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Effect of Viral Infection on the Nutrient Contents of two Forage Legumes

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Abstract

The use of *Stylosanthes guianensis* (I.164) and *Peuraria phaseoloides* as feed for ruminants in the tropics have been emphasised however information on the effect of viruses on their nutritive contents are scarce. In an investigation on the effects of viruses on the nutrient composition of these legumes, three viruses namely Bean common mosaic *(Potyvirus)* {BCMV}, Cucumber mosaic virus *(Cucumovirus)* {CMV}and Cowpea mottle virus {CPMoV}were singly inoculated on to these plants through the two youngest leaves. Leaves of inoculated plants were collected, dried and analysed for various mineral contents. The three viruses significantly increased ($p = 0.05$) the level of zinc, potassium and phosphorus in *P. phaseoloides*. However inoculation of sole CMV decreased the content of manganese in *P. phaseoloides* as compared with the uninoculated controls. Single viral inoculation of CMV and CPMV on to *S. guianensis* (I.164) showed significant increase in the level of zinc with 96.42 and 98.98 ppm as compared with 80.98 ppm recorded for healthy controls.

Keywords: Bean common mosaic; Cowpea mottle virus; Cucumber mosaic cucumovirus; Forages; Potyvirus,

Introduction

Incorporation of herbaceous legumes into animal feed brings about an increase in calf growth, milk production, viability and reduces cow live weight loss (Otchere, 1986). While some legumes such as soya bean (*Glycine max)*, lablab (*Lablab purpureus)*, velvet bean (*Mucuna spp*) and leucaena (*Leucaena leucocephala*) improve the average daily weight gain of dual-purpose Kenyan goats (Njarui *et al*., 2003). Red clover used for silage and white clover used in grazed swards in Europe led to enhanced growth rate and milk yield in comparison with pure grasses (Dewhurst *et al*., 2009). The basis of these improvement lies in the capacity of the legumes to fix atmospheric nitrogen and the improved animal productivity stems from higher quality feed containing the right proportions of protein, digestible energy and other mineral content (Dzowela, 1985; Musikira, 1988; Little and Agyemang, 1994). Mineral salts are very important in the diets of ruminants for optimum performance and production. Zinc (Zn), copper (Cu), iron (Fe), manganese (Mn) are among numerous mineral salts necessary for the performance of ruminants (Spears and Weiss, 2014).

The general effects of viral infection on the nutritional components of crops include a decrease in protein content (Goodman *et al.,* 1986). Just as Oben (1998) observed a decrease in the protein content of the African cassava mosaic virus-infected cassava tubers in contrast to uninfected tubers. However, sugar content and starch level of uninfected cassava tubers showed an inverse trend. Nutritional quality of virus-infected legumes is lessened (Jones, 1996); a predisposing factor which negatively affects the weight gain and milk yield of cattle in the tropics.

The yield of alfalfa (*Medicago sativa)* had been shown to decrease by Alfalfa mosaic virus in Britain. The virus reduced fodder yield by 14.8 to 22.8 % and by 15.0 to 18.1 % on a fresh and on a dry weight basis respectively (Baillis and Ollennu, 1986). It also impeded the growth of alfalfa as well as reduced reshooting rate after cutting (Miczynski and Hiruki, 1987). *Stylosanthes guianensis* (I.164) and *Peuraria phaseoloides* have been shown to be palatable and nutritive legumes for ruminants in the tropics (Ajayi *et al*., 2007; Monteiro *et al*., 2009; Guemour *et al*., 2010). However there is a dearth of information on the effects of viruses on their growth and nutritive content hence the purpose of this research.

Materials and Methods

Seeds of forage legumes namely *Lablab purpureus* (LP), *Stylosanthes guianensis* (I.164), *Peuraria phaseoloides* (PP) and *Centrosema pubescens* (Accession I.152) (CP) were collected from the Forage Legume Multiplication Unit of the International Livestock Research Institute (ILRI), Ibadan Centre. These seeds were planted on the field after treatment with 10% trisodium phosphate to destroy particles of the Tobacco Mosaic Virus (TMV) which might have adhered to the seed coats. Three (3) replicates of these accessions were planted in a field of 2.25m by 25m. Ten (10) seeds of each accession were planted in a row with each row duplicated. Rows were 1m apart and seeds were planted at 25cm intervals. Scoring for viral incidence and severity was done at 12 weeks after planting (WAP) using a 1 - 5 severity scoring scale in which 1 represents no observable symptom, 2 represents very mild mosaic or mottle symptoms, 3 represents moderate mosaic or mottle symptoms, 4 represents severe mosaic or mottle symptoms and 5 represents stunting or death of virus-infected plant.

Collection of leaf samples for serological analysis.

At 17 WAP, 188 leaf samples of 4 varieties of the planted herbaceous legumes comprising 49 *Centrosema pubescens*, 40 *Stylosanthes guianensis*, 48 *Peuraria phaseoloides* and 51 *Lablab purpureus* expressing viral symptoms were collected and serologically indexed for 4 spherical viruses, 2 potyviruses and a tobamovirus. The spherical viruses were cucumber mosaic virus (CMV), cowpea mosaic virus (CPMV), cowpea mottle virus (CPMoV) and southern bean mosaic virus (SBMV). The potyviruses were cowpea aphid-borne mosaic virus (CABMV), black eyed cowpea mosaic virus (BECMV) and a tobacco mosaic virus [tobamovirus] (TMV).

Serological analysis

Protein-A sandwich enzyme linked immunosorbent assay (PAS ELISA) described by Hughes (1986) and antigen coated plate (ACP) ELISA described by Hughes and Tarawali (1999) were used to detect the Tobamovirus and Potyviruses respectively. The antibodies used were polyclonal antibodies raised at the Virology Unit of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria.

Planting of healthy seeds for virus inoculations.

Seeds harvested from *S. guianensis* and *P. phaseoloides* on the field were separately sundried in paper envelopes under screen house conditions with temperature between 27 – 30°C. Seeds of each legume were planted as 2 seeds per pot and each legume was planted in three replicates with 5 potted plants in a replicate for each virus treatment. Seedlings were kept in a screen house belonging to the Virology Unit, IITA and watered daily throughout the period of the experiment.

Mechanical inoculations

Seedlings of each legume specie in a replicate were mechanically inoculated at three weeks after planting (WAP) with the respective viruses obtained from the Virology Unit of IITA. Infected leaves of cowpeas (*V. unguiculata* cv. IT82E-10, TVu 76 and Ife Brown) containing BCMV (black eyed strain) CMV and CPMoV respectively were separately ground as 0.1g/ml with inoculation buffer (0.1M phosphate buffer containing 0.01M ethylene diaminetetraacetic acid (EDTA) and 0.001M cysteine, pH 7.7). Carborundum (600 mesh) was dusted onto the leaves as an abrasive. After mechanical inoculations, plants were rinsed with tap water to wash off excess inoculums. Plants were observed regularly over a period of 4 weeks for symptom expression.

Analysis for mineral contents

At four weeks after inoculations (WAI), leaves were collected from the specific 5 virus-inoculated potted plants in a replicate and bulked. Each legume in a replicate was treated separately. The bulked leaves were oven-dried at 55°C overnight, ground in sterilised mortars before they were transferred to the Analytical Unit of IITA for protein and mineral content determination. The methods used were according to the methods of Hunter *et al*. (1984) and Novozamsky *et al*. (1983).

Statistical analysis

Results obtained for micronutrient analysis were statistically analysed using Generalized Linear Model (GLM) procedure (SAS, 2001). The analysis of variance (ANOVA) was calculated separately for each virus treatment and the controls.

Results

The rate of germination of the test legumes on the field ranged between 70.0% for *S. guianensis* (I.164) and 88.3% for *L. purpureus*. The mean of viral symptom severity was 1.0 for *S. guianensis*, 1.2 for *C. pubescens*, 1.4 for *L. purpureus* and 1.5 for *P. phaseoloides* (Table 1). When the leaves of the legumes planted on the field were serologically indexed, 20.7% of the total leaf samples of the four herbaceous legumes tested were positive. BCMV had the highest incidence with 11.7% of the tested samples being positive for the virus. This was followed by CMV with an incidence rate of 7.4% and CABMV which had an incidence rate of 6.9%. The virus with the lowest incidence was CPMoV which occurred in 1.1% of the tested samples. Other viruses tested and found positive included SBMV, CPMV and TMV. Their respective incidence rates were 4.8, 3.2 and 2.7% (Figure 1).

BCMV was detected only in *C. pubescens* and *L. purpureus*. CABMV, CMV and SBMV followed a similar trend. CPMoV was detected in *S. guianensis* and

Table 1: Mean symptom severity of virus incidence of four herbaceous legumes planted on the field

Herbaceous legumes	N	Percentage germination (%)	Mean of severity
Stylosanthes guianensis	60	70.0	1.0
Centrosema pubescens	60	86.7	1.2
Peuraria phaseoloides	60	80.0	1.5
Lablab purpureus	60	88.3	1.4

Key: N = number of samples

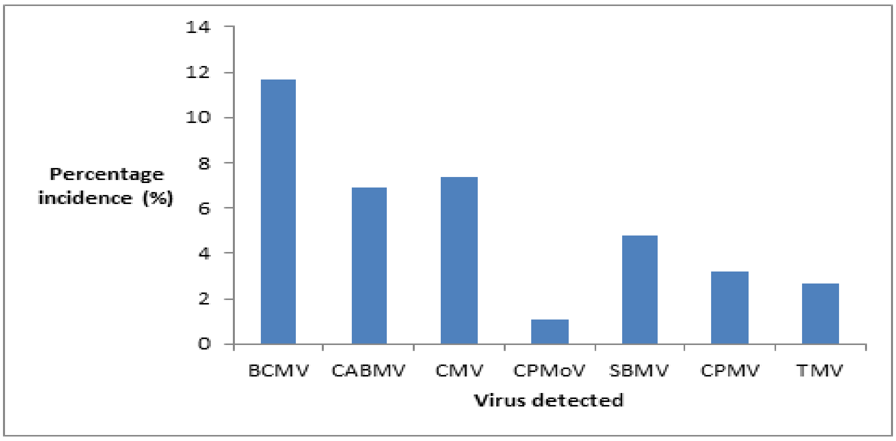

Figure 1: Incidence of viruses detected in leaf samples of four herbaceous legumes planted on the field

Table 2: Percentage of viral incidence on the planted herbaceous legumes

Legume species	No. pltd.	No. indexed	Percent. pos.	Percentage viral incidence					
				BCMV	CABMV	CMV	CPMoV	SBMV	CPMV
S.g	60	40	5.0	0.0	0.0	0.0	2.5	0.0	2.5
C.p	60	49	57.1	42.9	22.4	18.4	0.0	8.2	8.2
L.p	60	51	13.7	2.0	3.9	9.8	2.0	9.8	0.0
P.p	60	48	4.2	0.0	0.0	0.0	0.0	0.0	2.1

Key: S.g-*Stylosanthes guianensis*; C.p-*Centrosema pubescens*; L.p-*Lablab purpureus*; P.p-*Peuraria phaseoloides*. No. pltd.-number of seeds planted; Percent. Pos.-percentage positive. BCMV-*Bean common mosaic virus*; CABMV-*Cowpea aphid-borne mosaic virus*; CMV-*Cucumber mosaic virus*; CPMoV-*Cowpea mottle virus*; CPMV-*Cowpea mosaic virus*; SBMV-*Southern bean mosaic virus*; TMV-*Tobacco mosaic virus*.

L. purpureus. CPMV was detected in *S. guianensis*, *C. pubescens* and *P. purpureus* while TMV was detected in all the legumes with the exception of *S. guianensis* (Table 2).

Mixed viral infection was observed in some of the legumes tested. The legume in which there was highest occurrence of mixed infection was *L. purpureus* in which 42.9% of the leaf samples tested presented mixed viral infection of CMV and SBMV. Other types of mixed infection detected in *L. purpureus* was the occurrence of CABMV, CMV and SBMV (Table 3). Mixed infection was also observed in *C. pubescens* with the occurrence of BCMV and CABMV in 17.9% of the samples tested. There was no detection of mixed infection in *P. phaseoloides* and *S. guianensis*.

During the screen house experiment in which the selected legumes were mechanically inoculated, 100% germination was recorded for all the legumes but no symptom expression was observed for each of the 3 viruses inoculated even at 4 weeks after inoculation. No visible symptoms were observed after viral inoculations on *P. phaseoloides* and *S. guianensis* (I.164). However, some significant differences were noted in the calculated values of some of the nutrients tested for in these plants.

Values of nitrogen (N) content of virus-inoculated *P. phaseoloides* showed no significant difference when compared with those of uninoculated controls (Table 4). Furthermore, the Phosphorus (P) content of all the virus-inoculated plants of *P. phaseoloides* showed some significant increase when compared with that of the healthy control. The viruses inoculated on *P. phaseoloides* showed no significant reduction in the level of protein, calcium and magnesium contents of the infected plants. Inoculation of *P. phaseoloides* with BCMV, CMV and CPMoV resulted in a significant increase in the value composition of potassium when compared with those of the controls which had average values of 2.44 as compared with 3.12, 3.46 and 3.22 for BCMV, CMV and CPMoV-inoculated plants respectively. For other essential minerals such as sodium (Na), iron (Fe) and copper (Cu), there was no significant increase in the values of virus-inoculated *P. phaseoloides* as compared with the controls but CMV caused a significant reduction of Manganese (Mn) in the inoculated *P. phaseoloides* (Table 4). The content of zinc in *P. phaseoloides* was greatly increased when the plants were inoculated with each of the 3 viruses having average values of 93.57 ppm, 101.42 ppm and 99.42 ppm which were significantly different from the 69.31 ppm recorded for the healthy control plants (Table 4).

There were no significant differences in the values obtained for Nitrogen (N), Protein, Magnesium (Mg) and Potassium (K) when *S. guianensis* was singly inoculated with BCMV, CMV and CPMoV as the values of the uninoculated controls were not significantly different from the values of the inoculated plants (Table 5). Values obtained for level of zinc when *S. guianensis* was singly inoculated with CMV and CPMoV were significantly different ($P= 0.05$) from that of healthy control as there was significant increase in the value of zinc when the plant was singly inoculated with the viruses. Other minerals for which there was no significant difference included phosphorus, sodium, manganese, iron and copper.

Table 3: Mixed viral infections detected in leaves of forage legumes

Type of mixed infection	Legume in which detected:							
	Sg	% inc	Cp	% inc.	Lp	% inc	Pp	% inc.
BCMV +CABMV	-	0.0	+	7.9	-	0.0	-	0.0
BCMV+CMV	-	0.0	+	10.7	-	0.0	-	0.0
CMV+SBMV	-	0.0	-	0.0	+	42.9	-	0.0
BCMV+CMV+TMV	-	0.0	+	3.5	-	0.0	-	0.0
BCMV+CMV+SBMV	-	0.0	+	3.5	-	0.0	-	0.0
CABMV+BCMV+CPMoV	-	0.0	+	3.5	-	0.0	-	0.0
CABMV+CPMoV+CPMV	-	0.0	+	3.5	-	0.0	-	0.0
CABMV+CMV+SBMV	-	0.0	-	0.0	+	14.3	-	0.0
CMV+SBMV+TMV	-	0.0	+	7.1	-	0.0	-	0.0
BCMV+CABMV+CMV+SBMV	-	0.0	+	3.5	-	0.0	-	0.0
BCMV+CABMV+CMV+CPMoV	-	0.0	+	3.5	-	0.0	-	0.0
BCMVCABMVCMVSBMV	-	0.0	+	3.5	-	0.0	-	0.0
CABMV+CMV+CPMoV+SBMV+TMV	-	0.0	-	0.0	+	14.3	-	0.0

Key: Sg-*Stylosanthes guianensis*; Cp-*Centrosema pubescens*; Lp-*Lablab purpureus*; Pp-*Peuraria phaseoloides*; % inc.-Percentage incidence; BCMV-*Bean common mosaic virus*; CABMV-*Cowpea aphid-borne mosaic virus*; CMV-*Cucumber mosaic virus*; CPMoV-*Cowpea mottle virus*; CPMV-*Cowpea mosaic virus*; SBMV-*Southern bean mosaic virus*; TMV-*Tobacco mosaic virus*.

Table 4: Mean values for various minerals and protein contents in leaves of *Peuraria phaseoloides* after viral inoculations

	N	P	Protein	Ca	Mg	K	ppm Na	ppm Mn	ppm Fe	ppmCu
BCMV	3.79[a]	0.44[a]	23.69[a]	1.14[a]	0.26[a]	3.12[a]	1007.66[a]	97.23[ab]	79.54[a]	16.72[a]
CMV	4.05[a]	0.50[a]	25.31[a]	1.10[a]	0.28[a]	3.46[a]	1121.89[a]	88.21[b]	74.82[a]	19.78[a]
CPMoV	3.64[a]	0.45[a]	22.80[a]	1.06[a]	0.26[a]	3.22[a]	1032.75[a]	94.72[ab]	90.23[a]	21.70[a]
Control	3.37[a]	0.35[b]	21.06[a]	1.20[a]	0.26[a]	2.44[b*]	884.03[a]	105.82[a]	57.22[a]	13.60[a]
DMRT	NS		NS	NS	NS		NS		NS	NS

* Means with the same letter are not significantly different (? = 0.05)
Key: DMRT-Duncan multiple range test; NS-Not significant.

Table 5: Mean values for various minerals and protein contents in leaves of *Stylosanthes guianensis* after viral inoculations

	N	P	Protein	Ca	Mg	K	ppm Na	ppm Mn	ppm Fe	ppmCu
BCMV	4.14[a]	0.42[b]	25.92[a]	2.07[b]	0.27[a]	3.80[a]	4467.90[a]	87.99[a]	101.89[a]	15.14[a]
CMV	4.30[a]	0.48[a]	26.86[a]	2.29[a]	0.25[a]	3.41[a]	1831.39[a]	65.90[a]	30.08[a]	10.26[a]
CPMoV	4.10[a]	0.48[a]	25.62[a]	2.22[ab]	0.27[a]	3.80[a]	2166.32[a]	80.48[a]	57.36[a]	13.12[a]
Control	4.31[a]	0.44[ab]	26.95[a]	2.15[ab]	0.24[a]	3.51[a]	2047.28[a]	90.09[a]	68.70[a]	12.61[a]
DMRT	NS		NS		NS	NS	NS	NS	NS	NS

Note: Figures followed by same alphabets are not significantly different
Key: DMRT-Duncan multiple range test; NS-not significant.

Discussion

The detection of 7 viruses in these legumes during planting on the field showed their various levels of susceptibility to the tested viruses. While *S. guianensis* and *P. phaseoloides* had the lowest rates of viral infection, *C. pubescens* and *L. purpureus* had the highest rates of infection. Therefore, *C. pubescens* and *L. purpureus* could not be utilised for further investigations that this research work focused on. The selection of these legumes was due to their high nutritive value and importance as feed supplements for ruminant feeding in the tropics (Peters *et al.*, 1994). The 7 viruses have been earlier identified in related legumes such as cowpeas and desmodium (Shoyinka *et al.*, 1997; Hughes and Tarawali, 1999). BCMV is a potyvirus and had also been found to be of higher prevalence as shown in this study (Odedara *et al.*, 2007). The high prevalence of BCMV would have been attributed to the high incidence of the virus' vectors which include *Aphis gossypii* and *A. spiraecola* (Atiri *et al.*, 1984; French and Duncan, 2010). It could also have been due to the presence of many weeds which harboured the virus in adjacent fields since some of the common weeds that have been reported to harbour BCMV include *Macrotyloma axillare*, *Desmodium* sp. *Crotolaria spectabilis* (Lenne, 1998; Edeme and Hanson, 2000). Other viruses have also been shown to occur at varying levels of incidence in other plants.

The occurrence of mixed viral infection in *L. purpureus* and *C. pubescens* on the field has been earlier reported (Odedara *et al.*, 2007; 2008). This indicates that these legumes are susceptible to various viruses and their vectors. As vectors of potyviruses have been mentioned earlier, vectors of CMV are *Aphis craccivora* and *A. fabae* (Thottappilly and Rossel, 1992). For SBMV, the bean leaf beetle {*Ceratoma trifurcata* Forst (Fam: Chrysomelidae)}is an efficient vector (Kopek and Scott, 1983). In laboratory studies, SBMV was transmitted by *Ootheca mutabilis* which retained the virus for 13 days following a 24-hour acquisition feeding (Allen *et al.*, 1981). The asymptomatic expression of *S. guianensis* (I.164) and *P. phaseoloides* even after virus inoculations might have been due to the resistance and/or tolerance level of these plants to the inoculated viruses.

This could also have been due to the presence of some intrinsic chemicals which likely inhibited the symptomatic expression of these inoculated viruses in the legumes. However, there was an indication that *S. guianensis* showed higher resistance to the viruses than *P. phaseoloides* as values of mineral contents were affected by viruses in the latter than the former legume. This finding corroborates the detection of symptomless infection on alfalfa plants in a field experiment in Britain by Baillis and Ollenu (1986) where it was found that predominantly symptomless infection of alfalfa mosaic virus (AMV) was associated with yield decrease.

Some viruses have been reported to either increase or decrease levels of macro and micronutrients in forage legumes. Such examples include the decreased effect that Alfalfa Mosaic Virus (AMV) caused on the quantity of phosphorus, iron, copper, zinc and manganese in alfalfa plant (*Medicago sativa*) when inoculated with the virus in Turkey (Yardimci *et al*., 2007).

A decreased effect caused by viruses on legumes is of great concern as deficiencies of these minerals might lead to many malfunctions in the physiological system of the ruminants for example zinc deficit in ruminant forages have been shown to lead to reduced levels of zinc in milk in the United States of America (USA) and this had an unfavourable effect on the development of the young ruminants (Vrzgula *et al*., 1990). In addition, manganese deficiency reduces mucopolysaccharides in cartilage resulting in impaired skeletal development (Spears and Weiss, 2014). These mucopoly-saccharides are critical for the development of the bone matrix and play an important part in cartilage integrity.

In conclusion therefore, it is very important to reduce levels of viral infection in forages as infected forage legumes may not contain enough essential nutrients needed for maximum functioning of the body system of ruminants. This could be brought about through collaborative efforts between virologists and plant breeders to develop resistant varieties for maximum nutrition benefits for ruminants.

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