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Variation in the susceptibility of okra (*Abelmoschus esculentus* L. Moench) genotypes to okra mosaic virus and *Podagrica* species under field conditions

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A total of 21 okra (*Abelmoschus escuentus* L. Moench) genotypes were screened for their reactions against okra mosaic disease (OMD) and flea beetles (*Podagrica* species) infestations in field trials which were conducted from May to October, 2015 (wet season) and November 2015 to March 2016 (dry season), in order to identify sources of resistance and or tolerance. The trials were laid out in a randomised complete block design (RCBD) with four replications. Field resistance in the genotypes was assessed at 2, 6 and 10 weeks after planting using a 0 to 5 visual scale based on disease symptoms (where 1 denotes no symptom and 5, very severe symptom). Enzyme linked immunosorbent assay (ELISA) was performed to detect the presence of *Okra mosaic virus* (OkMV) in the okra genotypes. Populations of the flea beetle (*Podagrica* spp.), the vector of OkMV, and the associated leaf and fruit damage were also assessed. All the okra genotypes exhibited a varying range of disease symptoms and the flea beetle infestations, and lacked immunity. Genotypes GH2052, GH2063, GH2026, GH3760, GH5302, GH5332, GH5793, GH6105 and UCCC6 exhibited mild symptoms of OMD, and were less susceptible to flea beetle infestation and associated leaf damage during both seasons. Using ELISA, OkMV was detected in all the 21 genotypes. The mean number of fruits per plant and the mean fruit yield (t ha^{-1}) differed significantly (P<0.05) among the okra genotypes. Genotype GH5332 had the highest fruit yield of 11.88 t ha^{-1} followed by genotype GH6105 (9.34 t ha^{-1}). Percentage fruit damage due to the flea beetle infestation differed significantly among the okra genotypes, ranging between 43.7 and 91.2% and from 47 to 84% in both trials respectively.

Key words: Enzyme linked immunosorbent assay (ELISA), insecticides, *Abelmoschus esculentus*, okra mosaic disease, *Podagrica* species.

INTRODUCTION

Okra (*Abelmoschus esculentus* L. Moench) is a widely grown vegetable crop in the tropical and subtropical regions mainly for its immature edible green fruits, which are used as vegetable both in green and processed state (Lamont, 1999; Arapitsas, 2008; Saifullah and Rabbani, 2009). The fresh leaves can be used in the same manner as spinach while the seeds are said to be good sources of oil (Oyolu, 1977). Okra is a good source of carbohydrate, dietary fibre, fat, protein, calcium, iron, thiamine, riboflavin, nicotinamide and ascorbic acid (Tindall, 1986; Schippers, 2000; Asawalam et al., 2007).

The world okra production was estimated at 4.8 million tonnes (in 2007) with India leading in the production by 70% followed by Nigeria (15%), Pakistan (2%), Ghana (2%), Egypt (1.7%) and Iraq (1.7%) (Gulsen et al., 2007). Even though the West and Central African region including Ghana account for more than 75% of okra produced in Africa, yet the average productivity in the region is very low (2.5 t ha^{-1}) compared to East (6.2 t ha^{-1}) and North Africa (8.2 t ha^{-1}) (FAOSTAT, 2008).

In Ghana, okra is widely grown in both rainy and dry seasons mainly by small holder farmers and hence a major source of income for them. The yield potential of okra recorded in Ghana ranges from 2 to 3 t ha^{-1} (MoFA, 2007), depending on the cultivar, harvesting frequency and period for harvesting (Cudjoe et al., 2005). However, actual yields of okra are usually low and have also decreased over the past years (Asare-Bediako et al., 2014b).

Viral diseases are important constraints in the production of okra worldwide (Ndunguru and Rajabu, 2004; Asare-Bediako et al., 2014a, b). Okra is susceptible to at least 19 plant viruses with Okra mosaic virus (OkMV; genus *Tymovirus;* family Tymoviridae), Bhendi yellow vein mosaic virus (BYVMV, genus Begomovirus), Cotton leaf curl Gezira virus (CLCuGV, genus *Begomovirus*), and Okra leaf curl virus (OLCuV; genus *Begomovirus*) being the most common and well studied (Brunt et al., 1990; Swanson and Harrison, 1993; Tiendrebego et al., 2010; Sayed et al., 2014). Other begomoviruses such as Okra yellow crinkle virus (OYCrV) and Hollyhock leaf crumple virus (HoLCrV) have been reported to be infecting okra in Africa (Kon et al., 2009; Shih et al., 2007, 2009).

Okra mosaic disease (OMD) caused by OkMV (Koenig and Givord, 1974) is the most prevalent viral disease of okra in West Africa, with mean disease incidences ranging between 78 and 83% recorded in farmers' okra fields in Ghana (Asare-Bediako et al., 2014a,b). Incidence of OMD has also been reported in Ivory Coast (Givord et al., 1972; Fauquet and Thouvenel, 1987) and Nigeria (Koenig and Givord, 1974; Igwegbe, 1983; Alegbejo, 2001; Fajinmi and Fajinmi, 2010). Typical symptoms of OkMV infection include mosaic, vein chlorosis and vein-banding and plant stunting (Koenig and Givord, 1974; Brunt et al., 1990; Swanson and Harrison, 1993) as shown in Figure 1. Yield losses of up to 100% due to OkMV infection has been reported (Atiri, 1984; Alegbejo, 2001).

OkMV contains a single-stranded positive-sense RNA (approximately 6.2 kb) and it consists of isometric particles of 28 nm in diameter (Koenig and Givord, 1974). OkMV is transmitted in a non-persistent manner by the coleopteran *Podagrica* species (flea beetles) (Brunt et al., 1990, 1996). The virus is also sap-transmissible (Koenig and Givord, 1974. Besides being a vector for OkMV, flea beetles cause direct damage to plants and are the most important pest of okra in West Africa (Obeng-Ofori and Sackey, 2003). The feeding activity of flea beetles causes characteristic perforations of leaves leading to irregular holes reducing the photosynthetic surface area of the leaves. This can result in significant yield reductions (Echezona and Offordile, 2011). Such yield losses by infestation of flea beetles have been reported from Ghana (Obeng-Ofori and Sackey, 2003), Nigeria (Ahmed et al., 2007) and Burkina Faso (Dabiré-Binso et al., 2009).

Most of the research on management of OMD and its vector is oriented on chemical control. However, the flea beetle, and so OMD, is very difficult to control with insecticides due to development of resistance against the insecticides by the insect vector (Nono-Womdim, 2001). Breeding and planting of resistant varieties would be the most effective way of managing OMD, however, until today no host resistance has been identified against OMD (Nono-Womdim, 2001). Therefore, the study was conducted to screen different genotypes of okra for possible resistance or tolerance to OMD (Figure 1).

MATERIALS AND METHODS

Study area

The experiment was conducted at the Teaching and Research Farm of the School of Agriculture, College of Agriculture and Natural Sciences (CANS) of the University of Cape Coast from May to October, 2015 (wet season) and November 2015 to March 2016 (dry season). This location (5°10'N, 1.2°50'W) falls within the coastal savannah agro-ecological zone of the country with Acrisol soil type (Parker et al., 2010) and is a highly endemic site for OMD and flea beetle infestation. The area has a bi-modal rainy season from May to June and August to October with an annual rainfall ranging between 750 and 1000 mm (Parker et al., 2010) and is a highly endemic site for OMD and flea beetle infestation. The area has a bi-modal rainy season from May to June and August to

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Figure 1. Okra plant showing mosaic and leaf curl symptoms (Picture was taken by Elvis Asare-Bediako).

October with an annual rainfall ranging between 750 and 1000 mm (Parker et al., 2010) and temperatures ranging between 23.2 and 33.2°C with an annual mean of 27.6°C (Owusu-Sekyere et al., 2011).

Plant

Twenty-one genotypes of okra (both landraces and improved) were used for the study. The genotypes comprised of fifteen accessions from the Plant Genetic Resource Research Institute (PGRRI) at Bunso, Ghana, five farmers' varieties and a landrace. Accession names, accession numbers and sources of the 21 okra genotypes are shown in Table 1.

Experimental design and field layout

A randomized complete block design (RCBD) with twenty-one treatments and four replications was used. A total land area of 1344 m^2 measuring 84 × 16 m was ploughed and harrowed. The field was then divided into four blocks and each block was further divided into 21 plots, with each plot measuring 3 × 3 m. A distance of 1 m was left as walkway between the blocks and 1 m between the plots. A total of 21 okra genotypes representing the 21 treatments were sown directly at two seeds per hill at a planting distance of 0.6 × 0.6 m. Weed control was done as and when necessary using herbicides or hoe. NPK fertilizer (15:15:15) was applied at a rate of 250 kg ha^{-1}. Watering was done when necessary using sprinklers.

Data collection

Disease incidence and severity, population of flea beetles per plant and the associated leaf and fruit damage, mean number of fruits and mean fruit yield (t ha^{-1}) were recorded. Data was collected from nine plants per plot and the mean values were determined.

Severity of OMD was assessed at 2, 6 and 10 weeks after planting (WAP) based on the visual symptoms using 0 to 5 scale adopted from Alegbejo (1997) with modification as indicated in Table 2. Incidence of OMD, based on visual symptoms, was determined as the proportion of infected plants per plot, expressed as a percentage of total number of plants observed, as described by Galanihe et al. (2004). Flea beetle populations were taken from nine (9) plants per plot and the mean population per plant determined. The cumulative average number of adult beetle per plant was then determined as the beetle population that infested the crop during the experimental period (N'Guessan, 2001).

The severity of the pest damage was visually assessed at 10 WAP using a modified 0 to 5 scale (Kirsh, 1986) as indicated in Table 3.

Serological detection of Okra mosaic virus (OkMV) in the 21 okra genotypes

The presence of OkMV in the diseased okra leaf samples collected was tested by double antibody sandwich ELISA (DAS ELISA) as described by Clark and Adams (1977) using antiserum (rabbit polyclonal antibody) raised against OkMV (AC Diagnostics Inc. USA). Leaf samples were ground with mortar and pestle 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+0.05% v/v Tween 20 + 2% w/v PVP) at a 1:10 ratio (w/v) and tested in duplo.

The absorbance values at 405 nm (A_{405}) were recorded using an Anthosmicroplate reader (Biochrum Ltd, Cambridge, UK). Absorbance values of three (3) uninfected leaf samples were also measured. A test sample was deemed to be positive when the A_{405} was higher than 3 times the mean absorbance of the uninfected leaf samples (threshold value).

Table 1. Accession numbers, names and sources of the okra genotypes used for this study.

Accession number	Accession name	Country of origin
GH2026	Manshior	Togo
GH2052	Fetri (Ewe)	Togo
GH2057	Fetri	Togo
GH2063	Fetri	Togo
GH3731	Krotetenye	Ghana (Abortia Junction)
GH3734	Fetri	Ghana (Kpogadzi)
GH3760	Nkruma	Ghana (Nsapor)
GH4374	Nkruma	Ghana (Duabone No.1)
GH5302	Pebrenkruma	Ghana (Ayiogbe)
GH5321	-	Ghana (Pinihi)
GH5332	BropoAsontem	Ghana (Fententaa)
GH5786	Tuagya	Ghana (Koranten)
GH5793	Ogyeabatan	Ghana (Asikasu)
GH6105	Asontem	Ghana (Mankessim)
GH6211	Nkrumah	Ghana (Ashiaman)
UCCC1	Avalavi	Ghana (AssinAkonfodi)
UCCC2	Odumase	Ghana (FosuOdumase)
UCCC3	Antado	Ghana (Antado-KEEA)
UCCC4	Asontem	Ghana (AssinFosu)
UCCC5	Kakumdo	Ghana (Kakumdo)
UCCC6	UCC Campus	Ghana (UCC-Cape Coast)

Accessions GH2026-GH6211 were obtained from the Plant Genetic Resource Research Institute, Bunso, Ghana; UCCC1-UCCC5 are farmers' varieties, and UCCC6 is a landrace.

Table 2. Scale for visual rating of okra mosaic disease severity in farmers' okra fields.

Disease score	Description
0	Healthy, asymptomatic plant
1	Mild mosaic, mottle or chlorosis on leaves
2	Moderate chlorosis, mottle or mosaic without significant leaf distortion
3	Moderate chlorosis, mottle or mosaic with leaf distortion
4	Severe chlorosis, mottle or mosaic with leaf distortion plusstunting or dwarfing of the whole plant
5	Score 4 plus drying and leaf drop

Table 3. Scale for visually assessing okra for the severity of pest damage by *Podagrica* spp.

Damage score	Percentage damage	Description
0	0	No apparent damage
1	20	Approximately a quarter of total leaf area eaten
2	40	Approximately half of total leaf area eaten
3	60	Approximately three quarters of total leaf area eaten
4	80	Most leaves eaten, few leaves intact, stem green
5	100	All leaves and part of stem eaten

Data analyses

Data on mean severity scores were used to calculate Area Under the Disease Progress Curve (AUDPC) for each of the okra genotypes in Microsoft Excel according to Shaner and Finney (1977):

$$AUDPC = \sum [(Yi + 1 + Yi)/2] [Xi + 1 – Xi]$$

Table 4. Mean incidence of OMD on 21 okra genotypes during the rainy and dry seasons and detection of *Okra mosaic virus* (OkMV) by DAS-ELISA.

Genotype	Mean incidence of OMD (%) in the rainy season			Mean incidence of OMD (%) in the dry season			ELISA detection of OkMV
	2WAP	6WAP	10WAP	2WAP	6WAP	10WAP	
GH2026	0^{c}	63.3^{c}	90.0^{ns}	0.00^{ns}	42.6^{fgh}	90.00^{a}	++
GH2052	0^{c}	78.1^{abc}	90.0	0.00	28.9^{h}	83.98^{ab}	++
GH2057	0^{c}	69.3^{bc}	90.0	6.02	$60.3^{abcdefg}$	90.00^{a}	++
GH2063	0^{c}	78.1^{abc}	90.0	0.00	45.0^{efgh}	83.98^{ab}	++
GH3731	4.9^{c}	85.1^{ab}	90.0	0.00	41.1^{gh}	90.00^{a}	++
GH3734	4.9^{c}	90.0^{a}	90.0	12.05	81.2^{a}	90.00^{a}	++
GH3760	7.1^{c}	72.4^{bc}	90.0	0.00	47.4^{defgh}	77.95^{b}	++
GH4374	0^{c}	90.0^{a}	90.0	0.00	72.7^{abc}	90.00^{a}	++
GH5302	0^{c}	83.0^{ab}	90.0	6.02	45.0^{efgh}	77.95^{b}	++
GH5321	31.3^{abc}	90.0^{a}	90.0	6.02	69.1^{abcd}	90.00^{a}	++
GH5332	8.8^{bc}	76.3^{abc}	90.0	0.00	53.8^{bcdefg}	83.98^{ab}	++
GH5786	0^{c}	72.1^{bc}	90.0	0.00	52.7^{cdefg}	90.00^{a}	++
GH5793	0^{c}	73.2^{abc}	90.0	0.00	39.0^{gh}	83.98^{ab}	++
GH6105	4.9^{c}	90.0^{a}	90.0	0.00	53.8^{bcdefg}	90.00^{a}	++
GH6211	0^{c}	78.1^{abc}	90.0	0.00	66.3^{abcde}	90.00^{a}	++
UCCC 1	31.3^{ab}	90.0^{a}	90.0	12.05	66.3^{abcde}	90.00^{a}	++
UCCC 2	13.7^{bc}	90.0^{a}	90.0	0.00	81.2^{a}	90.00^{a}	++
UCCC 3	15.3^{abc}	90.0^{a}	90.0	6.02	75.2^{ab}	83.98^{ab}	++
UCCC4	37.8^{a}	90.0^{a}	90.0	14.84	64.3^{abcdef}	90.00^{a}	++
UCCC5	16.9^{abc}	90.0^{a}	90.0	17.27	69.1^{abcd}	90.00^{a}	++
UCCC 6	0^{c}	78.1^{abc}	90.0	0.00	45.0^{efgh}	77.34^{b}	++
Means	8.4	81.8	90.0	3.82	57.1	86.82	-
l.s.d	23.01	16.95	-	-	21.91	9.661	-
P value	0.013	0.018	-	0.058	<0.001	0.042	-

Means in the same column bearing identical letters are not significantly different ($P>0.05$). ns=not significant ($P>0.05$). Incidence data was transformed using arc sine transformation before ANOVA was done. $^{++}$Presence of *Okra mosaic virus* (OkMV) in leaf samples tested in both rainy and dry seasons.

where Y_i is the disease severity at the i^{th} observation, X_i is the time (weeks) at the i^{th} observation, n is the total number of observations.

Data on disease incidence and insect counts were transformed with angular and square root transformations, respectively in order to homogenise the variance before subjecting to ANOVA. All other quantitative data were subjected to one-way ANOVA and two-way ANOVA and the means separated by least significance difference method at 5% level of probability using GenStat Discovery version 4 (VSN International). Pearson's correlation coefficients among the parameters (disease incidence and severity, insect counts and associated leaf and fruit damage and yield data) were calculated using GenStat.

RESULTS

Mean incidence of OMD

Mean incidences (%) of OMD on 21 okra genotypes are shown in Table 4. Generally, for all 21 okra genotypes, the incidence of OMD increased from 2 to 10 WAP, with overall mean incidences is increasing from 8.4 to 90% in the rainy season and 3.82 to 86.82% in the dry season.

An analysis of variance (ANOVA) showed significant differences in mean incidence of OMD during the rainy season among the okra genotypes at 2 WAP ($F = 2.12$; df = 60; $P = 0.013$) and 6 WAP ($F = 2.03$; df = 60; $P = 0.018$) but all the okra genotypes showed symptoms of OMD at 10 WAP.

Similarly, in the dry season, ANOVA showed highly significant differences in mean incidence of OMD among the okra genotypes at 2WAP ($F=1.70$; df=60; $P=0.05$), 6 WAP ($F=3.68$; df=60; $P<0.001$) and 10 WAP ($F=1.80$; df=60; $P=0.042$). At 10 WAP, UCCC6 recorded the lowest mean incidence (77.34%) of OMD but this was not significantly different from GH3760, GH5302, GH2052, GH2063, GH5793 and UCCC3 with mean incidences of 77.95, 77.95, 83.98, 83.98, and 83.98% respectively but significantly different from the other genotypes (Table 4).

ELISA on leaf samples confirmed the presence of OkMV in all 21 genotypes in both the rainy and dry season trials (Table 4).

Table 5. Mean severity scores and mean area under disease progress curve (AUDPC) for 21 okra genotypes during wet and dry seasons.

Genotype	Rainy season			Dry season		
	Final severity	AUDPC	Host resistance	Final severity	AUDPC	Host resistance
GH2026	2.009^{cde}	8.86^{d}	R	2.104^{e}	7.77^{cde}	MR
GH2052	1.944^{de}	8.00^{d}	R	1.833^{ef}	4.75^{e}	R
GH2057	2.382^{abc}	8.91^{d}	R	2.875^{bc}	11.58^{ab}	S
GH2063	2.000^{cde}	8.78^{d}	R	2.042^{ef}	6.79^{cde}	MR
GH3731	2.194^{abcd}	10.17^{bcd}	S	2.292^{de}	7.79^{cde}	MR
GH3734	2.57^{a}	11.71^{ab}	S	3.708^{a}	13.87^{a}	S
GH3760	1.750^{e}	8.42^{d}	R	1.750^{ef}	5.83^{de}	R
GH4374	2.250^{abcd}	9.96^{bcd}	MR	3.188^{abc}	11.49^{ab}	S
GH5302	2.028 ^{cde}	7.97^{d}	R	1.917^{ef}	6.54^{de}	R
GH5321	2.306 ^{abcd}	13.47^{a}	S	3.208^{abc}	11.75^{ab}	S
GH5332	2.036^{cde}	9.10^{cd}	R	1.833^{ef}	5.92^{de}	R
GH5786	2.333^{abcd}	9.79^{bcd}	MR	2.750^{cd}	9.92^{bc}	MR
GH5793	1.972^{cde}	8.42^{d}	R	1.917^{ef}	6.25^{de}	R
GH6105	2.163^{abcde}	10.24^{bcd}	MR	2.083^{e}	8.00^{cd}	R
GH6211	2.472^{ab}	11.25^{abc}	S	3.208^{abc}	12.29^{ab}	S
UCCC 1	2.000^{cde}	11.33^{abc}	S	3.750^{a}	13.21^{a}	S
UCCC 2	2.333^{abcd}	12.81^{a}	S	2.958^{bc}	11.79^{ab}	S
UCCC 3	2.36^{abcd}	11.28^{abc}	S	2.958^{bc}	13.42^{a}	S
UCCC4	2.222^{abcd}	11.50^{ab}	S	3.042^{bc}	12.08^{ab}	S
UCCC5	1.958^{cde}	11.29^{abc}	S	3.375^{ab}	13.29^{a}	S
UCCC 6	1.972^{cde}	7.97^{d}	R	1.475^{f}	4.67^{e}	R
Mean	2.156	10.06	-	2.584	9.45	-
LSD	0.4272	2.224	-	0.5672	3.182	-
P-value	0.027	<0.001	-	<0.001	<0.001	-
$F_{20, 60}$	-	4.28	-	-	8.01	-

Means in the same column bearing identical letters are not significantly different (P>0.05). Host resistance status was based on the values of AUDPC where R=resistance, MR=moderately resistance, S=susceptibility. Difference in the overall mean AUDPC between dry and rainy seasons was significant (l.s.d= 0.701; d.f.=40; P=0.103). Diference in the overall final disease severity between dry and rainy seasons was significant (l.s.d=0.1203; d.f.=40; P<0.00).

Severity scores of OMD and area under disease progress curve (AUDPC)

Mean severity scores of OMD and AUDPC recorded at 10 WAP for the 21 okra genotypes during the rainy and dry seasons in field trials are shown in Table 5. There were significant differences in the final severity of OMD among the okra genotypes ($F_{20,60}$=1.93; *P*=0.027) during the rainy season (Table 5). GH3760 had the lowest mean severity score of 1.75, followed by GH2052, UCCC5, UCCC6, GH5793, UCCC1, GH2063, GH2026, GH5302 and GH5332, with mean severity scores of 1.944, 1.958, 1.972, 1.972, 2.00, 2.00, 2.009, 2.028 and 2.056, respectively. GH3734 had the highest severity score of 2.571. Similarly, in the dry season, the ANOVA showed highly significant differences in the mean severity of OMD at 10 WAP ($F_{20, 60}$=12.18; *P*<0.001). UCCC6 had the lowest mean severity score (1.475) of OMD, but was not significantly different from GH5793 (1.917), GH5332 (1.833), GH5302 (1.917), GH3760 (1.750), GH2063 (2.042) and GH2052 (1.833) and GH2026 (2.104).

The overall mean severity of OMD at 10 WAP recorded during the dry season trial (2.584) was significantly higher ($F_{20, 60}$=49.58; P=0.103) than that of the rainy season (2.156) as shown in Table 5.

There were significant differences in the AUDPC recorded for the 21 okra genotypes during the wet season ($F_{20, 60}$=4.28; *P*<0.001) and the dry season ($F_{20, 60}$=8.01; *P*<0.001). In the wet season, both GH5302 and UCCC6 had the lowest AUDPC which were not significantly different from that of GH2026, GH2052, GH2057, GH2063, GH3760, GH5332, GH5786 and GH5793, suggesting that they were tolerant to the OMD. Genotype GH5321 had the highest AUDPC which was not significantly different from that of UCCC2, GH3734, UCCC3, UCCC4 and UCCC5, indicating that they were very susceptible to the OMD (Table 5).

In the dry season, genotype UCCC6 had the lowest

AUDPC but was not significantly different from that of GH2052, GH3760, GH5302, and GH5332, indicating tolerance against OMD (Table 5). On the other hand, GH3734 had the highest AUDPC which was not significantly different from GH2027, GH5321, GH5211, UCCC1, UCCC2, UCCC3, UCCC4 and UCCC5, indicating that they were very susceptible to OMD (Table 5).

Two-way ANOVA indicated that the overall mean AUDPC recorded in the rainy season (10.06) was not significant different ($F_{20, 123}$= 2.69; *P*=0.103) from that of the dry season (9.48) as shown in Table 5, suggesting that the cropping season had no influence on the amount of OMD experienced by the okra genotypes.

Population of *Podagrica* spp. and associated leaf and fruit damage

***Cumulative average number of Podagrica* spp.**

The cumulative average number of flea beetles (CANFB) per plant recorded for the okra genotypes during the rainy and the dry seasons trials are shown in Table 6. An ANOVA showed significant differences among the okra genotypes both in the rainy season ($F_{20, 60}$=2.34; *P*=0.006) and in the dry season ($F_{20, 60}$=2.27; *P*=0.008). In the rainy season trial, genotype GH3774 had the highest CANFB per plant of 20.49 but this was not significantly different from that of UCCC2, GH5321 and UCCC3 with MCPFB of 19.12, 17.91 and 17.21 per plant respectively. GH5302 had the lowest MCPFB of 10.82 but it was not significantly different from that of GH2063, GH2052, GH4374, GH5332, GH5793, GH2057, GH3731, GH3760, GH6105, UCC4, UCCC5, UCCC6 and GH5786. In the dry season trial, genotype UCCC3 had the highest CAPFB per plant of 18.62, whereas genotype GH2026 had the lowest (5.6).

Two-way ANOVA revealed that the overall CANFB per plant recorded in the rainy–season trial (14.28) was significantly higher ($F_{20, 60}$=21.23; *P*=0.008) than that of the dry season (10.38) as shown in Table 6.

Severity of leaf damage by flea beetle

Mean severity scores of leaf damage by flea beetles during the rainy and dry seasons at 10 WAP are shown in Table 6. ANOVA showed that the severity of pest damage during the rainy season trial differed significantly among the okra genotypes ($F_{20, 60}$ = 15.91; *P*< 0.001). Genotype GH5332 had the lowest severity score of 1.818 which is not significantly different from GH2057, GH3731, GH3760, GH4374, GH5302, GH5786, GH5793, GH6105 and UCCC6 with mean severity scores of 2.09, 2.056, 1.833, 2.069, 1.833, 2.144, and 2.125 respectively (Table 6).

The ANOVA also showed highly significant differences in mean severity scores of leaf damage by the flea beetle among the okra genotypes during the dry season trial ($F_{20,60}$= 11.70; *P*<0.001). Genotype GH5332 had the lowest mean severity score of 0.896, but it was not significantly different from GH2063, GH2026, GH2052, GH3731, GH3760, GH5302, GH5786, GH5793, GH6105 and UCCC6 with mean damage severity scores of 0.958, 0.958, 0.979, 1.083, 1.042, 1.104, 1.104, 0.979, 1.00, and 1.067, respectively.

A two-way ANOVA indicated that overall pest damage on the leaves of okra plants recorded in the rainy season (2.317) was significantly higher ($F_{40, 123}$= 1439.36; *P*<0.001) than that of the dry season (1.223) (Table 6). This suggests a significant effect of the cropping seasons on severity of leaf damage due to beetle infestation.

Percentage fruit damage by the flea beetles

The percentage fruit damage due to the flea beetles infestation differed significantly among the okra genotypes during the rainy season trial (*P*<0.05), but did not differ significantly (*P*> 0.05) among them during the dry season (Table 6). It ranged from 43.7 to 91.2% with a mean pest damage of 72.1% in the rainy season, and ranged between 47 and 84% with a mean of 67.1% in the dry season. A two-way ANOVA, however, did not indicate significant difference (*P*>0.05) in the mean percentage fruit damage due to flea beetle infestation between rainy season (72.1%) and dry seasons (67.1%) as shown in Table 6.

Mean number of fruits per plant and mean fruit yield (t ha^{-1})

The mean number of fruits per plant and the mean fruit yield (t ha^{-1}) recorded during the rainy and the dry seasons differed significantly among the 21 okra genotypes (*P*<0.05) as indicated in Table 7. In both rainy and dry cropping seasons, the mean number of fruits per plant and mean fruit yield (t ha^{-1}) recorded for genotype GH5332 were significantly higher than the other 20 genotypes. Second best was genotype GH6105, of which the mean number of fruits per plant and mean fruit yield (t ha^{-1}) were significantly lower than GH5332 but significantly higher than the other 19 okra genotypes (*P*<0.05). Generally, both the mean number of fruits per plant and mean fruit yield (t ha^{-1}) recorded in the rainy season were higher than those in the dry season (Table 7).

DISCUSSION

This study revealed that all the okra genotypes tested in

Table 6. Mean severity of leaf damage and percentage fruit damage by *Podagrica* spp. during the two planting seasons.

Genotype	Cumulative average no. flea beetle per plant		Mean final severity of leaf damage		Mean percentage fruit damage (%)	
	Rainy season	Dry season	Rainy season	Dry season	Rainy season	Dry season
GH2026	13.19^{cde}	5.60^{g}	1.836^{f}	0.958^{c}	72.5^{abcdef}	56.1^{ns}
GH2052	11.50^{de}	7.37^{efg}	2.194^{d}	0.979^{bc}	73.5^{abcdef}	59.6
GH2057	13.30^{cde}	11.33^{bcdefg}	2.059^{def}	1.167^{b}	91.2^{a}	80.7
GH2063	11.19^{de}	10.50^{bcdefg}	2.153^{de}	0.958^{c}	69.4^{bcdefg}	62.7
GH3731	13.26^{cde}	8.08^{cdefg}	2.056^{def}	1.083^{bc}	81.4^{abcd}	67.1
GH3734	20.49^{a}	14.58^{abc}	2.964^{ab}	1.458^{a}	75.5^{abcdef}	70.7
GH3760	12.91^{cde}	7.83^{defg}	1.833^{f}	1.042^{bc}	87.0^{abc}	84.0
GH4374	11.72^{de}	6.70^{fg}	2.069^{def}	1.179^{b}	68.5^{cdefg}	58.1
GH5302	10.82^{e}	10.62^{bcdefg}	1.847^{ef}	1.104^{bc}	66.5^{defg}	79.5
GH5321	17.91^{ab}	14.00^{abcd}	2.611^{c}	1.500^{a}	68.6^{cdefg}	65.0
GH5332	12.81^{cde}	10.04^{bcdefg}	1.818^{f}	0.896^{c}	65.9^{defg}	62.3
GH5786	14.60^{bcde}	8.25^{cdefg}	2.049^{def}	1.104^{bc}	78.3^{abcde}	47.0
GH5793	12.37^{de}	8.12^{cdefg}	1.861^{ef}	0.979^{bc}	88.1^{ab}	72.3
GH6105	14.12^{cde}	6.88^{fg}	2.144^{def}	1.000^{bc}	82.2^{abcd}	70.4
GH6211	15.67^{b}	15.12^{ab}	2.764^{bc}	1.521^{a}	80.6^{abcd}	77.7
UCCC1	14.22^{cd}	13.33^{abcdef}	2.847^{bc}	1.604^{a}	59.9^{efgh}	54.9
UCCC2	19.12^{ab}	15.00^{ab}	2.833^{bc}	1.458^{a}	72.8^{abcdef}	64.0
UCCC3	17.21^{abc}	18.62^{a}	2.792^{bc}	1.458^{a}	78.8^{abcde}	81.4
UCCC4	14.68^{bcde}	14.04^{abcd}	2.611^{c}	1.542^{a}	58.7^{fgh}	74.6
UCCC5	15.32^{bcde}	12.54^{abcde}	3.194^{a}	1.625^{a}	43.7^{h}	57.8
UCCC6	13.58^{cde}	8.94^{bcdef}	2.125^{def}	1.067^{bc}	51.6^{gh}	62.3
Mean	14.28^{a}	10.83^{b}	2.317^{a}	1.223^{b}	72.1^{ns}	67.1
l.s.d	4.787	6.607	0.3113	0.2081	19.20	26.48
P value	0.006	0.008	<0.001	<0.001	<0.001	0.232

Means in the same column bearing different letters are significantly different ($P<0.05$). Overall means in the same row bearing different letters are significantly different from each other ($P<0.05$). Insect count data was transformed using square root transformation before ANOVA. Difference in the overall mean CANFB per plant between dry and rainy seasons trials was significant (l.s.d= 1.482;d.f.=40; P<0.05). Difference in the overall mean AUDPC between dry and rainy seasons' trials was significant (l.s.d= 0.441; d.f.=40; P<0.001).

both rainy and dry seasons were susceptible to OkMV. However, variation in the levels of incidence and severity were measured. Also variation was recorded in infestation by flea beetles and the associated damage to okra leaves and fruits. This finding is comparable to the work of Udengwu and Dibua (2014) where all 15 okra cultivars screened under field conditions were susceptible to OMD and OLCuD. Nataraja et al. (2013) also found that 23 cultivars of okra tested under field conditions were susceptible to okra yellow vein mosaic and sucking pests such as whiteflies, aphids, and leafhoppers.

Genotypes GH2052, GH2026, GH2063, GH3760, GH5302, GH5332, GH5793, GH6105 and UCCC6 exhibited mild symptoms with significantly low amount of OMD (AUDPC) in both rainy and dry seasons. This suggests that these accessions exhibited a steady state pathogen-host-environment interplay as described by Anneke et al. (2013). On the other hand, genotypes GH2057 and GH44374 exhibited mild symptoms (resistance) during the rainy season but became severe (susceptible) during the dry season. This indicates that their mode of resistance was not stable, but was influenced by varying environmental conditions. This is due to the interplay between the OkMV, host (okra genotypes) and environment (Anneke et al., 2013; Woolhouse and Gowtage-Sequeria, 2005; Barrett et al., 2008; Schrag and Wiener, 1995). Changes in the host-environment and disease ecology are key to creating novel transmission pattern (Anneke et al., 2013). The role of environmental factors such as temperature and humidity in virus survival and transmission, seasonality in abundance and distribution of flea beetle vector could account for the relatively higher disease incidence and severity in the rainy season trial than the dry season trial. In the rainy season, severity scores which ranged from 1.75 to 2.57, with overall mean of 2.156 were recorded for the 21 okra genotypes, whilst, in the dry season, the genotypes had severity scores ranging from 1.475 to 3.75 with a mean of 2.585 (Table 5).

Among the okra genotypes which showed mild

Table 7. Mean number of fruits per plant and mean fruit yield (t ha^{-1}).

Genotype	Mean no. of fruits/plant		Mean fruit yield (t ha^{-1})	
	Rainy season	Dry season	Rainy season	Dry season
GH2026	7.00^{cde}	3.75^{defgh}	3.17^{cdef}	1.517^{defg}
GH2052	3.32^{ef}	2.43^{efgh}	1.55^{ef}	0.900^{fg}
GH2057	5.55^{cdef}	4.33^{def}	4.41^{cd}	3.000^{bc}
GH2063	1.90^{f}	1.75^{gh}	0.86^{f}	0.708^{g}
GH3731	2.53^{f}	2.00^{fgh}	1.39^{ef}	0.973^{fg}
GH3734	5.65^{cdef}	3.21^{defgh}	2.85^{cdef}	1.269^{efg}
GH3760	4.55^{def}	3.83^{defgh}	3.43^{cde}	2.383^{cde}
GH4374	3.92^{ef}	2.71^{efgh}	1.68^{ef}	1.042^{fg}
GH5302	4.25^{def}	4.17^{def}	1.58^{ef}	1.535^{defg}
GH5321	8.05^{cd}	5.46^{bcd}	4.96^{c}	2.516^{cd}
GH5332	20.12^{a}	12.33^{a}	11.88^{a}	6.108^{a}
GH5786	3.22^{ef}	1.67^{h}	1.43^{ef}	0.684^{g}
GH5793	3.77^{ef}	2.33^{efgh}	1.50^{ef}	0.884^{fg}
GH6105	14.90^{b}	7.63^{b}	9.34^{b}	4.061^{b}
GH6211	3.25^{ef}	3.13^{defgh}	1.61^{ef}	1.291^{efg}
UCCC1	4.70^{def}	4.11^{defg}	2.36^{def}	1.651^{defg}
UCCC2	4.75^{def}	4.62^{cde}	2.49^{def}	2.017^{cdef}
UCCC3	3.87^{ef}	3.67^{defgh}	2.57^{cdef}	1.996^{cdef}
UCCC4	4.20^{def}	3.67^{defgh}	2.23^{def}	1.535^{defg}
UCCC5	5.10^{def}	3.35^{defgh}	2.90^{cdef}	1.509^{defg}
UCCC6	9.50^{c}	6.83^{bc}	3.75^{cde}	2.488^{cde}
Mean	5.91	4.14	3.23^{a}	1.908^{b}
l.s.d	3.977	2.387	2.394	1.2247
P-value	<0.001	<0.001	<0.001	<0.001

ns= not significant (P>0.05). Means in a column with the different letters are significantly different by l.s.d test at P<0.05. Overall means in the same row bearing different letters are significantly different. Difference in the overall mean fruit yields between dry and rainy seasons was significant (l.s.d=0.417; d.f.=40; P<0.001).Fruit yield was calculated as the cumulative of five harvesting done after 50% flowering.

symptom of OMD, GH5332 had the highest mean number of fruits per plot and mean fruit yield in tonnes per hectare (Table 3). This suggests that genotype GH5332 was tolerant to OkMV infection. On the contrary, genotype GH6105, even though it demonstrated severe symptom of OMD, it had the second highest fruit yield (Table 3), far above the national average of 2.5 t ha^{-1} (FAOSTAT, 2008), indicating that it was also tolerant to OkMV infection. Generally, the fruit yields recorded for the 21 okra genotypes in the rainy season were higher than that of the dry season. Thus, OkMV resistance/tolerance in GH5332 and GH6105 respectively, are not complete but can be influenced by environmental factors as reported by Juergens et al. (2010) when they screened oilseed rape cultivars against Turnip yellows virus (TuYV, genus *Polerovirus*). This type of resistance could be controlled by a single major gene together with additional contributing genes (Dreyer et al., 2001).

The cumulative average population of flea beetle and the associated leaf damage were significantly higher in the rainy season than in the dry season. These results thus corroborate the findings by Fasunwon and Banjo (2010) where higher populations of *Podagrica* spp. were recorded in early planting seasons than the late planting season. It has also been reported that the feeding activity of *Podagrica* spp. causes damage comprising of characteristic perforations of leaves, and irregular holes which reduce the photosynthetic surface area of the leaves leading to a great reduction of yield in okra (Echezona and Offordile, 2011). This may explain why leaf damage in terms of perforations in the leaves was higher in the rainy season when the beetle populations were also higher compared to the dry season.

The percentage of fruit damage due to the flea beetle infestation was extremely high in case of rainy season (43.7 and 91.2%) than the dry season (47 to 84%). Fruit damage affects the market value of the crop and could have a serious consequence on the profitability and farmers' income. This finding supports that of Obeng-Ofori and Sackey (2003) which states that, flea beetles are the most important pest of okra in West Africa.

The observed variation in disease severity and AUDPC could be due to different interaction effects between different host genotypes characteristics and OkMV and the biotypes or the species of flea beetles that were present. Similar reasons were assigned to the variations in the incidence and severity of Tomato yellow leaf curl virus (TYLCV, genus *Begomovirus*) among tomato genotypes tested (Aziziet al., 2008; Abu et al., 2011) and to variation in the susceptibility of *Arabidopsis thaliana* accessions to TuYV (Asare-Bediako, 2012). Plant characteristics are also known to affect vector population (Khan and Mukhopadhyay, 1986; Singh, 1990), and hence disease severity. Secondary plant metabolites (terpenoids, phenolics, flavonoids, quinones, alkaloids, cyanogenic glycosides, glucosinolates, etc.) and volatile substances (Karban et al*.,* 1997; Mello and Silva-Filho, 2002; Wu and Baldwin, 2010) are known to impart resistance to herbivore insects (Ehrlich and Peter, 1964).

Conclusion

The study has revealed that genotypes GH3760, GH2052, GH5332, UCCC6, GH5302, GH5793, GH2026 and GH2063 were tolerant to OkMV infection, flea beetle infestation and associated leaf damage during both rainy and dry season trials. However, among these, only genotype GH5332 had significantly higher yield, far above the national average yield, and can therefore be evaluated further for release to farmers. Genotype GH6105 which also had very high yield but very susceptible to virus and flea beetle damage could be incorporated into breeding programmes for subsequent release to okra farmers. With high percentages of fruit damage due to the flea beetle infestation, this insect is a serious pest of okra in Ghana besides transmitting OkMV and effort should be made to manage it.

CONFLICT OF INTEREST

The authors have not declared any conflict of interest.

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