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Seasonal and spatial variation in the prevalence of viral diseases and associated aphid-borne viruses in cucurbits in Cote d'Ivoire

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ABSTRACT

Viral diseases are a major constraint to cucurbit production worldwide. In 2014 rainy and dry cropping seasons, cross-sectional surveys were conducted in farmers' cucurbits fields in six agro-ecological zones (AEZs) of Côte d'Ivoire to assess prevalence and distribution of viral diseases. A total of 757 leaf samples were collected from melon, squash, cucumber, watermelon, gourd calabash, and pumpkin plants showing various virus-like symptoms. Enzyme linked immunosorbent assay (ELISA) using antisera against cucumber mosaic virus (CMV), zucchini yellow mosaic virus (ZYMV), papaya ringspot virus (PRSV), watermelon mosaic virus (WMV) and Moroccan watermelon mosaic virus (MWMV) were performed to detect viruses in the samples collected. Results revealed that viral diseases were prevalent in all the agro-ecological zones, with significantly higher mean prevalence in the dry season (53.6%) than in the rainy season (29.35%). The overall mean disease severity index was also significantly higher in the dry season (47.58%) than in the rainy season (28.17%). CMV, ZYMV and PRSV were found in all AEZs at varying rates between the rainy and dry seasons, whereas WMV and MWMV were not detected. CMV was more prevalent in the dry season (32%) whereas ZYMV was more prevalent in the rainy season (34%). This study has revealed that viruses infecting cucurbits are widespread and occurred in mixed infection which poses a potential threat to cucurbit crops production in Cote d'Ivoire.

Introduction

Cucurbits are well-known vegetables in West Africa because of their nutritive value and the incomes they generate. Indeed, cucurbits are rich in ingredients such as vitamins (A, B, C) and other dietary substances including protein, lipid, carbohydrates, mineral salts (calcium, iron, phosphorus) and lycopene having an antioxidant activity (Ozaslan et al., 2006). Cultivation of cucurbits ensures a substantial income to the farmers (Dje Bi et al., 2011).

Pests and diseases are major biotic constraints to the production of cucurbits worldwide (Loebenstein and Thottappilly, 2009). Among these, plant viral diseases are the most important limitation to cucurbit production (Lecoq and Katis, 2014; Ayo-John et al., 2014), causing physiological disorders and enormous losses all over the world in terms of quantity and/or quality of products (Nicaise, 2014). It is reported that up to 39 well characterized viruses of the genera *Begomovirus, Crinivirus*, *Polerovirus*, *Cucumovirus*, *Ipomovirus*, *Tobamovirus*, *Tospovirus* and *Potyvirus* are known to naturally infect cucurbits (Antignus et al., 2001; Knierim et al., 2010). In West Africa, viruses infecting cucurbits are reported in Nigeria (Ayo-John et al., 2014), Mali (Tsai et al., 2010) and Côte d'Ivoire (Fauquet and Thouvenel, 1987; Koné et al., 2010; Agneroh et al., 2012; Kone et al., 2015). Aphid-borne CMV, ZYMV, PRSV and pepo aphid-borne yellows virus (PABYV) infecting cucurbits have been found in Cote d'Ivoire (Fauquet and Thouvenel, 1987; Koné et al., 2010; Agneroh et al., 2012; Kone et al., 2015). These viruses are efficiently transmitted by several species of aphids notably *Myzus-persicae*, *Aphis gossypii*, *Macrosiphum euphobiaceae*, in a non-persistent manner, and are readily transmitted through mechanical activities (cell sap) (Franki et al., 1979; Gal-On, 2007).

Management of viral diseases is very important in order to improve yields and quality of cucurbits in Côte d'Ivoire. Information on viruses, their host plants and their prevalence at different cropping seasons across the various agro-ecological zones is an important prerequisite in developing effective control strategies. Such information is however limiting in Cote d'Ivoire. Fauquet and Thouvenel (1987) reported the presence of CMV and PRSV; on cucumber and squash. The study

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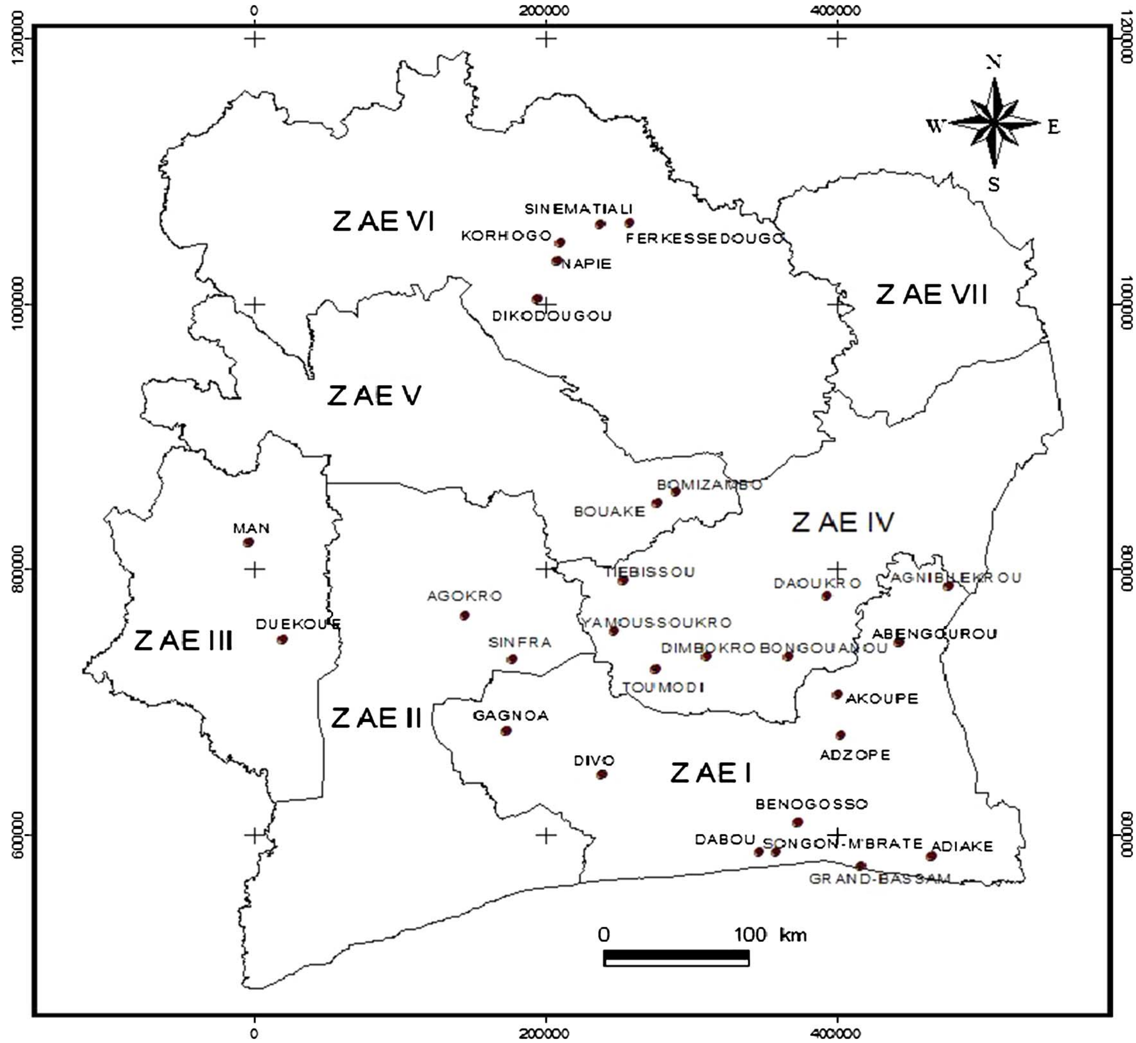

Fig. 1. Map of Côte d'Ivoire showing the six agro-ecological zones (AEZ) surveyed with sampling sites. AEZ = ZAE (Zone agro-ecologic) numbered from I to VI.

conducted by Koné et al. (2010) was in rainy season in the southern part of Côte d'Ivoire where ZYMV was reported for the first time in cucurbits. The study done by Agneroh et al. (2012) was conducted in three districts of the country and involved only two cucurbit species, *Lagenaria siceraria* and *Citrullus* sp. In view of these limitations, this study was conducted to assess the prevalence of virus disease in cucurbit crops during rainy and dry seasons in six out of seven agro-ecological zones (AEZs) in Cote d'Ivoire, and to identify the associated aphid-borne viruses.

Material and methods

Study areas

The study was conducted in 28 districts across six AEZs in Côte d'Ivoire (Fig. 1). The features of the various AEZs are shown in Table 1.

Field survey, data collection, sampling

The survey was conducted on several cucurbit crops including cucumber, squashes (zucchini and pumpkin), watermelon, melon and gourd (lagenaria) between February and August 2014. The dry season survey was done between February and March 2014, while that of rainy season was carried out between June and August 2014. Twenty-eight districts diverse in their climate and conditions under which cucurbits crops are grown were surveyed, and fifty-eight fields selected at intervals of 5 km were assessed. In each district, the fields were surveyed in a vicinity of 10–30 km.

Cropping patterns and field sanitations with regards to weedy status of the fields surveyed were observed and recorded.

Table 1
Features of the agro-ecological zones surveyed in Cote d'Ivoire in 2014.

Agro-ecological zones	Altitude (m)	Mean annual temperature (°C)	Mean annual rainfall (mm)	Vegetation
AEZ I	9–225	29 (5.6)	1400–2500	Humid dense forest of south
AEZ II	215–240	23.5(13.4)	1300–1750	Semi-deciduous forest
AEZ III	241–340	24.5 (7.7)	1300–2300	Humid green forest of West
AEZ IV	87–193	23.5(13.4)	1300–1750	Transitional, forest-savanna zone
AEZ V	243–322	23.5 (13.4)	1300–1750 (unimodal)	Transitional Savannah zone
AEZ VI	235–378	26.7(1.1)	1150–1350 (unimodal)	Savannah

Estimation of disease prevalence and severity

Plants were randomly evaluated for virus-like symptoms such as vein clearing, vein-banding, mosaic, and mottling, puckering, leaf and fruit malformation. About 10 plants were sampled per field for symptom scoring and disease prevalence. Disease prevalence (DP) was estimated for each farm based on 10 plants found along a 10 m diagonal transect set at the middle of the farm by determining the number of diseased plants expressed as a percentage of the total number of plants along the diagonal transect in the delimited area.

$$\mathrm{DP}(\%) = \frac{n}{N} \times 100$$

Where, n is the number of plants showing symptoms; N is the total number of plants assessed.

The plants were also scored for disease severity using a modified visual scale of 0–5, based on disease symptoms (Steel et al., 1980; Nelson et al., 1999), where 0 represents no disease symptom; 1, mild symptom on 10–25% of leaf surface; 2, mottling symptoms on 50% onset of mosaic; 3, chlorosis/leaf rolling/onset of downward and upward cupping; 4, severe symptoms; and 5, very severe symptoms/abnormal growth/dead of plant.

The disease severity index (DSI) was then determined for each field according to the formula (Steel et al., 1980; Nelson et al., 1999):

$$\text{Disease Severity Index (DSI)} = \frac{0*\mathrm{p0} + 1*\mathrm{p1} + 2*\mathrm{p2} + 3*\mathrm{p3} + 4*\mathrm{p4} + 5*\mathrm{p5}}{\mathrm{N(G{-}1)}} \times 100$$

where P0–P5 is the total number of observed plants in each disease symptoms grading per farm in each state within the agro-ecological zones (AEZs) surveyed, G = Number of grading = 6, N = Total number of observations.

Samples collection for virus indexing

Symptomatic cucurbit leaf samples were collected from 58 fields randomly selected from 28 districts across the AEZs. Between three-five young leaves were taken from each plant sampled. Both annual and perennial weeds which could serve as alternative host of cucurbit-infecting viruses were also collected and tested. A total of 757 samples were collected based on virus symptoms in order to identify viruses associated with them. The samples were dried over calcium chloride and stored at 4 °C until they were used.

Identification of viruses using DAS-ELISA

Virus identification was carried out using standard double antibody sandwiched enzyme-linked immunosorbent assay (DAS ELISA) as described by Clark and Adams (1977) using polyclonal antisera raised against ZYMV, CMV, PRSV, WMV and MWMV (DSMZ, Braunschweig, Germany). The dried leaf samples were homogenized (dilution 1:50 g/v) in extraction buffer (8.0 g NaCl, 0.2 g KH_2PO_4, 1.1 g Na_2HPO_4, 0.2 g KCl/L, pH 7.4) containing 0.05% v/v Tween 20, and 2% w/v polyvinyl-pyrolidone.

Each microtiter plate was coated with one of MWMV, CMV, WMV, PRSV, and ZYMV antisera. The IgG antibodies were diluted in the coating buffer ($Na_2CO_3 + NaHCO_3 + NaN_2$) at a recommended dilution of 1:1000 according to the manufacturer's instructions. Hundred microliters of the dilution was distributed in the wells and incubated at 37 °C for 3 h. The microplates were washed thrice with the phosphate buffer saline-Tween 20 (PBS-T). The homogenized leaf samples were added and incubated overnight at 4 °C.

After washing the plates three times with PBS-T, they were incubated with the enzyme conjugate (alkaline phosphatase conjugate, diluted at 1/1000 in PBS-T + BSA + NaN_2) at 37 °C for 2 h. After washing the plates three times with PBS-T, they were incubated for one hour at room temperature with freshly prepared phosphate substrate solution (100 μL per well). The substrate was p-nitrophenyl phosphate (pNPP) tablet (Sigma-Aldrich Co. LLC) applied at 1.0 mg Ml^{-1} in 9.87% diethanolamine, pH 9.8.

The absorbance values at 405 nm were measured using Anthos microplate reader (Biochrum Ltd, Cambridge, UK). Absorbance values of three uninfected leaf samples were measured. All samples were tested in two replicate wells and the absorbance value greater than three times that of a negative control (threshold value) and with a visually detectable yellow colour was rated as positive (Sevick and Akcura, 2013).

Data analysis

Data on disease prevalence and severity indices for the AEZs were subjected to analyses of variance (ANOVA) and the means were separated with the least significant difference (LSD) method at 5% level of significance. The incidences of CMV, ZYMV, and PRSV were compared by chi-square test among the AEZs and between the two seasons. All statistical analyses were performed using GenStat Discovery version 4 (VSN International).

Results

General observations

Farming practices assessed were cropping patterns and field sanitation (the presence of weeds and diseased plants). Mono-cropping was the predominant cropping pattern practiced by the majority of farmers in the various AEZs. Mixed cropping was only observed in AEZ II, where the cucurbit crops were associated with rice, maize, legumes, tomato and pepper.

Four levels of weeds were observed, i.e. level zero for clean field, level 1 for low weed density, level 2 for moderate weed density and level 3, very weedy fields (high weed density). Generally, the AEZs had a moderate level of weeds, indicating a sustained maintenance of the fields. AEZ VI followed by AEZV had the highest number of clean fields estimated at 75% and 55% respectively. AEZI followed by AEZ IV had the lowest number of clean fields estimated at 18% and 23% respectively. All the fields surveyed in AEZ II and AEZ III were weedy, indicating that they had been abandoned.

Symptoms of viral diseases were widespread in all the fields surveyed, suggesting that farmers were not practicing rogueing.

Disease symptoms observed on the cucurbit crops

The crops displayed a wide range of disease symptoms (Fig. 2). Five species of cucurbits namely cucumber, squashes (zucchini and pumpkin), watermelon, melon and gourd (lagenaria) were surveyed. The most commonly observed symptoms on all cucurbit crops were vein banding (Fig. 2a, b, f, h, j, l, o, p, w), downward leaves (Fig. 2m, r), shoe-string and colour breaking of leaf (Fig. 2c, d, i, t, u), leaf rolling (Fig. 2q, v, w). The other symptoms encountered were mottling, stunting, narrowing, leaf rolling and upward cupping (Fig. 2f, g, p, t,), yellowing (Fig. 2a, e, h, l), ringspots and blistering of fruit (Fig. 2g, l, s, x).

Prevalence of viral diseases of cucurbits in six agro-ecological zones (AEZs) of Cote d'Ivoire

The mean prevalence of viral diseases on cucurbit crops in the various AEZs during 2014 rainy and dry seasons are shown in Table 2. The ANOVA did not show significant difference ($p > .05$) in mean disease prevalence among the AEZs in both dry and rainy seasons. However, AEZ II had the highest prevalence of 79.7% whereas AEZ IV had the lowest (19.7%) during the dry season. In the rainy season AEZ III had the highest prevalence of 59.8% whereas AEZ V had the lowest (1.4%).

The ANOVA on mean severity indices in the rainy season revealed significant difference ($p < .05$) among the AEZs. AEZ III had the

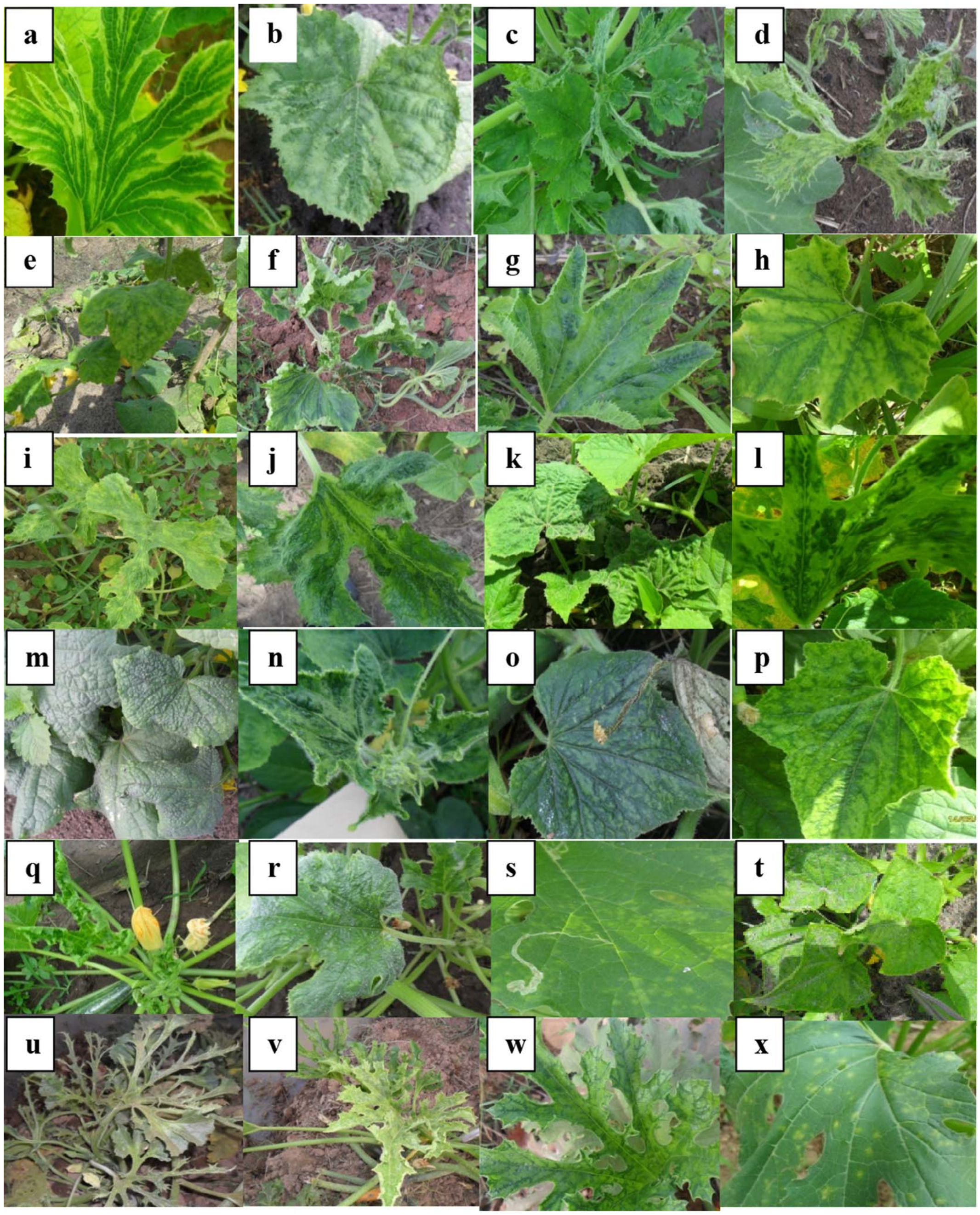

Fig. 2. (a–x): Disease symptoms observed on cucurbit crops surveyed on the field. (a, b, f, h, j, l, o, p, w) Vein banding; (m, r), downward curling of leaves; (c, d, i, t, u), shoe-string and colour breaking; (q, v, w), leaf rolling; (f, g, p, t,) mottling, stunting, narrowing, leaf rolling and upward cupping; (a, e, h, l), yellowing; (g, l, s, x), ringspots and blistering of fruit.

highest severity index of 51.7% whereas AEZ VI had the lowest (0.6%). The ANOVA however did not show significant difference ($p > .05$) among the AEZs during the dry season.

A two-way ANOVA also revealed that the mean prevalence of virus disease in the dry season (53.6%) was significantly higher ($p = .04$) than that of the rainy season (29.35%). The mean severity index recorded in the dry season (47.39%) was also significantly higher ($p = .003$) than in the rainy season (28.17%) as shown in Table 2.

Distribution of viruses on individual cucurbit crops sampled in dry season

Three different viruses namely CMV, PRSV and ZYMV were detected by DAS-ELISA from the plant samples in the dry season. Out of the 345 samples tested, 269 (78%) were positive at least to one of the three viruses identified (Table 3). The infection rate of virus per crop indicated the highest incidence of CMV in cucumber (57.2%), followed by zucchini (40.8%) and in melon (50%) whereas the lowest was in watermelon (5.55%). Moderate incidence of ZYMV was recorded in cucumber (34.5%), followed by zucchini (31.9%) and watermelon (11.1%) but none in melon (0%) and pumpkin (0%). PRSV was detected only in cucumber (8.3%) and zucchini (14.1%) but not in watermelon, melon and pumpkins.

Distribution of viruses on individual cucurbit crops sampled in the rainy season

CMV, PRSV and ZYMV were also detected in the samples collected in the dry season (Table 3). A total of 207 (67.7%) out of 306 samples were positive to at least one of the three viruses identified. CMV was detected in all four crops (cucumber, lagenaria, pumpkin, zucchini).

Table 2
Mean prevalence and severity index of viral disease in the dry and rainy seasons in the six agro-ecological zones (AEZs) in Côte d'Ivoire.

Agro-ecological zones	Prevalence (%)		Severity index (%)	
	Dry season	Rainy season	Dry season	Rainy season
AEZ I	51.5 ns	37.3 ns	53.0 ns	43.1a
AEZ II	79.6	37.8	52.1	27.7abc
AEZ III	78.7	59.8	56.8	51.7a
AEZ IV	19.7	33.3	26.3	34.0ab
AEZ V	36.8	6.5	47.8	11.9bc
AEZ VI	55.3	1.4	49.5	0.6c
Mean	53.60a	29.35b	47.58a	28.17b
LSD ($p \le .05$)	–	–	–	28.17

AEZ: Agro-ecological zone; Means in the same column having the same letters are not significant different by LSD at 5% level of probability; Means in the same row bearing different letters are significantly different ($p < .05$); ns: non-significant difference between the means ($p > .05$)

ZYMV was detected in cucumber, zucchini and pumpkin but not in lagenaria whereas PRSV was detected in cucumber only. With respect to virus infection rates per crop, CMV had 100% infection of lagenaria, followed by zucchini (42.7%), cucumber (30%) and pumpkin (25%). ZYMV was more prevalent in pumpkin (75%), followed by cucumber (63%) and zucchini (42.4%). Infection rate of PRSV was only 7.2% and was detected only in cucumber but not in the other three cucurbit crops.

Distribution of the viruses in agro-ecological zones during the rainy and dry season

Chi-square analysis revealed that the infection rate of CMV was significantly higher ($p < .05$) in the dry season than in the rainy season in AEZI (26.5% vs 15.3%) and AEZII (54.5% vs 20) (Table 4). In contrast, infection rate was significantly higher ($p < .05$) in the rainy season than in the dry season in AEZ III (60% vs 24%) and AEZ1V (44% vs 18%). However, chi-square analysis did not show any significant difference ($p > .05$) in the CMV infection rate between the rainy and dry seasons in AEZV and AEZ VI (Table 4).

Table 5 shows that the infection rate of ZYMV was higher in the dry season than in the rainy season in AEZIII (62% vs 53.5%), and AEZIV (28% vs 23.2%). It was however lower in the dry season than the rainy season in AEZI (27% vs 35%), AEZII (27% vs 28%), AEZV (0% vs 20%) and AEZ VI (12.2% vs 13.3%). Chi square test however did not show significant difference ($p > .05$) in the ZYMV infection rate between the two seasons for all the six AEZs (Table 5).

Chi-square test revealed that PRSV infection rates in the dry season were significantly higher ($p < .05$) than in the rainy season in AEZ I, AEZ II, and AEZ VI. On the contrary, the infection rates in the rainy season were significantly higher ($p < .05$) than in the dry season in AEZ IV (Table 6). However, chi-square test showed no significant difference ($p > .05$) in the infection rate between the dry and wet seasons in AEZIII. PRSV was not detected in AEZ V in both dry and rainy seasons (Table 6).

Table 4
CMV infection rate per agro-ecological zone in both dry and rainy seasons.

Zones	Infection rate (%)		χ^2	p-value
	Dry season	Rainy season		
AEZ I	26.5	15.3	5.14	.023*
AEZ II	54.5	20.0	4.65	.031*
AEZ III	24.0	60.0	3.72	.051*
AEZ IV	18.0	44.7	10.00	.001**
AEZ V	20.0	30.0	0.00	.50^{ns}
AEZ VI	22.4	20.0	0.02	.87^{ns}

AEZ: Agro-ecological zone.
* Significant at $p \le .05$.
** Significant at $p < .01$: ns: not significant ($p > .05$).

Table 5
ZYMV infection rate in both dry and rainy seasons in the six agro-ecological zones (AEZs).

Zones	Infection rate (%)		χ2	p-value
	Dry season	Rainy season		
AEZ I	28.2	35.0	2.79	.09^{ns}
AEZ II	27.3	28.0	0.07	.78^{ns}
AEZ III	62.0	53.5	0.1	.74^{ns}
AEZ IV	28.0	23.2	0.17	.68^{ns}
AEZ V	0	20.0	0.56	.45^{ns}
AEZ VI	12.2	13.3	0.11	.73^{ns}

AEZ: Agro-ecological zones; ns: not significant ($p > .05$).

Table 6
PRSV infection rate in both dry and rainy seasons in the six agro-ecological zones (AEZs).

Zones	Infection rate (%)		χ^2	p-value
	Dry season	Rainy season		
AEZ I	9.0	2.4	5.19	.022*
AEZ II	27.3	0.0	5.56	.01**
AEZ III	5.0	0.0	0.03	.856ns
AEZ IV	5.3	9.0	3.61	.05*
AEZ V	0.0	0.0	NA	NA
AEZ VI	16.3	0.0	3.8	.05*

AEZ = Agro-ecological zones; ns = non-significance.
NA = Not applicable.
* Significant at $p < .05$.
** Significant at $p < .01$.

Mixed viral infections of cucurbit crops

Apart from AEZ V, double and triple infections of cucurbit crops by CMV, ZYMV and PRSV were detected in all AEZs (Table 7). Co-infection with CMV and ZYMV was most prevalent, with a mean infection rate of 8.26% per AEZ, and occurred in all the AEZs except AEZV. This was followed by PRSV + CMV with a mean infection rate of 2.57%, and was detected in all AEZ except AEZIII and AEZV. Co-infection with ZYMV +PRSV was detected in only AEZI, AEZII and AEZ IV, and had mean infection rate of 1.92%. Co-infection with CMV+ZYM+ PRSV was

Table 3
Rate of infection of cucurbit crops by CMV, ZYMV and PRSV during wet and dry seasons.

Virus	Infection rate per crop (%) in the wet season					Infection rate per crop (%) in the dry season			
	cucumber	zucchini	watermelon	melon	pumpkin	cucumber	zucchini	lagenaria	pumpkin
CMV	57.2	40.8	5.55	50	0	30	42.7	100	25
ZYMV	34.5	31.9	11.1	0	0	63	42.4	0	75
PRSV	8.3	14.1	0	0	0	7.2	0	0	0

Cucumber mosaic virus; (CMV), *Zucchini yellow mosaic virus* (ZYMV); *Papaya ringspot virus* (PRSV).

Table 7
Mixed infections of viruses in cucurbit crops surveyed in six agro-ecological zones (AEZs) of Cote d'Ivoire during the 2014 growing seasons.

Zone/crops	Double infection (%)			Triple infection
	CMV + ZYMV	ZYMV + PRSV	PRSV + CMV	CMV + ZYM + PRSV
AEZ I	4.03	1.34	0.68	3.35
AEZ II	13.63	9.09	13.63	0
AEZ III	19.05	0	0	0
AEZ IV	10.87	1.09	1.09	2.17
AEZ V	0	0	0	0
AEZ VI	2	0	2	6
Mean	8.26	1.92	2.57	1.92

AEZ: agro-ecological zone; *Cucumber mosaic virus* (CMV); *Zucchini yellow mosaic virus* (ZYMV); *Papaya ringspot virus* (PRSV).

detected only in AEZI, AEZIV and AEZ VI, and had mean prevalence of 1.92.

Distribution of viruses within the weeds

CMV, ZYMV and PRSV were detected in the weed samples collected from the fields that were surveyed (Table 8). CMV had the highest prevalence of 64% in both annual and perennial weeds, followed by ZYMV with moderate prevalence of 28% whereas PRSV had the lowest prevalence of 4%. With respect to the viral infection of the annual weeds, CMV had the highest prevalence of 40%, followed by ZYMV with infection rate of 24%, whilst PRSV had the lowest infection rate of 4%. In respect of viral infection of the perennial weeds, CMV had the highest prevalence of 24%, followed by ZYMV with infection rate of 4% whereas PRSV was not detected (0%).

Discussion

Viral diseases have been reported to cause major losses in the quantity and quality of cucurbit crops worldwide and they represent one of the most important limiting factors for growers (Provvidenti, 1996). In the present study, symptoms of viral diseases were prevalent in all the six AEZs surveyed in both the rainy and dry seasons. This suggests that viral infection of cucurbits is widespread in all AEZs and at all cropping seasons in Côte d'Ivoire, and hence threatens cucurbit production in the country. The high prevalence of viral disease observed in the study could be due to farmers' poor agronomic practice such as poor farm sanitation (no rogueing, and weedy fields), continuous cropping, mono-cropping, and pest management methods (Hull, 2009; Thresh, 2003). It could also be due to the use of uncertified seeds by the farmers, the misuse of pesticides which causes the resistance of the aphid and whitefly vectors to the chemical and the lack of knowledge on viral diseases by the farmers (Afouda et al., 2013; Ayo-John et al., 2014).

Table 8
Growth habits, viral symptoms, and associated viruses detected by DAS-ELISA from weed samples.

Family	Growth habit	Symptoms	CMV	ZYMV	PRSV
Amaranthaceae	Annual	leaf rolling	+	−	−
Asteraceae	Annual	Downward cup	+	−	−
Asteraceae	Annual	Downward	+	+	−
Asteraceae	Annual	None	+	+	−
Asteraceae	Perennial	Leaf curl	−	−	−
Asteraceae	Annual	Leaf curl	+	−	−
Asteraceae	Annual	Mosaic	+	−	−
Asteraceae	Perennial	None	−	−	−
Capparidacea	Perennial	Necrosis	+	−	−
Commelinaceae	Perennial	None	+	−	−
Euphorbiaceae	Annual	Mosaic	+	+	−
Euphorbiaeae	Perennial	None	+	−	−
Euphorbiaeae	Perennial	Mosaic	+	−	−
Euphorbiaceae	Annual	None	+	+	−
Fabaceae	Annual	Mosaic	−	−	−
Fabaceae	Annual	Mosaic	+	+	−
Malvaceae	Perennial	Mosaic	+	−	−
Poaceae	Annual	None	+	+	+
Poaceae	Perennial	None	−	−	−
Poaceae	Perennial	Brownish	−	+	−
Portulaceae	Perennial	None	−	−	−
Portulaceae	Perennial	Narrowing	−	−	−
Rubiaceae	Annual	None	−	−	−
Solanaceae	Perennial	None	−	−	−
Verbenaceae	Perennial	None	+	−	−

(+): Sample infected; (−): non infected sample.

Mean prevalence and severity index of viral infection of cucurbits across the AEZs were significantly higher in the dry season than in the rainy season. This finding corroborates that of Kone et al. (2017), who reported of higher prevalence of virus disease in dry season than in rainy season in Cote d'Ivoire. It has been reported that seasonal changes can affect hosts, pathogens and vectors in ways that alter components of the basic reproductive numbers that determine the rate at which infected hosts are produced (Altizer et al., 2006). These mechanisms include those that influence parasite transmission, in part by altering the behaviour of hosts, the biology of vectors or parasite infectious stages in the environment (Altizer et al., 2006). In addition, high temperatures that are usually experienced in dry seasons, could increase the susceptibility of host plants to virus infection and rather accelerate the fitness of viruses to cause infection, as reported by Harvell et al. (2002) and Mitchell et al. (2005). The non-significant differences in the mean prevalence of virus disease amongst the six AEZ in both the dry and rainy seasons, further confirms the importance of the viral disease in all the AEZ in Cote d'Ivoire. This suggests that irrespective of the AEZ, cultivation of cucurbits in the dry season may result in higher incidence and severity of viral disease than rainy season planting, as has been reported by Kone et al. (2017).

The study revealed the prevalence of CMV, ZYMV and PRSV in all the AEZs surveyed. These three viruses have also been reported to be infecting cucurbit crops in Cote d'Ivoire (Fauquet and Thouvenel, 1987; Koné et al., 2010; Agneroh et al., 2012). This suggests that CMV, ZYMV and PRSV are widespread in all AEZs and constitute the most important viruses infecting cucurbit crops in Côte d'Ivoire. It has also been reported that CMV, ZYMV and PRSV are among the most frequent viruses infecting cucurbits worldwide (Massumi et al., 2007) causing up to 100% yield losses and up to 95% reduction in market value (Lecoq and Desbiez, 2012).

Variations in the infection rate of CMV, ZYMV and PRSV in the various AEZs were observed between the dry and wet seasons (Tables 7 and 8). This seasonal effect on the viral infection rate could be due to changing ecological conditions which in turn change the transmission rate of the virus, viral replication rates, the behaviour of the host plants and the biology of the vectors (Hull, 2009; Fajinmi, 2011). This result could also be due to intrinsic factors of the crops including susceptibility and agricultural practices employed within the AEZs (Juarez et al., 2013).

The various cucurbit crops (cucumber, lagenaria, pumpkin, zucchini, watermelon, melon) surveyed were susceptible to at least one of the three viruses identified (CMV, ZYMV and PRSV). This suggests that all the cucurbits constitute a potential and alternative virus reservoir as reported by Ali et al. (2012), and this, at least partly accounts for the high prevalence of viral disease observed in our study.

The rate of infection of various cucurbit crops by the three viruses (CMV, ZYMV and PRSV) varied from one cucurbit species to the other at various planting dates. For instance, in the dry season, CMV had 100% infection of lagenaria, followed by zucchini (42.7%), cucumber (30%) and pumpkin (25%) whereas ZYMV was more prevalent in pumpkin (75%), followed by cucumber (63%) and zucchini (42.4%) (see Tables 6). Thus, the predominant virus species varied according to the crop host and origin of the samples as observed by Barbosa et al. (2016). These variations in the host-virus interaction effects could be due to the differences in the genetic makeup of the different cucurbit crops and viral species as reported in tomato-Tomato yellows virus pathosystem (Delatte et al., 2005; Aziz et al., 2008; Madani et al., 2011).

Weeds play an important role as foci of infection from which there is spread into or within crops (Thresh, 1982). Hence the detection of CMV, ZYMV and PRSV in the weed samples collected from the cucurbit fields surveyed. This at least partly accounts for high prevalence of viral disease in the various AEZs. CMV was found to be the most infectious virus, infecting 62.5% of the weed samples, followed by ZYMV infecting 28%, and then PRSV infecting only 4%. The high incidence of CMV in the weed samples is not new. A relative study carried out in Nigeria indicated 78.6% incidence of CMV in weeds (Ayo-John et al., 2014). This finding is therefore consistent with the report of Van Regenmortel et al. (2000) which states that *Bromoviridae* including CMV, is one of the most important widespread viruses in the world infecting the largest number (approximately 1000) of plant species. CMV was reported to be seed-borne virus with a transmission rate estimated between 1% and 50% (Palukaitis and García-Arenal, 2003), hence its high incidence in the weeds as well as in cucurbit crops sampled in our study. The study also revealed that annual weed samples were more susceptible to the viruses than the perennials. This suggests that the cucurbit fields with higher density of annual weeds are more prone to virus infection than those with perennial weeds, since weeds are potential alternate hosts of viruses (Thresh, 1982). Effective weed management is therefore very important for preventing viral infection in cucurbit fields.

MWMV and WMV were not detected serologically, suggesting that for now, these two viruses do not infect cucurbit crops in Côte d'Ivoire.

Mixed virus infections in plants can exacerbate and increase the severity of disease symptoms, leading to significant yield losses (Malik et al., 2010). In the present study, mixed infections in cucurbit crops by CMV, ZYMV and PRSV were detected in all the six AEZs. Similar results were observed by Barbosa et al. (2016), based on molecular analysis of cucurbit samples collected from San Francisco valley in Brazil. It has been reported that mixed infections in cucurbits are frequently observed in natural conditions between viruses from Potyvirus genus and CMV (Barbosa et al., 2016). According to Syller (2012) multiple infections lead to a variety of intra host virus–virus interactions, many of which may result in the generation of variants showing novel genetic features, and thus change the genetic structure of the viral population. Hence, understanding the interactions between CMV, ZYMV and PRSV in cucurbits may be of crucial significance for the understanding of viral pathogenesis and evolution, and consequently for the development of efficient and stable management strategies, as suggested by Syller (2012).

Conclusions

The study revealed that viral diseases of cucurbits are widespread in all ecological zones in Cote d'Ivoire, with significantly higher prevalence in the dry season than in the wet season and are mainly associated with CMV, ZYMV and PRSV infections. Weeds samples belonging to 11 different families were infected with at least one of these three viruses. This information is therefore a valuable contribution to the knowledge of epidemiology of viral diseases of cucurbits in Cote d'Ivoire. Strategies for managing viral diseases of cucurbits should therefore consider the prevailing viral pathogens, seasons of severe infection and the weeds as alternate hosts.

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