

Research Article

Arinaitwe Abel Byarugaba*, Settumba B. Mukasa, Alex Barekye, Patrick R. Rubaihayo

Interactive effects of *Potato virus Y* and *Potato leafroll virus* infection on potato yields in Uganda

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Abstract: Potatoes are prone to attack by multiple viruses, which contribute greatly to yield and quality decline depending on the cultivar and the virus involved. This study investigated the effect of co-infection involving *Potato virus Y* (*potyvirus*) and *Potato leafroll Virus* (*pelero virus*) on productivity of five potato cultivars in Uganda and the nature of virus interaction during co-infection process. Variety response to virus infection by PVY, PLRV and co-infection (PVY + PLRV) varied across different varieties. The plants that were infected with PLRV had leaf rolling, stuntedness, leaf distortion, reduction in leaf size and mottling and light yellow mosaics, and in some cases, purple or red margins were observed, while single infection of PVY induced necrosis, leaf rugosity, crinkling, stunting, interveinal necrosis, blotching of the margins, leaf distortion and mottling. When the two viruses were combined during co-infection with PVY + PLRV, the symptoms were characterized by bright blotching and necrotic leaf margins with purpling of the leaf tips and leaf margins, stuntedness and leaf distortions. The virus disease severity was higher under mixed infected plants than single infected plants. The high disease severity culminated in a significant effect on yield, marketable tuber number per plant, plant growth height and plant vigor, which were different across the varieties. Co-infection involving PVY and PLRV caused a reduction in the marketable yield of 95.2% (Kinigi), 94% (Victoria), 89.5 (Rwagume), 45.3% (Royal) and 23.7% (Sifra). Single infection by PLRV caused a reduction in a marketable yield in Victoria (91.8%),

Kinigi (84.8%), Rwagume (73.3%), Royal (47.2%) and Sifra 22.1%, while PVY caused a marketable yield reduction in Victoria (87.2%), Rwagume (85.9.7%), Kinigi (85.1%), Royal (37.4%) and Sifra (14.1%). The effects associated with the co-infection of PVY and PLRV were lower than the combined value of the single infections, suggesting that the two viruses were interacting to affect the potato productivity. The high yield loss suggested that effective resistance strategy targeting PVY, PLRV and their combination was required to save the potato industry in Uganda.

Keywords: potato virus interaction effects, single virus infection, co-infection

1 Introduction

The occurrence of more than one virus species in a single plant is very common in many potato fields causing significant yield losses (Zhang et al. 2001). There are more than 40 viruses that infect potatoes in the fields (Jeffries 1998), and based on the distribution, pathogenic variability and yield loss, PVY and PLRV are the most important viruses (Palukaitis 2012). Survey studies conducted in potato fields in the neighboring countries of Kenya and Tanzania have reported that potato plants are co-infected with two or more viruses (Valkonen 2015; Okeyo 2017). Most of the potato varieties grown by farmers in Uganda are very susceptible to viruses (KAZARDI 2016) and are likely to be infected with several viruses. Plants with extreme resistance (ER) show no symptoms as with susceptible tolerant varieties or very limited necrosis in the form of pinpoint lesions in some varieties (Valkonen et al. 1996).

During the co-infection process, interactions may occur between viruses within the host cell and such viral interactions may be antagonistic, synergistic or neutral (Moreno and López-Moya 2019). In most cases, mixed viral infection may lead to greater disease severity than individual viral components, and this sometimes has been referred to as a synergistic disease (García-Cano et al. 2006). Conversely, antagonism usually occurs when the co-infecting viruses are related, resulting in interference, whereas in neutral

* **Corresponding author: Arinaitwe Abel Byarugaba**, Makerere University; College of Agricultural and Environmental Sciences (CAES), P.O Box 7062, Kampala, Uganda; Kachwekano Zonal Agricultural Research and Development Institute (KAZARDI), P.O Box, 421, Kabale, Uganda; Mbarara University of Science and Technology; Faculty of Interdisciplinary Studies (FIS), P.O Box 1410, Mbarara, Uganda, e-mail: abelarinaitwe@gmail.com

Settumba B. Mukasa, Patrick R. Rubaihayo: Makerere University; College of Agricultural and Environmental Sciences (CAES), P.O Box 7062, Kampala, Uganda

Alex Barekye: Kachwekano Zonal Agricultural Research and Development Institute (KAZARDI), P.O Box, 421, Kabale, Uganda

interactions also regarded as additive interaction, each virus acts independently from the other, so that the different disease phenotypes elicited in single infections are displayed in plants with mixed infections (Syller 2012). The interactive effects between specific viruses especially PVY and PLRV when in mixed infections have not been demonstrated before in Uganda varieties; however, PVY and PLRV effects have been cited to be variety dependent with high economic losses in potato varieties that lack resistance (Syller 2014). Therefore, this study was designed to examine the interactive effects between PVY and PLRV on potato productivity of three commonly grown susceptible potato varieties in Uganda and two varieties from Europe that show some level of field resistance to the viruses to ascertain a quantitative record of the effects of each of virus and their combined effects on Uganda common varieties.

2 Materials and methods

2.1 Plant materials

Five potato varieties comprising three widely grown susceptible varieties, Rwagume, Victoria Kinigi and two European varieties, Sifra and Royal, were used for this study. Healthy tubers were planted in trays containing sterilized loam soil in a greenhouse at Kachwekano Zonal Agricultural Research and Development Institute (KAZARDI). Watering was carried out throughout the growing period at 2 days interval using piped fresh water.

2.2 Virus inoculation

The isolates of PVY and PLRV collected from the virus-infected plant material of variety Rwagume and maintained in the laboratory at KAZARDI in single virus-infected tubers were used in the study. The leaves of Rwagume plants that had PVY or PLRV were first checked for the presence of virus by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) according to the procedure suggested by Clark and Adam (1977a) using antibodies against PVY or PLRV before using the plants as a source of inoculum in the study. Healthy potato plants grown from one-eye tuber were infected at 35 days after planting. PLRV infection was done using aphids that were first fed on the single PLRV-infected plants to acquire the virus for 2 days and later transferred to infect the healthy plants of the test varieties. PVY infection was done by

mechanical inoculation with the inoculum of PVY. Virus inoculum for PVY was prepared by grinding systemically infected leaves of Rwagume using sterilized mortars and pestles in 50 mM potassium phosphate buffer at pH 7.0 at a ratio of 1 g of tissue to 10 mL of buffer (10% w/v). Each plant was dusted with 600-mesh carborundum, and two leaflets were rubbed with a piece of sponge dipped in the viral inoculum. For PVY + PLRV combination, plants were mechanically inoculated with PVY, and then, viruliferous aphids with PLRV were transferred to infect the mechanically inoculated PVY plants. Noninoculated potato plants for the five varieties were included as healthy controls, and only distilled water was applied to these plants.

2.3 Virus symptoms development, disease severity and yield

Evaluation of effects of single and mixed infections of PVY and PLRV on plant growth characteristics and yield were recorded for symptoms, plant vigor, virus titer and total fresh weight of tubers per plant. Symptoms induced and their severity were assessed visually during successive crop growth stage inspections at 10, 20, 30 and 40 days after virus inoculation using a scale of 1–5 according to the procedure of Islam et al. (2015), where 1 = no symptoms, 2 = mild symptoms, 3 = intermediate symptoms, 4 = severe symptoms and 5 = very severe symptoms. Area under disease progress curve (AUDPC) was computed using the formula of Campbell and Madden (1990).

$$\text{AUDPC} = \sum_{i=1}^n [(X_i + X_{i-1} - 1)/2] [t_i - t_{i-1} - 1],$$

where X_i = present disease severity, X_{i-1} = previous disease severity and $t_i - t_{i-1} - 1$ = time difference between two consecutive disease severities.

A mean value for symptom severity ratings for each point of data collection was used to test for synergism, antagonism or neutral (additive) using Abbott's equation (Abbott 1925) following the procedure of Levy et al. (1986):

$$S_{\text{obs}} = (W + X + Y),$$

where W = mean disease rating for symptom phase 1; X = mean disease rating for symptom phase 2; Y = mean disease rating for symptom phase 3; and

$$S_{\text{obs}} = (W + X + Y + Z),$$

where Z = whole-plant disease phenotype evaluation for the disease caused by each viral treatment was determined at the end of the growing season.

In this case, the rating for Z was -1 , symptom development showed a remission in severity; 0 , symptom severity remained similar or constant throughout the experiment; or $+1$, symptoms continued to progress in severity with a continuation in plant decline.

At maturity, the plants were harvested, and tubers were counted and graded into four categories (large marketable size, >60 mm; medium, 45 – 60 mm; small, 30 – 45 mm; very small, <30 mm) and weighed. Yield loss was assessed by computing the % differences in weight, tuber number between the healthy plants and virus-infected plants according to Rahman *et al.* (2010) and % loss in yield = $H - D/H \times 100 = L(100) =$ percent loss at 100% incidence, where H and D are average weight of tubers per plant of healthy and virus-infected plants and $L =$ loss.

Percent reduction in growth characteristics, based on the growth and tuber weight per plant of healthy plants in each replication, was calculated for each plant. Percent reduction data were subjected to analysis of variance (ANOVA), and mean separation was done using Fisher's least significant difference with $P = 0.05$. Means were used to calculate the expected response (C_{exp}) from the co-infection of two viruses using equation developed by Gisi (1996):

$$C_{\text{exp}} = A + B - (AB/100),$$

where C_{exp} is the expected level of disease, A and B are proportional responses due to infection by virus A and B , respectively, as measured and AB is the response of the double virus infection.

C_{exp} was compared with the observed response (C_{obs}). The means of different growth traits (height and vigor) and yield components were used to quantitatively determine the degree of the observed reduction. This study adopted the cut offs by Gisi *et al.* (1985), where a response can be considered as follows: (1) synergistic, if the interaction of the ratio of the observed response (C_{obs}) to the expected response (C_{exp}) is greater than 1.5, (2) additive, between 0.5 and 1.5, and (3) antagonistic, less than 0.5.

2.4 Effects of interaction on relative virus titers in different potato varieties

The relative amount of viral titers in leaves was determined by the DAS-ELISA assay as described by Clarks and Adams (1977b). A sample, consisting of two inoculated leaves, was collected from each plant at 10, 20, 30 and 40 days after inoculation (DAI) and tested by DAS-ELISA. Samples were considered positive for the presence of PVY or PLRV when the absorbance value at 405 nm

was greater than twice the mean of comparable healthy control plants recorded 2 h after adding the substrate and was measured using a labdetect microplate reader running Capture 96, Inc software (Kohl and Ascoli 2017). Means of the viral titer were computed using Genstat and tested using nonparametric Kendall's tau rank correlation analysis at $P < 0.05$ to determine the correlation among severity scores and viral titer during the growth of the plant.

3 Results

3.1 Interactive effects on symptom development and virus disease severity

The results of the interactive effects of the viruses indicated that symptom development and severity were dependent on the virus type and the variety. Varieties differed in symptoms expressed by PVY and PLRV symptoms under single and co-infection (Figure 1).

In Victoria, PLRV induced light yellow chlorotic margins, and plant stem was observed to be smaller in size than the control. In Kinigi, PLRV induced mottling and leaf surface distortion as the leaves become more rugose. In Rwagume, the leaf tips and margin were purplish, and most of the leaves become rolled up coupled with stuntedness of the entire plant. Sifra remained clear of the PLRV typical symptom, while Royal showed reduced leaf size although remained green.

PVY symptoms also varied among the varieties. In Victoria, PVY induced mottling, leaf rugosity and crickling. In Kinigi and Rwagume varieties, PVY caused leaf distortion, blight yellow mottling's, stuntedness, necrosis and reduced leaf size, while in Sifra and Royal, the leaves remained green with the reduced plant size.

Co-infection with PVY + PLRV induced symptoms that were characterized by bright blotching and necrotic leaf margins with purpling of the leaf tips and leaf margins, stuntedness and leaf distortions. The degree of symptoms depended on the variety. Victoria showed prominence of leaf rolling with blotching of the margin and interveinal chlorosis.

Kinigi showed stuntedness with distinct necrotic blotches on the margins and leaf rolling while Rwagume showed severe blight blotching with leaf rolling and rugosity. Sifra remained green, while Royal showed the reduced plant size with leaves showing mild distortions.

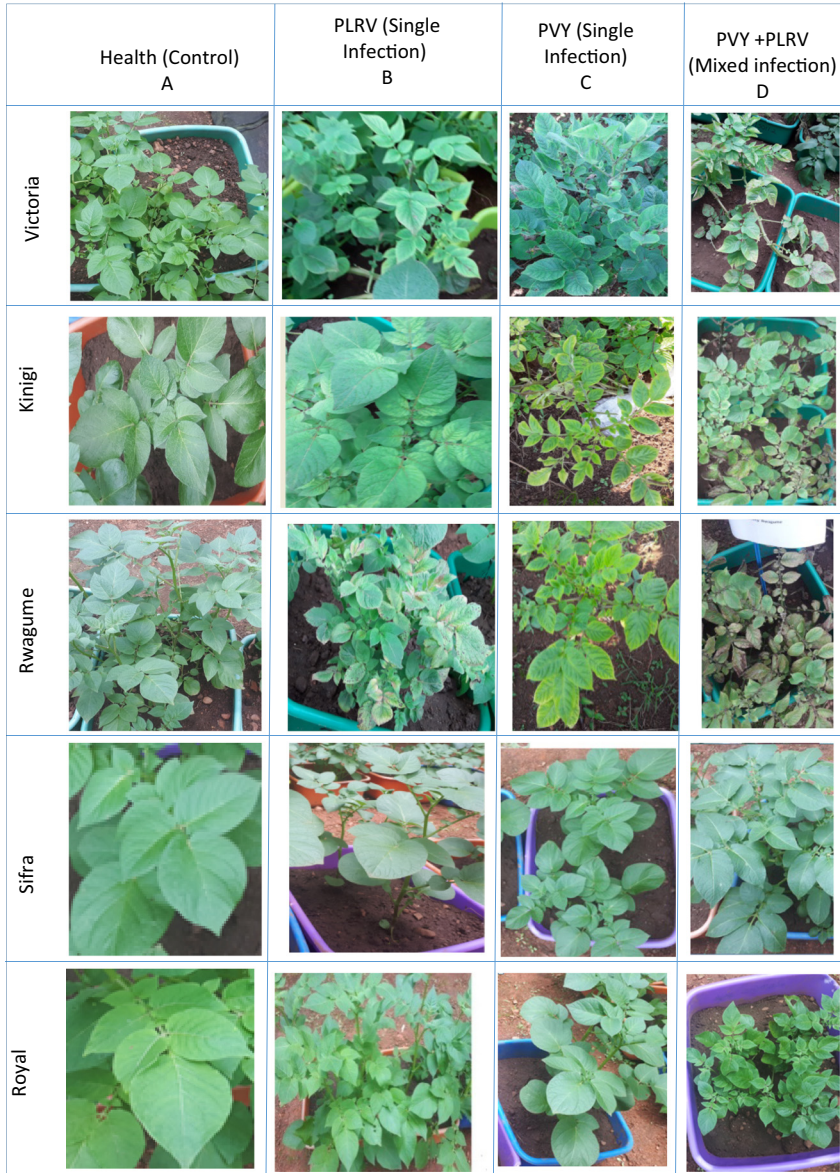
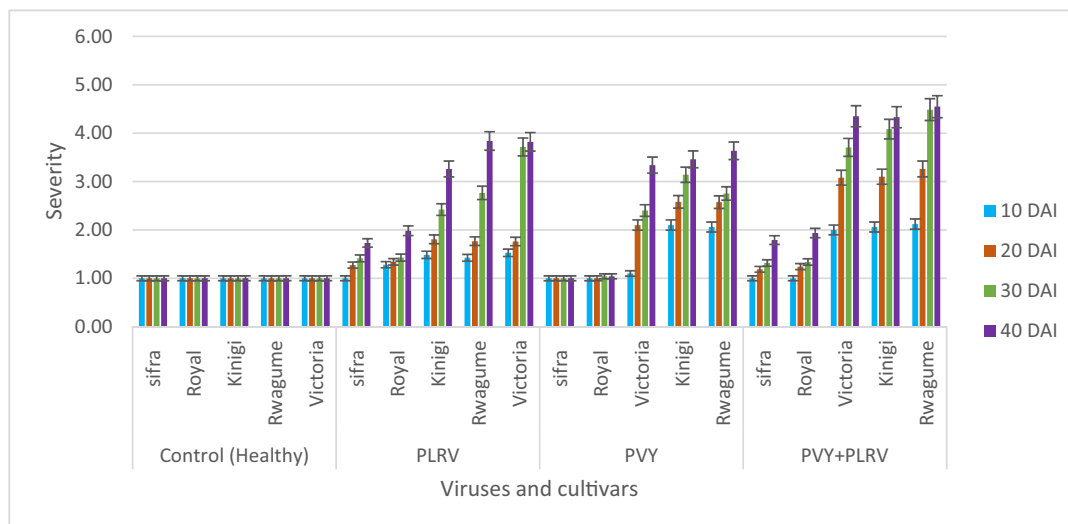


Figure 1: Symptom development caused by PVY and PLRV single infection and mixed infection.

Across all the varieties, Rwagume showed severe symptoms under single and co-infection with PVY and PLRV. Unlike Victoria, Rwagume and Kinigi varieties, the response of Sifra and Royal plants to co-infections by PVY and PLRV and single infection did not differ greatly from each other. It is important to note that Sifra and Royal contained the $R_{y_{adg}}$ for resistance to PVY, while Victoria, Rwagume and Kinigi did not have the R genes for resistance to either PVY or PLRV. The difference between the healthy control plants and the inoculated plants was obvious as control plants remained healthy and symptomless, while the inoculated plants developed symptoms.

The results of virus disease severity rating measured during the growing period from 10 days after inoculation (DAI) to 40 DAI are presented in Figure 2.

The results showed that co-infections of PVY + PLRV were associated with higher disease severity values compared to single infection. The severity rating under PLRV for the five varieties ranged from 1.73 to 3.84, with Rwagume recording the highest (3.84), followed by Victoria (3.82), Kinigi (3.26), Royal (1.98) and Sifra (1.73). The severity rating for PVY across the five varieties ranged from 1 to 3.64, with highest score observed in Rwagume (3.64), followed by Kinigi (3.46), Victoria (3.34), Royal (1.04) and Sifra (1.00). The severity rating under co-infection of PVY + PLRV



DAI= days after inoculation

Figure 2: Virus disease severity among the five varieties infected with PVY, PLRV and PVY + PLRV. DAI = days after inoculation.

ranged from 1.79 to 4.55, with highest recorded at 40 DAI in Rwagume (4.55), followed by Victoria (4.35), Kinigi (4.33), Royal (1.93) and Sifra (1.79). Across all the varieties, the most affected was Rwagume, followed by Victoria, Kinigi, Royal and Sifra.

The relative amount of the virus disease observed based on the relative area under the disease progress curve (rAUDPC) revealed significant ($P < 0.05$) effects in the level of symptom development for the viruses on different varieties. The results of the ANOVA for rAUDPC values are presented in Table 1.

The results showed that potato variety, virus type and interaction between variety and virus type were statistically significant ($P < 0.05$). The results of the rAUDPC values due to PLRV, PVY and PLRV infection in five potato varieties are presented in Table 2.

The results showed a significant ($P < 0.05$) difference in the rAUDPC values across the five varieties. The rAUDPC due to PLRV on five varieties ranged from 1.3 to 2.55 with Rwagume having 2.55, followed by Victoria (2.25), Kinigi (2.10), Royal (1.44) and Sifra (1.30).

The rAUDPC due to PVY infection ranged from 1 to 2.71 with Rwagume registering the highest disease area of 2.71 followed by Kinigi (2.63), Victoria (2.09), Royal (1.02) and Sifra (1.00). The rAUDPC due to the combination of PVY + PLRV ranged from 1.3 to 3.47 with highest observed for Rwagume (3.47), followed by Kinigi (3.26), Victoria (3.13), Royal 1.30 and Sifra (1.26).

Across the five varieties, the most affected with highest amount of virus disease area was Rwagume with the following rAUDPC values: PLRV (2.55), PVY (2.71) and

PVY + PLRV (3.47) followed by Kinigi with PLRV (2.1), PVY (2.09) and PVY + PLRV (3.26); Victoria with PLRV (2.25), PVY (2.63) and PVY + PLRV (3.13); Royal with PLRV (1.44), PVY (1.02) and PVY + PLRV (1.3) and finally Sifra with PLRV (1.3), PVY (1) and PVY + PLRV (1.26).

The results showed that co-infection of PVY + PLRV was associated with higher disease severity that culminated significant ($P < 0.05$) effects on plant growth and disease development based on rAUDPC values.

3.2 Effect of the single and mixed infections on growth and yield components

The results of single and mixed infections involving PVY and PLRV showed a significant ($P < 0.05$) effect on yield, tuber number, tuber size, plant height and plant vigor (Table 3).

Kinigi registered high yield loss with both single and mixed infections. The healthy control gave an average marketable yield of 22.35 T/ha, marketable tuber number of 5.5/plant overall total yield of 24.65 T/ha, maximum height of 60.3 cm and had vigor rating of 3.85. The PLRV-infected plants gave an average marketable yield of 3.45 T/ha, marketable tuber number of 1.5/plant, overall total yield of 5 T/ha, maximum height of 30.9 cm and had vigor rating of 2.7. When compared with healthy control plants, it translated into 84.8% loss in the marketable yield, 72.73% loss in marketable tuber number per plant, 79.8% loss of the total yield, 48.8% height reduction and 30.3% loss in plant vigor.

Table 1: ANOVA table for rAUDPC values for five varieties and types of virus infection

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	4	29.87344	7.468359	1113.46	<0.001
Type of virus infection	3	28.20486	9.401618	1401.69	<0.001
Variety X type of virus infection	12	14.48379	1.206983	179.95	<0.001
Residual	80	0.536589	0.006707		
Total	99	73.09867			

Table 2: Relative area under disease progress curve (rAUDPC) as a measure of disease severity for PVY, PLRV and combination on five potato varieties

Variety	Control (healthy)	rAUDPC			F.pr	L.s.d
		Single infection		Co-infection		
		PLRV	PVY	PVY + PLRV		
Kinigi	1.00	2.10	2.09	3.26	<0.001	0.144
Royal	1.00	1.44	1.02	1.30	<0.001	0.151
Rwagume	1.00	2.55	2.71	3.47	<0.001	0.058
Sifra	1.00	1.30	1.00	1.26	<0.001	0.054
Victoria	1.00	2.25	2.63	3.13	<0.001	0.102
F.pr		<0.001	<0.001	<0.001		
L.s.D		0.152	0.128	0.085		

Average rAUDPC values were obtained by dividing the AUDPC values with the values for the control (healthy plants).

PVY-infected plants gave average marketable yield of 3.25 T/ha, marketable tuber number of 1.67/plant, overall total yield of 5.35 T/ha, maximum height of 35.1 cm and had vigor rating of 2.75. When compared with healthy control plants, it translated into marketable yield loss of 85.1%, 69.7% loss in the marketable tuber number per plant, 77.9% loss of total yield, 41.8% height reduction and 30.3% reduction in plant vigor. Plants that were infected with virus combination of PVY + PLRV gave average marketable yield of 1.05 T/ha, marketable tuber number of 0.33/plant, overall total yield of 3.5 T/ha, maximum height of 22.1 cm and had vigor rating of 2.15 that translated into marketable yield loss of 95.2%, loss in tuber number per plant of 93.94%, total yield loss of 85.8%, height reduction of 63.3% and loss in plant vigor of 43.2%.

Royal was moderately affected by PVY, PLRV and PVY + PLRV. The healthy control plants gave an average marketable yield of 33.9 T/ha, marketable tuber number of 8/plant, overall total yield of 36.85 T/ha, maximum height of 49.3 cm and had vigor rating of 4. The PLRV-infected plants gave an average marketable yield of 17.9 T/ha, marketable tuber number of 5.83/plant, overall total yield of 24 T/ha, maximum height of 43.8 cm and had vigor rating of 3.85. When compared with control plants, it translated into marketable yield loss of 47.2%, loss of

marketable total tuber number per plant by 27.08%, total yield loss of 34.5, reduction in plant height of 11.2% and reduced plant vigor by 3.7%. The PVY-infected plants gave average marketable yield of 21.15 T/ha, marketable tuber number of 5.165/plant, overall total yield of 24.15 T/ha, maximum height of 45.7 cm and had vigor rating of 3.5, and when compared with healthy control plants, it translated into marketable yield loss of 37.4%, loss of marketable tuber number per plant by 35.42%, loss of total yield by 34.3%, height reduction of 7.4% and reduced plant vigor by 3.9%. Plants that were infected with the combination of PVY + PLRV gave average marketable yield of 18.55 T/ha, marketable tuber number of 4.665/plant, overall total yield of 24 T/ha, maximum height of 43.5 cm and had vigor rating of 3.3, which translated into marketable yield loss of 45.3%, marketable tuber number per plant of 41.67%, total yield loss of 34.8%, height reduction of 11.8% and reduced plant vigor by 17.5%.

Rwagume was severely affected by PVY, PLRV and PVY + PLRV. The healthy control plants gave an average marketable yield of 20.45 T/ha, marketable tuber number of 5/plant, overall total yield of 23.35 T/ha, maximum height of 56.5 cm and had vigor rating of 4.15. The PLRV-infected plants of Rwagume gave an average

Table 3: Effect of PVY, PLRV and the co-infection on potato growth and yield components

Parameter	Season	Virus	Kinigi	Royal	Rwagume	Sifra	Victoria	F.pr	LSD
Marketable yield (T/ha)	2018A	PLRV	2.7	18	5.4	21.6	0.7	<0.001	6.098
		PVY	3.3	20.1	2.3	19.8	2.3	0.003	10.16
		PVY + PLRV	0.9	19	1.9	15.9	1.9	<0.001	6.460
		Healthy control	19.2	34.9	19.8	26.1	20.5	0.007	8.14
	2019B	PLRV	4.2	17.8	5.5	17.5	2.8	<0.001	4.566
		PVY	3.2	22.2	3.5	22.9	3	<0.001	7.32
		PVY + PLRV	1.2	18.1	2.4	21.9	0.6	<0.001	5.630
		Healthy control	25.5	32.9	21.1	23.8	21.5	0.348	13.40
% Loss on marketable yield (observed)		PLRV	84.82	47.17	73.29	22.08	91.83	<0.001	12.96
		PVY	85.08	37.45	85.91	14.05	87.23	<0.001	21.34
		PVY + PLRV	95.23	45.28	89.48	23.65	94	<0.001	15.82
Expected loss on marketable yield due to both PVY + PLRV based on the model of Gisi (1996)			168.95	84.17	158.3	35.89	178.12	<0.001	28.65
Ratio of observed marketable yield loss due to PVY + PLRV/expected marketable yield loss			0.56	0.54	0.57	0.66	0.53		
Marketable tuber number/ plant	2018A	PLRV	1	5.33	1.67	6.33	0.33	<0.001	1.819
		PVY	1.67	5.33	1.33	5.33	0.67	0.004	2.739
		PVY + PLRV	0.33	5	1	4.67	1	<0.001	1.409
		Healthy control	5.67	8	5.67	7.33	6	0.795	4.228
	2019B	PLRV	2	6.33	2	5.33	1.33	0.002	1.819
		PVY	1.67	5	1.33	6.67	1.33	0.005	2.857
		PVY + PLRV	0.33	4.33	1.33	5	0.33	<0.001	1.243
		Healthy control	5.33	8	4.33	7.32	5.67	0.709	5.829
% Loss in marketable tuber number per plant (observed)		PLRV	72.73	27.08	63.33	20.45	85.71	<0.001	24.01
		PVY	69.7	35.42	73.33	18.18	78.8	<0.001	27.68
		PVY + PLRV	93.94	41.67	76.67	34.09	88.57	<0.001	23.74
Expected loss on marketable tuber number due to both PVY + PLRV based on the model of Gisi (1996)			141.49	62.08	135.89	38.29	163.62	<0.001	36.08
Ratio of observed marketable tuber number loss due to PVY + PLRV/expected marketable tuber number loss			0.66	0.67	0.56	0.89	0.54		
Total yield (T/ha)	2018A	PLRV	4.4	22.6	6.8	25.2	3.9	<0.001	5.733
		PVY	5.3	23.8	4.2	21.2	4.8	0.002	10.16
		PVY + PLRV	3.1	24	2.9	18.2	3.1	<0.001	4.073
		Healthy control	21.6	38.3	22.2	26.7	21.9	0.005	8.22
	2019B	PLRV	5.6	25.4	7.3	21	5.5	<0.001	4.903
		PVY	5.4	24.5	6.2	24.3	5.2	<0.001	6.992
		PVY + PLRV	3.9	24	2.9	26	2	<0.001	4.310
		Healthy control	27.7	35.4	24.5	24.8	22.6	0.330	13.80
Observed total yield loss (%)		PLRV	79.8	34.5	69.9	10.6	79	<0.001	12.73
		PVY	77.9	34.3	77.8	11.4	77.6	<0.001	20.35
		PVY + PLRV	85.8	34.8	87.5	13.6	88.4	<0.001	13.79
Expected loss on total yield due to both PVY + PLRV based on the model of Gisi (1996)			156.84	68.45	146.83	21.86	155.72	<0.001	28.1
Ratio of observed total yield loss due to PVY + PLRV/expected total yield loss			0.55	0.51	0.6	0.62	0.57		
Plant height (cm) at 60 DAP	2018A	PLRV	31.9	43	30.7	40.2	32	0.002	5.794
		PVY	36.1	44.7	32.5	43	29	<0.001	5.048
		PVY + PLRV	24.1	42.3	24.9	40.1	22.7	<0.001	4.612
		Healthy control	59.3	48.3	55.5	47.5	51.4	0.021	7.22
	2019B	PLRV	29.9	44.6	28.7	41.3	30	<0.001	5.838
		PVY	34.1	46.7	30.5	43.7	27	<0.001	5.134
		PVY + PLRV	20.1	44.7	21.2	40.6	19	<0.001	4.609
		Healthy control	61.3	50.3	57.5	48.7	53.4	0.009	
% Reduction in height due to PVY and PLRV (observed)		PLRV	48.8	11.21	47.44	12.19	40.85	<0.001	6.95
		PVY	41.83	7.43	44.14	6.83	46.51	<0.001	6.36
		PVY + PLRV	63.3	11.83	59.16	12.89	60.16	<0.001	6.43

Table 3: Continued

Parameter	Season	Virus	Kinigi	Royal	Rwagume	Sifra	Victoria	F.pr	LSD
Expected height reduction due to both PVY + PLRV based on the model of Gisi (1996)			89.99	18.52	90.99	18.89	86.75	<0.001	7.13
Ratio of observed height reduction due to PVY + PLRV/expected height loss			0.71	0.67	0.65	0.69	0.69		
Plant vigor score (1–5)	2018A	PLRV	2.7	3.7	3	2.7	3	0.351	1.151
		PVY	2.7	3.7	2.7	3	3	0.351	1.151
		PVY + PLRV	2.3	3.3	3.3	3	2.3	0.080	0.939
		Healthy control	3.7	3.7	4.3	4	3.7	0.452	0.939
	2019B	PLRV	2.7	4	3.3	3	2.7	0.022	0.814
		PVY	2.8	4	2.7	3	3.3	0.022	0.814
		PVY + PLRV	2	3.3	3	3	2	<0.001	0.4697
		Healthy control	4	4.3	4	4.3	4	0.871	1.050
Observed plant vigor reduction (%) due to PVY and PLRV		PLRV	30.34	3.86	23.69	-11.07	25.79	<0.001	18.52
		PVY	30.34	3.86	35.87	4.59	21.63	<0.001	16.01
		PVY + PLRV	43.21	16.1	24.01	9.13	43.21	<0.001	14.15
Expected vigor reduction (%) due to both PVY + PLRV based on the model of Gisi (1996)			60.24	7.55	59.32	-6.57	46.99	<0.001	28.33
Ratio of observed height reduction due to PVY + PLRV/expected height reduction			0.87	1.22	0.46	1.01	0.95		

marketable yield of 5.45 T/ha, marketable tuber number of 1.835/plant, overall total yield of 7.05 T/ha, maximum height of 29.7 cm and had vigor rating of 3.15 and when compared with healthy control translated into a marketable yield loss of 73.3%, loss of marketable total tuber number per plant by 63.33%, total yield loss of 69.9%, reduction in plant height of 47.4% and reduced plant vigor by 23.7%. The PVY-infected plants gave average marketable yield of 2.9 T/ha, marketable tuber number of 1.33/plant, overall total yield of 5.2 T/ha, maximum height of 31.5 cm and had vigor rating of 2.7, which translated into marketable yield loss of 85.9%, loss of marketable tuber number per plant of 73.3%, loss of total yield by 77.8%, height reduction of 44.1% and reduced plant vigor by 35.9%. Plants that were infected with the combination of PVY + PLRV gave the average marketable yield of 2.15 T/ha, marketable tuber number of 1.165/plant, overall total yield of 2.9 T/ha, maximum height of 23.05 cm and had vigor rating of 3.15, which translated into marketable yield loss of 89.5%, loss of marketable tuber number per plant of 76.67%, total yield loss of 87.5%, height reduction of 59.2% and reduced plant vigor by 24%.

Sifra was less affected compared to other tested varieties. The healthy control plants gave an average marketable yield of 24.95 T/ha, marketable tuber number of 7.3/plant, overall total yield of 25.75 T/ha, maximum height of 48.1 cm and had vigor rating of 4.15, while the PLRV-infected plants

gave an average marketable yield of 19.55 T/ha, marketable tuber number of 5.83/plant, overall total yield of 23.1 T/ha, maximum height of 49.75 cm and had vigor rating of 2.85, and when compared with the control plants, it translated into marketable yield loss of 22.1%, loss of marketable total tuber number per plant by 20.45%, total yield loss of 10.6%, reduction in plant height of 12.2% and reduced plant vigor by 26.3%. PVY-infected plants gave average marketable yield of 21.35 T/ha, marketable tuber number of 6/plant, overall total yield of 22.75 T/ha, maximum height of 43.35 cm and had vigor rating of 3, which translated into marketable yield loss of 14.1%, loss of marketable tuber number per plant (18.18%), reduced total yield by 11.4%, reduced plant height by 6.8% and reduced plant vigor by 21.6%. Plants that were infected with the combination of PVY + PLRV gave average marketable yield of 18.9 T/ha, marketable tuber number of 4.835/plant, overall total yield of 22.1 T/ha, maximum height of 40.35 cm and had vigor rating of 3, which translated into marketable yield loss of 23.7%, loss of marketable tuber number per plant of 34.09%, total yield loss by 13.6%, height reduction of 16.3% and reduced plant vigor by 27.1%.

Victoria was significantly affected by both single infection of PVY and PLRV and their combination. The healthy control plants of Victoria gave an average marketable yield of 21 T/ha, marketable tuber number of 5.83/plant, overall total yield of 22.25 T/ha, maximum height of 52.4 cm and had vigor rating of 3.85, while the PLRV-infected plants gave an average marketable yield of

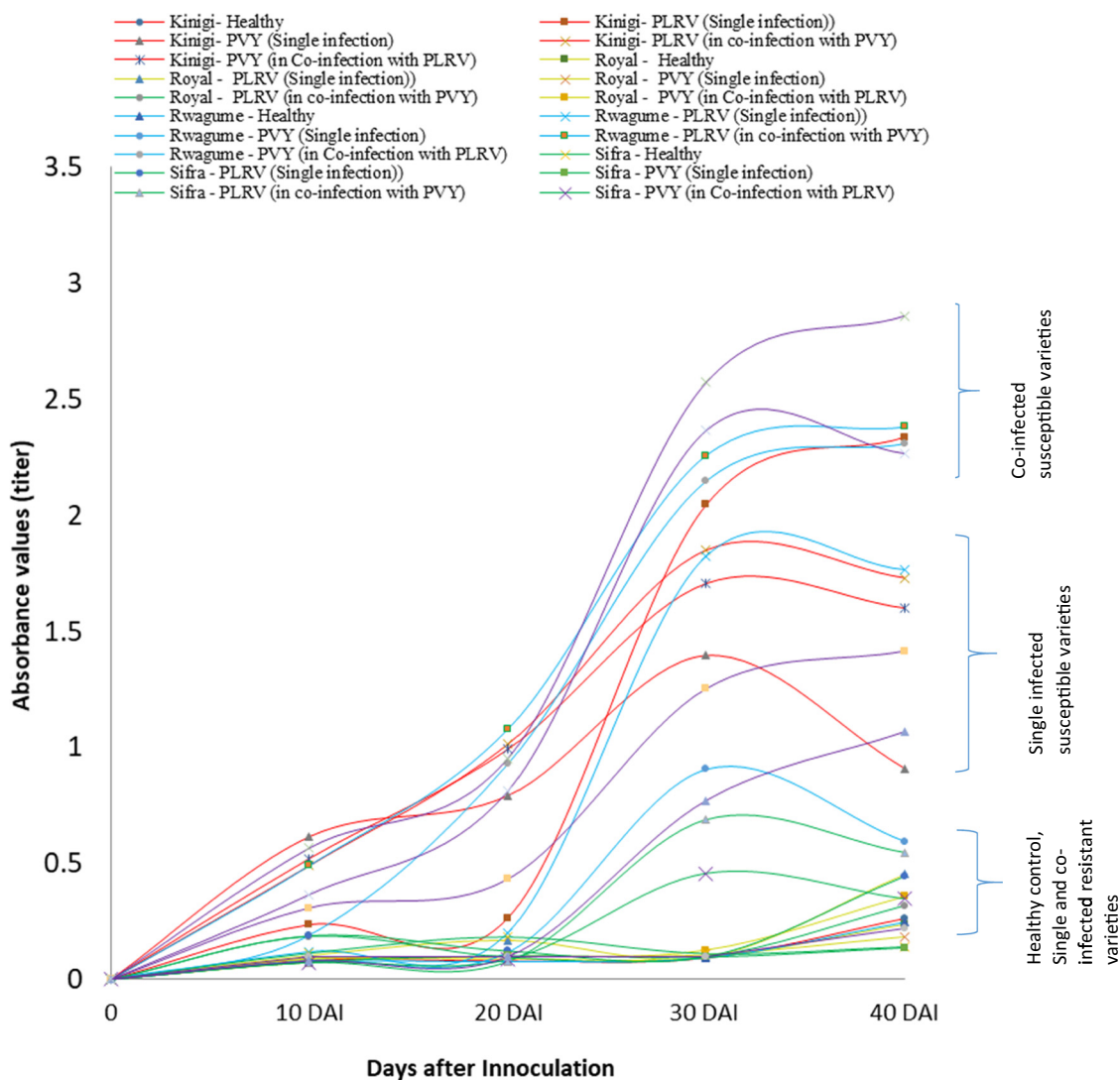


Figure 3: Relationship between viral titers for PVY, PLRV in single and mixed infection at different days after infection (DAI) among five potato varieties. DAI = days after inoculation.

1.75 T/ha, marketable tuber number of 0.83/plant, overall total yield of 4.7 T/ha, maximum height of 31 cm and had vigor rating of 2.85, and when compared with healthy control, plants translated into marketable yield loss of 91.8%, loss of marketable total tuber number per plant by 85.71%, total yield loss of 79%, reduction in plant height of 40.8% and reduced plant vigor by 25.8%. PVY-infected plants gave average marketable yield of 2.65 T/ha, marketable tuber number of 1/plant, overall total yield of 5 T/ha, maximum height of 28 cm and had vigor rating of 3.15, which translated into marketable yield loss of 87.2%, loss of marketable tuber number per plant of 78.8%, loss of total yield by 77.6%, height reduction of 46.5% and reduced plant vigor by 24.1%. Plants that were infected with the virus combination of

PVY + PLRV gave average marketable yield of 1.25 T/ha, marketable tuber number of 0.665/plant, overall total yield of 2.55 T/ha, maximum height of 20.85 cm and had vigor rating of 2.15 that translated into marketable yield loss of 94.0%, loss of marketable tuber number per plant of 88.57%, total yield loss of 88.4%, height reduction of 60.2% and reduced plant vigor by 43.2%.

The disease severity rating, yield loss and reduction in plant height and vigor as a result of mixed infection when subjected to the Abbotts equation of synergy: $C_{exp} = A + B - (AB/100)$, where C_{exp} was the expected level of disease effects, A and B are proportional responses due to infection by virus A and B , respectively, and AB is the response of the combination of viral infection. Based on threshold values of Gisi (1996), the results recorded

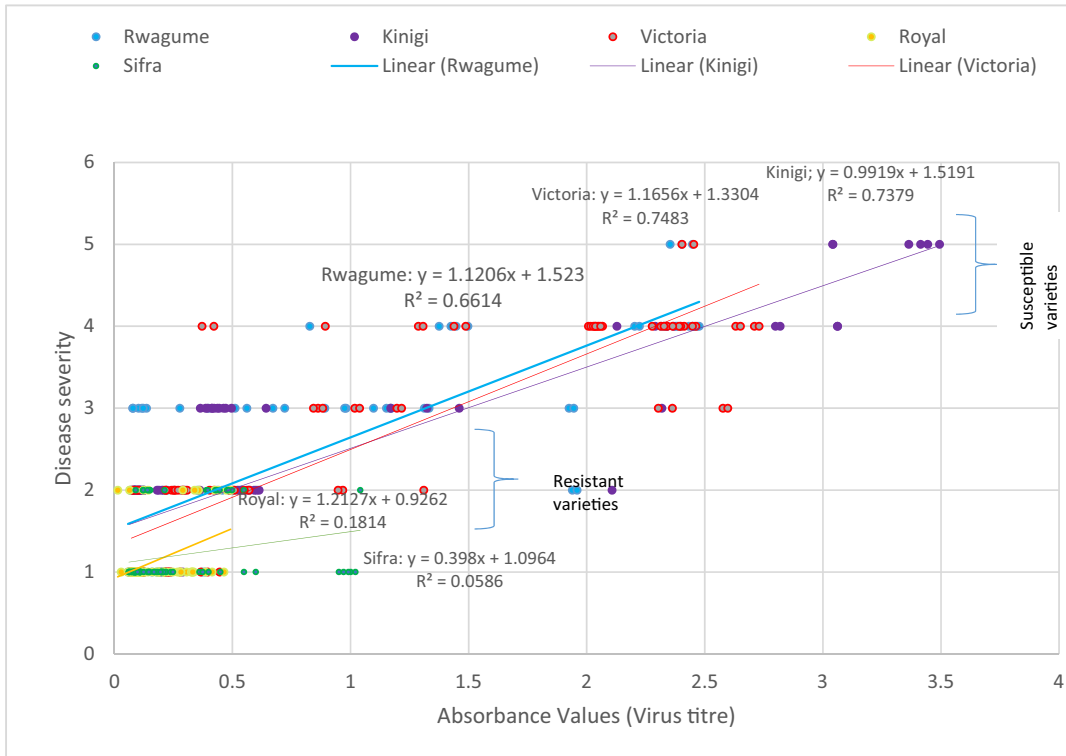


Figure 4: Relationship between the viral titer levels and disease severity for three susceptible and two potato-resistant varieties.

indicated that the ratio of the observed and the expected effects due to co-infection on the growth and yield component were in the range of additive threshold values of 0.5–1.5.

3.3 Virus titer during single and co-infection

The virus titers of PLRV and PVY were examined for single and co-infections in the five different potato varieties at 30 DAI and results showed significantly ($P < 0.005$) more viral titers of PLRV and PVY accumulating in co-infected than in singly infected plants (Figure 3).

The viral titer levels of the individual single infection increased exponentially and reached to maximum at 34 days after infection (DAI) in susceptible Kinigi, Rwagume and Victoria varieties and stabilized and then declined slightly. Among varieties, the viral titer build up was faster in Victoria, followed by Rwagume and Kinigi. The establishment of PLRV was faster compared to PVY among the susceptible varieties. The results also showed that despite the absence of *R* genes for resistance to PLRV in Sifra and Royal, the titers for PLRV in Sifra and Royal were lower than those of susceptible varieties.

While the virus-infected plants among the varieties were significantly different from the healthy control, the

viral titers for PLRV and PVY under co-infected plants was greater than single infection of PLRV and PVY in susceptible varieties as there was higher PLRV and PVY accumulation in susceptible varieties of Victoria, Rwagume and Kinigi compared to resistant varieties, Sifra and Royal. The viral titers and disease severity rating were correlated, and the results are presented in Figure 4.

The results showed that higher titer values were positively correlated ($P < 0.005$) with high disease severity. Susceptible varieties (Rwagume, Kinigi and Victoria) had higher viral titers that translated into higher disease severity, while the resistant varieties (Sifra and Royal) had low viral titers.

4 Discussion

PVY and PLRV have been reported to be expanding their geographical distribution and economic losses in contrast to most of other viruses infecting potato crops (Kreuze et al. 2020) with mixed viral infection involving PVY and PLRV becoming frequent (Kerlan and Moury 2008) as most potato varieties lack resistance to PVY and PLRV (Ahmadvand et al. 2012). The results of this study showed that varieties significantly differed in symptoms expressed by PVY and PLRV symptoms under single and co-infection,

which indicated the occurrence of variety-dependent virus interactions between PVY and PLRV during single and co-infection. The plants that were infected with PLRV alone had leaf rolling, stuntedness, leaf distortion, reduction in leaf size, mottling and light yellow mosaics, and in some cases, purple or red margins were observed, while single infection of PVY induced necrosis, leaf rugosity, crinkling, stunting, interveinal necrosis, blotching of the margins, leaf distortion and mottling. The symptoms observed for PLRV matched with those observed by Abbas *et al.* (2016), which indicated that primary infections of PLRV caused chlorosis of young leaves with an erect plant habit, while secondary symptoms consisted of the stunting of the shoots and the upward rolling of older leaves, which turned chlorotic, leathery and brittle while some varieties showed reddening or purpling of the top leaves.

The symptoms associated with PVY were similar to those observed by Chikh-Ali *et al.* (2013) and Szajko *et al.* (2014). When the two viruses were combined during co-infection with PVY + PLRV, the symptoms were characterized by bright blotching and necrotic leaf margins with purpling of the leaf tips and leaf margins, stuntedness and leaf distortions in the susceptible varieties, while the resistant varieties, Sifra, remained green and Royal showed reduced plant size with leaves showing mild distortions. The symptoms observed under co-infection had not been described earlier.

The results showed that symptom severity was dependent on the type of variety, which was similar to the study by Davidson *et al.* (2013), which indicated that PVY symptoms differed depending on the potato cultivar, but this study also found that severity was higher in plants with mixed infection than in plants with single infection, suggesting some level of interaction between the two viruses to influence the symptom development. The results showed variation in the symptom development of potato varieties to viral infection for the susceptible varieties; for example, plants of Victoria showed prominence of leaf rolling with blotching of the margin and interveinal chlorosis, Kinigi showed stuntedness with distinct necrotic blotches on the margins and leaf rolling, Rwagume showed severe blight blotching with leaf rolling and rugosity, while Sifra remained green and Royal showed reduced plant size with leaves showing mild distortions, which indicated variety differential responses to virus infection. Similar results were reported by Le Romancer and Nedellec (1997) and Draper *et al.* (2000), which indicated that a given variety can exhibit differential response to different viruses. The response of Sifra and Royal plants to co-infections by PVY and PLRV and single infection did not differ significantly from each other, indicating that the two varieties have the same level of resistance.

There was an increase in the symptom severity for PVY + PLRV co-infected plants relative to plants infected with each virus alone from 20 to 40 DAI, which demonstrated that mixed infections involving PVY and PLRV showed higher disease severity, which culminated in a significant effect on yield, marketable tuber number per plant, plant growth height and plant vigor, which were different across the varieties. The higher disease severity and effects on yield parameters due to mixed infections were a result of interaction between the two viruses. The significant effects on growth and yield due to PVY and PLRV suggested that these two viruses were a potential threat on potato production in Uganda.

The results also showed that the susceptible varieties produced very little commercial size tubers as indicated by the marketable tubers per plants with the most of the tubers being categorized as small (nonmarketable) compared to the healthy plants, suggesting that the viral infection led to tuber degeneration characterized by production of small-sized tubers, which is in line with the study by Crissman (1989), which indicated that the production of many small noncommercial value size tubers was a common feature in virus-infected potato.

The results for yield loss associated with PVY, PLRV and their combination on potato are the first one to be documented in Uganda, and the study established that yield losses due to PVY and PLRV were higher than the figures reported by Mariano (1989) in Philippine, which indicated that the yield loss of PLRV alone was ranging from 33.43% to 46.58% depending on the variety and those observed by Lung'aho *et al.* (2007) in Kenya (60.6–80.1%) for varieties Tigoni, Roslin Tana, Kenya Sifa and Dutch Robjyn. The reason for the high losses observed in this study was attributed to the variety differences as the varieties used were very susceptible except Royal and Sifra.

The varieties (Victoria, Rwagume and Kinigi) had higher viral titers than Sifra and Royal when inoculated with PVY, PLRV and PVY + PLRV, and the presence of PVY did not affect the titer of PLRV in the co-infection, suggesting that the PLRV was not able to reduce the titers of PVY and vice versa. However, during co-infection, the titers of both PLRV and PVY were 0.5- to 1.5-fold higher than the ones observed in single infection in susceptible varieties and increased as the crop grew with maximum peak titers registered between 30 and 35 days after inoculation as a result of direct effects of host–virus interactions due to the absence of the *R* genes and could have also been attributed to the stable co-existence between PVY and PLRV. The fact that PLRV is Phloem-limited virus (Taliensky *et al.* 2003) while PVY is not, could have promoted niche specialization that increased the

severity of the two viruses permitting co-existence in these susceptible varieties, while the low level of viral titers for PVY and PLRV observed in the two resistant varieties (Sifra and Royal) under single and co-infection were attributed to the direct effects of interactions between the viruses and the *R* genes contained in these two varieties, implying that the two viruses could have interacted by stimulating a common host defense response that regulated both of them to maintain the relatively low titer levels.

The high virus titer levels in susceptible cultivars correlated well with the high disease severity that translated in higher yield losses, suggesting that viral titer influences the crop yield performance. Van (2017) reported that high viral titer in plants substantially affected the plant yield and disease severity, and Killick's (1979) report observed that the yield loss was strongly related to viral load when infected with PLRV.

The results also indicated that there was a high yield loss due to single and co-infection of PVY and PLRV with an increase of titer levels that correlated positively with the increase in disease severity for the susceptible varieties, but the ratios of the observed virus effects to the expected when both viruses were co-infecting were in the range of 0.5–1.5, suggesting additive interaction. Gisi et al. (1985) reported that when the ratio of the observed effects to the expected effects is in the range of 0.5–1.5, additive interaction was indicated. The results showed that mixed virus symptoms in co-infected plants suggested additive model of interaction, which was similar with the report of Syller (2012), which highlighted that additive interaction as a form of neutral interactions where each virus became transmitted independently from the other (s), so that the different disease symptoms elicited in single infections were also displayed in plants with mixed infections. This study is first to report additive interaction for PVY and PLRV in potato, but additive interaction have been reported in co-infection involving *Cowpea aphid-borne mosaic virus* (CABMV), *Cowpea mottle virus* (CMeV), *Southern bean mosaic virus* (SBMV) (Nsa and Kareem 2015), *Tobacco mosaic virus*, *Cucumber mosaic virus* (Garces-Orejuela and Pound 1957), *Lucerne mosaic virus* and *Potato aucuba mosaic virus* (Ross 1959).

This results showed differences in titer level between single and co-infection with high titer levels and severity noted within the susceptible varieties (Victoria, Kinigi and Rwagume) and low titers with the resistant varieties (Sifra and Royal), which was contrary to Srinivasan and Alvarez (2007) who reported that co-infections of PVY and PLRV had showed no difference in viral titer between doubly and singly infected plants, suggesting that viral

titers for PVY and PLRV during co-infection in this study were dependent on virus and resistance level of the infected variety as reported by Lana and Adegbola (1977). The results also showed a slight decline in viral titer levels at 40 DAI, which was attributed to the fact that the plants had aged and were beginning to senesce, which lead to a slight a drop in titer levels. This behavior of decline in titer of viruses was a characteristic of old plant tissues as described by Peterschmitt et al. (1992) and was supported by the report of Gibson (1991) that indicated that potato plants were more susceptible to viruses when young and become less susceptible as they mature.

The results also showed that PVY and PLRV infections caused significant effects on potato productivity, which has provided a better understanding of the effects of the viruses: PVY and PLRV on potato varieties in single and mixed infections, and indicated that these two viruses acted independently during co-infection and could stably co-exist inflicting additive effects on growth and productivity of potato. The high yield losses observed due to PVY, PLRV and co-infection of PVY + PLRV suggested the need to use virus-free seed potato or varieties with resistance to the viruses.

5 Conclusion

The results demonstrated the contribution of individual virus and co-infection effects on the yield of five selected varieties and revealed that PVY and PLRV contributed greatly to the yield loss in Uganda and that the interaction was host dependent with additive interactive effects on growth and yield parameters. The results revealed that yield loss, plant vigor, disease severity, and titer values associated with the mixed infection were lower than the combined value from the single infections.

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