INFLUENCE OF TWO NIGERIAN STRAINS OF *MOROCCAN WATERMELON MOSAIC VIRUS* ON THE GROWTH AND YIELD PARAMETERS OF CUCUMBER (*CUCUMIS SATIVUS* L.)

[a]Eyong, O. I. and [b]Owolabi, A. T.

[a]Department of Forestry & Wildlife Resources, Cross River University of Technology, Calabar

[b]Department of Plant & Ecological Studies University of Calabar, Calabar

SUMMARY

The effects of two Nigerian strains of MWMV namely MWMV-cor and MWMV-lag on growth and yield parameters of cucumber were studied in two separate experiments. Plants were inoculated singly and with a combination of both strains at 9 days after planting (DAP) and 21 DAP in a randomized complete block design. Plants inoculated with the buffer only served as controls. The results of the study showed that infection of cucumber by the virus strains singly and in mixed inoculation led to significant reduction in all the yield parameters at 9 DAP, with reduction ranging between 41.1% - 66.7% for MWMV-cor, 43.3% – 61.9% for MWMV-lag and 42.7% – 71.8% for mixed inoculation. The results obtained for 21 DAP also revealed that there were losses of between 17.7% – 36.2 % caused by MWMV-cor, between 22.9% – 40.0% for MWMV-lag while losses in the yield parameters due to mixed infection were between 21.5% – 38.1%. No apparent synergistic effect was observed with mixed inoculation of the virus strains on both growth and yield parameters as the values obtained for the growth and yield parameters at both inoculation ages were comparable to those singly inoculated by the two strains. The results presented here provide evidence that MWMV strains constitute a threat to cucumber production and particularly so by the more virulent MWMV-cor, which caused comparatively greater yield loses than MWMV-lag. Sourcing for and planting of resistant varieties of cucumber and preventing early aphid introduction can be helpful.

Keywords: Cucumber, *Moroccan watermelon mosaic virus* (MWMV), growth, yield

CUCUMBER (*Cucumis sativus* L.) is believed to have originated from the Himalayas, possibly in India where a great many varieties have been observed (1, 2). It is cultivated worldwide for its edible fruits and belongs to the Cucurbitaceae family, which also include squashes, pumpkins, luffas, melons, and watermelons.

Cucumber fruit is eaten raw or consumed fresh in salad. Some varieties are grown specifically for pickling. Nutritionally, it is a rich source of vitamins B1, B2, B5, B6, folic acid, and vitamin C and mineral nutrients such as calcium, iron, magnesium, phosphorus, potassium and zinc (3). According to Abiodun (4), cucumber consumption provides an anti-inflammatory flavonoid, called fisetin which is believed to play an important role in brain health by protecting nerve cells from age-related decline. Cucumber has also been reported to contain lignans and cucurbitacins which have been implicated in lowering the risk of breast, uterine, ovarian, and prostate cancer **(**5, 6**).** According to Szalay (7), the fruits contain numerous antioxidants, including the well-known vitamin C and beta-carotene which provide additional health benefits.

Morocco watermelon mosaic virus (MWMV), though not so called, was first reported by Fischer and Lockhart (8) in Morocco. A potyvirus in the family *Potyviridae*, MWMV has since been reported in different parts of Europe including Spain, Italy, France and Greece (9, 10, 11, 12). There are also records of its occurrence in East, Central and Southern and West Africa (13, 14, 15, 16, 17). The virus has been considered a potential threat to cucurbit production, causing severe crop decline and large-scale yield losses by several authors (12, 14, 18).

Out of about 59 viruses that have been reported infecting cucurbits (19), few have actually been investigated with respect to yield losses in these crops, though they have been acknowledged as constituting potential danger to their production world-wide (20, 21, 22). Blua and Perring (23) reported that inoculation of zucchini by *Zucchini yellow mosaic virus* (ZYMV) during vegetative growth and early flowering stages caused 94% and 76% reduction in marketable fruits respectively. Plants inoculated after fruit set and healthy control plants produced comparable number of marketable fruits. For quality as indicated by fruit size and percentage of soluble solid, reduction caused by ZYMV infection occurred during vegetative and early flowering states but not when plants were infected after fruit set. In another experiment involving two trials,

Fletcher *et al*. (24) found that inoculation of the 'Delica' buttercup squash (*Cucurbita maxima* Duch.) variety at early, mid, and late stages of growth with ZYMV and *Watermelon mosaic virus 2* (WMV-2), [now called *Watermelon mosaic virus* (WMV)], caused serious yield losses and adverse quality effects in comparison with yields from uninoculated plots. Total yield losses from ZYMV were 48% for early-season and 26% for mid- season infections. Yield losses from WMV-2 were found to be statistically significant only after early infection (38%). Numbers of marketable fruit were reported to be similarly affected as was marketable fruit weight due to fruit blistering. They also documented that early season single or combined infections of ZYMV and WMV-2 reduced the mean marketable yield of 'Delica' from 84 to 23% of the total yield of uninoculated plots. In both trials, mixed infections of ZYMV and WMV 2 caused yield effects that were similar to those associated with ZYMV alone.

Jones *et al*. (25) reported that in individual plants of chicken pea (*Cicer arietinum* L.) that developed *Cucumber mosaic virus* (CMV) symptoms at different growth stages following the introduction of the virus by aphid vector, shoot and pod dry weight were reduced by 60–65% and 77–79% (for early infection) and 44 and 66% (for late infection) respectively. Seed yield losses were 78–80% (for early infection) and 65–67% (for late infection) and reduction in 50-seed weight was 20–25% regardless of time of infection. Infection reduced seed quality and in addition to smaller seed size, seed coats were discoloured and malformed. The proportions of malformed seeds were 9–11% (early), 3–6% (late) and 0·5% (healthy). Seed yields decreased by 44–45% when CMV incidence reached 61–74% at final assessment in plots with simulated 1–2% initial incidence. Seka *et al.* (26) have also reported that infection of yam by CMV caused reduction in the mass of tuber. Earlier report by Lutham **(**27), in field experiment, showed that CMV diminished shoot dry weight by 72–81%, seed yield by 80–90% and individual seed yield by 17–25% in lentil (*Lens culinaris* Medikus). Delmiglio and Pearson (28) who evaluated the effect and incidence of CMV, WMV and ZYMV on *Sicyos autralis* L. also reported a general reduction in growth up to 72% by CMV NZ100 and up to 65% for CMV NZ119 but ZYMV was not found to cause significant growth reduction in crop.

The cultivation of cucumber has become a popular and striving business in Odukpani and Akamkpa Local Government Areas of Cross River State, Nigeria providing a source of livelihood for the local farmers and meeting the consumption needs of Calabar (the state capital) metropolis. However, its cultivation could be frustrated by MWMV which has been found to be widespread in the region from a recent survey (data to be published later).

Two strains of the virus, isolated from *Lagenaria breviflora* (Benth.) Roberty and *Coccinia barteri* (Hook. f.) Keayin Nigeria (17), have been shown to be easily mechanically transmissible and readily transmitted in a fore-gut manner by *Myzus persicae*, *Aphis spiraecola* and *A. gossypii*. Symptoms commonly found on infected cucurbits by the virus strains are mosaic, green vein-banding, rugosity, severe leaf malformation and reduction in size, and stunting when infected by the two virus strains independently (18).

Previous studies involving MWMV have centered on characterization and identification of the virus (12, 13, 14, 15, 16, 17) transmission efficiency of the two strains of the virus reported in Nigerian by two clones of *A. spiraecola* (29) and effect of the virus on some physiological aspects of infected plant (30). From the review of literature, there is no empirical data on the effect of MWMV on the performance of any of the susceptible cucurbits. Since a virus becomes economically relevant if it causes significant reduction in the quantity and quality of the host or other host products, this study was undertaken to examine the economic significance of the virus with respect the growth and yield of *C. sativus*.

MATERIALS AND METHODS

Source of seeds and virus strains used

Cucumber seeds used for this study were sourced from Okurikang, a community reputed for cucumber growing in Odukpani LGA of Cross River State, Nigeria. Seeds were planted in plastic pots of 16 cm diameter and subsequently inoculated mechanically with the two strains of MWMV for susceptibility test. The inoculated plants exhibited symptoms characteristic of infection by the virus. The strains, designated as MWMV-cor and MWMV-lag in this study, were previously described by Owolabi *et al* (19) and are maintained in the screen house of the university.

Experimental design

Randomized complete block design as described by Little and Hill (31) was used. There were four (4) blocks

each containing 30 polyethylene bags (120 bags in all) of 15 cm diameter and 21 cm long, filled with 5 kg of rich garden soil (about ¾ filled). The bags were arranged in six (6) rows of five. Three seeds were sown per bag but later thinned to one before inoculations were carried out. Stakes were provided for the developing seedlings.

Inoculation of plants

Inoculations were carried out twice, at 9 days after planting (DAP) when the primary leaves had become well expanded and at 21 DAP, just before the onset of flowering. There were four treatments viz: MWMV-lag, MWMV-cor, MWMV-lag + MWMV-cor, (a combination of both strains in mixed inoculation) and the control (inoculation with the buffer only). Within each block, the treatments were randomized using a table of random numbers. Leaves to be inoculated were dusted with 600-mesh carborundum. Virus inocula (the treatments) were prepared by triturating symptomatic MWMV-infected leaves in 0.03 mM disodium hydrogen orthophosphate (Na_2HPO_4 $2H_2O$) buffer, pH 8.0 and were applied mechanically. The inoculated leaves were then rinsed with distilled water. Evaluations of the effects of the virus stains on the plant performance on growth parameters and yield quantification were carried out 4 weeks after planting, when the study was terminated.

Effect of virus strains on plant height and leaf number

The effect of the virus inocula on plant height was determined by measuring each of the plant from the soil level to the tip of the shoot using a metre rule. For the leaf number, the leaves were counted per plant. Data were also obtained for the controls.

Effect of virus strains on fresh and dry leaf shoot weights

The shoots were severed from the root system with a sharp knife and the fresh weight determined *in situ*. The samples were later oven-dried to constant weight at 70^{o}C. They were then weighed using Blauscal weighing balance (DHB 9053A, Ocean Med, England).

Effect of virus strains on fresh and dry leaf weights

Leaves harvested were weighed separately for each of the treatments as described above to determine the virus effect on fresh leaf weight. Dry weight was obtained by drying the leaf samples to constant weight at 70^{o}C for 24 hr before data were taken.

Effect of virus strains on fruit number, length and weight

Evaluation of the virus strains on yield parameters was carried out using the same experimental design and employing the same inoculation dates and procedure. The experiment was terminated three (3) months after planting and fruits were harvested. Influence of virus strain on fruit number was determined by counting the number of fruits per inoculated plant, and measuring the fruit with the metre rule to obtained values for fruit length. Fruit weight determination was carried out using a Blauscal weighing balance (DHB 9053A, Ocean Med English).

Data analysis

All data presented were means of 4 replicates for each parameter and for each treatment. Data collected were analyzed statistically using SPSS version 20 and Duncan multiple range test to establish significant differences between means.

RESULTS

Effect of virus strains on growth parameters

The results showed that MWMV-cor significantly reduced ($p<0.05$) plant height, leaf number, fresh and dry shoot weight, fresh and dry leaf weight of cucumber when inoculated at 9 DAP by 50.4%, 69.6%, 49.1%, 84.5%, 72.3% and 88.7% respectively compared to the values obtained for the corresponding controls (Table 1). For 21 DAP, values obtained for leaf number, dry shoot weight, fresh leaf weight and dry leaf weight were also significantly lower with percentage reduction ranging from 36.9% for leaf number to 53.0% for dry shoot weight compared to the corresponding controls. However, values obtained for plant height and fresh shoot weight, though reduced, were statistically insignificant ($p<0.05$). The results also showed that values obtained for all the growth parameters at 9 DAP were lower and differed significantly from those obtained when plants were inoculated at 21 DAP.

Similar to the results obtained for MWMV-cor, inoculation of the crop with MWMV-lag at 9 and 21 DAP led to reductions in all the growth parameters investigated when compared to the controls. Reductions ranging from 51.1% for fresh shoot weight to 83.6% for dry leaf weight caused by the virus strain at 9 DAP after inoculation differed significantly ($p<0.05$) from those obtained at 21 DAP (Table 1) in all the growth parameters. The values obtained at 21 DAP were also significantly lower than the corresponding controls. Percentage reductions ranged from 27.1% for

fresh shoot weight to 45.9% for dry leaf weight.

Generally, infection of the crop when doubly inoculated with MWMV-cor + MWMV-lag engendered significant reduction in the growth parameters at both inoculation stages when compared to the corresponding values for the controls. For all of the parameters, values obtained

at 9 DAP differed significantly from those obtained at 21 DAP, which in turn differed significantly ($p<0.05$) from those of the corresponding controls (Table 1). The least reduction of 51.3% was recorded for plant height while the highest of 92.5% was observed for dry leaf weight.

Table 1: Effect of two strains of *Moroccan water melon mosaic virus* on growth parameters of cucumber

	Growth parameters[¥]												
Treatments[†]	Inoculation time (Days after planting)	Plant height (cm)	Reduction (%)	Leaf number	Reduction (%)	Fresh shoot weight (g)	Reduction (%)	Dry shoot weight (g)	Reduction (%)	Fresh leaf weight (g)	Reduction (%)	Dry leaf weight (g)	Reduction (%)
MWMV-cor	9	49.13a	50.4	11.44a	69.6	49.47a	49.1	5.48a	84.5	13.48a	72.3	2.30a	88.7
	21	73.5b	25.7	23.81b	36.9	73.89b	24.0	16.56b	53.0	28.61b	41.2	9.68b	52.5
	Control	99.07b		37.75c		97.24b		35.26c		48.63c		20.37c	
MWMV-lag	9	46.75a	52.8	19.50a	55.5	44.53a	51.1	5.19a	64.8	12.18a	74.7	3.35a	83.6
	21	70.69b	28.5	29.19b	33.4	65.55b	27.1	8.92b	39.6	28.99b	39.8	11.00b	45.9
	Control	99.07c		43.83c		90.99c		14.76c		48.13c		20.37c	
MWMV- cor +	9	48.25a	51.3	11.44a	63.7	36.31a	59.9	4.69a	73.2	12.04a	68.8	1.52a	92.5
MWMV-lag	21	71.68b	27.6	22.00b	30.2	64.59b	28.6	9.29b	46.9	26.71b	30.9	11.58b	43.2
	Control	99.07c		31.50c		90.49c		17.51c		38.63c		20.37c	

[†] MWMV-cor = *Moroccan watermelon mosaic virus* (Coccinia strain). MWMV-lag = *Moroccan watermelon mosaic virus* (Lagenaria strain);

[¥]Each value is a mean of 4 replicates. In each column of specific treatment, means followed by the same letter are not significantly different according to Duncan multiple range test. Percentage reduction was calculated by expressing the difference between the control and the treatment as a percentage of the value for the control.

For inoculation at 21 DAP percentage reduction ranged from 27.6% to 46.9% for plant height and dry shoot weight respectively.

Effect of virus strains on fruit number

The results presented in Table 2 showed that the mean fruit number obtained from plants inoculated with MWMV-cor at 9 DAP was significantly lower ($p<0.05$) than the number obtained for 21 DAP which in turn differed significantly from the corresponding controls. Percentage reductions were 66.7% and 36.2% for both inoculation stages. For MWMV-lag, there was a significant reduction in the number of fruits obtained at 9 DAP by 61.9% and at 21 DAP by 40.0% compared to the corresponding controls. Mixed inoculation with both virus strains significantly reduced the fruit number regardless of the stage of inoculation in comparison with the control. Losses stood at 71.4% and 38.1% at 9 DAP and 21 DAP, respectively.

Effect of virus strains on fruit length

Inoculations with MWMV-cor at 9 DAP and at 21 DAP led to 51.1% and 29.3% reduction respectively in fruit length (Table 2). The values obtained for both inoculation stages were significantly lower compared to the corresponding value for the control. For MWMV-lag, the values obtained at 9 DAP was significantly reduced ($p<0.05$) compared to the value obtained at 21 DAP. This in turn was significantly lower that the values obtained for the corresponding control. Percentage reductions were 51.9% at 9 DAP and 25.9 % at 21 DAP (Table 2). The results also showed that there was significant reduction in fruit length when the plant was doubly inoculated with MWMV-cor and MWMV-lag at both 9 and 21 DAP, with percentage reduction of 49.5% for the former and 28.6% for the latter in relation to the corresponding controls.

Effect of virus strains on fruit weight

For MWMV-cor, fruit weight obtained at 21 DAP was comparable to that of the control, but inoculation at 9 DAP caused significant reduction in fruit weight compared to the values obtained for 21 DAP and the control (Table 2). Fruit weight was significantly reduced by as much as 43.3% by MWMV-lag at 9 DAP compared to 22.9% when inoculation was carried out at 21 DAP. Infection at 21 DAP also engendered significant reduction in fruit weight compared to the corresponding control. The result also showed that mixed inoculation engendered significant weight loss of 42.7%

when plant was inoculated at 9 DAP. However, the mean value obtained at 21 DAP with 21.5% loss did not differ significantly from that of the corresponding control.

Table 2: Effect of the virus inocula on yield parameters: Fruit weight, number and length of cucumber inoculated with *Moroccan watermelon mosaic virus* at two inoculation stages

	Yield parameters[¥]						
Virus inocula[†]	Inoculation time (Days after planting)	Fruit number	Reduction (%)	Fruit length (cm)	Reduction (%)	Fruit weight (g)	Reduction (%)
MWMV-cor	9	1.75a	66.7	4.40a	51.3	47.68a	41.1
	21	3.35b	36.2	6.38b	29.3	65.07b	17.7
	Control	5.25c		9.03c		81.02b	
MWMV-lag	9	2.00a	61.9	4.33a	51.9	48.17a	43.3
	21	3.15b	40.0	6.68b	25.9	65.53b	22.9
	Control	5.25c		9.02c		85.02c	
MWMV- cor +	9	1.50a	71.4	4.56a	49.5	48.70a	42.7
MWMV-lag	21	3.25b	38.1	6.45b	28.6	66.72b	21.5
	Control	5.25c		9.03c		85.02b	

[†]MWMV-cor = *Moroccan watermelon mosaic virus* (Coccinia strain). MWMV-lag = *Moroccan watermelon mosaic virus* (Lagenaria strain);

[¥]Each value is a mean of 4 replicates. In each column of specific treatment, means followed by the same letter are not significantly different according to Duncan multiple range test. Percentage reduction was calculated by expressing the difference between the control and the treatment as a percentage of the value for the control.

DISCUSSION

The effects of two Nigerian strains of MWMV namely MWMV-cor and MWMV-lag on growth and yield parameters of cucumber were studied in two separate experiments. Plants were inoculated singly and with a combination of both strains at 9 DAP and 21 DAP in a randomized complete block design. Plant inoculated with the buffer only served as controls.

The results showed that the virus strains, singly and in mixed infection, led to significant reduction in nearly all the growth parameters of cucumber when inoculated at 9 DAP and at 21 DAP when compared to the uninfected plants. Greater losses were recorded from inoculation at 9 DAP than at 21 DAP. The results obtained in this study confirm previous reports that virus infection reduces growth performance of crops (32), with greater loses associated with early infections (27, 28, 33, 34).

This study also showed that there were significant reductions in all yield parameters examined at both 9 DAP and 21 DAP compared to the healthy controls. Greater losses occurred when the crop was inoculated early compared to vegetative state (21 DAP) inoculation. Similar observation has been reported in earlier studies investigating the relationship between time of virus infection of plants and yield losses in some cucurbits infected by cucurbit or non-cucurbit viruses. Blua and Perring (23) had documented heavier reduction in marketable fruits of zucchini inoculated with ZYMV during vegetative growth than observed for early flowering stage. Fletcher *et al* (24) have also demonstrated greater and significant losses in the yield of "Delica" variety of *C. moschata* engendered by ZYMW and WMV-2 in single and mixed infection and chicken pea infection by CMV (25) during early season than mid-season. In both cases heavier losses were found associated with early infection than when plants were inoculated at mid and late seasons.

Mixed virus infection of plants often leads to greater disease severity than individual viruses acting alone, a phenomenon known as synergism. Synergism has been demonstrated to cause greater losses in various plant growth parameters such as plant height, weight, and yield compared to single inoculation (35, 36) sometimes lethal, killing off the inoculated plants (36, 37**)**. In such instances the viruses involved were unrelated. The result of the study showed that there was no indication of synergism between the virus strains when cucumber was doubly inoculated with two strains of a virus (MWMV), as the values obtained for the growth and yield parameters at both inoculation ages were comparable with either of the two strains. This could be explained by the submission that stains of the same virus do cross protect (38).

CONCLUSION

In conclusion, the results obtained in this study have provided empirical data that the two MWMV strains pose

serious danger to cucumber cultivation, and particularly so by the more virulent MWMV-cor, which caused comparatively higher yield loses than MWMV-lag. Growers of the crop are at the risk of incurring significant yield losses if infection occurs early during the vegetative growth which could come through transmission by the ubiquitous *A. spiraecola* which has been established as an efficient vector of the virus strains (29). It is recommended that sourcing for and planting of resistance varieties of cucumber, monitoring and control of aphid vectors may be helpful to stem this.

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