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Full Length Research Paper

Cowpea viruses: Quantitative and qualitative effects of single and mixed viral infections

M. A. Taiwo* and O. J. Akinjogunla

Department of Botany and Microbiology, University of Lagos, Akoka, Lagos, Nigeria.

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Multiple viral infections have been reported on cultivated commercial cowpeas (*Vigna unguiculata*) in Nigeria. In this study, the effect of inoculating two commercial cultivars (cvs) ("Oloyin" and "Olo II') and two lines from IITA (Ife Brown and TVu-76) with buffer, *Cowpea aphid-borne mosaic* (CABMV), *Cowpea mottle* (CMeV) and *Southern bean mosaic* (SBMV) viruses individually as well as in mixtures (CABMV+ CMeV, CABMV+ SBMV, CMeV+SBMV, and CABMV+ CMeV+SBMV) at 10 and 28 days after planting (DAP) on the growth, yield and nutritive content of seeds from infected plants were evaluated. The age of the plants at time of infection and the different viral treatments significantly affected the different parameters assessed. The average height of plants inoculated 10 DAP were significantly shorter than those of plants inoculated 28 DAP. Inoculating with single, double and triple viruses (10 DAP) resulted in 19-34%, 31-46% and 42-53% reductions in plant height, respectively. Viral infections also resulted in significant reductions in the number of pods and seeds produced. Plants inoculated with the three viruses 10 DAP produced the least number of pods and seed. Viral treatments resulted in the production of seeds with a lower protein content of 24.8-28.9% compared with the 28.5-30.4% protein in seeds from the control plants. Plants inoculated 10 DAP with the triple viruses produced the seeds with the least protein content (24.8-27.1%). The carbohydrate, fat and moisture contents of seeds from virus infected plants were however slightly higher than those of the control plants while the ash contents were lower. Generally, the commercial cowpea cvs were more severely affected by the viral treatments. These results indicate that infection at an early age and by multiple viruses can have devastating effects on the growth, yield and the nutritional quality of cowpea.

Key words: Cowpea aphid-borne mosaic virus, cowpea mottle virus, southern bean mosaic virus, cowpea virus-induced yield loss, cowpea seed protein content.

INTRODUCTION

Cowpea (*Vigna unguiculata* Walp-Holl) is an important food and fodder legume in the sub-humid tropics of Africa. It provides an extremely significant portion of the dietary protein of the people of sub-Saharan Africa (Rachie, 1985), where about 8,902,085 ha are under cowpea grain cultivation, and 2,907,091 tones were produced in 2001 (FAOSTAT 2003). Nigeria which produced about 70% of this is reputed as the world's largest producer of cowpea. However, cowpea yields are low in Nigeria, due to attack by pests and infection by pathogenic microorganisms, especially viruses. Viruses cause devastating effects and they are a major constraint to increased production (Thottappilly and Rossel, 1992).

Over 140 viruses infect cowpeas world-wide (Hughes and Shoyinka, 2003), but only nine have been reported in Nigeria (Taiwo, 2003). However, only *Cowpea aphid-borne mosaic virus* (CABMV) genus *Potyvirus* and *Cowpea yellow mosaic virus* (CYMV) genus Comovirus are considered important because of their geographical distribution and the economic losses attributable to them (Thottappilly and Rossel, 1992). More recently CABMV, *Southern bean mosaic virus* (SBMV) genus *Sobemovirus* and *Cowpea mottle virus* (CMeV) genus *Carmovirus* were reported to be fairly prevalent and of moderate incidence on cultivated cowpeas in Nigeria. Mixed infections by two viruses were prevalent, but multiple infect-

*Corresponding authors E-mail: monitaiwo@yahoo.com. Tel: +2348033127850. Fax: +23414445391.

ions caused by four or five viruses were also reported in individual samples of the commercially cultivated cowpeas (Shoyinka et al., 1997).

Mixed viral infections are not uncommon in nature, apart from cowpeas they have also been reported in other commercial vegetables such as pumpkin, watermelon, pepper, Irish potatoes, tomatoes and wheat (Bowen et al., 2003; Murphy and Bowen., 2006; Murphy et al., 2000; Sikora et al., 1998). Mixed viral infections usually result in a more severe disease symptom culminating in significant reductions in quantitative parameters such as plant height, weight and subsequently yield and at times causing plant death.

Losses attributable to individual virus infection are fairly well documented (Thottappilly and Rossel, 1992), but there is only limited information on the effects of mixed viral infections on cowpeas in Nigeria. Owolabi et al. (1988) reported a 78-100% reduction in the pod number of cowpeas (Ife Brown and Nigeria B7) inoculated with *Blackeye cowpea mosaic virus* (BlCMV) genus *Potyvirus* and CYMV at 9 days after planting while the complete loss of irrigated cowpeas in northern Nigeria has been attributed to dual infection of CABMV and *cowpea golden mosaic virus* genus *Begomovirus* (Rossel, 1977). Viruses have also been reported to affect plants qualitatively. Increased amount of protein has been reported in barley infected by *Wheat streak mosaic virus*(WSMV) genus *Tritimovirus* and *Barley stripe mosaic virus* genus *Bymovirus* (White and Blakke, 1982), and in spring wheat infected by WSMV (Langham and Glover, 2005).

This study was therefore designed to evaluate the effects of single and multiple viral infections with three cowpea viruses (CABMV, SBMV and CMeV) on the growth, yield and nutritive content of the seeds of two of Nigeria's commercial cowpea cultivars (cvs) ("Oloyin" and "Olo II") and two experimental lines from the International Institute for Tropical Agriculture (IITA) (Ife Brown and TVu 76).

MATERIALS AND METHODS

Sources of viruses and cowpea cultivars/lines

The three viruses used during this investigation: *Cowpea aphid-borne mosaic virus* (CABMV), *Cowpea mottle virus* (CMeV) and *Southern bean mosaic virus* (SBMV) were obtained from IITA as dried samples stored over $CaCl_2$. They were propagated and maintained individually on Ife Brown, by mechanical inoculation. Seeds of the commercial cowpea cvs ("Oloyin" and "Olo II") were obtained from and confirmed as released cultivars at the Federal Ministry of Agriculture, Moor plantation, Ibadan, while seeds of Ife Brown and TVu 76 were collected from IITA. The seeds were planted in labeled plastic pots and maintained in a greenhouse at the University of Lagos at a temperature of 28-32°C.

Virus treatments and inoculation procedures

Mechanical inoculations were performed on the seedlings at 10 and 28 days after planting (DAP) with the following inocula: CABMV, CMeV, SBMV, CABMV+CMeV CABMV+SBMV, CMeV+SBMV, CABMV+CMeV+SBMV and buffer. At least four plants were inoculated per treatment, inoculations were performed on the 6th and 24th of May 2004, during the rainy season. In a previous experiment, inoculations were performed in October and November 2002, during the dry season.

Viral inocula were prepared in 0.05 M K_2HPO_4 pH 7.5, (1 g of tissue to 10 ml of buffer) and the plants were dusted with carborundum before inoculation. For mixed viral infections, saps from the relevant infected plants were mixed at a ratio of 1:1 (v/v) just before being used as inocula. The pots were labeled before the plants were inoculated and they were then arranged in a randomized complete block design in the greenhouse located at the University of Lagos. The greenhouse was sprayed weekly with cypermetrin 10% E.C.

The effect of viral treatment on quantitative parameters

The effect of the different treatments on plant height was determined by measuring stem length in centimeters from soil level to the tip of the stem for all the four plants that received the same treatment at 10, 20 and 30 days after inoculation (DAI). The averages for the measurements taken were later calculated and recorded. The effect of the different treatments on the yield of cowpeas was also evaluated by harvesting, counting and recording the total number of pods produced by plants that received similar treatment. The seeds were later counted, sun-dried for seven days, weighed and stored in labeled envelopes until when needed.

The effect of viral treatment on qualitative parameters

The effect of the various viral treatments and the age of the plants at the time of inoculation on the protein, fat, moisture and ash contents of the harvested cowpea seeds were determined according to the methods described by the Association of Official Analytical Chemists (AOAC, 1975), in the Department of Chemistry, University of Lagos, Akoka, Lagos, Nigeria. The seeds harvested from plants that received the different treatments were individually ground into fine powder, which were poured into clean dry glass bottles that were tightly covered. The protein contents of the different samples were determined by the Macro-Kjeldahl method as described by the AOAC (1975). The fat content was determined by the Soxhlet method, using petroleum ether as extraction medium. The moisture content was estimated as the loss in weight of a specific portion of the ground cowpeas after it was placed in an oven at 105°C for 5 h. Ash was determined by dividing the weight of the residue from a 5 g sample of ground cowpea dried at 100°C for 2 h, 300°C for 1 h., and 550°C for 5 h in a furnace, by the weight of the original sample, multiplied by one hundred. The carbohydrate (CHO) content was estimated as suggested by the AOAC (1975), using this formula % CHO = 100-{(% moisture) + (% protein) + (% fat) + (% ash)}.

The data obtained were analyzed with the statistical package for social scientists and Duncan multiple range analysis was used to test for significance between the various virus treatments.

RESULTS

Quantitative effects of viral treatment

The age of plant at the time of infection and the various viral treatments had significantly different effects on the heights of the cowpea plants (Figure 1). Generally the

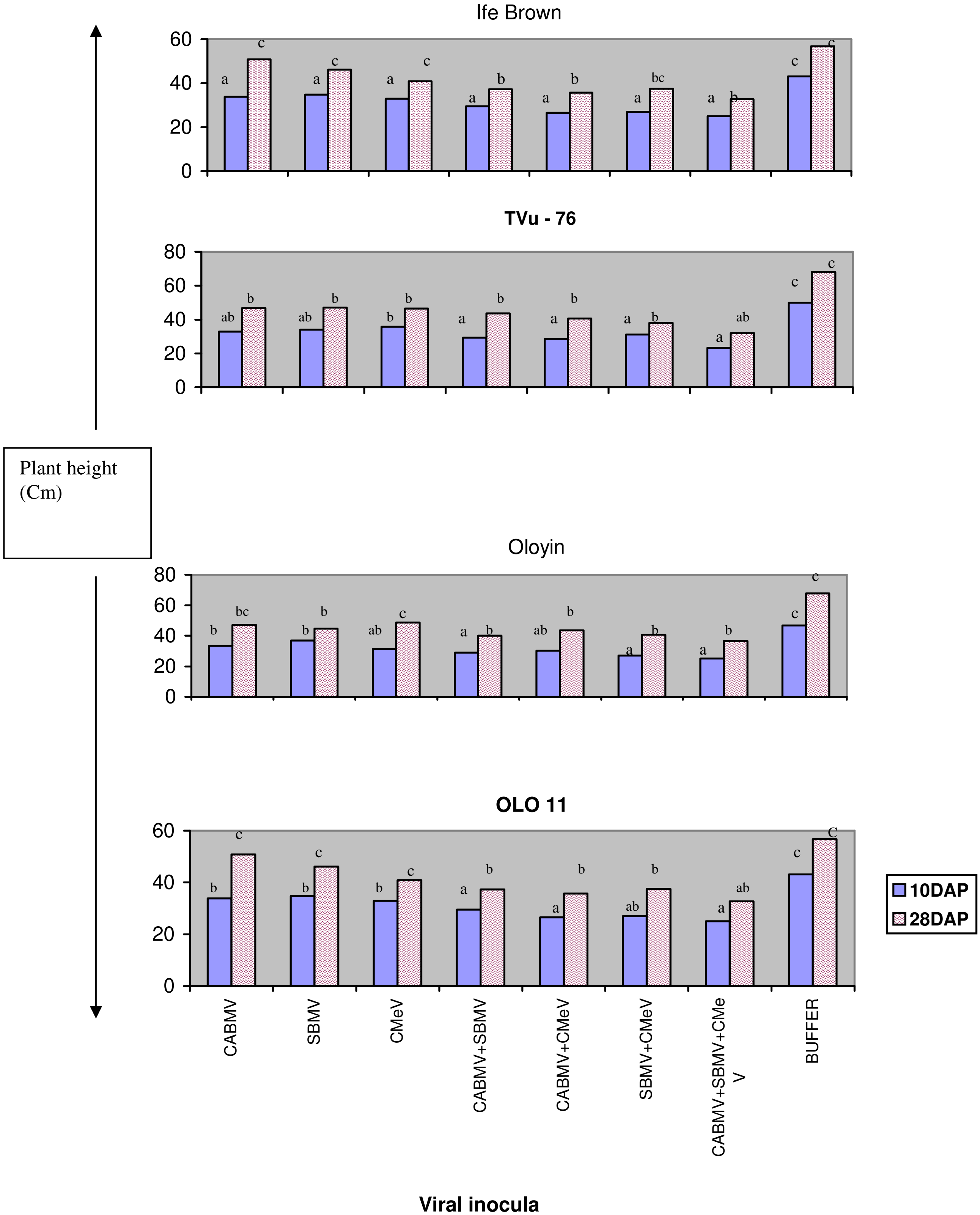

Figure 1. The effect of single and mixed viral treatments and plant age at time of infection on the height of cowpeas. CABMV: *Cowpea aphid borne mosaic virus,* SBMV: *Southern bean mosaic virus,* CMeV: *Cowpea mottle virus.* Each value is the mean of 4 replicates. In each column, means followed by the same letter are not significantly different (P=0.05) according to Duncan's multiple range test.

Table 1. Percentage decrease in the height of cowpeas infected by single and mixed viruses.

Viral Inocula	Cowpea Cultivar							
	Ife Brown		TVu76		Oloyin		Olo II	
	A	B	A	B	A	B	A	B
CABMV	30	11	34	31	33	30	22	11
SBMV	32	15	32	31	26	34	19	19
CMeV	30	15	28	32	37	28	24	28
CABMV+ SBMV	39	28	41	36	42	40	31	34
CABMV+ CMeV	39	30	43	40	39	36	39	37
SBMV+ CMeV	39	26	38	44	46	40	37	34
CABMV+ CMeV+ SBMV	46	33	53	52	50	46	42	42

A=Data from plants inoculated 10 days after planting compared with those from buffer inoculated plants
B= Data from plants inoculated 28 days after planting compared with those from buffer inoculated plants
Viral inocula: CABMV= *Cowpea aphid-borne mosaic virus,* SBMV= *Southern bean mosaic virus,* CMeV= *Cowpea mottle virus.*

Table 2. Effect of single and mixed viral infections and plant age at time of infection on the number of pods produced by cowpeas.

Viral Inoculum	Api (Days)	Compea cultivars			
		Ife Brown	TVu76	Oloyin	Olo II
CABMV	10	2.0ab	1.8a	1.5ab	1.5a
	28	2.3ab	2.5ab	2.3ab	2.5ab
SBMV	10	1.8a	2.0a	1.8ab	2.0ab
	28	2.3ab	2.5ab	2.0ab	2.5ab
CMeV	10	2.0ab	1.8a	1.8ab	1.5a
	28	2.5ab	2.3a	2.3ab	2.3ab
CABMV + SBMV	10	1.5a	1.3a	1.3a	1.5a
	28	2.0ab	1.8a	1.5ab	2.0ab
CABMV +CMeV	10	2.0ab	1.3a	1.5ab	1.3a
	28	2.5ab	2.3a	2.0ab	1.8ab
SBMV + CMeV	10	1.8a	1.8a	1.3a	1.8ab
	28	2.3ab	1.8a	1.5ab	2.5ab
CABMV+SBMV + CMeV	10	1.3a	1.0a	1.3a	1.3a
	28	1.8a	1.3a	1.3a	1.8ab
Buffer (control)	10	3.5b	4.0c	3.8c	3.5c
	28	3.5b	4.0cc	3.3c	3.8c

CABMV: *Cowpea aphid borne mosaic virus,* SBMV: *Southern bean mosaic virus*
CMeV: *Cowpea mottle virus,* API: Age of plants at inoculation in days.
Each value is the mean of 4 replicates. In each column, means followed by the same letter are not significantly different (P=0.05) according to Duncan's multiple range test.

average height of plants inoculated 10 DAP were shorter than those inoculated 28 DAP for all the cvs/lines. The reductions were most obvious in Ife Brown were the heights recorded for plants inoculated 10 DAP were significantly different from those inoculated 28 DAP as well as from those of the buffer inoculated control (Figure 1). Inoculating with single, double or triple viruses 10 DAP resulted in 19-34%, 31- 46% and 42-53% reductions in plant height, respectively (Table 1).

The various viral treatments resulted in the production of fewer pods, which were significantly different from those of the buffer inoculated control plants, especially in cvs "Oloyin", "Olo II", and TVu 76 (Table 2). The number of pods produced by Ife Brown inoculated 28 DAP were however not significantly different from those of buffer inoculated control except for plants inoculated with triple viruses. Plants inoculated with a mixture of the three viruses especially 10 DAP produced the least number of pods. The percentage reduction in pod number for plants at this early stage of growth was 65-75 %, however, the age of plant at the time of viral inoculation did not significantly affect the number of pods produced (Table2).

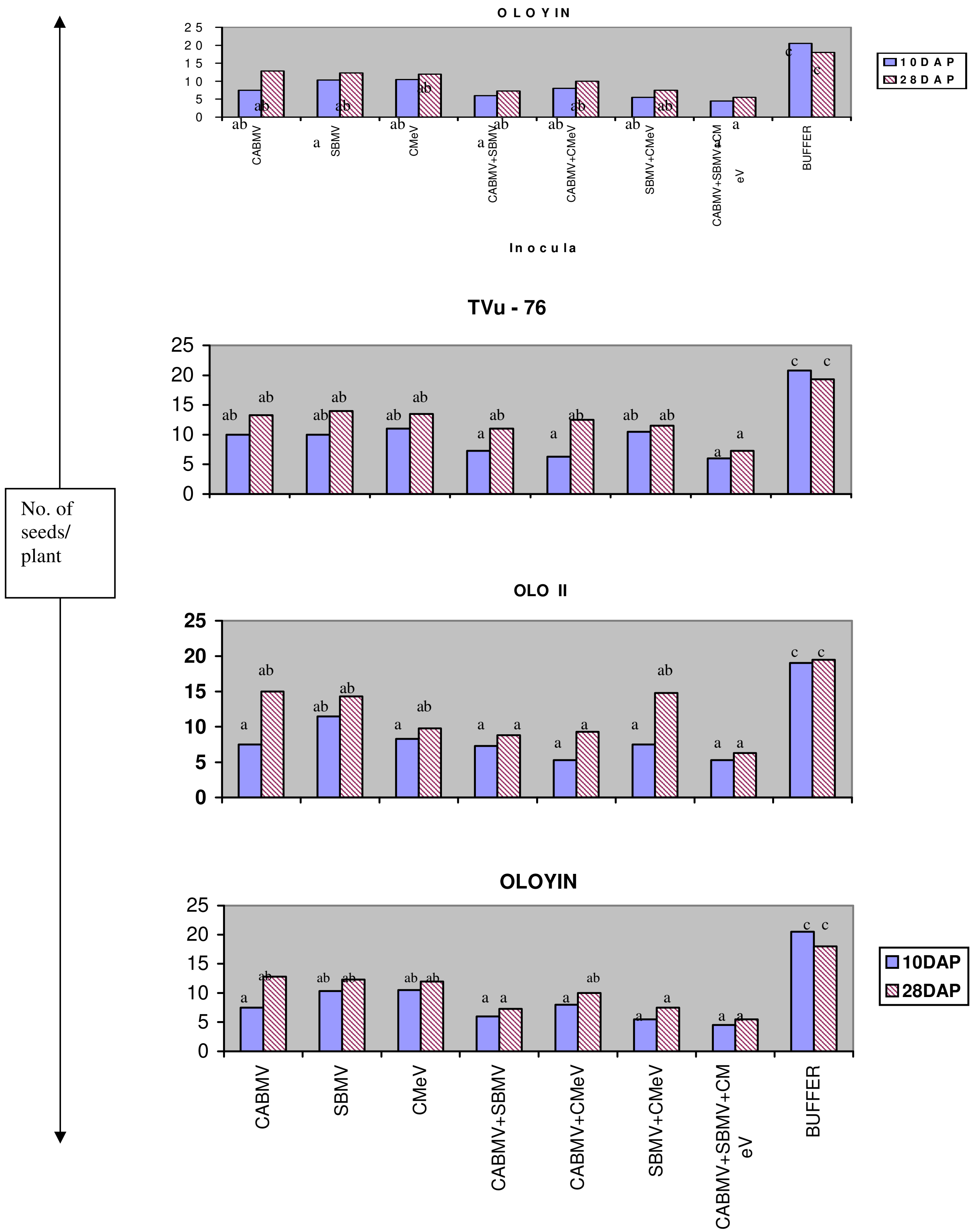

Figure 2. The effect of single and mixed viral treatments and plant age at time of infection on the number of seeds produced by cowpea. CABMV: *Cowpea aphid borne mosaic virus,* SBMV: *Southern bean mosaic virus*, CMeV: *Cowpea mottle virus.* Each value is the mean of 4 replicates. In each column, means followed by the same letter are not significantly different (P=0.05) according to Duncan's multiple range test.

Table 3. Percentage decrease in the number of seeds produced by cowpeas infected by single and mixed viruses.

Cowpea Cultivar								
Viral Inocula	**Ife Brown**		**TVU76**		**Oloyin**		**Olo II**	
	A	**B**	**A**	**B**	**A**	**B**	**A**	**B**
CABMV	45	31	52	31	63	29	61	23
SBMV	57	26	52	27	50	32	40	27
CMeV	43	18	47	30	49	33	57	50
CABMV+ SBMV	60	47	65	43	71	60	62	55
CABMV+ CMeV	42	28	70	35	61	44	73	53
SBMV+ CMeV	49	34	50	40	74	58	61	24
CABMV + CMeV + SBMV	64	52	71	63	78	69	73	68

A= Data from plants inoculated 10 days after planting compared with those from buffer inoculated plants.
B= Data from plants inoculated 28 days after planting compared with those from buffer inoculated plants.
CABMV= *Cowpea aphid-borne mosaic virus*, SBMV= *Southern bean mosaic virus*, CMeV= Cowpea mottle virus

Table 4. The effect of single and mixed viral treatments and the age of plant at time of infection on the protein content of cowpea.

Cowpea cultivar								
Virus Inoculum	**Ife Brown**		**TVU76**		**Oloyin**		**OloII**	
	A	**B**	**A**	**B**	**A**	**B**	**A**	**B**
CABMV	27.1	28.2	28.4	28.9	25.4	26.1	26.1	27.2
SBMV	27. 4	28. 7	28. 9	28.0	27.1	26.2	26.3	26
CMeV	27. 0	28	28.0	28.9	26	25.9	27	26.8
CABMV+ SBMV	27	28	27.3	28.9	26.6	26.1	23.3	26.1
CABMV+ CMeV	27.1	28	27.1	27.6	25.2	25.6	25	25
CABMV+ CMeV+ SBMV	26.4	27	26.4	27.1	25	25.2	24.8	25.5
BUFFER	29.9	29.6	30.4	29.9	29.9	28.8	28.5	29

A= Data for plants inoculated 10 days after planting, B= Data for plants inoculated 28 days after planting. CABMV= Cowpea aphid-borne mosaic virus, SBMV= Southern bean mosaic virus, CMeV= Cowpea mottle virus.

Similarly, the number of seeds produced by plants that received the various viral treatments was significantly different from those produced by the controls (Figure 2). In single virus infections, reductions caused by CABMV were higher in the commercial cvs than in IITA lines. The greatest reduction in this category (63%) was observed in cvs "Oloyin" inoculated at 10 DAP. In dual infections, greater reductions were observed in "Oloyin" (74%) and "Olo II" (73%) than in Ife Brown (60%) and TVu 76 (70%) (Table 3). Generally, infections at 10 DAP especially with triple viruses resulted in the greatest reduction in yield. The percentage reduction in seed yield in triple virus infections (10 DAP) ranged from 64 to 78%, compared with the 43-63% reductions recorded for single virus infections (Table 3).

Generally, the commercial cultivars ("Oloyin" and "Olo II") were more severely affected by the viral treatments than the IITA lines, as greater reductions in seed number were observed in these groups of plants. Plants inoculated during the dry season especially at 10 DAP died prematurely and therefore produced no seeds.

Qualitative effects of viral treatment

The various viral inocula caused reductions in the protein content of the seeds harvested from infected plants. The protein content of such seeds ranged from 24.8 to 28.9% while those from buffer inoculated plants ranged from 28.5 to 30.4% (Table 4). Generally, seeds from plants inoculated 10 DAP had lower protein content than those from plants inoculated 28 DAP. Mixed viral infections also affected the protein content of the seeds. The plants that were inoculated with a mixture of the three viruses produ-

Table 5. The effect of viral treatment on the nutritive content of cowpeas inoculated 28 days after planting.

Inoculum	Nutritive Content	Ifebrown %	Tvu-76 %	Oloyin %	Olo II %
CABMV	Carbohydrate	61.07	61.87	63.33	62.39
	Fat	1.69	1.40	1.63	1.50
	Moisture	5.85	4.58	5.82	5.71
	Ash	3.22	3.22	3.11	3.25
SBMV	Carbohydrate	61.15	62.64	63.25	63.13
	Fat	1.41	1.46	1.50	1.68
	Moisture	5.46	4.89	6.01	5.93
	Ash	3.29	3.00	3.06	3.23
CMeV	Carbohydrate	61.99	62.22	63.72	ns
	Fat	1.44	1.44	1.69	1.53
	Moisture	5.37	4.47	5.48	ns
	Ash	3.19	2.98	3.19	ns
Buffer (control)	Carbohydrate	60.38	61.58	60.99	61.36
	Fat	1.42	1.24	1.55	1.49
	Moisture	5.92	4.12	5.58	4.81
	Ash	3.30	3.17	3.04	3.36

CABMV= Cowpea aphid-borne mosaic virus, SBMV= Southern bean mosaic virus, CMeV= Cowpea mottle virus.
ns= No sample for analysis.

ced seeds with the least protein content (24.8-27.1%). Seeds from IITA's lines Ife Brown and TVu 76 had slightly higher protein content than the commercial cvs (Table 4). The carbohydrate content of seeds from virus infected plants were higher (61.07-63.72%) than those from buffer inoculated controls (60.38-61.58%).

Seeds from virus infected commercial cowpea cvs had higher carbohydrate content than IITA's lines. The fat and moisture contents of seeds from the virus infected plants were also slightly higher than those of the buffer inoculated plants, while the ash contents were generally lower (Table 5).

DISCUSSION

The results of this study indicate that the age of plant at the time of viral infection have quantitative and qualitative effects on cowpeas. Infection at an early age (10 DAP) resulted in a greater reduction in the yield and the nutriative content of the cowpea seeds than infection at 28 DAP. These results agree with reports of previous studies which indicate that the younger the plants at the time of infection, the more severe the disease symptoms and the greater the effects on yield (Uyemoto et al., 1981; Agrios et al., 1985; Langham et al., 2005). Infection at a later stage (28 DAP) resulted in reduced effects because at that stage, the plants were more matured and the virus had a less deleterious effect on them. These results are similar to those of Beniwal and Chaubey (1980) and Owolabi et al. (1988) who worked with urdbean leaf crinkle virus, BICMV and CYMV on cowpeas respectively. It is also noteworthy that the time of the year in Nigeria when the experiment was conducted, also influenced the type of results obtained. A preliminary experiment conducted during the dry season of 2002 resulted in complete loss in pod and seed yield especially in plants inoculated 10 DAP. This may be attributed to the very hot and dry weather associated with the dry season. Hughes and Shoyinka (2003) have also indicated that yield losses due to viral infection in sub-Saharan Africa depends on the time of infection, virus strain, possible virus mixtures, cultivars and environmental interactions especially climate.

Multiple viral infections resulted in a greater reduction in growth and yield parameters than single viral infections. Several studies involving mixed viral infections have demonstrated synergistic interactions in dual viral infections using growth parameters such as plant height, weight, yield and effect on seeds in soybean, cowpea, sugar beets and peppers (Anjos et al., 1992; Calvert and Ghabrial, 1983; Kuhn and Dawson, 1973; Pio-riberio et al., 1978; Wintermantel, 2005; Murphy and Bowen, 2006).

The results of this investigation suggest that triple virus infection caused greater reductions in growth and yield parameters than single and double viral infections. This result is noteworthy, as most previous studies were restricted to dual viral infections. However, the possibility of 3-5 viruses infecting a single plant is not uncommon in nature (Shoyinka et al., 1997).

The results of this study also demonstrated that viral infections reduced the protein content of the seeds of all the cowpea cvs and lines. This result is similar to that reported by Thind et al. (1996) working with a yellow mosaic virus infection of Mung bean (*Phaseolus aureus* L.). However, some other studies with cucumber mosaic virus in pea plants have indicated increases in the protein content of leaves, stems and roots of virus infected plants (Shukla and Rao, 1994). Also, Langhams and Glover (2005) reported 0.2-18.5% increase in seed protein of winter wheat inoculated with WSMV.

The increase in protein levels could be attributed to the diversification in the types of proteins synthesized by the host, as a result of viral infection. These include the coat protein, inclusion bodies, genome-linked proteins, enzymes involved in viral replication and poly-protein processing, and proteins involved in vector transmission and cell to cell movement (Shukla et al., 1994). The reduction in the protein content of cowpea seeds reported in this study suggests that the effect of the virus overwhelmed the increases in virus-induced proteins associated with infections.

This study has once again confirmed the susceptibility of Nigeria's commercial cowpea cvs to the three viruses used in this investigation. The results also indicate that losses in yield as well as in the nutritive value of cowpea seeds occur as a result of infection at an early stage of growth, especially with multiple viruses. The reduction in seed protein as a result of viral infection would worsen the protein deficiency problem of the populace who depend on cowpea as the cheapest means of obtaining their daily protein requirement. There is therefore the need to prevent viral infection of cowpea in order to ensure high yield and guarantee its nutritive value. Presently, the use of resistant varieties is the only practicable means of controlling the viruses (Thottappilly and Rossel, 1992), but the rate of adoption seems rather slow. Alternatively, biotechnological research that will result in the production and deregulation of virus-resistant cowpeas through coat- protein gene transfers should be intensified.

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