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Ability of systemic insecticide dimethoate to prevent aphid colonisation and the spread of aphid transmitted viruses in *Solanum tuberosum* Lin.

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Abstract

Seven potato (*Solanum tuberosum* Lin.) varieties previously indexed against potato leaf roll virus (PLRV), potato virus Y (PVY), potato virus S (PVS) and potato virus X (PVX) were grown in the field for two seasons at Namulonge representing warm, mid-altitude tropics and, for one season at Kalengyere representing cool highland areas. One set of the seven potato varieties was sprayed with dimethoate at a rate of 1.19 kg a.i. ha^{-1} at weekly intervals to prevent aphid infestation and spread of aphid transmitted viruses. The second set was not treated. Seed tuber indexing before planting showed the absence of aphid transmitted viruses (PLRV, PVY and PVS) in seed potato obtained from Kalengyere during both seasons. Weekly collection of aphids at both sites showed a predominance of *Myzus persicae* (Sul.), the principle vector of most potato viruses. Dimethoate controlled aphids at Kalengyere but not at Namulonge. As a result, seed potatoes obtained from Kalengyere and grown at Namulonge acquired the aphid-transmitted PLRV in the first season of growth whether they were treated with the insecticide or not. However, all the varieties were free from PVY and PVS. At Kalengyere, all the varieties grown in the field were free from aphid-borne viruses irrespective of the insecticide treatment. These results indicate that there is probably *M. persicae* resistance to dimethoate at Namulonge.

Key words: Dimethoate, virus latent infection, aphid-borne viruses, *Myzus persicae*, ELISA.

Introduction

Aphids are the main natural vectors of most plant viruses (Hooker, 1982). The spread of aphid-borne viruses is related to the size of aphid populations and presence of infection sources (Raman, 1985). In a field initially planted with virus-free seed potato, virus infection may be introduced by infective alate aphids. Similarly, if virus-free alate aphids colonise a field with some few virus infected plants, they will spread infection to formerly uninfected plants (Jayasinghe, 1988; Auclair, 1989; Harrewijn 1989).

In certified seed, the number of virus infection sources may be low or absent, especially where seed plots are isolated (Raman, 1985; Jayasinghe, 1988). In a typical crop, the strategy of virus control is to reduce the number of virus vectors within the crop (Schepers, 1972; Gibson and Rice, 1989). This can be achieved by use of aphicides, especially systemic ones. A combination of systemic insecticides with initial virus-free seed stocks will greatly reduce or completely prevent the spread of persistently aphid transmitted viruses in a potato crop.

One of the aphicides that has been used to curtail the spread of aphid-borne viruses in *Solanum* potato is dimethoate (0,0-dimethyl-S-(N-methyl carbamoyl)-methyl) (Kibata, 1982; Raman and Radcliffe, 1992). It has both contact and systemic properties (Matthews, 1984) and, a capacity to reduce the number of short trial probes by *Myzus persicae* and *Aphis fabae* (Scop.) on various hosts (Gibson and Rice, 1989). These attributes probably reduce the chances of transmission of non-persistent viruses and may completely stop the spread of persistently transmitted potato viruses, for instance PLRV.

In this regard, a study was undertaken to determine the effectiveness of dimethoate in preventing or reducing the spread of aphid-borne viruses in virus-indexed certified seed potato tubers. Attention was paid to potato leaf roll virus (PLRV), potato virus Y (PVY) and potato virus S (PVS) which are aphid-borne. Potato virus X (PVX) which is only transmitted by contact was also included because its presence in some *Solanum* potato varieties increases their susceptibility to other potato viruses especially PLRV (Jayasinghe et al., 1989).

Materials and Methods

Seven potato varieties, Rutuku, Kabale, Kisoro, Victoria, Sangema, 382171.4, and 381403.1 were field grown at Namulonge (1150 m above sea level, and 32° 27'1E 0° 32' N) during the 1994 short rains (October - December) and 1995 long rains (February - May). The same varieties were grown at Kalengyere (2400 m above sea level, and 32° 27' E 0° 32' N) during the 1995 long rains. The first five entries are released varieties, and popular with farmers Among them, Rutuku and Kabale are late maturing clones mainly for highlands, while Kisoro, Victoria and Sangema are clones with wide adaptability. All the clones have shown high levels of resistance to PLRV, PVY, and, PVS in the highlands of south-western (Kabale) and eastern (Mbale) Uganda. However, variety trials in low elevations have not involved highly sensitive and specific serological tests for particular viruses. The other clones, 382171.4 and 381403.1, are pre-released varieties whose potential as future varieties is quite high.

The experimental arrangement was a split plot design with three replications, such that the insecticide treated and unsprayed blocks constituted the main plots while varieties were the sub-plots. Each main plot was 270 m^2, devided into seven sub-plots of 4.8 m long and 3.0 m wide. The sub-plots were separated from each other by 1.5 metre-wide alleys, and the main plots by 2-metre wide strips planted with maize (var. Longe 1) at Namulonge and wheat (cult. U0036) at Kalengyere to act as barrier against insecticide drift during spraying. In addition, a polythene paper barrier was erected between the main plots during each spray application. In each sub-plot were four rows each planted with 12 tubers. A spacing of 75 cm between rows and 40 cm within a row, giving approximately 33,300 plants ha^{-1}, was adopted, rather than the recommended spacing of 70 cm by 30 cm (Anon., 1994). This was to prevent aphids moving from plant to plant early in the season through early plant contact.

The tubers were planted by placing them in a furrow about 10 cm deep and covered with soil in ridges. In one main plot, dimethoate (Rogor) was sprayed at a rate of 1.19 kg a.i. ha^{-1} once every week for ten weeks, while the other main plot was not sprayed. Spraying started in the third week (approximately 21 days) after planting or 7 days after emergence when more than 95 % of the seed tubers had germinated and emerged out of the soil.

The plots were kept weed-free and given three fungicidal spray of Dithane M-45 (Mancozeb) at a rate of 400 g a.i. ha^{-1} to prevent fungal attack. The fungicides were applied at 30, 50 and 70 days after planting. All spray applications were done using a 15-litre capacity knapsack spray pump.

Seed-borne potato virus latent infection indexing

Before planting, 20 medium sized (100-120 g) seed tubers out of 100 tubers of pre-basic seed from each of the 7 potato varieties. These tubers were healthy in the previous season but their health status needed to be validated in the current season. They were induced to sprout using rindite (7:3:1 parts of ethylene chlrohydrin, ethylene dichloride and carbon tetrachloride, respectively; Burton, 1966). Three weeks after application of rindite, all the seed tubers had sprouted. For each tuber, a sprout and part of the tuber flesh from the heel end were scooped out and indexed for latent infection with PLRV, PVY, PVX and PVS using the Direct Double Antibody Sandwich Immunosorbent Assay (DAS-ELISA) in duplicate wells (Converse and Martin, 1993).

The virus antigen-antibody reaction for each of the above viruses was detected using the respective virus antibodies conjugated to a phosphatase enzyme. The enzyme substrate used was P-nitrophenyl phosphate adjusted to pH 9.8 in 10% diethanolamine stock solution (Converse and Martin, 1993). The antigen-antibody reaction for each virus was quantified using the Multiskan Plus ELISA plate reader (Version 2.03) through a 405 nm wavelength light filter. The negative-positive threshold (cut-off value for healthy and infected samples, respectively) for each virus was set at the mean absorbance value of 10 healthy (control) samples plus three standard deviations ($\pm$ 3sd) (Satula et al., 1986; Flanders et al., 1990). Any sample whose absorbance value was greater than the threshold value for a particular virus was declared to be infected with that virus. The mean absorbance values for each variety in both seasons of acquiring seed from Kalengyere were worked out and tabulated.

Incidence and abundance of potato infesting aphid species on field grown potatoes

Aphid data were collected from 20 randomly selected and marked potato plants per sub-plot. For each of the sun-plot, five plants from each row, were inspected for aphid infestation once every week beginning from the fourth week after planting. Before the plants attained 12 compound leaves per main stem, all the leaves on all the available stems per plant were inspected and aphids counted *in situ*. Thereafter, three main stems per plant were examined, all leaves were inspected and aphids counted. At every count, aphid samples were collected from each sub-plot in 10 ml specimen bottles containing 95% ethanol. The samples were taken to the laboratory, and aphid sub-samples removed and standardised using a graduated 1.5 ml vial. A maximum of 1 ml of aliquoted aphids containing not more than 450 was used per sub-plot where aphids existed. In these samples, aphid species that colonize potatoes were identified with the help of a binocular microscope using identification keys (Anon., 1979a, 1979b; Blackman and Eastop, 1984) and individuals per aphid species counted. These activities were done for 9 and 6 weeks at Namulonge during the 1994 short rains and 1995 long rains, respectively, and for 9 weeks at Kalengyere during the 1995 long rains. The population build-up of each aphid species on dimethoate sprayed and unsprayed potatoes were plotted against time (weeks).

Virus incidence and severity in field grown insecticide treated potatoes

Two fully expanded apical leaflets (Flanders et al., 1990) were picked from each of the plant in the middle two rows per sub-plot, at 75 days after planting (DAP). The samples were taken to the laboratory and tested for the presence of

PLRV, PVY, PVX and PVS using DAS-ELISA protocol. The virus antigen-antibody reactions were quantified as before. Virus infection quantification was based on the principle that the intensity of the yellow colour of the virus antigen-antibody reaction is proportional to the virus antigen concentration (Salazar, 1983; Converse and Martin, 1993), and therefore, to the severity of virus infection. The negative-positive thresholds for healthy and infected samples, were set as described earlier. Differences between treatments were tested using analysis of variance (ANOVA) of Mstatc statistical software package, the means were where the "F" statistics significance, indicated using the Least Significant Difference (LSD) test at the alpha level of 0.05.

Results

Virus latent infection of seed potato tubers

Virus indexing of seed potato tubers obtained from Kalengyere during the 1994 short rains and 1995 long rains were free from PLRV, PVY and PVS which are all transmitted by aphids.

The effect of dimethoate on potato infesting aphids

At Namulonge, dimethoate spraying stimulated the development of *M. persicae* population but controlled other aphid species (Fig. 1). At Kalengyere, all the aphid species were controlled in sprayed potatoes while their numbers increased with time in the unsprayed plots(Fig. 1).

In unsprayed potato at both sites, the populations of the other potatoes - infesting aphids species increased progressively during the season. At both locations, *M. persicae* was the main aphid species infesting potato (Fig. 1). There was generally higher aphid pressure at Kalengyere than at Namulonge in unsprayed plots. At Namulonge, Dimethoate-sprayed potato were more infested than the unsprayed crop beginning from the fifth week of sampling (Fig. 2).

There was a rapid rise in the number of aphids per plant in the fifth week of sampling of the sprayed and unsprayed potatoes at Namulonge and Kalengyere, respectively (Fig. 2). Conversely, at Namulonge, aphid numbers declined in the unsprayed potatoes. At Namulonge, there was more infestation in the 1995 long rains than the 1994 short rains (Fig. 2)., and the varieties used in the study were highly susceptible to aphid infestation. Considering the 1995LR season for both sites, there was higher aphid pressure at Kalengyere than at Namulonge (Fig. 2).

Virus incidence and severity in field grown dimethoate treated *Solanum* potatoes

Virus indexing at 75 DAP, of potatoes grown at Namulonge during the 1994SR showed no significant differences in infection with PLRV, PVY and PVS, between sprayed and unsprayed potatoes (Table 1). For PVX infection however, differences among the varieties

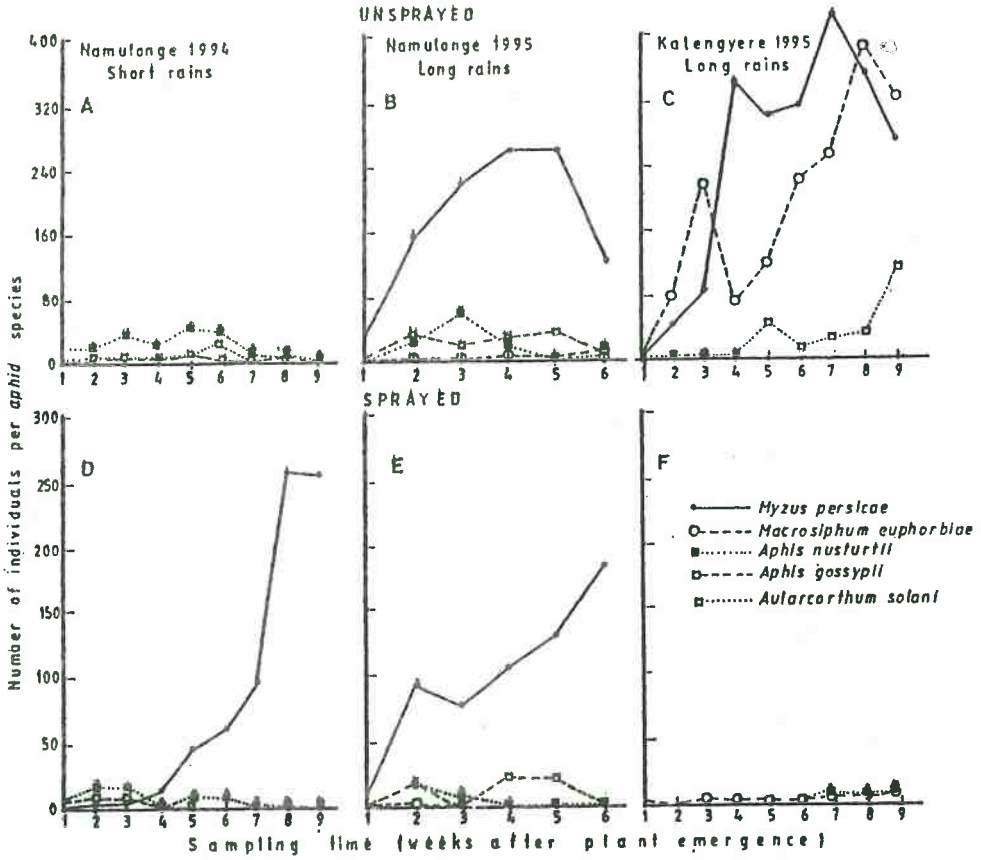

Figure 1. Aphid species composition in sprayed and unsprayed potato plots at Kalengyere and Namulonge,1994-1995.

Table 1. Mean squares from ANOVA tables of Multiskan Plus ELISA plate reader values for four viral infections in seven Solanum potato varieties under two insecticide spray treatments at Namulonge during the short rains of 1994 and at Kalengyere during the 1995 long rains

Source of Variation	Df	Namulonge				Kalengyere			
		Potato leaf roll virus	Potato virus Y	Potato virus X	Potato virus S	Potato leaf roll virus	Potato virus Y	Potato virus X	Potato virus S
Replication	2	0.003	0.222	0.004	0.002	$1x10^{-5}$	0.001	$1x10^{-4}$	$1x10^{-5}$
Spray (A)	1	0.002	$1x10^{-5}$	0.001	0.002	$5x10^{-4}$	$1x10^{-5}$	0.001	$1x10^{-4}$
Error	2	0.003	$5x10^{-4}$	$5x10^{-4}$	$3x10^{-4}$	$3x10^{-4}$	$5x10^{-5}$	0.005	$4X10^{-5}$
Variety (B)	6	0.001	$3x10^{-4}$	0.189**	$4x10^{-4}$	0.001	0.001	0.098**	0.006
AB	6	0.003	$2x10^{-5}$	0.003	$1x10^{-4}$	0.001	$2x10^{-4}$	0.008	$7X10^{-5}$
Error	24	0.0004	$8x10^{-5}$	0.002	$2x10^{-4}$	0.001	0.001	0.008	$9X10^{-5}$
C.V. (%)	-	7.62	6.20	19.01	8.15	36.6	35.0	41.2	34.2

** significant at P = 0.01

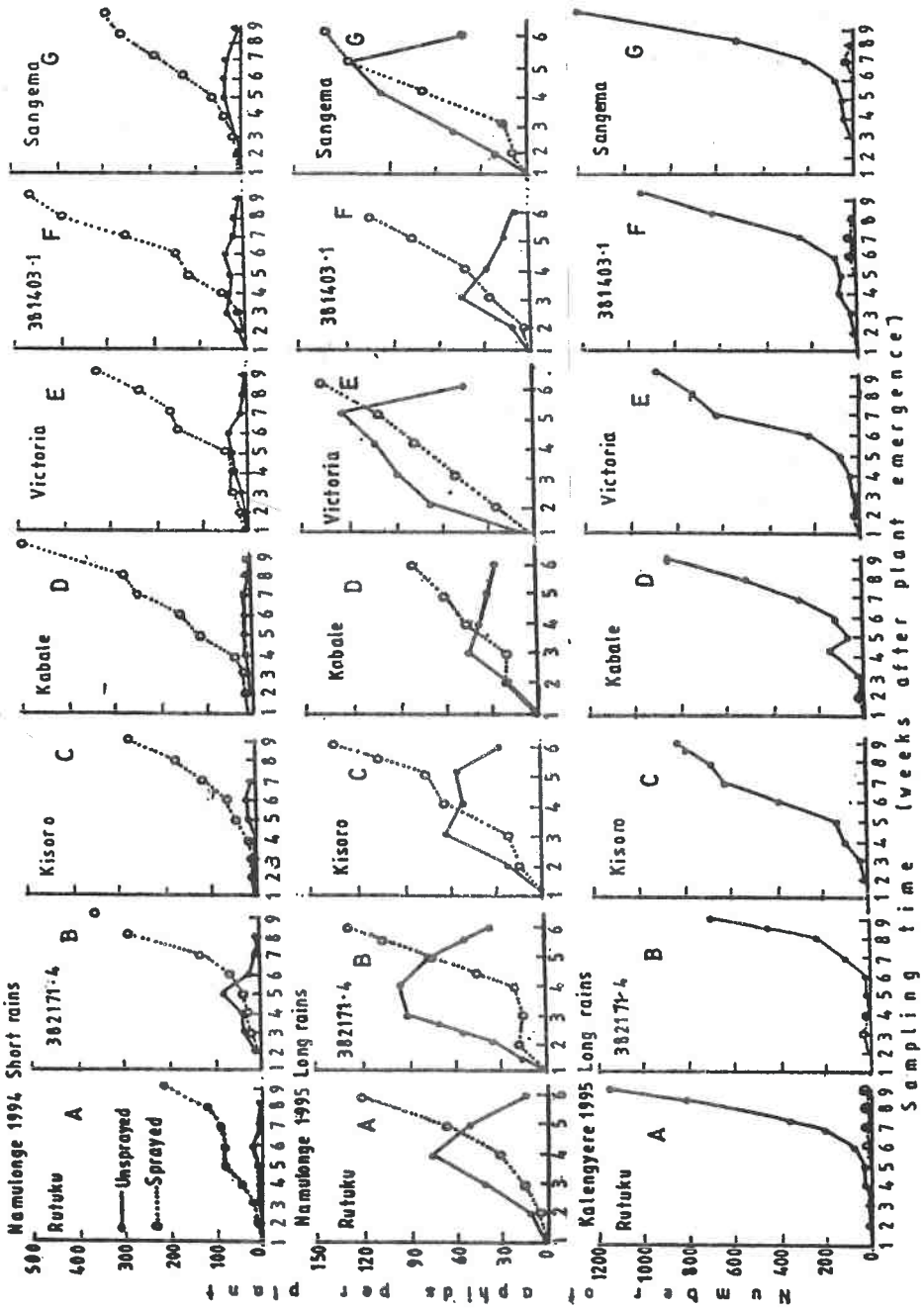

Figure 2. Aphid population development on Dimethoate-sprayed and unsprayed plots of three potato varieties at Namulonge and Kalengyere, 1994-95

was significant. A similar pattern was observed at Kalengyere in the 1995LR.

At Namulonge in the 1995LR, no significant effect of insecticide treatments on virus infection of field grown potatoes was observed. As in the 1994SR, significant differences were observed among the varieties for PVX infection (Table 2). Virus testing of leaf samples showed that during both seasons at Namulonge, all the potato varieties were infected with PLRV but free from PVY and PVS irrespective of the insecticide treatment. At Kalengyere, all varieties were free of PLRV, PVY, and PVS, irrespective of the insecticide treatment (Table 2).

Discussion

The original seed stocks obtained from Kalengyere were free from aphid-transmitted viruses. However, the use of dimethoate insecticide on potato at Namulonge did not deter the colonisation and population development of *M. persicae*, but controlled other potato infesting aphids, and the spread of PLRV. The absence of aphid-borne viral infections in sprayed potatoes at Kalengyere demonstrated that dimethoate can probably prevent the spread of some aphid-borne viruses in *Solanum* potato under same environments.

Table 2. Mean Multiskan Plus values for Potato leaf roll virus (PLRV), Potato virus Y (PVY) and Potato virus S (PVS) infection in field grown Solanum potato 75 days after planting at Namulonge and Kalengyere, 1994-95.

Variety	Mean absorbance values (Relative virus titre)					
	Unsprayed			Sprayed		
	PLRV	PVY	PVS	PLRV	PVY	PVS
(a) Namulonge 1994 Short rains						
Rutuku	0.258	0.139	0.212	0.256	0.127	0.200
382171.4	0.256	0.128	0.201	0.252	0.127	0.186
Kisoro	0.237	0.126	0.195	0.235	0.127	0.195
Kabale	0.263	0.132	0.205	0.249	0.134	0.205
Victoria	0.278	0.126	0.198	0.248	0.122	0.193
381403.1	0.251	0.126	0.202	0.237	0.126	0.213
Sangema	0.256	0.127	0.192	0.242	0.124	0.207
LSD (0.05)	0.034	0.015	0.037	0.034	0.015	0.037
Threshold[a]	0.178	0.147	0.235	0.178	0.147	0.235
(b) Namulonge 1995 Long rains						
Rutuku	0.611	0.157	0.143	0.578	0.160	0.183
382171.4	0.577	0.141	0.176	0.461	0.133	0.150
Kisoro	0.610	0.160	0.174	0.569	0.139	0.159
Kabale	0.645	0.150	0.158	0.614	0.147	0.169
Victoria	0.586	0.149	0.169	0.611	0.152	0.169
381403.1	0.675	0.160	0.169	0.595	0.149	0.169
Sangema	0.600	0.166	0.183	0.577	0.132	0.128
LSD (0.05)	0.147	0.037	0.036	0.147	0.037	0.036
Threshold[a]	0.184	0.246	0.245	0.184	0.246	0.245
(c) Kalengyere 1995 Long rains						
Rutuku	0.076	0.074	0.071	0.047	0.053	0.061
382171.4	0.038	0.056	0.051	0.073	0.072	0.070
Kisoro	0.087	0.091	0.134	0.089	0.097	0.126
Kabale	0.094	0.094	0.137	0.089	0.093	0.115
Victoria	0.069	0.080	0.121	0.085	0.094	0.106
381403.1	0.090	0.093	0.123	0.089	0.099	0.109
Sangema	0.073	0.077	0.110	0.088	0.095	0.132
LSD (0.05)	0.053	0.041	0.016	0.053	0.041	0.016
Threshold[a]	0.103	0.105	0.144	0.103	0.105	0.144

[a] Cut-off values for healthy and infected samples.

The indexing of seed potatoes obtained from Kalengyere before planting indicated the absence or a probable rare occurrence of PLRV at Kalengyere. This virus was last detected at this site in 1990 (Anon., 1993). Therefore, the absence of PVY is not surprising because it has not been detected at Kalengyere since 1988 while PVS was last detected in 1989 (Anon., 1993). Therefore, the insect-transmitted potato viral infections that were detected in field grown potatoes at Namulonge must have been introduced by virus arthropod vectors during growth.

In unsprayed potato at Namulonge, infestation started declining between the fifth and sixth week of potato growth. By the ninth week, infestation was at a minimum in unsprayed plots but maximum in sprayed ones. The decline in aphid infestation from the fifth week in unsprayed potato at Namulonge could have been due to the setting-in of critical densities of aphids and aphid disease epizootics which caused the aphid population to crash (Broadbent, 1957; Devonshire, 1989). In sprayed potatoes, the sharp increase in aphid density and plant infestation in the sixth week could have been due to the failure of dimethoate to control *M. persicae* (Devonshire, 1989; Quaglia *et al.*, 1993). Alternatively, the effectiveness of this insecticide could have been reduced by environmental or plant growth factors unique to the area, as has been observed by other researchers (Broadbent, 1957; Devonshire, 1989; Atiri and Jimoh, 1990).

At Namulonge, dimethoate did not prevent the infection of potato with PLRV despite the initial low aphid pressure. This could have been due to high virus infection pressure in the area inspite of the low vector numbers. In addition, the failure of dimethoate to suppress *M. persicae* the principle vector of PLRV, could have contributed to the virus spread in both sprayed and unsprayed potatoes.

At Kalengyere, no aphid-borne virus was detected in both sprayed and unsprayed potatoes in spite of the high aphid pressure. This implied that there is a low virus infection pressure at Kalengyere regardless of the high vector numbers.

Virus screening of field grown potatoes at Namulonge indicated that all the varieties were susceptible to PLRV. However, Rutuku and Kabale seemed to be the most susceptible. This may have been due to their initial infection with PVX because when some potato cultivars are infected with PVX, they tend to be more susceptible to PLRV than those that are free of PVX (Jayasinghe *et al.*, 1989).

The study has shown that a high aphid pressure may not necessarily lead to virus spread, especially in areas of low virus infection pressure. According to Powell and Mondor (1973), a large number of aphids in a potato field without a source of virus infection cause little or no danger of virus spread compared with few aphids on or from virus infected plants. This could have been the case for Kalengyere. The absence of PLRV or any aphid-borne viruses, despite the high aphid abundance at this location calls for stringent measures to enforce the "flush out system" that will always ensure seed flow from high to low elevations but not the other way round. This will ensure the supply of virus-free seed potato from the highlands to potato farmers else-where.

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