# GROWTH AND YIELD RESPONSES OF SWEET PEPPER (*Capsicum annuum* L.) CULTIVARS TO INFECTIONS WITH CUCUMBER MOSAIC VIRUS DISEASE

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## ABSTRACT

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*A screenhouse study was conducted to determine the effects of early and late infections of Cucumber mosaic virus (CMV) disease on the growth and reproductive characteristics in two cultivars of sweet pepper (Cayenne and Yolo Wonder). Each cultivar was evaluated in three independent trials for early infection (infected at 4-leaf stage), late infection (infected at 8-leaf stage), and control (uninoculated) under screenhouse conditions. Sweet pepper seeds were planted in plastic pots using Completely Randomised Design (CRD) with 20 replicates. One hundred percent disease incidence was observed in both early and late infected plants of the two cultivars. In the plants infected at 8-leaf stage, disease severity was suppressed by 36.4 and 23.3% in Cayenne and Yolo Wonder, respectively. Late infection decreased height, leaf diameter, fruit length and fruit weight by 7.1, 6.4, 2 and 15.3%, respectively in Cayenne. Conversely, late infection suppressed these traits by 6.7, 8.2, 16.7, and 17.1%, respectively in Yolo Wonder. This study revealed that early CMV infection of Cayenne and Yolo Wonder pepper was more devastating than late infection. Therefore, pepper farmers should adopt strategies that would prevent early infection of pepper plants in CMV endemic areas so as to ensure appreciable yield.*

**Keywords**: *Sweet pepper; Cucumber mosaic virus; incidence; infections; growth; yield*

## INTRODUCTION

The origin of pepper has been traced to Central and South America (Grubben and El Tahir, 2004) where not less than 30 species have been identified. Of these, the most commonly cultivated are *Capsicum annuum* L., *C. frutescens*, *C. chinense*, *C. pubescens* and *C. baccatum* (Wang and Bosland, 2006). Pepper is cultivated for various reasons ranging from food source and income to medicinal purposes. Fresh pepper is an excellent source of vitamins A, C and dietary fiber. It is now recognized as a high value crop and occupies a pride of place among vegetables because of its delicacy and pleasant flavour (Kurubetta and Patil, 2009). In addition, pepper is rich in minerals like iron, potassium, calcium, magnesium, phosphorus, sodium and selenium. It is consumed in various dishes in mixture with tomato, onion, potato, carrots and other spices.

In West Africa majority of the pepper farmers are concentrated in the rural areas where plots are mostly less than one hectare (Dagnoko *et al*., 2013). The crop is cultivated in all agro-ecologies (Adigun, 2001) both in dry and wet seasons. However, productivity in African countries is usually low, owing to an array of biotic and abiotic stresses. Amongst these, poor soil fertility, weed infestation and diseases are prominent (Idowu-Agida *et al*., 2010). Pepper production is seriously constrained by about 68 viruses (Pernezny *et al*., 2003). Of these, *Cucumber mosaic virus* (CMV) is one of the most economically important as it is capable of reducing fruit yield and quality. *Cucumber mosaic virus* belongs to the genus *Cucumovirus* in the family *Bromoviridae*. It is a tripartite virus with high sequence variability, classified into three subgroups with 80 to 97% identical nucleotides in their coat protein. The virus induces symptoms such as necrosis and severe stunting (Zitter and Murphy, 2009). In susceptible varieties, the leaves become narrow and no longer expand and affected leaves may drop prematurely. However, late infected plants may show foliar mottling or no symptoms.

*Cucumber mosaic virus* is difficult to control partly because it is transmitted by several species of aphid. This insect vector is capable of acquiring the virus shortly after landing on infected plant and quickly transmits it the next time it feeds. Application of insecticide is ineffective since the virus is transmitted in a non-persistent manner. It has also been observed that insecticide sprays make the insect vector to jump from plant to plant thereby resulting in rapid spread of the virus. The use of resistant cultivar is one of the most effective and economically viable control options. However, since it has been confirmed that strategies that would delay infection could provide some level of tolerance to virus diseases (Zitter and Murphy, 2009), this study was conducted to determine the growth and reproductive responses of two sweet pepper cultivars to early and late infections with CMV.

## MATERIALS AND METHODS

### Study location

The experiment was carried out under screenhouse conditions (35 to 40 °C) at the Department of Crop Production, Federal University of Technology, Minna (between latitudes 8° 20´ and 11° 30´ N, and longitudes 3° 30´ and 7° 20´ E of the prime meridian, and 212 m above sea level). Minna is located in the Southern Guinea

Savanna agroecology of Nigeria. The mean annual rainfall is 12000 mm. The rainy season normally begins in April, peaks in September and ends in the first week of October.

**Virus source and multiplication**

The CMV inoculum used was obtained from the stock in the Department of Crop Production, Federal University of Technology, Minna. This initial inoculum was multiplied by mechanical transmission onto healthy cowpea seedlings in order to ensure adequate inoculum for subsequent inoculations. It was accomplished by sowing virus free-seeds of TVU-76 cowpea variety in plastic pots (30 cm in diameter and 30 cm high) containing two kilogrammes of steam sterilized loamy soil. Virus extract for inoculation was prepared by grinding the initial CMV inoculum (CMV-infected leaf) with inoculation buffer. At 10 days after sowing (DAS), the upper leaf surface of each cowpea plant was rubbed with virus extract after dusting with carborundum powder (600 mesh). Symptomatic leaves were harvested at 3 weeks after inoculation (WAI) and stored over non-absorbent cotton wool in airtight vial bottles containing silica gels (5 g per leaf)

**Treatments, experimental design and inoculations**

The two commonly grown sweet pepper cultivars (Cayenne and Yolo Wonder) in Minna were evaluated. Each cultivar was evaluated in three independent trials for early infection (infected at 4-leaf stage), late infection (infected at 8-leaf stage), and control (uninoculated). The experiment was arranged in Completely Randomized Design with 20 replicates. Pepper seeds were sown in perforated plastic pots (30 cm in diameter and 30 cm high) containing two kilogrammes of steam sterilized loamy soil. Seedlings were thinned to three plants per pot at one week after emergence. Virus extract for inoculation was prepared by homogenizing (1g per 1mL) CMV-infected leaves in inoculation buffer (0.1M sodium phosphate dibasic, 0.1M potassium phosphate monobasic, 0.01M ethylene diamine tetra acetic acid and 0.001M L-cysteine per litre of distilled water, adjusted to pH 7.2) using cold sterilized mortar and pestle. The upper leaf surface was dusted with carborundum powder (600-mesh) and 2μL of β- mercapto ethanol was added to the virus extract. A piece of cheesecloth was dipped into the virus extract and then rubbed on the dusted leaf surface. The inoculated plants were rinsed with distilled water and maintained in a screenhouse for symptom development.

**Data collection and analysis**

Pepper plants were observed for disease incidence at 1, 2 and 3 weeks after inoculation (WAI), disease severity (3, 4, and 5 WAI), and growth and reproductive characteristics. Disease severity was based on a scoring scale described by Arif and Hassan (2002): 1 = healthy plant, 2 = slight mosaic 3 = moderate mosaic, 4 = severe mosaic (leaf distortion and stunting) and 5 = severe mosaic (stunting and plant death). Data were subjected to Analysis of Variance at $p \leq 0.05$. Means of significant differences were separated using the Least Significant Difference (LSD). Statistical analysis was performed using PROC GLM of SAS version 9.2 (Statistical Analysis System, 2008).

## RESULTS

**Incidence and severity of *Cucumber mosaic virus* disease**

In the early infected plants, symptoms of CMV disease were first noticed at four days after inoculation (DAI), particularly on Yolo Wonder. In the late infected plants, symptoms were first observed at 6 DAI, also on Yolo Wonder. Early and late infected plants of Cayenne elicited mild mosaic at 1 WAI. At 3 WAI, early infection resulted in 100% disease incidence in both cultivars whereas late infection decreased CMV incidence by 39.5 and 20.1% in Cayenne and Yolo Wonder, respectively (Fig. 1A). In contrast, control (non-inoculated) plants were apparently healthy. At 3 and 4 WAI, disease severity decreased by 25% in the late infected plants of Cayenne while in Yolo Wonder, it was reduced by 8 and 10.7%, respectively (Fig. 1B). At 5 WAI, late infection suppressed CMV severity was suppressed by 36.4 and 23.3% in the late infected Cayenne and Yolo Wonder, respectively.

**Effects of *Cucumber mosaic virus* disease on the vegetative traits**

Non-infected plants were significantly ($p<0.05$) taller than early and late infected plants (Fig. 2). At 3 WAI, early infection of Cayenne reduced plant height by 23.4% while 7.3% height reduction was induced by late infection. In Yolo Wonder, early infection caused 29.1% height reduction which was different from a reduction of 10.6% associated with late infection. Height reduction caused by early and late infections marginally decreased and at 7 WAI, the infected plants of Cayenne exhibited 12.4% reduction in height while late infected plants showed 7.1% height reduction. The early infected plants of Yolo Wonder had 16.5% height reduction, contrary to 6.7% observed in the late infected plants.

Non-infected plants produced broad leaves with normal shape unlike the CMV-infected plants with characteristic narrow leaves. The leaves of the control plants were significantly ($p<0.05$) wider than those of early and late infected plants (Fig. 3). At 1 WAI, early infection of Cayenne reduced leaf diameter by 25% while a reduction of 9.4% was induced by late infection. In Yolo Wonder, the late infected plants showed 28.6% reduction in leaf diameter, contrary to 16.7% observed in the late infected plants. At 7 WAI, early infection of Cayenne reduced leaf diameter by 17%, whereas leaf diameter of the late infected plants decreased by 6.4%. Leaf diameter decreased by 22.5 and 8.2%, respectively in early and late infected plants of Yolo Wonder. At 4 and 5 WAI, there were no significant ($p>0.05$) differences in number of branches per plant of the control, early and late infected plants of both cultivars (Fig. 4). Also, at 6 WAI, there were no reductions in the number of branches from late infected plants of both cultivars. In contrast, number of branches per plant decreased by 66.7 and 75% in the early infected plants of Cayenne and Yolo Wonder, respectively. At 7 WAI, both the control and late infected produced the same number of branches per plant (4 branches) while early infection resulted in 50% reduction in number of branches of the two cultivars.

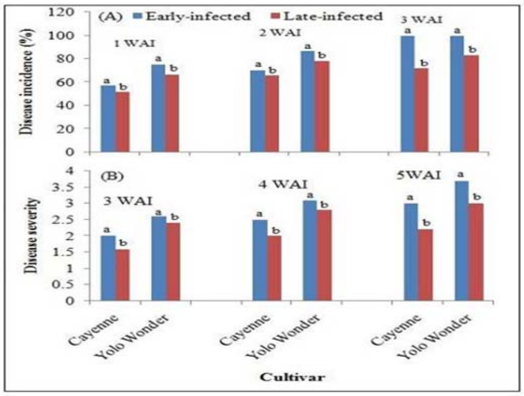


Fig. 1: Disease incidence (A) and severity (B) in pepper plants inoculated with *Cucumber mosaic virus* at 4-leaf stage (early infected) and at 8-leaf stage (late infected) at different weeks after inoculation (WAI) Means of the vertical bars with different superscript letter differ significantly at p≤0.05 according to the Least Significance Difference (LSD)

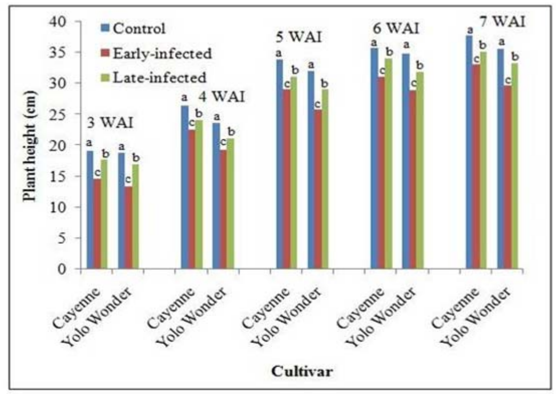


Fig. 2: Height of pepper plants inoculated with *Cucumber mosaic virus* at 4-leaf stage and 8-leaf stage) compared with healthy plants at different weeks after inoculation (WAI); Means of the vertical bars with different superscript letter differ significantly at p≤0.05 according to the Least Significance Difference (LSD)

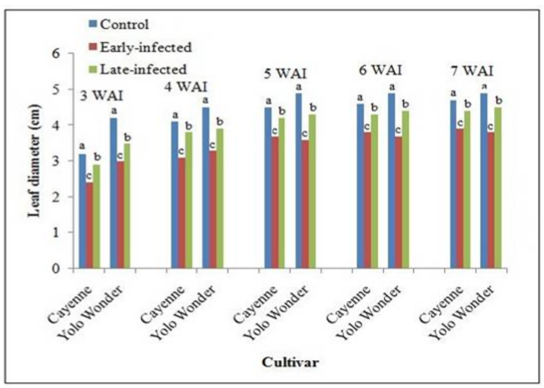


Fig. 3: Leaf diameter of pepper plants inoculated with *Cucumber mosaic virus* at 4-leaf stage (early infected) and 8-leaf stage (late infected) compared with healthy plants at different weeks after inoculation (WAI); Means of the vertical bars with different superscript letter differ significantly at p≤0.05 according to the Least Significance Difference (LSD

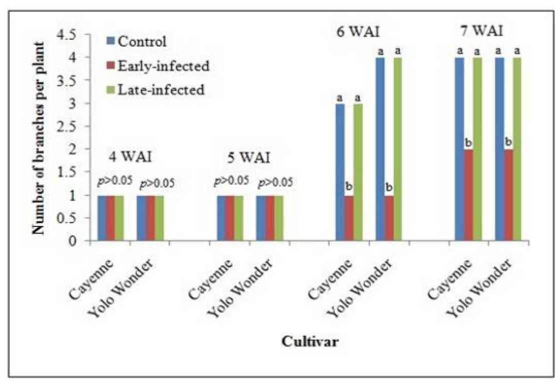


Fig. 4: Number of branches per plant in pepper plants inoculated with *Cucumber mosaic virus* at 4-leaf stage (early infected) and 8-leaf stage (late infected) compared with healthy plants at different weeks after inoculation (WAI). Means of the vertical bars with different superscript letter differ significantly at p≤0.05 according to the Least Significance Difference (LSD

In Cayenne, fruiting of the control and late infected plants commenced on the same day (80 DAS) (Table 1). On the other hand, early infection delayed fruiting for 7 days. In Yolo Wonder, fruiting of the control and late infected plants began at 79 and 80 DAS, respectively, while fruiting commenced at 89 DAS in the early infected plants. The control and late infected plants of Cayenne produced the same number of fruits per plant which was significantly ($p<0.05$) higher than number of fruit from the early infected plants. A similar trend was observed in Yolo Wonder. However, early infection of Cayenne and Yolo Wonder decreased the number of fruits per plant by 42.9 and 66.7%, respectively. The lengths of fruits from non-infected (7.6 cm) and late infected (7.5 cm) plants of Cayenne were similar but significantly ($p<0.05$) longer than the fruits obtained from early infected (5.5 cm) plants. Early and late infections decreased fruit length by 27.6 and 2%, respectively. In Yolo Wonder, fruit length decreased by 26.2 and 2% in the early and late infected plants, respectively. In Cayenne, early infection reduced fruit weight by 44.2%, whereas late infection suppressed it by 15.3%. In Yolo Wonder, early and late infection decreased fruit weight by 46.7 and 17.1%, respectively.

Table 1: Reproductive and yield characteristics of pepper plants inoculated with *Cucumber mosaic virus* at 4-leaf stage (early infected) and 8-leaf stage (late infected) compared with healthy plants

| Cultivar/Treatment | Days to fruiting | Fruits per plant (no.) | Fruit length (cm) | Fruit weight per plant (g) |
|---|---|---|---|---|
| Cayenne | | | | |
| Control | $80^{b}$ | $7^{a}$ | $7.6^{a}$ | $15.6^{a}$ |
| Early infected | $87^{a}$ | $4^{b}$ | $5.5^{b}$ | $8.7^{c}$ |
| Late infected | $80^{b}$ | $7^{a}$ | $7.5^{a}$ | $13.2^{b}$ |
| ±SEM | 0.2 | 0.4 | 0.4 | 0.2 |
| YoloWonder | | | | |
| Control | $79^{b}$ | $6^{a}$ | $6.5^{a}$ | $10.5^{a}$ |
| Early infected | $89^{a}$ | $2^{b}$ | $4.8^{c}$ | $5.6^{c}$ |
| Late infected | $80^{b}$ | $6^{a}$ | $5.7^{b}$ | $8.7^{b}$ |
| ±SEM | 0.4 | 0.4 | 0.2 | 0.2 |

Means followed by common superscript letter along the same column are not significantly different at $p \leq 0.05$ according to the Least Significance Difference (LSD)

## DISCUSSION

Symptoms of disease appeared earlier in the early-infected plants due to low level of resistance at that growth stage. The intensity of symptoms manifested in the early infected plants also confirmed that infection at the early growth stage was more devastating than late infection. Similar observation was made by Chung *et al*. (2015) when Chinese cabbage (*Brassica campestris*) plants were inoculated with *Turnip mosaic virus* (TuMV). However, the fact that both the early and late infected plants elicited symptoms of CMV revealed the destructive nature of the pathogen. It also implied that both varieties were susceptible to infection although Cayenne variety appeared to be more tolerant when infected at later growth stage. The susceptibility of Yolo Wonder to CMV agrees with the findings of Nono-Womdim *et al*. (1991) when some pepper lines were infected with the virus. The height of early infected plant was drastically reduced as a consequence of the severity of virus infection. This was possibly caused by virus interference with plants' physiological processes (Senthil *et al*., 2005). Growth is attained through cell enlargement, division and differentiation, which are most sensitive to physiological processes in stressed condition being expressed in diseased plants (Mushtaq *et al*., 2014). Taiz and Zeiger (2010) reported that severe viral symptoms inhibited plant growth by obstructing the flow of water to the adjacent elongating cells from the xylem. Plant height was more adversely affected in the early than late-infected plants due to impairment of roots and vascular systems of the diseased plants (Dufour *et al*., 1989). The same reason might hold for the effects of early and late infections on leaf diameter. However, in the early infected plants, leaf diameter was more prone to deleterious effect of CMV probably due to invasion of chloroplast and retarded photosynthesis.

Balachandran *et al*. (1994) reported that leaves were deformed and failed to expand due to the destruction of chloroplasts in yellow leaf areas of *Nicotiana tabacum* cv. *xanthi* infected with *Tobacco mosaic virus* (TMV). Reductions in morphological traits of the diseased plants decreased with increase in plant age probably because virus attack activated defense mechanism of the plants. Upon entry of the virus, biochemical compounds in the host-plants were triggered, which resulted in continuous battle (Pierce and Rey, 2013). As plants advanced in age, their innate defense mechanisms provided better protection and possibility to overcome cell-to-cell movement of the pathogen. Studies have shown that in host – pathogen interaction, a resistant genotype is able to prevent virus replication and establishment, whereas a susceptible variety succumbs to infection (Whitham *et al.,* 2006). The lower values observed with respect to growth characters in the infected plants were due to the deleterious impact of the virus, which corroborated the findings of Kollmann *et al*. (2007). The cumulative effects of early infection on plants' vegetative traits probably resulted in poor yield and severe losses at harvest (Mushtaq *et al*., 2014).

Fruiting was delayed in the early infected plants probably as a consequence of virus – host plant interaction. This possibly arose from the fact that infected plants expended more time and energy to overcome the stress imposed by the pathogen. Herms and Mattson (1992) reported that the stresses imposed by pathogens could induce a complex cellular and molecular response system to prevent damage and ensure plant's survival. However, in most cases such defense response is at the detriment of growth and yield. The late-infected plants produced significantly more and longer fruits than early infected plants probably due to combined effects of poor growth and underutilization of growth resources in the latter. The lower number of fruits and fruit weight observed in virus-infected plants supported the findings of Damiri (2014) in which some pepper plants were evaluated under CMV, TMV and *Potato virus Y* (PVY).

## CONCLUSION

This study revealed that early CMV infection of Cayenne and Yolo Wonder pepper was more devastating than late infection. Therefore, strategies that would delay infection in CMV endemic areas could guarantee tolerance and appreciable yield.

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