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Influence of planting date on incidence and severity of viral disease on cucurbits under field condition

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ABSTRACT

Three field experiments were conducted to assess the effect of planting date on the incidence of viral diseases and the severity and the susceptibility of the cultivars. Two cultivars of cucumber (Hybrid Tokyo F1 and Poinsett) and one local variety of zucchini (Bolle) were used for the evaluation in May-July 2014, September-November 2014, and February-April 2015. A randomized complete block design with three replications was used for the experiments. Data were collected on disease incidence, severity, and time until first symptoms occurred. Area under severity index progress curve (AUSIPC) and area under disease progress curve (AUDPC) were calculated respectively for disease severity as well as the incidence on each cultivar. The results demonstrate the susceptibility of all cultivars to the tested viral diseases. The effect of planting dates on cultivars was significantly different ($P < 0.05$) at the different growing stages whereas there was no significant difference ($P > 0.05$) in planting date-variety interaction.

Introduction

Viral diseases are the major problem of cucurbits production in Ivory Coast. *Cucumber mosaic virus* (CMV) and *Zucchini yellow mosaic virus* (ZYMV) have been reported as the most prevalent viruses in these crops (Fauquet and Thouvenel, 1987; Koné et al., 2016; Agneroh et al., 2012; Kone, 2016). They are transmitted by aphids in a non-persistent manner. These viruses cause mosaic, mottling, enation, and puckering of foliage; mosaic and distortion of fruits; and plant stunting (MacNab et al., 1983). Significant yields losses due to ZYMV infections have been reported, ranging from 50 to 94% (Blua and Perring 1989; Müller et al., 2006).

Hence, management of these viral diseases is of utmost importance in order to safeguard yields of cucurbit crops in Ivory Coast. Various strategies have been employed in the management of cucurbit viral diseases, including removal of weeds and volunteer cucurbit crop plants (Sharma et al., 2016), use of super reflective plastic mulch (Stapleton and Summers, 2002; Barbercheck, 2014); the use of beneficial insects to control aphids (Kos et al., 2008), and the application of insecticides (Sharma et al., 2016). However, all these methods have not been very effective in the management of viral diseases. One reason is that the aphid populations developed resistance against the frequently applied insecticides. In addition, non-persistently transmitted viruses can already be transmitted before the aphid vector is killed by the insecticide (Jayasena and Randles, 1985; Maelzer, 1986; Simons, 1957; Webb and Linda, 1993). A limitation for the successful us of reflective films in cucurbits has been that plant growth rapidly covers the mulch and thereby lessens reflectivity (Damicone et al., 2007).

The most effective and simplest strategy of controlling viral diseases is growing resistant varieties. Breeding for host resistance is difficult due to the incompatibility among different cucurbit species (Zitter and Murphy, 2009). Therefore, evaluation of other cultural methods to control viral diseases such as the planting date could help to develop alternative strategies (Hull, 2013). It is

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known that the planting date is important in tropical climates (Hull, 2013). Planting date based strategies have been successfully used in the avoidance or management of tungro virus disease in rice (Manwan, 1987) and to control of groundnut affecting viruses in African countries by sowing groundnut at the start of the main rainy season (Hull, 2009). However, adequate information on the use of specific planting dates in managing viral diseases in cucurbits in Ivory Coast is lacking. Therefore, in this study we attempted to evaluate the effectiveness of different planting dates for the management of virus diseases in cucumber and zucchini.

Materials and methods

Study site

The field study was conducted in Dabou-Agneby, situated 45 km west of Abidjan. This location (04°16 W; 05°16 N) experiences two rainy seasons interrupted by two dry seasons. The long rainy season spans from February to June while the short rainy season is from September to November. The annual mean rainfall is between 1500 and 1600 mm distributed through the year. The area has sub-equatorial climate characterized by hot and humid weather, with temperatures varying around 28° C and a relative humidity of 85% (Table 1). The vegetation is of coastal type dominated by small mangroves (Avenard et al., 1971; Comoé et al., 2009).

Plant material

Two cucumber varieties (Tokyo hybrid F1 and Poinsett) obtained from a seed- shop and a farmers' preferred variety of zucchini ("Bolle", local variety) obtained from a local market were used for this study.

Experimental design and field layout

A randomized complete block design with three replications was used. The treatment comprised three planting dates (May 2014, September 2014 and February 2015) and two cucurbits (cucumber and zucchini). The field was divided into three blocks with three plots of 20 plants of each cultivar. Plots were 1 m x 10 m and each contained two 5-m long rows of cucumber or zucchini spaced by 60 cm. Sowing was done with 2 seeds per hill at intra-row spacing of 40 cm and inter-row spacing of 60 cm. The number of plants per hill was later reduced to one plant when seedlings reached the two leaves stage. Manure was incorporated into the soil prior to sow cucumber and zucchini.

Table 1
Microclimate of the site during the three trials.

Trial	Month	Temperature (°C)	RH (%)	Rainfall (mm)
Trial 1: 2014	May	26.4	86.6	102.3
	June	25.4	88.8	174.1
	July	27.1	87.2	260.6
Mean		26.3	87.5	179
Trial 2: 2014	September	26.3	83.7	20.57
	October	27.7	80.57	39.83
	November	26.13	81	93.33
Mean		26.71	81.75	51.24
Trial 3: 2015	February	27.1	84	16
	March	28	78	0
	April	25.6	90.6	3.2
Mean		26.9	84.2	6.4

Cultural practices

Granular fertilizer (12-22-22 kg/ha N-P-K) was incorporated two weeks after sowing (WAS). Compost was applied 28 days after germination at a rate of 10 L per 10 m^2 plot. The plots were watered when necessary. Fungicide Mancozan Ivory 80 WP (content: 800 g/kg), Callicuivre (content: 50%) (fungicide available in Ivory Coast) and the nematicide Diafuran 5G (Carbofuran: 5%) were applied with the doses recommended by the manufacturer on the plants to prevent fungial and nematode infections. The insecticides K-optimal 35 EC (Content: Lambda-cyhalothrine: 15 g/l, Acétamipride : 20 g/l), and Decis 12 EC (active molecule: Deltaméthrine: 12.5 g/l) were applied twice per week, starting one week after sowing when the first insects (aphids and whiteflies) were observed on the plants.

Data collection in the field

Data were collected on disease incidence and severity, the occurrence of the first symptoms and yield.

Disease incidence was determined based on the symptoms on diseased plants.

The proportion of diseased plants was estimated by: **IC** $= \frac{n}{N} \times 100$ (IC = incidence; n = number of diseased plants; N = total number of plant assessed).

The severity index of the disease described the damage caused by the diseases on plants leaves. A modified 0–5 visual scale of Nelson et al. (1999) and Steel and Torrie (1980) based on disease symptoms, was used to score the diseased plants as follows: 0: No disease symptoms; 1. Mild mottling on 10% of leaf area; 2. Mottling on 50% of leaf area/light downward cupping; 3. Pronounced downward or up cupping of leaf/chlorosis/75–100% leaf mottling; 4. Severe mosaic/severe distortion of leaf/crinkled leaf/stunting of entire plant/leaf bunching; 5. Severe leaf distortion/necrosis/narrowed or shoes-string leaf.

Disease severity index was then determined for each treatment using the formula according to Nelson et al. (1999) and Steel and Torrie (1980) as shown below:

$$\text{Disease Severity Index (DSI)} = \frac{0*P0+1*P1+2*P2+3*P3+4*P4+5*P5}{N(G-1)} \times 100$$

Where P_0 to P_5 = Total number of observed plants in each disease symptom grading per farm site in each state within the agro ecological zone surveyed.

G = Number of grading = 6 and N = Total number of observations.

Areas under disease progress curve

The areas of disease progress on the cultivars or varieties were calculated using the incidence and disease severity index.

Thus, the Area under severity index progress curve (AUSIPC) for disease severity was calculated using the modified formula described by Shaner and Finney (1977) as below:

AUSIPC = $\sum_{n=1}^{n-1}(\text{DS1} + \text{DS2}/2)\text{x}(\text{t2} - \text{t1})$ where, DS1 is disease severity recorded in time 1 and DS2 the disease severity recorded in time 2.

Area under disease progress curve (AUDPC) for disease incidence was calculated using the formula described by Muengula-Manyi et al. (2013):

AUDPC $\sum_{i=1}^{n}[(\text{Xi} + \text{X}(\text{i}+1)/2)]\text{x}(\text{t})$ where, Xi is the incidence of disease at time i, Xi + 1 is disease incidence recorded at the time i + 1, n, the number of registration on the incidence, and t, days between the registration of Xi and Xi + 1.

Data analysis

Mean disease incidence and severity index data were tested for homogeneity of variance before analyses. They were transformed using arcsine transformation described by Legendre and Legendre (1998), in order to homogenize the variances. Data were subjected to the analysis of variance (ANOVA) and the mean effects were separated by least significant differences (LSD) method at 5% level of probability using GenStat Discovery version 4 (VSN International).

Results

Incidence of disease

The field evaluation on the effect on planting date and cultivar on the mean incidence of virus disease at different growth stages showed interesting results (Table 2). ANOVA showed significant difference among the planting dates in terms of mean disease incidence at 14 DAS ($F = 4.12$; $df = 2$; $P = 0.036$); 28 DAS ($F = 7.37$; $df = 2$; $P = 0.005$); 42 DAS ($F = 11.58$; $df = 2$; $P < 0.001$) and 56 DAS ($F = 13.53$; $df = 2$; $P < 0.001$).

Disease incidence on cucurbit varieties increased with the growth stage. At the final growth stage (56 DAS), mean incidence recorded for planting dates 2 and 3 (90% each) was not significantly different ($p > 0.05$) between them but significantly higher than the panting date 1 (73%).

ANOVA also showed significant difference ($P < 0.05$) in incidences among the cultivars at all growth stages (Table 2). At the final growth stage (56 DAS), the mean incidence recorded for Bo (84.9%) was not significantly different from P7 but significantly higher than TF1 (54.2%).

Disease severity

ANOVA on the effect of planting date on mean disease severity index showed significant difference among them at 14 DAS ($F = 3.99$; $df = 2$; $P = 0.039$); 28 DAS ($F = 29.97$; $df = 2$; $P < 0.001$); and 56 DAS ($F = 72.06$; $df = 2$; $P < 0.001$) but was not significant at 42 DAS ($F = 0.01$; $df = 2$; $P = 0.98$) as shown in Table 3. At 56 DAS, the mean severity index recorded for planting date 2 (82%) was significantly higher ($P < 0.05$) than that of the planting date 1 (51.5%) and planting date 3 (52.1%).

The mean disease severity recorded for the different cultivars was not significantly different at 14, 42 and 56 DAS but was significantly different at 28 DAS. In general, disease severity increased steadily from 19.7% at 14 DAS to 62% at 56 DAS, indicating that disease severity increased with growth stages. At the end of the observations (56 DAS), the variation of disease severity showed the highest value of 82% that was recorded in minor season (T2) whereas the lowest value of 51.5% was recorded in major rainy season (T1). However, this was not significantly different from that one registered in dry season (T3) with a value of 52.1%.

Disease incidence among the cultivars was not significantly different ($P > 0.05$) at 28 and 56 DAS, but differed significantly among them ($P > 0.05$) at 14 and 42 DAS. At 42 DAS, mean incidence on cultivar P7 was not significantly different from that of TF1 but was significantly higher ($P < 0.05$) than for Bo.

Areas under disease progress curve using incidence

ANOVA for the AUDPC values based on disease incidence showed no significant difference ($P > 0.05$) among the planting dates as well as the cultivars (Fig. 1). This suggests that the disease has spread at all planting dates and then all the cultivars were affected by the virus disease. However, with respect to Bolle cultivar, planting date 1 had the highest AUDPC value whilst planting date 2 had the lowest. In respect of Poinsett cultivar, planting date 2 had the highest AUDPC value whilst planting date 1 had the lowest, and in Tokyo F1 cultivar, planting date 2 had the highest AUDPC value whilst planting date 1 had the lowest.

Table 2
Mean disease incidence in three varieties of cucurbits at different growth stages.

Planting date	14 DAS				28 DAS				42 DAS				56 DAS			
	TF1	P7	Bo	Mean	TF1	P7	Bo	Mean	TF1	P7	Bo	Mean	TF1	P7	Bo	Mean
1 (May 2014)	13.7	1.6	36.2	22.2 a	35.3	40.7	54	43.3 b	46	58.7	67.0	57.4 b	72.5	72.8	74.6	73.3 b
2 (Sept, 2014)	18.9	0.0	19.9	12.9b	69.0	90.0	34.9	64.7a	77.3	90.0	37.1	68.2 b	90.0	90.0	90.0	90.0 a
3 (Feb 2015)	19.3	8.6	15.0	14.3 b	45.6	46.2	34.0	41.9 b	73.9	90.0	90.0	84.2 a	90.0	90.0	90.0	90.0 a
Mean	17.3a	8.4c	23.7 a	16.46	50.0b	59.0a	41.0b	50.0	65.7ab	79.6a	64.8ab	70.0	54.2 b	84.3 a	84.9 a	84.43

Means in the same row within a growth stage bearing the same letters are not significantly different by LSD at 5% level of probability.
Means in the same column within a growth stage, bearing identical letters are not significantly different by LSD at 5% level of probability.
TF1: Tokyo F1 hybrid; P7: Poinsett; Bo: Bolle.
DAS: Days after sowing.

Table 3
Mean disease severity index on different cucurbits planted at different dates at different growth stages.

Planting date	14 DAS				28 DAS				42 DAS				56 DAS			
	TF1	P7	Bo	Mean	TF1	P7	Bo	Mean	TF1	P7	Bo	Mean	TF1	P7	Bo	Mean
1	26.6	30.1	29.4	28.7 a	39	38.7	41.3	39.7b	44.6	42.7	46.9	44.7a	52.2	49.8	52.5	51.5b
2	21.1	10.0	15.0	15.4 b	46.9	49.2	28.6	41.6a	51.6	54.3	27.3	44.4a	90.0	90.0	65.9	82.0a
3	16.9	12.3	15.7	15 b	24.4	24.7	21.6	23.6c	34.4	47.1	52.9	44.8a	43.1	52.6	60.5	52.1b
Mean	21.5a	17.5a	20.0a	19.7	36.8a	37.5a	30.5b	34.96	43.5b	48.0a	42.4c	44.63	61.8a	64.1a	59.6a	62

Means in the same row within a growth stage bearing the same letters are not significantly different by LSD at 5% level of probability.
Means in the same column within a growth stage, bearing identical letters are not significantly different by LSD at 5% level of probability.
TF1: Tokyo F1 hybrid; P7: Poinsett; Bo: Bolle.
DAS: Days after sowing; Data on the severity index was arcsine transformed before ANOVA was done.

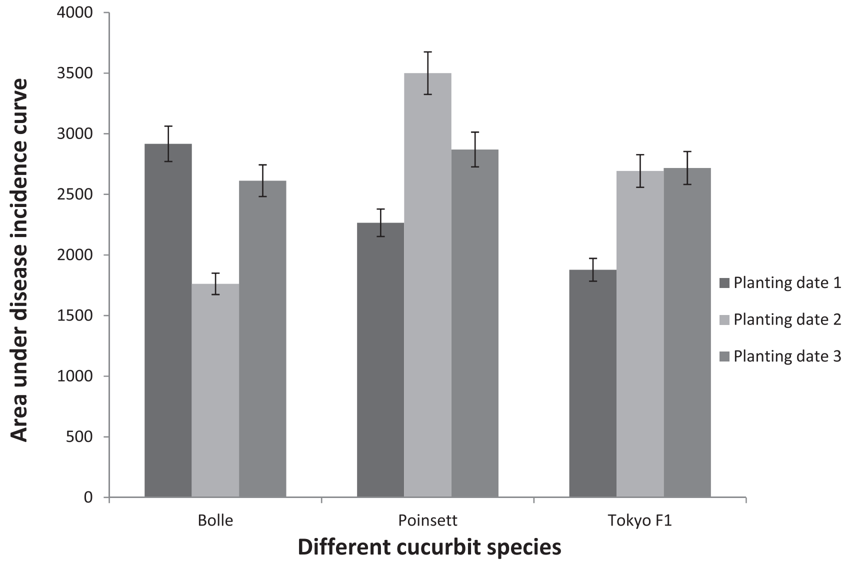

Fig. 1. Area under disease progress curve using disease incidence on zucchini cultivar (Bolle) and cucumber cultivars (Tokyo F1 and Poinsett) during three planting dates.

Areas under severity index progress curve

The total amount of disease that occurred on each variety at the different planting dates was calculated and expressed as the AUSIPC as shown in Fig. 2. ANOVA showed a significant difference among the planting dates (F = 9.30; df = 2; P = 0.002) and the planting date - variety interaction effects (F = 7.32 df = 4; P = 0.002), but was no significantly different among the varieties (F = 1.76; df = 2; P = 0.205).

AUSIPC value for the planting date 1 was significantly higher ($P < 0.05$) than planting dates 3 which was significantly higher than planting date 2. In Poinsett cultivar planting date 2 had significantly higher values of AUDSIC than planting dates 1 and 3. Similarly, AUDSIPC values recorded for Tokyo F1 cultivar at planting date 2 was significantly higher ($P < 0.005$) than that of planting date 1, which was also significantly higher than planting date3 (Fig. 2).

Discussion

This study demonstrated the susceptibility of all cucurbit cultivars used in this field experiments to virus infections. Even though the zucchini variety showed the first symptoms on the leaves (planting dates 2 and 3), the incidences between the varieties were the same after 56 DAS. The occurrence of the first symptoms was approximately between 10 and 14 DAS. Assuming that symptoms already occur one week after infection, this means that virus transmission must have taken place already when seedlings were just one week old. Consequently, insecticides need to be applied immediately after emergence or already to the seed coat before sowing if systemic insecticides are used. The disease incidence was low in planting date 1 corresponding to the major rainy season and high in planting dates 3 and 2, corresponding to the minor and dry seasons, respectively. The low infection rate during the planting date 1 could be due to the fact that during this period the high rainfall

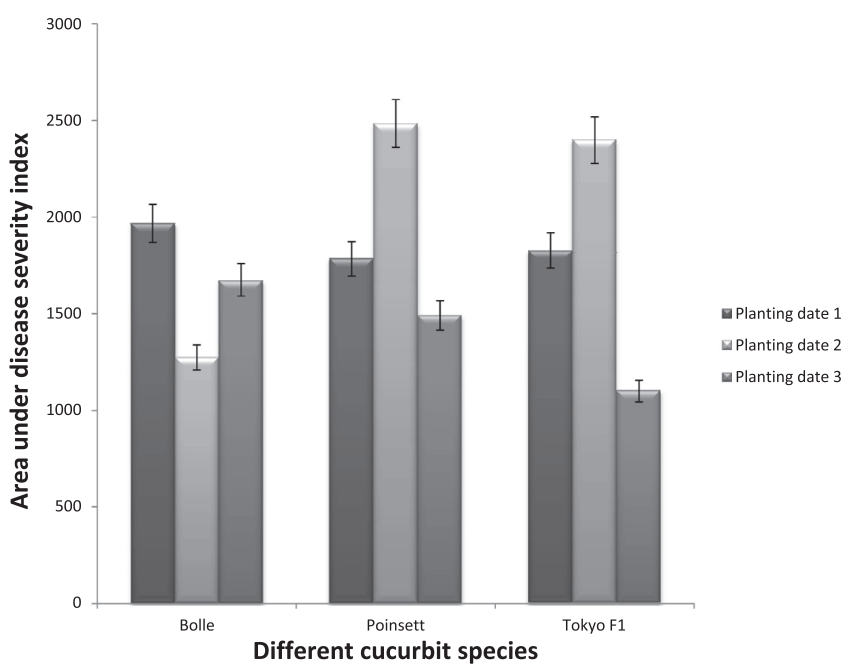

Fig. 2. Area under disease progress curve using disease severity index on zucchini cultivar (Bolle) and cucumber cultivars (Tokyo F1 and Poinsett) during three planting dates.

could negatively affect vector populations and their mobility preventing the spread of the viral disease. The planting dates 2 and 3 coincided with the period of moderate rainfall (100–200 mm) coupled with warm and humid weather which could be favourable for vector development and spread, resulting in increased disease development. The increase of the disease may be a secondary development of virus disease in the field (Vanderplank, 1963; Astier et al., 2001; Hull 2009).

The study has demonstrated a varied host-virus-environment interaction effect in terms of AUDSIPC, as have been reported by other scientists (Schrag and Wiener, 1995; Woolhouse and Gowtage-Sequeria, 2005; Barrett et al., 2008; Engering et al., 2013). For instance, the highest AUDSIPC were recorded for Porsette and Tokyo F1 cultivars during planting date 1, whilst Bolle had the lowest during the same period (Fig. 2). It has been reported that host-environment and disease ecology are key to creating novel transmission pattern (Engering et al., 2013).

Disease incidence increased with the increase in growth stage. This suggests that the susceptibility of the plants to the virus infection was enhanced when the plants became older. This observation is in contrast to the finding of Fargette et al. (1993) on the pathosystem cassava-ACMV where the susceptibility of aging cassava plants to virus infection reduced with age. It was reported elsewhere (Gibbs and Harrison, 1974; Matthews, 1991; Silhavy et al., 2002) that an increase of infection rate may depend on aphids' preference. In fact, the concentration of solutes in plant sap has an attracting effect on aphids. It has also been reported that populations of aphids can reach pest proportions (caused damage) rapidly, producing parthogenetic generations, where the females can be able to produce eggs without sexual cycle (Blais et al., 2003). Consequently, if the asexual generations are produced during the planting dates 2 and 3, that can contribute to the increase of disease development because of the abundance of aphids.

Farmers' agricultural practices have a major impact on viral infections in many crops (Hull, 2009). The farming practices such as inadequate or lack of fertilizer application and the presence of weeds and volunteer cucurbit plants within and around the fields favour development of virus diseases (Anderson et al., 2004; Jones, 2009). Overlapping cropping seasons where the old maturing crop constitutes the virus reservoirs for the newly planted one are also critical (Thresh, 2003; Hull 2009). In addition, irrigation of plants by spraying might also increase development of virus diseases (Afouda et al., 2013).

High infection rates may also be due to the use of unimproved seeds (locally cultivated varieties or non-resistant variety) by farmers (Ayo-John et al., 2014). This facilitates the survival and perennation of viruses and their vectors, and can provide a 'green bridge' between successive growing seasons (Hull, 2002). Seed transmission, although the infection rate is known to be low for most viruses, may be another source of infection. Approximately 20% of viral plant pathogens are known to be seed-transmitted, and possibly one-third will eventually be shown to be transmitted in this manner (Johansen et al., 1994). In particular, seed transmissibility has been demonstrated for species of the family *Potyviridae* (e.g. ZYMV) (Simmons et al., 2011). For ZYMV, the seed to seedling transmission rate has been calculated to be ca. 1.6% (Simmons et al., 2011).

Conclusions and recommendation

The variation of incidence and severity index with changing weather conditions give evidence that the physical factors through the planting dates have an influence on disease incidence and severity. The susceptibility to virus diseases of the cucurbit varieties grown in Ivory Coast indicates the need to look for improved varieties to minimize yield losses and safeguard a sustainable cucurbit production. However, this study underlines that the planting date is an important factor which should be taken into account when developing an integrated strategy to manage viral diseases.

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