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DEVELOPMENT AND DETERMINATION OF LARVAL STAGES OF FALL ARMYWORM ON SOME STAPLE CROPS

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ABSTRACT

Fall armyworm (FAW), Spodoptera frugiperda Smith (Lepidoptera: Noctuidae), is a serious emerging pest of maize (Zea mays L.) and many other alternative crop hosts in sub-Saharan Africa. Adequate knowledge on the development of S. frugiperda on maize and other alternative host crops is important in the development of integrated pest management programmes. The objective of this study was to determine the larval developmental stages of FAW using head capsule and other body morphometrics of FAW on maize and other alternative host crops in Nigeria. Food hosts (maize, cassava and cowpea) were replicated five times and arranged in a Completely Randomised Design. The results showed that mean growth ratio of larval development on maize, cassava and cowpea were 1.51, 1.54 and 1.50, respectively; and all conformed to Dyar's rule. Head capsule width of larval instars showed six frequency peaks, representing six larval instars. Mean width of head capsule from the first to sixth larval, in the three crops, were significantly different. The shortest (14 days) and longest (17 days) developmental periods were recorded on maize and cassava. Pupal weight and length were not significantly different among the crops. There was a linear and significant correlation (maize = 0.98, cassava = 0.98 and cowpea = 0.99) between the stages of larval development and head capsule width. The number of larval instars of FAW, duration of their developments and the weights of larva and pupa on maize, cassava and cowpea are useful information in determining the number of generations of FAW on each crop. This information could, therefore, be applied in decision making on the appropriate time and duration of application of control actions when these crops are infested.

Key Words: Dyar's rule, growth ratio, head capsule, larval instars

RÉSUMÉ

La chenille légionnaire d'automne (FAW), *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae), est un important ravageur émergent du maïs (*Zea mays* L.) et de nombreuses autres cultures hôtes alternatives en Afrique subsaharienne. Des connaissances adéquates sur le développement de *S. frugiperda* sur le maïs et d'autres cultures hôtes alternatives sont importantes dans le développement

O.O. ODEYEMI et al.

de programmes de lutte intégrée contre les ravageurs. L'objectif de cette étude était de déterminer les stades de développement larvaire de la chenille légionnaire d'automne en utilisant la capsule céphalique et d'autres morphométries corporelles de la chenille légionnaire d'automne sur le maïs et d'autres cultures hôtes alternatives au Nigeria. Les hôtes alimentaires (maïs, manioc et niébé) ont été répliqués cinq fois et disposés selon un plan complètement aléatoire. Les résultats ont montré que le taux de croissance moyen du développement larvaire sur le maïs, le manioc et le niébé était respectivement de 1,51, 1,54 et 1,50; et tous se conformèrent à la règle de Dyar. La largeur de la capsule céphalique des stades larvaires a montré six pics de fréquence, représentant six stades larvaires. La largeur moyenne de la capsule céphalique de la première à la sixième larve, dans les trois cultures, était significativement différente. Les périodes de développement les plus courtes (14 jours) et les plus longues (17 jours) ont été enregistrées sur le maïs et le manioc. Le poids et la longueur des pupes n'étaient pas significativement différents entre les cultures. Il y avait une corrélation linéaire et significative (ma $\ddot{s} = 0.98$, manioc = 0.98 et niébé = 0.99) entre les stades de développement larvaire et la largeur de la capsule céphalique. Le nombre de stades larvaires de la chenille légionnaire d'automne, la durée de leur développement et les poids des larves et des pupes sur le maïs, le manioc et le niébé sont des informations utiles pour déterminer le nombre de générations de la chenille légionnaire d'automne sur chaque culture. Cette information pourrait donc être appliquée dans la prise de décision sur le moment et la durée appropriés d'application des actions de contrôle lorsque ces cultures sont infestées.

Mots Clés : La règle de Dyar, le taux de croissance, la capsule céphalique, les stades larvaires

INTRODUCTION

The fall armyworm (Spodoptera frugiperda Smith (Lepidoptera: Noctuidae) is an increasingly damaging polyphagous pest on major food security crops in sub-Saharan Africa (SSA). The pest originated from the sub-tropical and tropical regions of America and feeds mostly on leaves and stems of more than 80 crop species (CABI, 2017). It feeds and causes severe damages mostly to the cultivated grasses of economic importance such as maize, sorghum, sugar cane and other crops like legumes and cotton. The pest was first reported in 2016 in Nigeria, Sao Tomé, Benin and Togo (IITA, 2016, IAR&T, 2016), but currently spans the whole SSA region (FAO 2017; FAO 2018). Losses due to confirmed and suspected infestations of fall armyworm in maize, sorghum, rice and sugarcane in African countries have been estimated at USD13.38 billion (CABI, 2017). A suggestion has been made for a coordinated regional approach and cross boundary cooperation in surveillance and monitoring, diagnostics, epidemiology, containment and management of FAW in order to avert the menace caused

by this emergency plant health matter (FAO, 2017).

Control of FAW is usually achieved through the application of synthetic insecticides (Blanco et al., 2010), but it involves high cost, potential environmental contamination, and development of resistance to chemicals, and often pest resurgence. Smallholder farmers in the Sub-Saharan Africa demand environmentally friendly and cost-effective management method for FAW. Therefore, sustainable management of FAW requires efficient and cost effective management strategies that suits smallholder farmers. Several findings show that there are multiple cost-effective control options involving cultural (CABI, 2017; Assefa and Ayalew, 2019; Kumela et al., 2019), biological, botanical (Murua et al., 2006; Blanco et al, 2014), push-pull (Kumela et al., 2019) and integrated FAW management (Haftay, 2020) approaches relevant to smallholder farmers. However, little success in applied ecology and pest monitoring will be achieved without a better understanding of the phenology and dynamics of insects' life cycle on its candidate hosts.

446

In order to establish rational pest management strategies, it is essential to first determine the basic life cycle of a pest, including the number of larval instars on suspected host crop species. Technical information on larval instars has been reported to be essential for mortality-survivorship studies based on life tables, population modeling and determination of community structure, each of which inform the development of pest management strategies (Gold et al. 1999; Alencar et al., 2001) especially in pest species where population management targets the larval stage (Gray et al., 1996). One method for establishing the number of larval instars involves measuring the width of the larval head capsule throughout development and applying Dyar's rule (Panzavolta 2007; Cazado et al. 2014). This method is based on the assumption that the size of the head capsule is consistent throughout a given instar but undergoes a regular geometric increase as the larva passes from one instar to the next (Dyar, 1890).

The developmental stage of fall armyworm that causes damage is the larval stage. Larvae of fall armyworm are voracious in nature and cause huge damage by defoliating host plant. First instar larvae scrape leaves and shows pinhole symptoms like stem borer attack and windowpane feeding symptoms like European corn borer attack (Sisay et al., 2019). In the later vegetative stages, damage results in skeletonised leaves and heavily windowed whorls (Goergen et al., 2016). The matured larvae present in the whorls of older plants can feed on maize cob or kernels, reducing yield and quality (Abrahams et al., 2017; Capinera, 2017). The damage caused by young larvae as a result of feeding on young leaf wheels, ear and tassel sometimes leads to total yield loss (De Almeida Sarmento et al., 2002). This implies that this pest could cause approximately 100% crop loss in maize if not managed with due consideration (CABI, 2019). Therefore, any meaningful management strategy for ameliorating the

effect of fall armyworm on its host crops must target the larval stage, which causes the damage.

Information on the incidence of FAW has been documented; but there is a paucity of information on the developmental biology of FAW on its major host crops in the SSA region. The objective of this study was to determine the larval developmental stages of FAW using head capsule and other body morphometrics of FAW on maize and other alternative host crops in Nigeria.

METHODS

A culture of S. frugiperda was raised from ten batches of eggs each containing 100 and 150 eggs collected from infested cultivated maize fields, established by the Institute of Agricultural Research and Training (IAR&T), Ibadan, southwestern Nigeria. The maize leaves with attached eggs were cut from the parent plant using scissors. The eggs were surface-sterilised by dipping them in 0.1% freshly prepared sodium hypochlorite for 15 minutes; rinsed carefully using distilled water, and then dried on paper towel. The surface sterilised egg masses were transferred in a lunchbox on moist paper towel. The box was covered with a well-ventilated top, and placed in the oviposition cage. Relative humidity of 80-90% was maintained in the lunchbox by placing a Petri dish with water soaked cotton wool at the bottom of the box, below the paper towel. The eggs were left for about 4-6 days to develop into a blackhead stage, and then hatched into neonate larvae.

The newly hatched caterpillars were separated into three groups. Larvae in groups I, II and III were fed daily with maize, cowpea and cassava leaves, respectively.

Five larvae were picked from each group and preserved in sample bottles containing 70 % ethanol. The preservation continued until the larvae began to pupate. The larvae were subsequently taken out for measurement of the head capsule width (at the vertex), body

RESULTS

length (from the tip of head to tip of the abdomen), and body width (at the prothorax). Young larvae were measured under a stereomicroscope fitted with an eyepiece micrometer (x10); while mature larvae were measured with a plastic metric ruler. The histograms of the head capsule widths were plotted and separated into groups using the highest point of each group to determine the number of larval instars in the ontogeny of *S. frugiperda*.

The head capsule groupings, body length and body width were subjected to analysis of variance and their means separated using Tukeys HSD. The grouping was further tested for conformity with Dyar's rule (Dyar, 1890) by comparing the observed and calculated average head capsule widths of each instar using the Student's t test. The calculated average head capsule width of an instar was obtained as the product of the mean head capsule width or the succeeding instar and its mean growth ratio. The growth ratio was calculated as the quotient or the observed mean head capsule width of the succeeding instar and the observed mean head capsule width of the previous instar (Opoosun and Odebiyi, 2009). The relationships between the head capsule widths of all larval instars and duration of each instar were subjected to regression analysis.

Body length and width. The body length of FAW reared on maize, cassava and cowpea leaves were not significantly different at larval instars I (F = 0.35, df = 2, P<0.71), III (F = 2.65, df = 2, P<0.0902), IV (F = 2.39, df = 2, P < 0.1084) and V (F = 3.27, df = 2, P < 0.0504). The length of larval instar II recorded on cassava leaf was significantly (F = 9.28, df = 2, P = 0.0021) different from the length observed on maize and cowpea leaves. The length of larval instar VI on cowpea was not significantly different from the corresponding length on maize, but was significantly (F =3.75, df = 2, P<0.0307) different from that on cassava (Table 1). The width of the FAW larval instars I, IV and V were not significantly different on maize and cassava leaves, but were significantly smaller (F = 4.44, df = 2, P< 0.0193; F = 49.92, df = 2, P<0.0001; F = 23.66, df = 2, P < 0.0001) on cowpea leaf. The width of the larval instar II on cassava was significantly (F = 4.26, df = 2, P = 0.0343) greater than on maize and cowpea leaves. At the 3rd larval instar, the body widths were not significantly different when fed on maize, cassava and cowpea leaves (F = 1.49, df = 2, P = 0.2454). The width of instar VI was significantly different among the three crops (F = 12.36, df = 2, P < 0.0001) (Table 2).

TABLE 1. Body length (mm) \pm SE of the life stages of *S*. *frugiperda* on different crops (24-30 °C; 60.0 \pm 10% RH; 12 hr photoperiod)

Food host		Larval instars							
	1st instar	2nd instar	3rd instar	4th instar	5th instar	6th instar			
Maize	2.91±0.10a	5.69±0.41b	10.80±0.59a	17.63±0.18a	20.90±0.65a	25.69±0.57ab			
Cassava Cowpea	2.95±0.31a 2.68±0.22a	8.50±0.50a 5.82±0.39b	10.78±0.52a 9.22±0.52a	15.00±1.83a 14.27±0.60a	20.90±0.71a 19.41±0.29a	26.88±0.63a 24.31±0.71b			

Numbers followed with the same alphabet in the column are not significantly different at P < 0.05 Tukey's HSD

Food host	Larval instars								
	1st instar	2nd instar	3rd instar	4th instar	5th instar	6th instar			
Maize	0.49±0.03a	0.83±0.09b	1.37±0.08a	2.40±0.04a	3.27±0.3a	3.95±0.12a			
Cassava	0.46±0.05a	1.18±0.04a	1.41±0.07a	2.31±0.09a	3.31±0.13a	3.48±0.06b			
Cowpea	0.32±0.04b	0.75±0.06b	1.24±0.06a	1.63±0.05b	2.30±0.11b	3.22±0.05b			

TABLE 2. Body width (mm) \pm SE of the life stages of *S*. *frugiperda* on different crops (24-30 °C; 60.0 \pm 10% RH; 12 hr photoperiod)

Numbers followed with the same alphabet in the column are not significantly different at P < 0.05 Tukey's HSD

Growth ratios. The growth ratios varied across the larval instar stages, ranging from 1.38 to 1.81 on maize, 1.34 to 1.70 on cassava and 1.35 to 1.64 on cowpea, with mean growth ratios of 1.51, 1.54 and 1.50, respectively (Table 3). Table 3 also showed the conformity of the head capsule measurement to Dyar's rule, and based on a *t*-test for the differences between the observed average head capsule width and the calculated average (d), a *t*-value greater than 2.57 was needed to depict a significant difference between the observed and the calculated averages. However, t-values of 1.7, 2.24 and 1.79 were obtained when FAW larvae were reared on maize, cassava and cowpea, respectively.

The head capsule. The head capsule width of each successive instar differed significantly on maize (F = 4115.56, df = 5, P<0.0001), cassava (F = 3397.69, df = 5, P<0.0001) and cowpea (4411.72, df = 5, P < 0.0001) (Table 4). The development of first instar larva took 3 days before it molted into the second instar, irrespective of the food host; while second and third instar larvae had 2 days each on maize, cassava and cowpea. The duration of fourth larval instar was 2, 2 and 3 days on maize, cassava and cowpea, respectively. The fifth instar completed its development on maize, cassava and cowpea at 2, 2 and 4 days, respectively; while the sixth instar duration on maize, cassava and cowpea were 3, 5 and 3

days, respectively. Consequently, the total larval developmental periods of FAW on maize, cassava and cowpea were 14, 16 and 17 days, respectively (Table 4). The daily measurement of head capsule width of larval instars on each food host showed six frequency peaks as confirmed by Dyar's rule representing six larval instars (Figs. 1 - 3).

Regression analysis. There was a positive and significant relationship between the larval developmental period and mean head capsule width on maize (Fig. 4A), cowpea (Fig. 4B) and cassava (Fig. 4C). The food hosts did not affect both the pupal weight (F = 1.00, df = 2, P = 0.4058) and pupal length (F = 1.27, df = 2, P = 0.3261) significantly (Table 5).

DISCUSSION

Body length and body width. The progressive increase in body length and width of *S. frugiperda* on the three food hosts indicate that the food hosts are suitable for the development and survival of the insect. This is an indication that in the absence of maize, the major host, the insect can sustain its pest status on cassava and cowpea. Hence, for effective management FAW in maize production in sub-Saharan Africa, the existence of alternative host crops, most of which are co-inhabitants with maize, need to be integrated within the strategy.

Larval instar	Maize			Cassava			Cowpea					
	OA (mm)	GR	CA (mm)	D (d)	OA (mm)	GR	CA (mm)	D	OA (mm)	GR	CA (mm)	D
I	0.36				0.33				0.36			
П	0.53	1.47	0.54	-0.01	0.56	1.7	0.51	0.05	0.58	1.61	0.54	0.04
Ш	0.97	1.81	0.8	0.17	0.95	1.7	0.86	0.09	0.95	1.64	0.87	0.08
N	1.37	1.41	1.46	-0.09	1.38	1.45	1.46	-0.08	1.31	1.35	1.43	-0.12
V	1.99	1.45	2.07	-0.08	2.04	1.49	2.13	-0.09	1.98	1.51	1.97	0.01
VI	2.74	1.38	3	-0.26	2.73	1.34	3.14	-0.41	2.71	1.37	2.97	-0.26
Mean growth ratio		1.51				1.54				1.5		
Average difference			-0.05				-0.09				-0.05	
Standard deviation of differences		0.16				0.2				0.14		
t calculated			1.7				2.24				1.79	
t tabulated			2.57				2.57				2.57	

TABLE 3. Head capsule width for larval instars of *S. frugiperda* and test for conformity to Dyar's rule

Reject Ho if *t* calculated >*t* tabulated

Decision: do not reject Ho; growth ratio conforms to Dyar's rule

Note: growth ratio-the mean head capsule width of a succeeding instar divided by the mean head capsule width of a preceding instar

Calculated average—observed mean head width of a preceding instar multiplied by the mean growth ratio

OA = Observed Average, GR = Growth ratio, CA = Calculated Average, D = Differences between OA and CA

O.O. ODEYEMI et al.

	ADD	e	5	L	10	14	17
ea	SI (day)	e	0	0	б	4	Э
Cowp	Range	0.3 - 0.4	0.5 - 0.6	0.9 - 1.0	1.3 - 1.4	1.95 - 2.3	2.6 - 2.8
	HCW (mm)	0.36±0.01a	$0.58\pm0.01b$	0.95±0.01c	1.31±0.01d	1.98±0.02e	2.71±0.01f
	ADD	e	S	Г	6	11	16
ava	SI (day)	e	0	0	0	0	5
Cass	Range	0.3 - 0.4	0.5 - 0.6	0.9 - 0.95	1.3 - 1.4	1.95 - 2.3	2.7-2.8
	HCW (mm)	0.35±0.01a	0.56±0.01b	0.95±0.01c	1.38±0.01d	2.04±0.04e	2.73±0.01f
	ADD	e	5	Г	6	11	14
ce.	SI (day)	e	7	7	7	7	Э
Maiz	Range	0.3-0.4	0.5 - 0.6	0.9 - 1.2	1.3 - 1.4	1.9 - 2.1	2.7-2.8
	HCW (mm)	0.36±0.01a	0.53±0.02b	0.97±0.03c	$1.37\pm0.02d$	1.99±0.02e	2.74±0.01f
Larval instar			Π	Ш	N	٧	M

HCW = Head capsule width, SI = Stadium of Instar, ADD = Accumulated days of development

TABLE 4. Head capsule \pm (SE) measurement and developmental period of larval instars of S. frugiperda on three different crops

Growth ratios. The results of the head capsule size showed that it increased at each molt by an average of 1.51, 1.54 and 1.50) on maize, cassava and cowpea, respectively, indicating that the molting of FAW on the three crops progresses almost at the same rate. Information about growth rates of FAW on different crops can be used to predict when the insects will be most abundant during the growing season and, consequently, when crops are most at risk. There was no significant difference between the observed and the calculated average growth rates, showing conformity to Dyar's rule, thus, approximating the expected constant ratio of 1.4 for lepidopterous larvae (Wigglesworth, 1974).

Head capsule. Observations on the life stages of S. frugiperda with respect to measurement of larval head capsule showed six larval instars on maize, cassava and cowpea. This is confirmed from the frequency distribution of head capsule width of successive instars, which showed multimodal curves with six distinctive modal peaks. This is similar to the report of Hardke et al. (2015) that S. frugiperda passed through six larval instars during its development. There was no intraspecific variability in the number of larval instars in response to the different crops used. This may be suggesting that the food quantity and quality in the crops supported the development of the insect. This is also a signal that FAW is a threat not only to the production of maize but also be to cassava and cowpea.

Regression analysis. A linear relationship obtained between head capsule width and larval developmental period with a high regression coefficient of 0.98 (maize), 0.99 (cowpea), and 0.98 (cassava) was highly significant. The excellent fit of the linear model suggests that there was nearly no overlap in the width of the head capsules among the different instars. The linearity of the relationship between the head capsule width and larval developmental period further indicated that variation in the developmental period for each larval instar was



Figure 1. Frequency distribution of the head capsule width of larval instars of S. frugiperda.on maize.



Figure 2. Frequency distribution of the head capsule width of larval instars of *S. frugiperda on* cassava.



Figure 3. Frequency distribution of the head capsule width of larval instars of *S. frugiperda* on cowpea

Food host	Pupal weight	Pupal length
Maize Cassava Cowpea	0.15±0.01a 0.16±0.01a 0.13±0.01a	1.42±0.06a 1.38±0.03a 1.30±0.06a

TABLE 5. Mean pupal weight and length $(\pm S.E)$
of S. frugiperda on three crop plants

Figures followed with the same alphabets in the column are not significant (Tukey's HSD, P<0.05

not strong enough to deviate from Dyar's rule; and also that the longer the stadium the greater the amount of growth (Oke and Odebiyi, 2010).

Larval and pupal developmental period. The total larval developmental periods of FAW on the tree crops were very close, however, 14 days recorded on maize as against 16 and 17 days on cassava and cowpea, respectively may be preference related. The relatively shorter larval developmental period of FAW observed on maize may be as a result of maize being its primary host reported in Africa (Prassana *et al.*, 2018). However, the successful development and survival of *S. frugiperda* on maize, cassava and cowpea is a



Figure 4. Relationship between head capsule width and larval developmental stages of *S. frugiperda* on maize (A), cassava (B) and cowpea (C).

warning signal that *S. frugiperda* could use other crops like cassava and cowpea as alternative hosts.

Implications of new developments on the strategic management of FAW. The development of FAW larvae on other host crops such as cassava and cowpea implies that these alternative host crops cannot be intercropped with maize nor used as succeeding crops to maize in crop rotation in the management of FAW because they are important staple crops. However, other non host crops could be considered in combination with FAW host crops to reduce the incidence and severity of FAW infestation.

CONCLUSION

It is clear that *S. frugiperda* completes its larval development in six larval stages on maize, cassava and cowpea. It is evident from the relatively shorter larval developmental period of *S. frugiperda* on maize could confer a high susceptibility of maize to FAW infestation compared to cassava and cowpea. Strategic management of FAW should, therefore, be targeted at the larval developmental period. The development of crop varieties with the ability to delay or deter the development of FAW so as to reduce population build up could also be incorporated into the integrated pest management components of FAW.

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O.O. ODEYEMI et al.

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456

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