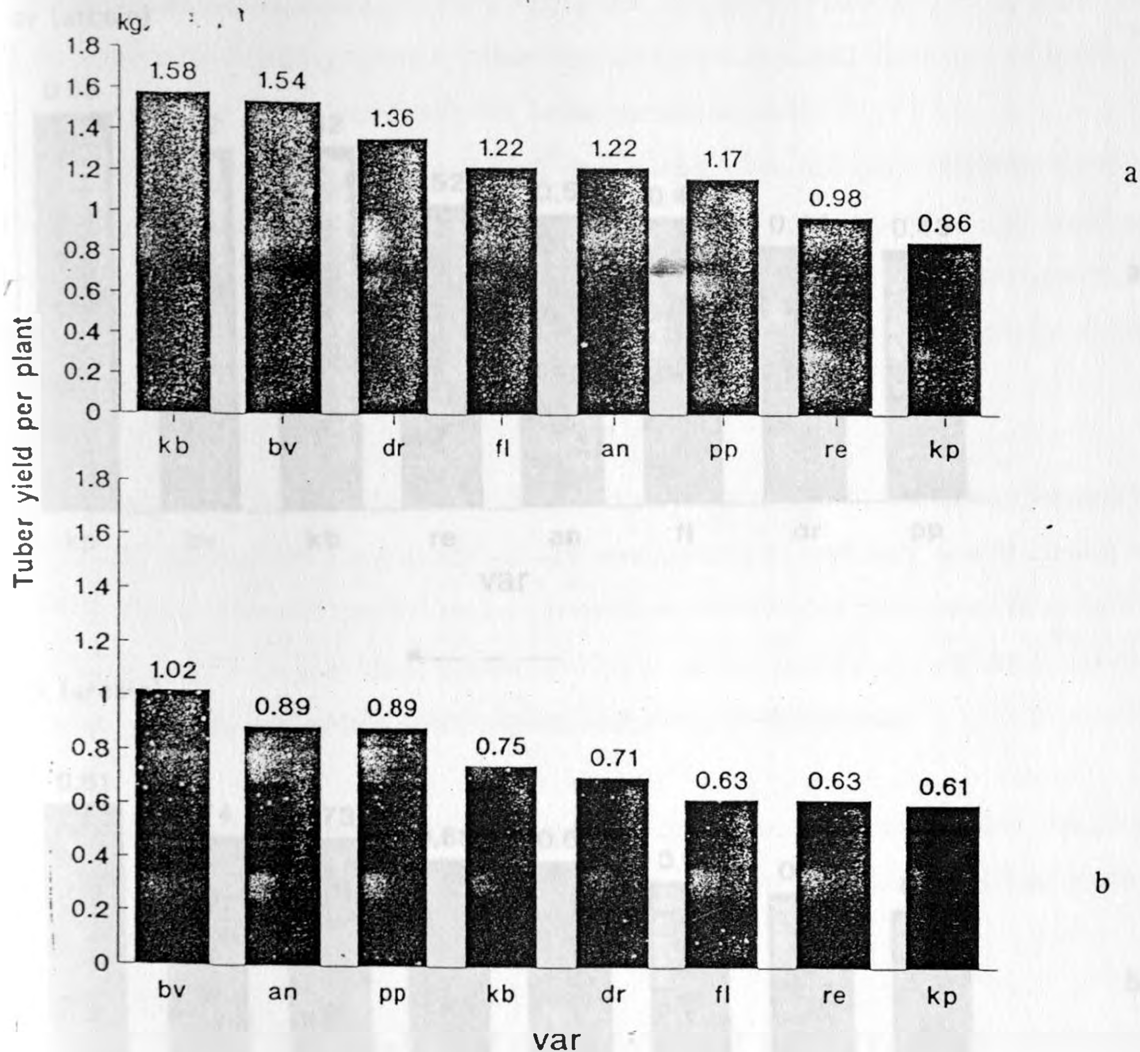


Fig. 23. The coefficients of variation (CV) observed on tuber yield per plant within each variety during the long season(a) and short season(b)

cv (arcsin)
Tuber yield CV
0.68 0.62 0.62 0.52 0.5 0.49 0.44 0.43
kp bv kb re an fl dr pp
var
a

cv (arcsin)
Tuber yield CV
0.81 0.74 0.73 0.68 0.67 0.62 0.59 0.55
kp re dr an pp kb fl bv
var
b

DISCUSSION

Tree-tomato (*Cyphomandra betaceae*) is widely grown as garden fruit shrub in most potato-growing areas in the Central Province of Kenya. In most cases, the majority of these shrubs are infected and show virus-like symptoms characterized by dark green or brown vein-banding, mosaic, blistering, malformation and distortion of leaves. Severe stunting and plant death has been noticed as well.
These symptoms are caused by a PVY strain which has not been characterized and identified in Kenya. The aim of this study was partially to determine and confirm the identity of this virus and to assess its importance, its potential as a pathogen to more important crops grown in Kenya by the small scale farmers as an intercrop with the tree-tomato.

Tree-tomato is not commonly infected by many viruses, probably due to its tannin-rich sap. Smith (1967) reported that this species selects only very severe strains of PVX. Other viruses reported on tree-tomato worldwide are Cucumber Mosaic Virus (CMV) and PVY. The identification of this virus was carried out on the basis of host range, physical properties, transmission and electron-microscopy.

The host range showed that only species of Solanaceae were susceptible. Species in the families Chenopodiaceae, Leguminosae, Cucurbitaceae, Amaranthaceae and Compositae were not susceptible.

The fact that none of the Cucurbitaceae plant species were susceptible confirms that the virus prevailing in tree-tomato in Kenya is not a CMV strain. In addition, electron-microscopic observation showed that the virus has flexuous or filamentous particles and not polyhedral ones as CMV. Observation of tested indicator plants showed that the virus could not infect *Datura stramonium, Gomphrena globosa* and *Phaseolus vulgaris*. The first two are susceptible to PVX and immune to PVY (Berks, 1970; de Bokx and Huttinga, 1981). In addition, the modal length of the virus extracted from tree-tomato was 738 nm, which is longer than the modal length of 545 nm reported for PVX (Berks, 1970).

The host range and the modal length related this virus to PVY strains. Symptoms induced on *Nicotiana tabacum* var "White Barley", "Kentucky", "Samsun" and "Xanthii" such as vein clearing, veinal necrosis, green vein banding, stunting and extreme white mottling, are common symptoms reported as caused by various PVY strains. In addition, symptoms such as chlorotic or necrotic ring spots caused by PVX on tobacco species were not observed. *Nicotiana rustica, N. glutinosa* and *N. debneyi*, all reacted with symptoms described on PVY strains. These species are known to react also with ring spots and severe necrosis or mere mottling to PVX. These symptoms were not observed. *Physalis floridana* and *Nicandra physaloïdes* reacted with mottling and necrotic blotches. However, this strain showed a severe infection on *P. floridana* by causing flower abortion.

The relationships to PVY was mostly confirmed by symptoms induced on potato varieties. Six out of the nine varieties reacted with green vein banding characteristic to PVY strain. Other varieties showed just mild mottling or leaf puckering. However, the virus could not infect any *Chenopodium* species. Most PVY strains are able to cause chlorotic local lesions on *Chenopodium amaranticolor* and *C. quinoa*. However, these two species were reported as not reliable assay hosts to all PVY strains and it is only 'Aquila 6' and *Solanum demission* Y which are considered as suitable assay hosts to all reported PVY strains (Delgado-Sanchez, 1966; de Bokx and Huttinga, 1981).

These few criteria confirmed that the virus extracted from tree-tomato is a strain of PVY. This was confirmed by its host-range restricted to Solanaceae, except *Datura stramonium*, its relatively low stability in crude sap and its modal length of 738 nm. The identity of the virus was confirmed by an ELISA test against PVY.

Transmission tests showed that the PVY strain isolated from tree-tomato was transmissible through the seed of that plant and could spread easily through aphid among tree-tomato plants in the field. These two factors explain the endemic state of this virus disease on tree-tomato found in farmers home-gardens. The role played by

Myzus persicae and *Macrosiphum* sp. in the spread of this virus was confirmed in the greenhouse. The virus was also shown to be highly transmissible through potato tubers. Such high rate of transmission through seeds, tubers and aphids show that tree-tomato kept in home-gardens represents a high risk to potato crops as primary source of PVY strains. The risk should be considered also for pepper, where it is commercially grown, as the virus was shown to be virulent on pepper.

In addition to the tree-tomato virus, this study looked at other viruses that cause infection on potatoes in the field, particulary strains of PVY .The study carried out on 36 virus isolates presumed to be PVY strains as by the observed symptoms on potato varieties revealed many complexities on the forms of PVY occurrences because of mixed infections with other viruses in the field. The various symptoms observed on differential hosts as described here showed the following deviation from PVY strains.

1. Ring spots. These symptoms have never been reported as caused by any PVY strain on tobacco species. However, these symptoms were observed on *Nicotiana tabacum* var 'White Burley', and *Nicotiana rustica.* A leaf dip preparation in electron-microscopy showed only aggregated elongated particles.

2. Chlorotic local lesions. These were induced on *Chenopodium album.* This common assay host is known to be immune to PVY(de Bokx and Huttinga, 1981).

3. *Datura stramonium*, generally immune to PVY, reacted with mottling and mosaic to some isolates. This indicated the presence of another virus.

4. *'Hybrid Aquila 6'.* This is known as the most reliable assay host for PVY strains. The plants showed a systemic mottling and necrotic blight.

The above symptoms were expressed by one or another group among the 24 isolates originally collected. Symptomatology on differential host could not be considered as a suitable parameter to assess the frequencies of occurrence of PVY in the field. Although most of the isolates were collected on potato plants showing typical symptoms of PVY, symptom expressions on diagnosis showed such great deviations that it was obvious that most of the isolates caused or initiated symptoms which are not related to potato virus Y. Thus, 20 isolates were able to initiate infection in *Gomphrena globosa* by inducing necrotic lesions , 16 isolates caused various infection types in *Datura stramonium* . such as mottling, mosaic and ring spots. There were only 7 isolates which did not cause any symptoms on these two varieties. This represented only 11.11% of the total samples and did not really reflect the actual rate of occurrence of PVY in the field or its importance among other potato virus diseases. The fact is that PVY does not necessarily occur as a single infection in the field but most often in a complex with other aphid or manually transmitted potato viruses, such as PVA, PVS and PVX. The positive reaction of *D. stramonium* and *Gomphrena globosa* indicated the presence of PVS. The next step or approach to obtain a more accurate figure representing PVY importance in the field will either be a number of serial manual inoculation on differential hosts immune to PVX, PVS and PVA. Such approach may be suitable only for PVX, but not for PVS which was shown to be able to induce chlorotic and necrotic greyish lesions, surrounded by reddish or pinkish halo on *G. globosa*. Thus, the sole observation of symptoms could not be the criteria for diagnostic or strain differentiation. Such symptoms could have been caused either by PVX or PVS or by a synergetic action of the two viruses. Some isolates producing such symptoms produced also symptoms similar to PVY on other differential hosts such as tobacco species, *Physalis floridana* and *Nicandra physaloïdes*.

As demonstrated by reactions of differential hosts, PVY strains were rarely found as single infections of potato crops in the field. Thus, any studies on the extent of variation of PVY need, as a pre-requisite condition, to determine in which form the PVY occurs. The collection of isolates on the basis of symptoms on potato plants could not help much in assessing both the variation and the rate of occurrence of

potato virus Y in potato crops. To determine in which form and at which rate potato virus Y strains occurred in the field, ELISA tests were conducted on all differential hosts, which reacted positively.

The fact that results of ELISA tests showed that when collecting infected potato plants samples in the field, around 85.7% of the samples were to contain potato virus Y. However, only 16.7% of the sample will be made of pure PVY infection. These results show that PVY can be found at the rate of 69% in the form of mixed infection with one, two or three other viruses. Association with one virus (X or A) was estimated at 26.2%, that with two different viruses at 33.3% (PVY-X-A; PVY-X-S and PVY-S-A), and that with all three other viruses (PVY-X-S-A) at 9.5%.

These results showed that it will be an impossible task to estimate some major parameter in field conditions:

- It is almost impossible to assess which crop losses are attributed to each virus in field conditions.
- Assessment of PVY or other potato virus strains differentiation can be misleading if ELISA test is not used to determine the purity of each isolate.
- Although some differential hosts are selective to one virus only, such procedure is costly and time-consuming as compared to ELISA tests.

Results in Table 11 showed that 13 PVY strains could be separated into two groups. The common strains PVY^{o}, not causing veinal necrosis on tobacco varieties and the necrotic strains PVY^{N}, causing necrotic symptoms on *Nicotiana tabacum* var 'White Burley', 'Samsun' and 'Kentucky'. In addition, within each group, 'strains' showed differences in their ability to infect pepper cv 'Long Red Cayenne'. The difference between these 13 strains can be summarized as shown in Table 11. This summary also shows that there is almost equal proportion of the common strains PVY^{o} (7 strains) and necrotic strains PVY^{N} (6 strains). It equally shows that most tested

strains are virulent on pepper (9/13). Thus, when not grown for commercial purposes, pepper should be considered as potential reservoir host for most Kenya PVY strains.

Considering the choice of assay hosts when studying PVY strains, these studies confirmed earlier findings that *Chenopodium* species are not always suitable assay hosts.

No work has been done in East Africa to determine the correlation between aphid infestation and rate of PVY or other aphid transmitted viruses under field conditions and the impact of such correlation on the choice or breeding potato varieties in the country. Results obtained on aphids showed a wide variation among varieties. A ratio of 3.5:1 between the most infested variety ('Kerr's Pink') and the less infected ('Kenya Baraka') was observed. The same high ratio (8:1) was also observed during the second crop between 'Kerr's Pink' (7044) and 'Bvumbe' (884). Such high ratio reflect a variation in the resistant level against aphids among potato varieties. Results on the first crop (rainy season) show that four varieties, 'Kenya Baraka', 'Bvumbe', 'B.53' and 'Pimpernel', were different from the two most infested varieties. The second experiment (short rains season) shows that all varieties are significantly different from 'Kerr's Pink' and that among the five varieties with less infestation and with no differences among them, they are three of the four varieties which had good performance during the first season.

The weather conditions are an important factor to consider before assessing the susceptibility or resistance of potato varieties to aphid. The comparison of the average population per variety between the second season (short rains) and the first season (long rains) shows a ratio of 5:1. Such ratio means that there was five-times more aphid pressure on potato varieties during the short rains. This seasonal difference has an effect on the variety behaviour as it was observed on Kenya Baraka. This variety has the lowest rate in the long rain season but performed poorly by showing the second highest infestation rate during the second crop. Therefore,

such experiments should be mostly carried out during short rains season, where, due to less heavy rains, less wind and relatively high temperature, which boost aphid population and create maximum pressure on potato varieties. Under such conditions, results are bound to be more meaningful than several experiments carried out during the long rains season.

Nevertheless, data on both first and second cropping seasons show consistency, as the same three varieties 'Bvumbe', 'B.53' and 'Pimpernel' are always the less infested varieties. In both seasons, the variety 'Annet' maintains a middle position and showed no significant difference from the less infested three varieties. Thus, after the two cropping seasons, under different weather conditions and different pressure of aphid populations, it can be concluded that the three varieties, 'Bvumbe', 'Pimpernel' and 'B.53' are less attractive to aphid and that the variety 'Annet' is moderately attractive to aphid. A more analytical study on their mechanism of resistance could lead to a more elaborate choice of initial parent for breeding programme.

The basic mechanism of potato plant resistance to aphid has been described (Johnson, 1953, 1956; Ryan *et al*, 1983; Ave *et al*, 1986). Epidermal hairs have been recognized as an effective means of plant resistance to aphid infestation. The secretion from these hairs has a gumming effect on aphids and prevents movement and ultimately causes death by starvation. Such secretions have been identified as polyphenolic compounds. Thus, the resistance to aphid will depend on both the quality and the type of secretion. Therefore, the PPO activities were assessed and the results showed that there was no difference among varieties during the first crop (long rains), but there was a significant difference between 'Bvumbe' with the least %T (or higher PPO activity) than the rest. However, there was no difference between 'Bvumbe' and 'B.53'. Apparently, these two varieties have the highest PPO activity and therefore are more likely to be resistant to aphid than others. The relationship between PPO activities and aphid population on each variety was assessed.

During the first cropping season, the correlation between PPO and aphid population was negative ($r2 = -35$) and the regression curve ($y = 8.5 - 4x$) did not reflect the fact that an increase in %T means an increase in the susceptibility to aphid. However, the same correlation was relatively higher ($r2 = 0.72$) in the second crop and the regression curve ($y = 24.7x - 7$) reflected the normal trend that the lower the %T, the higher the PPO will be and the more the plant will be resistant to aphid. The opposite trend observed in the first crop could be attributed to the fact that there was no significant difference in the PPO activity among varieties. Considering the correlation observed after two crops and the comparison of means, it can be suggested that the varieties 'Bvumbe' and 'B.53' have a relatively higher PPO activity than other varieties. The same two varieties have shown a lower aphid infestation in both cropping season. The susceptibility level to PVY infection also showed a wide variation among varieties (ratio of 7:1 for the 1st crop and 3:1 in the second crop). Such variation should mainly be attributed to the following factors:

- The genetic resistance to the virus itself
- The genetic resistance to vectors
- The initial infection rate in potato tuber seeds
- The transmission potential of the most predominant vector.

The mechanism of resistance or susceptibility to PVY has been described by several authors. However, the comparison of data on PVY rate and aphid infestation rate showed that there is a strong correlation between the two parameters. The first shows that there is a weak correlation ($r2 = 0.25$) and the regression curve reflected by the equation $y = 13.5 + 0.43x$ confirms that an increase of aphid population on variety tends to increase PVY infection rate. The same fact was observed in the second trial with a high coefficient of correlation ($r2 = 0.72$) and a regression curve reflected by the equation $y = 18.5 + 0.15x$.

However, the behaviour of 'Kenya Baraka' (lowest aphid population and highest rate of PVY) suggests that a higher susceptibility to PVY cannot be correlated by the

inclusion of aphid-resistant gene into it. This means a variety should have a combination of both types of resistance to give satisfactory performance.

The other important parameter to consider is the effect of PVY infection on tuber yield. It appears that the rule that higher virus infection means a reduction on yield does not apply with a uniform intensity on a set of varieties. This means that two varieties may have the same rate of virus infection but be affected differently on their yield. Another parameter to consider here is the genetic production potential of a variety. Among the eight varieties tested, some are classified as high-yielding and some as medium- or low-yielding. From that assumption, a mere comparison of yields cannot be a sufficient criteria to assess the pressure of virus infection on the variety yield. Among the eight varieties, 'Annet', 'Kenya Baraka', 'Feldeslohn', 'Dutch Robjin' and 'Bvumbe' are considered as high producers and can yield over 40 tonnes per ha, while 'Kerr's Pink', 'Pimpernel' and 'B.53' are considered as medium and yield between 20 to 40 tonnes per hectare. The obtained production shows that varieties with the highest PVY rate during the second crop, namely 'Kenya Baraka', 'Dutch Robjin' and 'Feldeslohn' have recorded the highest yield reduction varying from 47.8 to 52.5%, while varieties with the lower PVY rate, notably 'Pimpernel' and 'Annet' have a lower yield reduction varying between 22.3 - 23.9. The intermediate level shows the same trend, this group includes 'Bvumbe' and 'B.53' with a reduction of 33.8 to 35.7.

The variety 'Kerr's Pink' is in a group of its own where we find the highest PVY rate (52%) corresponding to a comparatively low reduction (29.6%) in yield as compared to the highest and the average group. Such behaviour in 'Kerr's Pink' explains why small scale farmers, obliged to use their own seed, preferred this variety. The anecdote in most visited areas is that whether there is rain or not or whether under higher aphid infestation, 'Kerr's Pink' never fails to give some acceptable yield. The conclusion is that reduction should be considered as a more reliable parameter to assess than the yield itself.

GENERAL CONCLUSION

Early works described the occurrence of PVY in potato, pepper and tobacco in Kenya (Bock, 1976; Bondole, 1986). However, its occurrence and importance on tree-tomato (*Cyphomandra betaceae*) has never been reported in the country.

A flexuous virus was isolated from tree-tomato and identified by host range, physical properties, electron-microscopy and ELISA test. The host range included species in the families of Solanaceae, Leguminosae, Chenopodiaceae, Cucurbitaceae, Compositae and Amaranthaceae, and it showed a close similarity to the PVY host range. However, the strain could not induce local lesion on any *Chenopodium* species. This lack of virulence on *Chenopodium* sp. differentiated it from strains of PVY described by Bock (1963), isolated from potatoes and pepper and a necrotic strain isolated by Bondole (1986) from Nicotiana tabacum var Speight.8. The virus showed a relative stability in crude sap; a thermal inactivation of point of 65 C, a dilution end point of 10 -3 and a longevity in vitro of 66 hr.The modal length of 738 nm was assessed on electron microscope micrographs obtained from partially purified preparation. The virus was positively identified as PVY through ELISA test.

The virus was readily transmitted through seed of tree-tomato and tuber of potato varieties. The epidemiological intensity on tree-tomato was seen to be high. The infection rate in a field of tree-tomato ranged from 0% to 100% after a period of 7 months. Considering that tree-tomato is a perennial fruit tree found in most small-holdings in Kiambu District and other potato-growing areas, it is evident that this fruit tree is a major source of inoculum and should be regarded as a danger to potato crops. It is wise to alert authorities on the need of destroying this species of non economic importance in the areas. The PVY strain from tree-tomato also proved to be virulent to pepper (*Capsicum annuum*). It suggested that where pepper is not grown for economic reason, it must be regarded as a potential danger to potato crops in small holdings.

An attempt to differentiate PVY strains from tree-tomato and potato varieties collected from three locations (Thika, Kabete and Molo), revealed the fact that PVY occurs mostly in association with other elongated potato viruses (PVX, PVA and PVS). Among the 36 isolates that were studied in details, only 7 (16.7%) were pure PVY strains, while the rest were in association with one, two or three other viruses. Among them PVA, PVX, and PVS. The mixed infection showed how difficult it is to assess crop losses due to each virus under field conditions. Some differential hosts reacted by the production of symptoms that could be masked due to the interaction with another virus. The ELISA test showed that PVY symptoms are often masked. These observations showed that differential hosts should not be considered as the sole criteria for differentiating viruses and that a great deal of time can be saved if a preliminary ELISA test of isolates is carried out before strain differentiation tests.

Among the 36 isolates, 13 isolates were confirmed to be pure PVY strains through ELISA test and were distributed in the groups PVY^o and PVY^N respectively. PVY^o strains were more virulent to pepper than strains of PVY^N group.

The study of relationships between susceptibility to aphids and PVY infection revealed the following observations:

- Potato varieties show a wide variation in their attraction to aphids. These differences were reflected by ratios of 4:1 and 7:1 between the highest and lowest infestation during the first and second cropping seasons, respectively.
- The curve of an average aphid population showed that aphids start infecting potato after 6 to 7 weeks and the peak for most varieties is reached between the 8th and 12th week after planting. This observation suggests that trials on planting times could lead to an escape approach in the aphid control. However, such trials or approach should take into consideration the rainfall patterns.

- The two cropping seasons showed a big difference in total aphid population. The average population of aphid per variety was 5 times higher during the short rains season than during the long rains season. Such pattern was expected as aphids are mostly washed away by heavy rains on the one hand (long rains) and multiply intensively under relatively high temperatures and less rains and winds. The latter factors are obtained during short rains season. This suggests a rotation approach where potato should come between March and April and other less susceptible crops during the short rains season.

The correlation study among various parameters showed that:

- There is a strong correlation between aphid infestation and PVY infection rate. However, the behaviour of 'Kenya Baraka' during the first crop suggests the best approach is the combination of both resistance to aphids and PVY.

- The PPO activities considered as the major factor controlling aphid infestations did not show a consistent difference between varieties or with aphid infestation. This suggests that the eight tested varieties cannot be considered as good material for further breeding programme for aphid resistance.
- The correlation between aphid and PVY resistance was further confirmed by the fact that the same varieties showed relatively good performance for both parameters. These were 'Pimpernel', 'Bvumbe', 'Annet' and 'B.53'.

- The effect of PVY infection on tuber yield could be assessed through two factors, the coefficient variation of tuber weight per plant within each variety and the reduction of yield after two to four cropping seasons rather than through the comparison of variety yield per unit area.

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ANNEXES

…1. Whole number of weekly aphids counts on eight potato cultivars during the long rains season (April-July 1987)

…s/weeks	22 May/6 wks	28 May/7 wks	4 June/8 wks	11 June/9 wks	17 June/10 wks	24 June/11 wks
…plicates	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4
…r's Pink	34 08 02 06	39 03 06 33	15 151 17 14	07 11 10 29	34 18 48 77	55 72 68 74
…deslohn	09 11 07 07	02 10 12 09	16 46 03 14	10 16 10 13	43 19 22 74	48 57 62 55
…ya Baraka	10 08 19 06	00 05 03 02	01 02 07 03	04 05 01 01	17 07 18 02	15 06 18 24
…ch Robjin	07 10 17 15	15 26 01 05	34 23 10 88	05 11 24 39	45 48 27 44	60 42 58 38
…et	12 14 06 11	19 20 02 16	119 19 34 23	03 14 02 20	03 46 18 09	36 57 62 25
…pernell	06 05 03 07	04 05 14 00	07 15 05 07	02 01 08 02	17 18 16 16	35 48 31 47
…53 (Roslyn …) Eburu	05 07 08 20	09 15 10 08	03 26 05 05	21 00 00 06	22 09 16 05	18 21 41 17
…ale	09 22 18 08	18 11 35 06	08 26 09 15	17 00 02 08	01 13 33 27	12 16 19 34

…/Weeks	1 July/12 wks	8 July/13 wks	15 July/14 wks	Total per variety
…plicates	1 2 3 4	1 2 3 4	1 2 3 4	
…r's Pink, C	14 24 73 17	04 26 03 09	12 09 09 07	1038
…deslohn	17 30 134 06	09 04 05 24	12 14 02 11	843
…ya Baraka	26 14 13 10	15 02 04 05	05 11 06 14	309
…ch Robjin	20 34 14 11	10 11 09 20	12 07 04 04	848
…et	54 09 06 53	05 04 11 14	09 11 08 05	776
…pernell	24 68 46 20	00 13 06 05	10 12 17 16	556
…53 (Roslyn …) Eburu	14 09 27 32	03 03 01 03	11 05 35 05	418
…ale	01 11 02 40	04 07 04 10	01 00 11 11	489

...nsformed value (N) of the weekly aphid counts per variety and per replicate observed during the long rains season (April - July 1987)

	22.05/6 wks				28.05/7 wks				4.06/8 wks				11.06/9 wks				17.06/10 wks			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
	5.8	2.8	1.4	2.4	6.2	1.7	2.4	5.7	3.9	12.3	4.1	3.7	2.6	3.3	3.2	5.3	5.8	4.2	6.9	8.8
	3.6	3.3	2.6	2.6	1.4	3.2	3.5	3.0	4.0	6.7	1.7	3.0	3.2	4.0	3.2	3.6	6.6	4.4	4.6	8.6
	3.2	2.8	4.3	2.4	1.0	2.2	1.7	1.4	1.0	1.4	2.6	1.7	2.0	2.2	1.0	1.0	4.1	2.6	4.2	1.4
	2.6	3.2	4.1	3.8	3.8	5.1	1.0	2.2	5.8	4.7	3.2	9.3	2.2	3.3	4.9	6.2	6.7	6.9	5.2	6.6
	3.5	3.7	2.5	3.3	4.4	4.5	1.4	4.0	10.9	4.4	5.8	4.8	1.7	3.7	1.4	4.4	1.7	6.8	4.2	3.0
	2.4	2.2	1.7	2.6	2.0	2.2	3.7	1.0	2.6	3.8	2.2	2.6	1.4	1.0	2.8	1.4	4.1	4.2	4.0	4.0
	2.2	2.6	2.8	4.4	3.0	3.8	3.1	2.8	1.7	5.1	2.2	2.2	4.5	3.6	1.0	2.4	3.2	3.2	2.4	2.4
	3.0	4.7	4.2	2.8	4.2	3.3	5.9	2.4	5.3	5.1	3.0	3.9	4.1	1.0	1.4	2.8	1.4	3.6	5.7	5.2

	24.06/11 wks				1.07/12 wks				8.07/13 wks				15.07/14 wks				
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	
	7.4	8.4	8.2	8.6	3.7	4.9	8.5	4.1	2.2	5.1	2.0	3.2	3.5	3.2	3.2	2.8	1038
	6.9	7.5	7.8	7.4	4.1	5.4	11.5	2.4	3.2	2.2	2.4	4.9	3.5	3.7	2.3	3.0	843
	3.9	2.6	4.2	4.9	5.1	3.7	3.6	3.2	3.8	1.7	2.2	2.4	2.4	3.3	2.6	3.7	309
	7.7	6.5	7.6	6.2	4.5	5.8	3.7	3.3	3.2	3.3	3.2	4.5	3.5	2.8	2.2	2.2	848
	6.0	7.5	7.8	5.0	7.3	3.2	2.6	7.2	2.4	2.2	3.3	3.7	3.2	3.3	2.8	2.2	776
	5.9	6.9	5.6	6.9	4.9	8.2	6.8	4.5	1.0	3.8	2.6	2.4	3.2	3.4	4.1	4.0	556
	4.2	4.5	6.4	4.1	3.7	3.2	5.2	5.6	2.0	2.0	1.4	2.0	3.3	2.4	5.9	2.4	418
	3.5	4.0	4.4	5.8	1.4	3.3	1.7	2.2	2.2	2.8	2.2	3.2	1.4	1.0	3.3	3.3	489

ily total aphids count observed on eight potato cultivars during the long season (April to July 1987)

DATES											
1 22/4/87	7 8/5	14 3/6	21 10/6	28 17/6	35 24/6	42 1/7	47 8/7	56 15/7	63 22/7	29/7	Total/ Season
50	81	183	57	176	269	128	42	37			1023
49	47	155	79	164	198	75	50	27			849
44	57	192	39	76	180	122	34	33			767
43	10	13	11	44	63	48	26	36			294
20	23	34	13	67	161	158	22	55			553
40	28	39	40	39	70	82	10	56			404
57	70	78	27	101	81	54	25	23			516
34	33	79	49	158	222	187	42	39			843
42.12	43.6	96.6	39.4	103.1	155.5	106.7	30.1	38.0			561.1

...4: Anova table for weekly aphid count during the long rains season, analyzed as the V N (April-July, 1987)

Source	df	Ss	Ms	F
Week	8	327.45	40.93	18**
Block	3	2.7	0.88	0.4
Variety	7	123.02	17.57	7.7**
Error	269	612.94	2.28	
Total	287	1066.07		

CV = 38.2% Sdweek = 0.377; Sdblock = 0.252;
Sdvariety = 0.356
Ft = 2.01 (0.05%) and 2.64 (0.01%)

Abbreviation used: CV = Coefficient of variation
Sd = Standard error difference
N = Weekly aphid count

Annex 5 Comparison of Aphid Population Means (Transformed Values N) on potato varieties Assessed by Using the Duncan new multiple range test (p = .05%, p = .01%)

T1	Var	KB	BV	B.53	PP	AN	FL	DR	KP
Var	Mean	2.89	3.41	3.42	3.66	4.30	4.44	4.60	4.88
KP	4.88	1.99**	1.47**	1.46**	1.22**	0.58	0.44	0.28	–
DR	4.60	1.77**	1.19*	1.18*	0.94*	0.30	0.16	–	–
FL	4.44	1.55**	1.03	1.02	0.78	0.14	–	–	–
AN	4.3	1.41**	0.89	0.88	0.64	–			
PP	3.66	0.77	0.25	0.24	–				
B.53	3.42	0.53	0.01	–					
BV	3.41	0.52	–						
KB	2.89	–							

Major differences: (#)

0.05% => KP = KB, BV, B.53 and PP
=> DR = KB
=> FL = KB
=> An = KB

0.01% => KP = KB, BV
=> DR = KB
=> FL = KB

6. Comparison of aphid population means (transformed value N) by Using the single Dunnet (value).

Control Mean	Treatment means						
KP	DR	FL	AN	PP	B53	BV	KB
4.88	4.60	4.44	4.30	3.66	3.42	3.41	2.89
Differences 0.95				*	*	*	*
Differences 0.99				*	*	*	*

' => .95   2.6   2 (2.2786)/36 = 0.95

=> .99   3.18   2 (2.2786)/36 = 1.13

Whole numbers (N) of weekly aphid counts per variety and per replicate recorded on potato varieties during the short rains season (October 1987 - January 1988)

Date / Replicate	23.12.87				30.12.87				6.01.88				13.01.88				20.01.88				27.01.88		
[illegible]	3	0	0	17	0	12	2	7	1	17	89	178	35	344	98	1074	478	324	160	271	225	523	164
[illegible]	0	0	1	7	0	0	0	5	14	27	23	69	65	80	86	81	142	292	6	8	12	35	27
[illegible]	0	0	0	2	0	0	41	0	4	30	55	12	194	144	597	8	30	79	135	206	120	105	91
[illegible]	10	2	27	4	0	0	0	2	109	38	9	52	347	29	31	154	456	177	22	145	220	10	7
[illegible]	0	0	1	1	0	1	0	3	6	5	37	8	107	33	36	72	43	14	32	29	300	82	23
[illegible]	0	0	0	0	0	1	0	2	2	6	0	6	88	116	36	51	381	275	130	93	289	6	33
.53	0	3	1	2	0	0	0	1	3	23	5	9	3	125	113	83	548	381	84	125	203	12	176
[illegible]	0	1	5	1	0	0	0	1	16	10	9	21	25	31	58	47	110	29	61	19	42	12	84

Week	4.02.88				12.02.88				19.02.88				Total
KP	1038	745	75	170	383	208	120	219	13	1	0	0	7044
FL	47	40	22	23	6	16	12	8	2	1	2	1	1235
KR	215	91	353	34	117	207	174	14	2	2	6	8	3131
OR	210	21	33	78	210	63	50	91	12	3	3	5	2661
AN	26	85	9	47	82	190	90	29	2	0	2	4	1446
PP	242	66	304	108	149	23	82	15	1	0	1	1	2520
.53	61	13	72	127	3	13	29	2	0	10	9	2	2355
V	122	17	82	29	2	0	2	2	2	0	0	1	884

8. Transformed values (N + 1) of the weekly aphid counts per variety and per replicate recorded during the short rains season (October 1987 - January 1988)

Week	23.12.88				30.12.87				6.01.88				13.01.88				20.01.88			
Replicate	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
KP	2	1	1	4.2	1.0	3.6	1.7	2.8	1.4	4.2	9.5	13.4	6.0	18.6	9.9	32.8	21.9	18.0	12.3	16.0
FL	1	1	1.4	2.8	1.0	1.0	1.0	2.4	3.8	5.3	4.0	8.4	8.1	9.0	9.3	9.1	11.9	17.1	2.6	3.0
KB	1	1	1.0	1.7	1.0	1.0	6.4	1.0	2.2	5.6	7.4	3.6	13.9	12	24	3	5.6	8.9	11.6	14.4
DR	3.3	1.7	5.3	2.2	1.0	1.0	1.0	1.7	10.5	6.2	3.2	7.2	18.7	5.5	5.7	12.5	21.4	13.3	4.8	12.1
AN	1.0	1.0	1.4	1.4	1.0	1.4	1.0	2.0	2.6	2.4	6.1	3.0	10.4	5.8	6.1	8.5	23.4	19.5	9.2	11.2
PP	1.0	1.0	1.0	1.0	1.0	1.4	1.0	1.7	1.7	2.6	1.0	2.6	9.4	10.8	6.1	7.2	19.5	16.6	11.4	9.7
R. 53	0	2.0	1.4	1.7	1.0	1.0	1.0	1.4	2.0	4.9	2.4	3.2	2.0	13.6	10.7	9.2	6.6	3.8	5.7	5.4
RV	1.0	1.4	2.4	1.4	1.0	1.0	1.0	1.0	4.1	3.3	3.2	4.6	5.1	5.7	7.7	6.9	10.5	5.5	7.8	4.5

Date	27.01.88				4.02.88				12.02.88				19.02.88				Total/Season
Replicate	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	
KP	15.0	22.9	12.8	18.9	32.2	27.3	8.7	13.0	19.6	14.5	11.0	14.9	3.6	2.0	3.7	2.4	7044
FL	3.6	6.0	5.3	8.7	6.9	6.4	4.8	4.9	2.6	4.1	3.6	3.0	1.7	1.4	1.7	1.4	1245
KB	11.0	10.3	9.6	7.5	14.7	9.6	18.8	5.9	10.9	14.4	13.2	3.9	1.7	1.7	2.6	3.0	3131
DR	14.9	3.3	2.8	4.6	14.5	4.7	5.8	6.9	14.5	8.0	7.1	9.5	3.6	2.0	3.7	2.4	2661
AN	17.3	9.2	4.9	6.9	5.2	9.3	3.2	6.9	9.1	13.8	9.5	5.4	1.7	1.0	1.7	2.2	1446
PP	16.9	2.6	5.8	4.0	15.6	8.2	17.5	10.4	12.2	4.9	9.1	4.0	1.4	1.0	1.4	1.4	2520
R.53	14.3	3.6	13.3	13.7	7.9	3.7	8.5	11.3	2.0	3.7	5.5	1.7	1.0	3.3	3.2	1.7	2355
RV	6.6	3.6	9.2	6.7	11.1	4.2	9.1	5.5	1.7	1.0	1.7	1.7	1.7	1.0	1.0	1.0	884

Annex 9. Weekly total aphid counts observed on eight potato cultivars during the short rains season (Oct 87-Jan 88)

	DATE (WEEK)									
Variety	WK5	WK 6	WK 7	WK 8	WK 9	WK 10	WK 11	WK 12	WK 13	Total/ season
DR	43	2	208	256	800	258	342	414	33	2661
B.53	6	1	40	384	1004	579	273	47	21	2355
Annet	2	4	56	248	1181	452	167	391	8	1446
Kerr's P.	20	21	285	1551	1233	1266	2028	630	14	7044
Kenya B.	2	41	101	943	450	371	693	512	18	3131
Feldslohm	8	5	133	312	448	149	132	42	6	1235
Bvumbe	7	0	56	161	219	182	250	6	3	884
Pimpernell	0	3	14	291	879	341	720	269	3	2520
Total/Week										
Average/ Week	11	9.6	111.6	518.3	776.75	449.8	575.6	288.9	13.3	

Annex 10. Anova table for the analysis of aphid counts (transformed value) during the short rains season (October 1987 - January 1988)

Source	df	ss	mss	f
Week	8	42221	527.64	32.7
Block	3	101.06	33.69	2.1
Varieties	7	1260.6	180.09	11.2
Error	269	4338.9	16.13	
Total	287	9921.66		
CV = 62%				

Annex 11. Comparison of the aphid population means (N + 1) recorded on potato varieties during the short rains season by using the Duncan new multiple range test.

		Variety							
		BV	FL	AN	B.53	PP	DR	KB	KP
Variety	Means	4.06	4.74	4.97	6.24	6.29	6.92	7.39	11.25
KP	41.25	7.19*	6.38*	6.28*	5.01*	4.9*	4.33*	3.86*	-
KB	7.39	3.33	2.65	2.42	1.15	1.1	0.47	-	-
DR	6.92	2.86	2.18	1.95	0.68	0.61	-		
PP	6.29	2.23	1.55	1.32	0.05	-			
B.53	6.24	2.18	1.5	1.27	-				
AN	4.97	0.91	0.23	-					
FL	4.74	0.68	-						
BV	4.06								

Annex 12. Comparison of the aphid population means (N+1) per variety recorded during the short rains season by using the single Dunnet value

Control mean	Treatment means						
KP	KB	DR	PP	B.53	AN	FL	BV
11.25	7.39	6.92	6.29	6.24	4.97	4.74	4.06
Difference 0.95	*	*	*	*	*	*	*
Difference 0.99	*	*	*	*	*	*	*

The significant difference given by the Dunnet equation for two-sided comparisons between KP and all treatment is 2.56 and 3.04 at p = .95 and p = .99 respectively.

Annex 13. PVY infection rate (%R) recorded on potato varieties submitted to strong initial inoculum in the field assessed through ELISA test one month before crop lifting

Varieties	Virus infection rate				
	1	2	3	4	Average %
Kerr's Pink	0	33.3	55.6	33.3	30.6
Dutch Robjin	44.4	44.4	22.2	33.3	36.1
Annet	11.1	0	11.1	22.2	11.1
Kenya Baraka	44.4	22.2	44.4	44.4	38.9
Pimpernell	11.1	0	11.1	0	5.6
B. 53	0	22.2	11.1	0	8.3
Bvumbe	0	22.2	11.1	33.3	16.7
Feldeslohm	22.2	11.1	44.4	44.4	30.5
Average	22.2				

sd = 13.3 CV = 59.7

Annex 14. Anova table for the analysis of PVY infection rate recorded during the long rains season and analyzed as transformed value (Arc Sin %R)

Source	df	ss	ms	f
Block	3	0.17	0.06	1.1
Variety	7	0.08	0.15	3.1
Error	21	1.05	0.051	
Total	31	2.30		
CV = 52.2%	SD Block 0.112		SD var = 0.158	
Ft = 2.49 (0.05%) and 3.64 (0.01%)				

Abbreviations used: CV = coefficient of variation
SEdiff = standard difference for block or varie
r: PVY infection rate

Table 15. Comparison of the means of PVY infection rate recorded during the long rains season and analyzed as tranformed value (Arc sin %R)

		PP	BB	AN	RV	KP	FL	DR	KR
	Means	0.17	0.21	0.29	0.36	0.51	0.57	0.64	0.67
KR	0.67	0.50*	0.46*	0.38*	0.31	0.16	0.10	0.03	-
DR	0.64	0.47*	0.43*	0.35	0.28	0.13	0.07	-	
FL	0.57	0.40	0.36	0.28	0.21	0.06	-		
KP	0.51	0.34	0.30	0.22	0.15	-			
RV	0.36	0.19	0.15	0.07	-				
AN	0.29	0.12	0.08	-					
.53	0.21	0.04	-						
PP	0.17	-							

Annex 16. Comparison of PVY infection means between the control (KP) and other variety by using the single Dunnet value after the first cropping season.

KP Control	Treatment Means						
	KB	DR	FL	BV	AN	B.53	PP
0.81	0.67	0.64	0.57	0.36	0.29	0.21	0.17
Differences at 0.05					*	*	*
Differences at 0.01						*	*

Annex 17. Secondary PVY infection rate (%R) recorded on potato varieties during the **short rains** season through ELISA.

	Varieties							
	KP	DR	AN	KB	PP	RE	BV	FL
I	58.0	25.0	16.6	33.3	8.3	25.0	66.0	33.3
II	33.3	16.6	33.3	25.0	0	8.3	0	50
III	41.7	50	25	16.6	16.6	8.3	16.6	41.7
IV	75.0	41.7	25	58.0	33.3	41.7	8.3	25.0
	52.0	33.1	25.0	33.2	64.5	20.8	22.7	37.5

Annex 18. Anova table for the comparison of PVY infection means among potato varieties for the short rains season

Source	df	ss	ms	f
Block	3	0.28	0.094	2.3
Variety	7	0.64	0.092	2.2
Error	21	0.87	0.041	
Total	31	1.79		

CV = 36.9% SD Block 0.102 SD var = 0.144

Ft = 2.9 (0.05%) and 3.64 (0.01%)

Annex 19. Comparison of means of PVY infection rate recorded during the short rains season and analyzed as transformed value (Arc sin %R) by using Duncan new multiple range test.

		PP	BV	B.35	AN	KB	DR	FL	KP
	Mean	0.33	0.42	0.45	0.52	0.61	0.61	0.66	0.81
KP	0.81	0.48**	0.39*	0.36*	0.29	0.20	0.20	0.15	–
FL	0.66	0.33	0.24	0.21	0.14	0.05	0.05	–	
DR	0.61	0.28	0.19	0.16	0.09	–	–		
KB	0.61	0.28	0.19	0.16	0.09	–			
AN	0.52	0.19	0.10	0.07	–				
B.53	0.45	0.12	0.03	–					
BV	0.42	0.09	–						
PP	0.33	–							

Major differences: KB = PP, BV and B.53

20. Comparison of secondary PVY infection means among varieties by using the single Dunnet test.

KP	FL	DR	KB	AN	B.53	BV	PP
0.81	0.66	0.61	0.61	0.52	0.45	0.42	0.33
ff. at 5%							*
ff. at 0.01%							*

Table 21. Average per replicate of Polyphenol oxidase (PPO) activity expressed as the percentage transmittance (%T) assessed through a wavelength 440 mm with a spectronic 20 R spectrophotometer during the long rains season (April - July 1988)

	Date/Replicate											
	Day 1				Day 2				Day 3			
Variety	1	2	3	4	1	2	3	4	1	2	3	4
Kerr's Pink	73.0	84.0	53.0	73.7	89.0	70.3	85.0	61.0	81.0	51.3	74.7	54.3
Annet	65.0	60.7	68.7	54.7	62.7	75.0	64.0	72.0	69.7	68.3	70.0	60.7
Kenya Baraka	80.3	85.0	65.3	74.0	86.0	84.0	75.3	80.3	74.0	74.3	79.0	70.3
Feldeslohn	65.0	72.3	88.0	58.0	71.0	76.0	87.0	69.0	57.3	69.0	87.7	71.7
B.53	71.6	67.0	53.0	56.0	66.3	66.3	73.3	66.7	50.3	79.3	63.3	63.7
Bvumbe	73.0	56.0	40.0	43.0	63.7	73.3	64.3	66.7	50.3	79.3	63.3	63.0
Dutch Robjin	68.0	66.0	43.0	50.0	67.0	80.0	81.0	77.0	50.3	75.3	80.3	75.0
Pimpernell	53.0	72.0	78.0	38.0	70.3	63.7	74.6	56.7	56.3	73.3	68.0	67.0
Pimpernell	53.0	83.7	46.7	54.6	61.3	75.3	85.1	56.7	68.3	82.7	66.7	54.7

Annex 22. Average per date of measurement of Polyphenol oxidase (PPO) activity (%T) assessed during the long rains season

Variety	D1	D2	D3
Kerr's Pink	70.9	76.3	65.3
Annet	62.3	68.4	67.2
Kenya Baraka	76.1	81.4	74.4
Feldeslohm	68.6	75.8	71.4
B.53	62.1	69.1	76.4
Bvumbe	53.0	67.0	64.2
Dutch Robjin	56.8	76.3	72.2
Pimpernell	60.3	71.3	66.2

Table 23. Polyphenol oxidase activity (PPO) expressed as the percentage transmittance (%T) assessed through a wavelength 440 nm with a spectronic 20 R spectrophotometer during the short rains season (October 1987 - January 1988)

	Date/Replicate											
	Day 1				Day 2				Day 3			
Variety	1	2	3	4	1	2	3	4	1	2	3	4
Kerr's Pink	86.3	86.3	73.0	80.7	85.0	-	80.6	56.0	85.7	-	81.0	57.3
Annet	69.0	55.0	65.0	72.7	75.7	71.0	75.0	67.7	57.7	67.0	66.5	67.0
Kenya Baraka	79.3	78.0	69.3	80.7	77.0	80.3	84.0	73.3	81.7	80.3	70.7	74.3
Feldeslohn	62.0	76.3	92.3	56.0	63.0	76.7	92.0	66.7	65.0	73.0	83.7	62.7
B.53	74.7	73.7	59.0	59.7	49.7	61.7	76.7	59.0	58.3	73.7	60.3	62.7
Bvumbe	81.0	58.0	45.0	51.3	50.6	81.3	90.0	50.3	57.7	70.3	61.0	60.7
Dutch Robjin	84.0	73.0	54.7	62.3	51.3	74.0	80.7	73.0	39.7	73.7	85.0	73.3
Pimpernell	74.0	83.7	46.7	54.6	61.3	75.3	85.1	56.7	68.3	82.7	66.7	54.7

Annex 24. Average of PPO activities (%T) per date of measurement during the short rain season (October 1987 - January 1988).

Varieties	Day 1	Day 2	Day 3	Average
Kerr's Pink	82.4	76.1	74.7	77
Annet	66.5	72.3	64.1	67.6
Kenya Baraka	76.8	78.1	76.8	77.4
Feldeslohm	73.1	74.6	69.4	72.4
B.53	66.8	61.8	63.6	64.1
Bvumbe	58.8	62.6	62.4	61.3
Dutch Robjin	67.5	71.3	73.0	70.6
Pimpernell	64.7	69.7	68.2	67.5

Annex 25. Tuber yield per plant (kg) observed on eight varieties submitted to aphid infestation and PVY infection during the long rain season (April - July 1987)

	KP	DR	AN	KB	PP	B.53	BV	FL
	1.050	1.440	1.750	2.600	.910	.790	1.280	1.070
	.990	1.200	1.410	2.000	1.240	.780	1.990	.730
	.180	1.780	1.200	1.900	1.480	1.370	1.710	1.290
	1.310	1.340	1.410	1.720	1.190	.950	2.000	1.410
BLOCK I	1.000	1.100	1.490	1.950	1.240	1.130	2.140	1.140
	1.250	1.520	1.400	1.820	1.060	1.100	1.720	1.230
	.950	1.540	.850	.810	.960	1.110	1.290	1.480
	.850	1.330	1.240	1.350	1.310	1.370	.530	1.040
	.170	1.480	1.250		2.340	1.380	1.120	1.000
x	.97	1.41	1.33	1.83	1.19	1.08	1.51	1.19
Sd	.33	.19	.25	.52	.18	.21	0.53	.23
CV	34.1	14.1	18.3	28.5	15.8	19.8	35.1	19.1
	1.270	1.250	1.520	2.100	1.380	1.180	2.120	.910
	.960	1.240	1.250	.720	1.260	1.060	2.220	.890
	1.300	1.050	.920	1.200	1.090	.830	1.390	1.390
	.990	1.460	.960	1.520	.960	1.200	.890	.820
	.920	1.420	1.030	1.020	1.050	1.450	1.010	.920
BLOCK II	.790	1.830	1.000	1.520	1.030	.960	1.470	1.950
	1.350	1.080	1.050	1.510	1.120	1.270	2.850	1.710
	.400	2.000	1.060	1.500	1.010	2.050	2.050	1.380
	1.530	1.320	1.450	2.100	1.010	.850	.800	1.480
x	1.06	1.41	1.13	1.47	1.12	1.12	1.64	1.27
Sd	.34	.32	.22	.45	.13	.21	.7	.41
CV	32.6	22.9	19.1	30.9	11.9	18.6	42.8	31.9
	.470	1.160	1.880	1.800	1.160	.880	1.580	1.040
	1.150	1.290	1.050	.900	1.000	.770	1.300	.900
	1.100	1.630	1.220	2.210	1.100	1.110	1.700	.960
	1.190	1.000	.910	2.290	1.570	.890	2.260	1.540
BLOCK III	.880	1.390	1.430	1.300	.890	.820	1.100	1.050
	.270	1.480	1.140	1.830	1.110	.780	2.240	1.140
	.390	.840	1.030	1.510	.880	1.300	1.210	1.070
	.260	1.660	1.320	1.940	.810	1.020	2.030	1.170
	1.340	1.300	.930	.610	1.420	.560	1.410	1.300
x	1.01	1.31	1.21	1.59	1.10	.90	1.65	1.13
Sd	.39	.27	.30	.57	.25	.22	.44	.19
CV	39.3	20.9	25.1	35.9	22.9	23.9	26.7	17
	.250	1.800	1.000	1.900	1.440	.330	1.000	1.400
	.430	1.400	.730	1.200	.809	.750	.500	1.950
	1.080	1.070	.920	.850	.560	.860	.740	1.800
	.650	1.200	1.830	1.250	1.100	.770	.890	1.690
	1.070	1.380	1.210	1.100	.690	.840	.660	1.660
BLOCK IV	.940	1.220	1.320	1.210	.680	.810	.910	1.120
	1.350	1.350	1.010	.890	.810	.870	.510	.940
	.520	1.160	1.210	1.260	.780	.800	.750	1.310
	.260	1.290	1.590	1.150	1.120	.790	.670	.770
x	0.73	1.32	1.20	1.37	1.32	.76	1.36	1.32
Sd	.39	.21	.34	.54	.26	.29	.43	.27
CV	54.5	15.9	28.5	39.6	19.8	37.9	31.4	20.5

Annex 26. Anova for the comparison of tuber yield per plant among varieties for the long rains season

Source	df	SS	MS	F
Plant	8	59.6	7.4	0.5
Block	3	55.4	18.5	1.4
Variety	7	1593.9	227.7	16.8**
Error	269	3652.1	13.6	
Total	287	5360.9		

CV = 29.7% Sdplant = 0.921 Sdblock 0.614
Sdvariety = 0.868 Ft = 2.01 (0.05%) and 2.64 (0.01%)

Annex 27. Comparison of the tuber yield means per plant among potato varieties for the long rains season

		Variety							
		KP	B.53	PP	AN	FL	DR	BV	KB
Varieties	Means	0.86	0.98	1.17	1.22	1.22	1.36	1.54	1.58
KB	1.58	0.72**	0.6**	0.41**	0.36**	0.36**	0.22*	0.04	-
BV	1.54	0.68**	0.56**	0.37**	0.32**	0.32**	0.18*	-	
DR	1.36	0.5**	0.38**	0.19*	0.14	0.14	-		
FL	1.22	0.36**	0.24**	0.5	-	-			
AN	1.22	0.36**	0.24**	0.5	-				
PP	1.17	0.31**	0.19*	-					
B.53	0.98	0.12	-						
KP	0.86	-							

Annex 28. **Anova for the comparison of coefficients of variation of tuber yields per plant observed within each variety during the long rains season**

Source	DF	SS	MS	F
Bloc	3	0.03	0.01	2.1
Var	7	0.24	0.03	7.1
Error	21	0.10	0.004	
Total	31	0.38		

CV = 13.0%
Se.d bloc = 0.035 Se.d var = 0.049

Annex 29. Comparison of the coefficient of variation (CV) means of tuber yield per plant recorded during the long rains season, analyzed as transformed values (Arc sin CV) of by using the Duncan new multiple range test.

		PP	DR	FL	AN	B.53	KB	BV	KP
Variety	Means	.43	.44	.49	.50	.52	.62	.62	.68
KP	.68	.25*	.24*	.19*	.18*	.18*	.16	.06	.06
BV	.62	.19*	.18*	.13*	.12*	.10	-		
KB	.62	.19*	.18*	.13*	.12	.10			
B.53	.52	.09	.08	.03	.02	-			
AN	.50	.07	.06	.01					
FL	.49	.06	.05	-					

major differences: KP vs PP, SE, DL, N, V.53
BV vs PP, DR, FL, AN
KB vs PP, DR, FL

Annex 30. Tuber yield (kg) per plant recorded on potato varieties observed during the second crop

	Plant	KP	DR	PP	KB	B.53	AN	BV	FL
Block I	1	0.26	1	0.9	0.5	1.3	0.45	0.6	50.3
	2	0.3	1.1	1	0.75	0.6	1.1	0.8	0.57
	3	0.5	0.75	0.25	0.3	0.75	0.75	0.6	0.7
	4	0.25	1.1	0.5	0.48	0.61	0.65	1	0.7
	5	0.75	0.5	0.25	0.75	0.77	0.6	0.75	0.53
	6	0.25	0.75	0.8	0.8	0.9	0.9	0.5	0.6
	7	0.76	1	0.6	1.11	0.75	0.5	0.6	0.55
	8	1.26	0.5	0.75	0.87	0.58	0.5	0.71	0.75
	9	1.01	0.25	1.2	0.75	0.9	1.25	1.02	0.37
Means		0.593	0.689	0.694	0.701	0.795	0.74	0.736	0.56
SD		0.372	0.397	0.325	0.240	0.223	0.28	0.178	0.15
CV%		62.9	57.6	46.9	34.3	28.1	38.1	24.3	0.27
Block II	1	0.51	0.61	1	0.9	0.9	0.9	0.5	0.45
	2	0.27	0.54	1.25	1	1	0.51	1	0.5
	3	0.6	0.38	1.1	1.2	0.27	0.47	1.1	0.6
	4	1	1	1.1	0.6	0.73	1.12	0.95	0.55
	5	0.8	0.3	0.9	1	0.51	0.75	0.7	0.75
	6	0.78	0.32	0.4	0.81	0.47	0.51	0.5	0.56
	7	1.33	0.76	1.75	0.4	0.52	0.51	1.2	0.61
	8	1	1	1.25	0.5	0.5	0.75	0.8	0.6
	9	0.61	1.1	0.6	0.9	0.25	1.4	0.98	0.5
Means		0.766	0.667	1.038	0.812	0.572	0.77	0.858	0.56
SD		0.314	0.311	0.391	0.261	0.258	0.32	0.250	0.86
CV		41.1	46.7	37.6	32.2	45.1	0.42	29.2	15.3
Block III	1	0.375	0.42	0.725	1	0.38	1.5	1.65	0.45
	2	0.675	0.825	0.89	1.1	0.45	1.11	0.9	0.75
	3	1.25	1.05	1.515	0.8	0.83	0.68	1.5	0.98
	4	0.72	0.75	0.73	1	0.47	1.89	0.75	1.11
	5	0.78	0.36	1.15	0.4	0.92	1.5	1.5	1.05
	6	1.065	0.75	1.3	1.14	0.63	1.125	1.05	1.13
	7	0.15	0.9	0.63	1.02	1.12	0.9	1.22	1.13
	8	0.39	1.125	1.05	0.94	0.45	1.85	2.62	0.38
	9	0.48	1.13	0.7	0.2	0.18	1.2	1.41	0.38
Means		0.653	0.811	0.97	0.844	0.430	1.306	1.400	0.82
SD		0.349	0.279	0.319	0.327	0.328	0.411	0.547	0.33
CV%		53.4	34.5	32	38.7	76.3	31.5	39.1	40.6
Block IV	1	0.255	0.38	1.38	0.3	0.75	0.45	1.01	0.38
	2	0.375	0.23	0.75	0.87	0.6	1.11	0.77	0.6
	3	0.38	0.6	0.6	0.92	0.66	0.86	1.23	0.3
	4	0.3	0.75	1.38	0.8	0.53	0.75	1.35	0.42
	5	0.36	0.6	0.77	0.54	0.56	1.17	0.9	1.065
	6	1.05	0.45	0.63	0.6	0.45	0.45	1.23	0.71
	7	0.39	0.77	0.87	0.48	0.38	1.14	1.065	0.39
	8	0.45	0.93	0.96	0.59	0.24	0.6	1.095	0.75
	9	0.4	0.435	0.405	0.6	0.7	0.15	1.26	0.62
Means		0.440	0.571	0.860	0.635	0.541	0.748	1.1	0.581
SD		0.235	0.221	0.340	0.197	0.162	0.359	0.19	0.24
CV%		53.5	38.7	38.9	31.3	30.1	48.4	16.9	41.4

Annex 31. Anova for the comparison of tuber yield means per plant among potato varieties after the short rains season.

Source	df	ss	ms	f
Plant	8	0.45	0.06	0.6
Block	3	2.63	0.87	9.0**
Variety	7	5.81	0.83	8.5**
Error	269	28.06	0.1	
Total	287	36.04		

CV = 40.8% sd plant = 0.78 sd block = 0.052
sd variety = 0.074 ft = 2.01 (.05%) and 2.64 (.01%)

32. Comparison of the tuber yield per plant (kg) among potato varieties after two cropping seasons (short rain season: October '87 - January '88)

		Variety							
		KP	B.53	FL	DR	KB	PP	AN	BV
rieties	Means	0.61	0.63	0.63	0.71	0.75	0.89	0.89	1.02
BV	1.02	0.41**	0.39**	0.39**	0.31**	0.27**	0.13*	0.13	-
AN	0.89	0.28**	0.26**	0.26**	0.18*	0.14	0.05	-	
PP	0.89**	0.28**	0.26**	0.26**	0.18*	0.14	-		
KB	0.75	0.149	0.12	0.12	0.04	-			
DR	0.71	0.10	0.08	0.08	-				
FL	0.63	0.02	-	-					
B.53	0.63	0.02	-						
KP	0.61	-							

jor differences: (0.05) BV = KP, B.53, FL, DR, KB, PP
AN = KP, B.53, FL, DR
PP = KP, B.53, FL

(0.01) BV = KP, B.53, FL, DR, KB
AN = KP, B.53, FL
PP = KP

Annex 33. Anova for the comparison among the coefficient of variation observed on the tuber yield variations per plant during the short rains season.

Source	df	Ss	Ms	F
Block	3	0.027	0.009	0.6
Variety	7	0.21	0.030	2.0
Error	21	0.31	0.015	
Total	31	0.55		
CV = 18.2%	Sdreplicates = 0.061 Sdvarieties = 0.086 Ft = 2.4 g (0.05%) and 3.64 (0.01%)			

Annex 34. Comparison of the coefficient of variation (%CV) of tuber yield per plant within each variety recorded during the short rains season and analyzed as ArcSin %CV by using the Duncan new multiple range test

		BV	FL	KB	PP	AN	DR	B.53	KP
		0.55	0.59	0.62	0.67	0.68	0.73	0.74	0.81
KP	0.81	0.27*	0.23*	0.20	0.15	0.14	0.09	0.08	-
B.53	0.74	0.19	0.15	0.12	0.07	0.06	0.01	-	
DR	0.73	0.18	0.14	0.11	0.06	0.05	-		
AN	0.68	0.13	0.09	0.06	0.01	-			
PP	0.67	0.12	0.08	0.05	-				
KB	0.62	0.07	0.03	-					
FL	0.59	0.04	-						
BV	0.55	-							

Difference KP ≠ BV and FL

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Annex 35. Materials included in the polivalent ELISA kit

CODE	NAME	CHEMICAL COMPOSITION	QUANTITY	PHYSICAL STATE	FOR 1 PLATE	REMARKS
I	BUFFERS					
1	Coating 0.73 g $NaHCO_3$	0.4 g Na_2CO_3 50.0 ml 0.05 g NaN_3 50.0 ml distilled water	1 bottle	Liquid: transparent	Mix 5 ml of buffer 1 with 200 ml distilled water	Store the bottle with the concentrated solution in cold and dark place
2	PBS	8.0 g NaCl 0.2 g KH_2PO_4 0.2 g KCl 2.9 g Na_2HPO_4 $2H_2O$	12 packets	Powder: white	Dissolve 1 pack in 1.0 litre distilled water	Stable at room temperature
2B	PBS-Tween	Tween 20 or Tween 80	1 bottle 10 ml	Liquid: yellow	PBS-Tween = mix 0.5 ml of Tween with 1.0 litre of PBS buffer	Stable at room temperature
3	Extraction buffer	4.0 g PVP - 40,000 2.0 g Egg albumen	12 packets	Powder: milky	Dissolve 1 packet in 200 ml of PBS + Tween	Dissolve the powders, first with a very small amount of distilled water
4	Conjugate buffer	0.4 g PVP - 40,000 0.2 g Egg albumen	12 packets	Powder: milky	Dissolve 1 packet in 20 ml of PBS + Tween	Same as of 3
5	Substrate buffer	29.1 ml of Diethanolamine 16.9 ml distilled water 4.0 ml HCl (37%)	1 bottle 50.0 ml	Liquid: transparent	Mix 5.0 ml of buffer 5 with 25 ml of distilled water	Store the concentrated solution in a cold and dark room
6	0.2M Glycine for partial dissociation	0.75 g Glycine 10 ml HCl (1 : 1)	10 packets	Powder: white	Dissolve 1 packet in 30 ml of distilled water. Add 0.6 ml of HCl	Prepare immediately before use. Never add water after the HCl
7	10% NaOH for total dissociation	20.0 g NaOH 20.0 ml distilled water	1 bottle	Liquid: transparent	Dilute 3.0 ml of buffer 7 with 27 ml of distilled water	Stable at room temperature for many months
II	ELISA plates	Polystirene	4	Solid plastic		Reuse: 2 times. PLRV: parital dissociation. PVY, PVX and PVS: total dissociation