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Influence of Seedling Age at Inoculation and Cultivar on the Pathogenicity of a Virus Causing Yellow Mosaic Disease of *Commelina Benghalensis* L. on Cowpea

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Abstract

A screenhouse experiment was conducted to evaluate the influence of seedling age at inoculation and cultivar on pathogenicity of the virus causing yellow mosaic disease of *Commelina benghalensis* L, a broad leaf weed, on cowpea. Three cowpea varieties namely Vita 5, IT84S2246D and Ife Brown were grown in pots and inoculated with sap extracted from leaves of *C. benghalensis* infected with yellow mosaic disease at 7, 14, and 21 days after germination (DAG). It was found that inoculation of cowpea seedlings at 7 DAG subsequently led to the most severe symptoms, which were manifested by mosaic and yellowing of leaves and eventual poor growth and yield attributes. On the other hand, plant growth and yield attributes that were comparable to those of the healthy control plants were recorded for plants inoculated at 21 DAG. Specifically, in regards to the interaction effects, cv. Vita 5 that were sap-inoculated at 7 DAG had the lowest yield attributes, while cv. IT84S2246D inoculated at 21 DAG had the highest yield attributes. The results put together showed that although the yellow mosaic virus of *C. benghalensis* was sap-transmissible and pathogenic to cowpea causing characteristic yellow mosaic disease symptoms and reduction in yield attributes, severity of the disease is less if infection occurs at older stage of cowpea growth.

Keywords: Yellow mosaic virus, *Commelina benghalensis,* Pathogenicity, Seedling age cowpea

1. Introduction

Cowpea, *Vigna unguiculata* (L) Walp (Fabaceae), is an important grain legume in tropical countries of Africa and a veritable source of dietary protein, calcium and iron for the teeming population of human and livestock (Murdock *et al.*, 1997). In Nigeria, the cultivation of this legume extends from the rain forest zone, where its production is marginal, to the Northern Savanna grassland, where over 90% of total seed is produced (Ebong, 1968). The situation remains little changed even now. Practically, most of the cowpea produced is grown in the multiple cropping systems. However, the crop continues to be highly susceptible to a wide variety of pests and diseases both in the field and during storage (Adedire and Ajayi, 2003).

The relationship between crops and disease organisms is generally a complex annual cycle involving crops, vectors and weeds. Weeds are known to play important roles in the spread and epidemiology of virus diseases (Dufus, 1971; Sidek *et al.,* 1993). Many insect and nematode pests, which infect crops with viruses causing major diseases, have often been

shown to acquire the viruses initially from weeds (Zimdahl, 1980). The presence of okra mosaic tymovirus in three malvaceous weeds in Nigeria appeared to be an important source of the virus for crop plants (Atiri, 1984). Cowpea chlorotic mottle bromovirus (CCMV), which causes severe damage in susceptible cowpea cultivars alone or in mixed infections, has been isolated from two weed species, *Clitoria ternatea* and *Desmodium heterocarpon* also in Nigeria (Thottappilly *et al.,* 1993). In the United States of America, cucumber mosaic virus, which causes epidemics of disease in Cantaloupe melons, lettuce and sugar beet, is carried by at least two aphid vectors from several weeds including species of *Brassica*, *Sisymbrium* and *Physalis*. Certain garden ornamentals and several crops also carry the virus in Arizona (Rice, 1974).

Commelina benghalensis L., Family- Commelinaceae, an annual /perennial weed, occurs widely in the tropics where it grows in a wide range of situations in grassland arable crops including cowpea fields. *C. communis,* a close relative of *C. benghalensis* has been reported as a naturally infected weed host of CMV (Zitter, 2001), which is also an important pathogen of cowpea both experimentally and in the field. The *Commelina* yellow mottle virus is a DNA virus and it has been isolated from this weed. Apart from the allelopathic activity of this weed on crops it appeared to be an important virus reservoir, thereby constituting a major constraint to efforts aimed at increased and sustainable food production.

The age of plant at inoculation, genotype, season of growth, planting density among other factors have also been found to exert varying degree of influence on the pathogenicity of disease agents in different pathosystems. Many specific relationships between weeds, crops and their pests and pathogens have been established, sometimes enabling action to be taken against weeds to prevent or reduce damage to crops (Mattews, 1991). The objective of this study, therefore, was to determine if the pathogenicity of the mosaic disease of *C. benghalensis* on cowpea was influenced not only by the host cultivar but also by the age of seedling at inoculation as well as their possible interactive effects.

2. Materials and Methods

2.1 Experimental design and plant propagation

Experiments were conducted in the screenhouse of the Faculty of Agriculture, University of Ilorin, Nigeria situated in the Southern Guinea Savannah ecological zone, to evaluate the influence of age of seedling at inoculation on the pathogenicity of the causal agent of a yellow mosaic disease of *C. benghalensis* (weed host) in 3 cultivars of cowpea. The cowpea cultivars were inoculated at 7, 14, and 21 days after germination (DAG). It was factorial a 3x4 factorial experiment in a completely randomized design with 6 replications. The three cultivars of cowpea were Vita 5, IT84S2246-D and Ife Brown, which were obtained from the Teaching and Research Farm of the University of Ilorin, Nigeria. They were raised in stands of two in 5-liter (25 cm diameter) plastic pots, filled with sandy-loam soil sterilized at 121^{o}C for 30 minutes prior potting.

2.2 Collection of diseased leaf samples and inoculation

Diseased leaf samples of *C. benghalensis* naturally manifesting yellow mosaic disease symptom were collected from farms adjoining the Faculty of Agriculture buildings, University of Ilorin. Leaf sap was extracted by homogenization of leaves using mortar and pestle, in 0.05 M phosphate buffer, pH 7.2, at the rate of 1 g leaf sample to 1 ml of the buffer.

The first and second leaves from the stem base of cowpea plants were lightly dusted with carborundum and rubbed with the extracted sap using cotton wool at the 2-3 leaf stage (7 DAG), 4-5 leaf stage (14 DAG) or 6-7 leaf stage (21 DAG). The leaves were rinsed with running water. The control plants were mock –inoculated with phosphate buffer only. Plants were watered once per 3 days to avoid water stress during the growth period of the plants.

2.3 Data collection and analysis

Initially, observations were made daily and then on weekly basis for symptoms manifestation i.e. type and nature of symptoms, number of days to appearance of symptoms after inoculation and position on plant of the leaf with first symptoms. Growth parameters such as plant heights, number of leaves and mean leaf size using leaf area meter, were taken on a weekly basis after each inoculation and at flowering and podding stages. Yield parameters including the number of pods per plant, total pod weight per plant, average weight per plant, percentage grain weight per pod, and percentage yield loss per variety and inoculation regime were determined at or after harvest. All collected data were subjected to analysis of variance (ANOVA). Treatment means were separated using the New Duncan's multiple range test at 5% level of significance.

3. Results and Discussion

3.1 Symptoms manifestation

Symptoms of the disease caused by virus extracted from weed host, *C. benghalensis* appeared on sap- inoculated cowpea plants. Mock- inoculated plants were however free from the visible symptoms of the disease. Initially, the symptoms manifested as mild mottling graduating to pronounced yellow mosaic pattern with increased age of infection (Plate 1). These were similar to the symptoms observed on naturally infected weed host (Plate 2).

First symptoms appeared on cowpea plants inoculated at 7 DAG at 6.3 days after inoculation while it appeared at 9.0 and 11.0 days after inoculation respectively on cowpea plants inoculated at 14 and 21 DAG (Table 1). The three cowpea cultivars varied significantly in respect of days to appearance of symptoms on plants after inoculation. The disease symptoms appeared at 6.6, 9.0 and 11.0 days after inoculation on cultivars Vita 5, IT84S2246D and Ife Brown, respectively (Table 1). The interaction effect between cowpea cultivar and seedling age at inoculation was significant. On all cowpea cultivars, symptoms appeared earliest in seedlings inoculated at 7 days after germination, followed by those inoculated at 14 days and 21 days after germination. Cultivar Vita 5 inoculated at 7 dag was the first to manifest symptom in 5 days after inoculation, while Ife brown inoculated at 21 DAG was the last in13.8 days after inoculation.

3.2 Plant growth response

The combined effects of age of plant at inoculation and variety on plant height, number of leaves and leaf size of 3 cowpea cultivars over the first 5 weeks following inoculation is shown in Table 2. The values for cv. Vita 5 inoculated at 7 DAG were the lowest whereas cv. IT84S2246-D inoculated both at 14 and 21 DAG were the highest among inoculated plants. In all cases, the mock-inoculated plants of all varieties had the highest values followed by those inoculated at 3 weeks after germination. Plate 3 compares the appearance (plant height, number of leaves, size of leaves etc) of infected and healthy control cv. Vita 5 plants at 5 weeks after inoculation with the viral agent.

3.3 Yield response

The main effect of age and variety as well as their interaction effect on yield responses of cowpea are shown in Table 3. For all recorded parameters, which included total number of pods per plant, total and average weight of pods, total and average grain weight as well as % weight of grain per pod, the general trend was similar to that recorded for growth parameters. Significantly lower values were recorded for plants that were inoculated at the earliest seedling age compared to those inoculated later. Analysis of the varietal effect showed cv. vita 5 with significantly lower yield values than the other two cultivars for most of the parameters while Cv IT84S2246D had the highest values. Specifically, cv. Vita 5, sap-inoculated at 7 DAG, had the lowest yield value while cv. IT84S2246D inoculated at 21 DAG had the highest yield values for most parameters.

3.4 Comparative percentage yield loss

The percentage loss of yield based on grain weight, pod weight and number of pods, in the three cultivars of cowpea is shown in Fig. 1. The three cowpea cultivars significantly differed with regard to each parameter. In all cases, cultivar IT84S2246-D had the lowest percentage of losses, while the highest percentage loss was recorded in cultivar Vita 5.

It is apparent in this study that the degree of susceptibility varied with the cultivars in terms of time of appearance of symptoms, and the subsequent growth and yield responses. Inter cultivar comparison showed that cv. Vita 5 was the most susceptible at all inoculation stages. Relative to the control in each cultivar, grain yield loss due to early infection was in the range of 40 to 60 %, while late infections caused between 10-15 %. Cowpea plants inoculated early (7 days after germination) with the sap from infected weed, manifested early mosaic symptoms, while plants inoculated late (21days after germination) manifested symptoms much later. Such plants that manifested early infection symptoms subsequently had lower growth and yield components than those recorded for plants that had late symptom manifestation, owing to inoculation at relatively older age. This observation has further highlighted the role of physiological maturity conferred on the host plant system by age. A similar observation to that observed here was made long ago (Owusu *et al.*, 1968), who reported that the earlier tobacco plant was infected with the tobacco ring spot virus, the higher the pathogenic effect of the virus on the tobacco plant. Of recent, similar observations were also made on some tomato cultivars mixed infected with the tomato mosaic virus and potato virus X (Balogun, 2008).

A reduction in total fruit yield is a common feature, and an important economic aspect of virus diseases (Mathews, 1991). Yield reduction manifesting in various forms is therefore normally expected with increased disease severity in virus diseases, as was the case in this study, in which the severity was enhanced by infection at early growth stage. Reduction in size and number of pods was most noticeable and this was probably a result of impairment of pod and seed initiation, as well as increased abortion as had been observed by Walkey *et al.* (1985). Hampton (1975) had noted that lower yields in virus infected cowpea may sometimes be due to a reduction in both the size and number of fruits.

The results in the present study support earlier observations and reports of Balogun and Aliyu (2005) that the broad-leaf weed, *C. benghalensis,* naturally infected with the yellow mosaic disease could serve as a potent reservoir for the causal agent and a source of inoculum for transmission to susceptible arable crops. More over, the fact that the viral agent was easily transmitted by rubbing the juice from the infected weed, on cowpea leaf, under ambient screenhouse conditions makes it highly plausible that transmission through farm operations, such as manual weeding, which is still widely practiced under the vastly subsistent farming system in sub-Saharan Africa, was a possibility.

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Table 1. Days to appearance of first symptoms in 3 cowpea cultivars after inoculation with sap extract from C. *benghalensis* naturally infected with a yellow mosaic disease

- = Did not manifest symptoms.

Cultivar / Days after germination	Days after inoculation to appearance of first symptoms[1]			
	Vita 5	**IT84S2246D**	**Ife Brown**	**Mean[2]**
Mock inoculation	-	-	-	-
Inoculation at 7 DAG	5.0g	7.6de	6.4f	6.3C
Inoculation at 14 DAG	7.0ef	11.5b	8.8c	9.0B
Inoculation at 21DAG	7.8d	13.8a	11.5b	11.0A
Mean[3]	6.6γ	11.0α	8.8β	

[1]Figures within columns and rows having the same small letter(s) do not differ significantly (P>0.05).

[2]Means within the column having different capital letter differ significantly (P<0.05).

[3]Means within the row having different Greek letter are significantly different at P< 0.05) using the New Duncan's Multiple Range test.

Table 2. Combined effect of age of plant at inoculation and cowpea cultivar on some growth parameters at 5 weeks after final inoculation

Cultivar x Seedling age at inoculation	**Plant height (cm)**	**No. of leaves per plant**	**Average leaf size (cm^2)***
Cv. Vita 5, Mock- inoculated at 7 DAG	30.0a	17.5a	52.6a
Cv. Vita 5, Inoculated at 7 DAG	14.4f	13.8d	21.2d
Cv. Vita 5 Inoculated at 14 DAG	14.9f	14.0d	25.4d
Cv. Vita 5 Inoculated at 21 DAG	24.3b	17.0ab	31.0c
Cv. IT84S2246D Mock- inoculated at 7 DAG	31.9a	16.3abc	54.2a
Cv. IT84S2246D Inoculated at 7 DAG	20.9cd	15.8bc	37.1b
Cv. IT84S2246D Inoculated at 14 DAG	22.8bc	14.0d	38.1b
Cv. IT84S2246D Inoculated at 21 DAG	24.6b	16.0bc	48.0a
Cv. Ife Brown Mock-Inoculated at 7 DAG	31.2a	16.5abc	53.4a
Cv. Ife Brown Inoculated at 7 DAG	17.9e	15.5c	24.5c
Cv. Ife Brown Inoculated at 14 DAG	19.5de	13.8d	33.8b
Cv. Ife Brown Inoculated at 21 DAG	23.8bc	15.8bc	37.1b

[1]Means within a column followed by the same letter(s) are not significantly different using the New Duncan's Multiple Range Test at P= 0.05.

* Size of a trifoliate leaf measured by a leaf area meter

Table 3. Effect of age and variety and their combination effects on some yield parameters in cowpea under mock or sap inoculation with yellow mosaic virus disease of *Commelina benghalensis*

Seedling age / inoculation	No of Pods Per Plt	Total Pod Wt(g)	Mean Pod Wt(g)	Total Grain Wt/Plt	% Grain Wt/Pod
Mock Inoculated at 7 DAG	13.5a	15.3a	1.15a	13.5a	88.1a
Inoculated at 7 DAG	7.8d	7.9d	0.97b	6.3d	76.7c
Inoculated at 14 DAG	9.4c	9.4c	0.98b	7.9c	82.6b
Inoculated at 21 DAG	10.7b	10.8b	1.0b	9.2b	82.9b
S.E	0.24	0.25	0.03	0.38	1.31
Cowpea Variety					
Cv. Vita 5	6.6c	6.3c	0.9c	5.1c	76.7b
Cv. IT84S2246-D	13.4a	13.9a	1.0b	12.1a	86.9a
Cv. Ife brown	11.0b	12.4b	1.1a	10.5b	84.1a
S.E	0.2	0.3	0.03	0.3	1.1
Treatment Combinations					
Cv.Vita5 mock-inoculated	9.8de	11.5c	1.2ab	10.3c	89.5a
Cv.Vita 5 inoculated 7 DAG.	4.5g	3.4f	0.74e	2.2e	66.4d
Cv.Vita 5 inoculated 14 DAG	5.5fg	5.0ef	0.84de	3.5e	76.4c
Cv.Vita 5 inoculated 21 DAG.	6.5f	5.6e	0.87de	4.2e	74.5c
Cv.IT84S2246-D mock- inoc.	16.8a	17.1a	1.0bc	15.4a	89.7a
Cv.IT84S2246-D inoc 7 DAG.	10.0d	11.1c	1.1abc	9.4c	84.6ab
Cv.IT84S2246-D inoc 14 DAG	12.5c	12.4c	0.99cd	10.8c	87.1a
Cv.IT84S2246-D inoc 21 DAG.	14.5b	14.9b	1.0bc	12.9b	86.3ab
Cv. Ife brown mock-inoculated	14.0b	17.4a	1.3a	14.8a	85.0ab
Cv. Ife brown inocd 7 DAG.	8.8e	9.1d	1.0bc	7.2d	79.2bc
Cv. Ife brown inocd 14 DAG.	10.3d	11.2c	1.1abc	9.5c	84.4ab
Cv. Ife brown inocd 21 DAG	11.0d	12.0c	1.1abc	10.4c	87.8a

*Means in the same column followed by the same letter(s) are not significantly different at P= 0.05 using the New Duncan's Multiple Range Test.

DAG: Days after germination.

Plate 1. A: Mock-inoculated (control) Cowpea cv. Vita 5 remained without symptoms even at the fruiting stage.
B: Cowpea cv Vita 5, inoculated at 7 days after germination with sap from infected leaf of *Commelina benghalensis*, manifesting severe yellow mosaic symptoms a few weeks after inoculation.

Plate 2. A: Wild *Commelina benghalensis* without virus (mosaic) symptoms (apparently healthy)
B: Naturally infected *Commelina* plant manifesting yellow mosaic symptoms

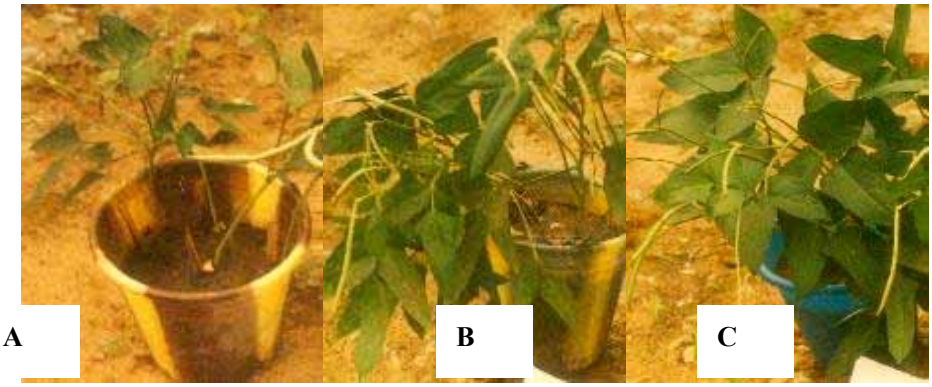

Plate 3. Differential growth and yield response of cv Vita 5 cowpea plants following mock or sap inoculation at different seedling age with buffer or sap extracts from *Commelina benghalensis* weed plants manifesting yellow mosaic disease syndrome.
Seedlings were sap- inoculated at (A) 7 days after germination (DAG) (B) 21 DAG. (C) Mock-inoculated with buffer only at 7 DAG (healthy control). Plants were photographed at the reproductive stage (8 weeks after germination i.e. 5 weeks after the last inoculation).

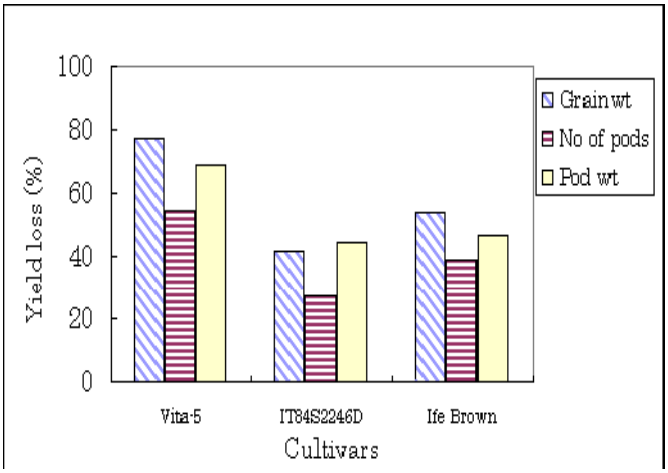

Figure 1. Comparative yield loss in three cultivars of Cowpea under infection with a virus isolate from *Commelina benghalensis,* a weed host