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Assessing New Scouting Approaches for Field Sampling of *Spodoptera frugiperda* and Its Parasitoids †

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Abstract: The efficient control of fall armyworm (FAW) *Spodoptera frugiperda* depends on timely and reliable detection of its egg masses and early larval stages. A range of tools exist for field scouting of FAW among which the newly developed Farmer Interface App (FIA). The current experiments were conducted under the hypothesis that scouting pattern relevance determine the significance of FAW and parasitoids oviposition data collected. Seven scouting patterns were compared during intensive sampling of FAW and two parasitoid species in maize plots. The FIA - being the simplest model among them, and the one which can be easily implemented by low-literate farmers – gave precision levels statistically comparable to those of more complex models. The pest oviposition data, the egg parasitoid *Telenomus remus* and the egg-larval parasitoid *Chelonus* sp. were modelled in this study.

Keywords: species monitoring; sampling accuracy; scouting method; application algorithm

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1. Introduction

The rapid spread of fall armyworm (FAW) *Spodoptera frugiperda* (J E Smith) (Lepidoptera: Noctuidae) in maize cropping areas of Africa [1] forced pest control practitioners to a comprehensive re-assessment of an appropriate integrated pest management strategy adapted to smallholder farm conditions [2,3]. However, such a strategy cannot be implemented without proper surveillance, monitoring and scouting efforts [3,4]. Scouting a maize field for assessing the FAW infestation level is key to the decision of deploying curative control methods. This enables early alerts and promotes effective use of insecticides by indicating to farmers when and most importantly when not to apply insecticides based on calculations of intervention thresholds, which to the best of our knowledge are only based on expert opinion for FAW in countries of recent invasion [3-5] in African, Asian and Australian continents.

Two common semi-systematic FAW scouting patterns are used in maize production systems: the "W" and the "Ladder" models (Figure 1) depending on maize growth period. The "W" scouting pattern is frequently used during initial maize growth stages until the plants become taller, dense and the "W" pattern difficult to implement. The "Ladder" model is suitable at maize tasseling stage and beyond [4,6].

A few initiatives were launched post FAW invasion in Africa and other invaded continents with different scouting protocols for FAW [3,4]. These approaches can be implemented by advanced frontline and extension staff but difficult for use by low-literate farmers. Therefore, one of the tools assembled to help farmers scout their fields and support decision for timely intervention was the Farmer Interface Application (FIA) developed by the International Institute of Tropical Agriculture (IITA) and partners. The FIA scouting protocol is built on a "Diagonals" model (Figure 1) which is deemed simplest for a tool intended to be used by low-literate farmers.

The current experiments were conducted under the assumption that the highest the plant infestation or parasitoid oviposition on a set of plot quadrats the relevant they are for consideration for field scouting. This means that the scouting pattern able to capture most oviposition sites on a maize plot should be the most accurate. In this study, we hypothesized that FAW and parasitoids field oviposition rate varies over maize growth period. We also assumed that scouting pattern accuracy is dependent on the maize growth period. Two new scouting patterns were proposed and examined in this work.

2. Materials and methods

2.1. Experimental procedure and plot layout

All experiments were conducted on-station at the International Institute of Tropical Agriculture (IITA-Benin), 06° 25.05′N and 02° 19.89′E, in southern Benin. The rainfall in southern Benin has a bimodal regime (March – July and September – November). The early maize variety EVDT 99 W STR was sown during preliminary trials at a spacing of 80 cm between rows and 40 cm between plants. The extra-early maize variety 2009 TZEW DT STR was planted for the second growing season experiments at same spacing modalities. NPK-fertilizer application was done at the dosage of 100 kg*ha^{-1} two weeks post maize emergence and just after the first weeding. Urea was applied at the dosage of 50 kg*ha^{-1} two weeks after the first fertilizer application. In total three weedings were done on a 2-4-week intervals depending on weed density.

Preliminary trials were conducted from June 19 to August 16, 2019, on two different fields of 1 ha and 0.5 ha, separated by 50 m of natural fallow. The fields were divided into 25 plot quadrats, whose dimensions were 10 x 10 m and 6.5 x 5.5 m for the 1 ha and 0.5 ha fields, respectively. On the 1 ha field, the quadrats were 10 m apart and the outer quadrats were 5 m from field border to avoid edge effects. On the 0.5 ha field, the quadrats were 6.5 m apart over rows and 5.5 m apart over columns; the outer quadrats were 2-3 m from field border. All the quadrats were numbered following the same principle for easy of reference. Rows were north-south oriented and columns east-west, the first row being the most eastern and the first column the most northern (Figure 1). As by example, the north-easternmost quadrat was coded 1.1 (Row 1 and Column 1). The south-westernmost quadrat was numbered 5.5 (Row 5 and Column 5) and the one in the middle 3.3 (Row 3 and Column 3).

Experiments were also established during the second growing season from October 11 to November 29, 2019 on three 1 ha fields separated by 40-70 m natural fallow. Each field contained four 40 x 40 m experimental plots. The experimental plots were demarcated with a distance of 20-40 m within the fields. Twenty-five (25) 4 x 4 m quadrats were defined on each experimental plot for data collection. The quadrats were 4 m apart and outer quadrats were 2 m from plot border to avoid edge effects. The quadrats were numbered similar as described above.

2.2. Data collection

Intensive sampling of FAW egg masses and larvae was performed on three-day intervals throughout the experiments. Observations on plant infestation were done on 20 random plants on all the 25 quadrats per experimental plot. The same sample plants were followed throughout to count the number of FAW egg masses and larvae per plant.

Likewise, FAW eggs and caterpillars were collected during first season's trials on other 10 random plants and conditioned in the laboratory for further identification of emerging parasitoids. Hatching adult parasitoids were screened and counted per species.

2.3. Scouting patterns

All scouting patterns examined consisted of 5 quadrats each. "Diagonals" shape was compared to the "W" and "Ladder" patterns [4] including other-way-round models of "W" and "Ladder" (Figure 1). In this study we proposed two additional patterns "Cross" and inverted "T" consisting of 4 new quadrats not considered in previous forms (Figure 1); totaling 7 patterns compared.

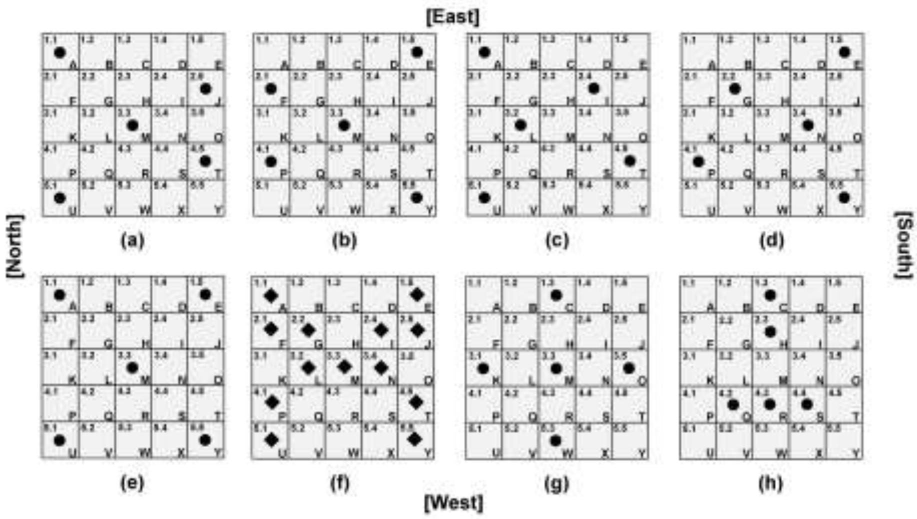

Figure 1. Diagram of quadrats and scouting patterns measured. Quadrat location was defined with Row#.Column# and coded A-Y (quadrat 3.3 coded M is the middle unit); detailed description is provided in the text. Each scouting pattern (a-h) consisted of 5 quadrats (dots); (a) "W" [4], (b) Other-way-round "W" (adapted from [4]), (c) "Ladder" [4], (d) Other-way-round "Ladder" (adapted from [4]), (e) Diagonals [7], (f) all target quadrats (diamond squares) of patterns a-e, (g) "Cross" pattern, and (h) Inverted "T" pattern; (g) and (h) were the new scouting patterns proposed and estimated in this study.

2.4. Statistical analyses

Analysis were firstly performed to determine the most significant oviposition quadrat hotspots. Secondly, measurements on the above-defined scouting patterns (Figure 1) were done using the datasets of corresponding quadrats. The statistical significance of the patterns was analyzed for the different responses i.e. the numbers of infested plants (with FAW eggs and/or larvae) and the numbers of adult parasitoids emerged in the laboratory. Comparisons were done over three scouting periods matching three maize growth periods: early whorl stage (0-21 days), late whorl stage (22-49 days) and beyond tasseling (> 50 days) [4]. All observation dates were grouped into the three periods. Data on FAW larvae, FAW-infested plants and emerging adult parasitoids were log-transformed before analysis to meet the assumptions of normality and equal variance. The data were then analyzed using a linear model analysis of variance (ANOVA) type II sum of squares with field size (preliminary trials) and scouting quadrat/pattern design as fixed effect factors, and maize growth period as a categorical variable. Tukey's post hoc tests at the 5% significance level were used to examine differences among the groups, followed by pairwise comparisons [8].

3. Results

3.1. FAW oviposition and scouting patterns

FAW infestation was higher during the first season (Figure 2). The highest FAW oviposition occurred during the early maize whorl stage (0-21 days) (Figure 2a) during the first growing season. For the second growing season there was an additional peak of oviposition during the late maize whorl stage (22-49 days) and specifically on the 24th day post maize plant emergence (Figure 2b). Still, the greatest larval infestation rates of FAW was confined to the early maize whorl stage (0-21 days) for both seasons. FAW oviposition decreased significantly at post-tasseling stage (> 50 days) (Tables 1 & 2; Figure 2). The highest oviposition rate was recorded on the larger plot (1 ha) during the first growing season. Quadrat, field and maize growth period were all highly significant effects for FAW oviposition except quadrat factor for all the responses during the first growing season (Table 1). The top 5 scouting quadrats of FAW infestation hotspots during the second planting period were O, T, E, Q, Y and S, B, N, G, X (Figure 1) for egg masses and for both larvae and infested plants, respectively. None of these sets of quadrats matched exactly any of the seven studied scouting patterns. Consistently, the scouting pattern effect was not statistically significant for all the responses regarding FAW oviposition during the first growing season (Table 2). However, the factor was significant for the numbers of FAW larvae and plants infested by the pest during the second season. The top 5 patterns for both responses were inverted "T" (Figure 1h), other-way-round "Ladder" (Figure 1d), "Ladder" (Figure 1c), "Cross" (Figure 1g) and "W" (Figure 1a).

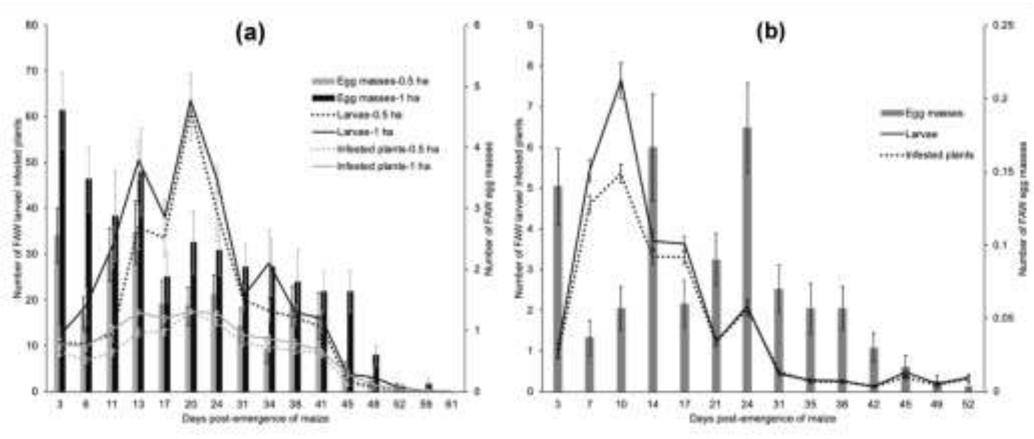

Figure 2. Average FAW egg masses, larvae and infested plants fluctuation on all scouting quadrats over (a) first and (b) second planting season and on different field size during (a) first planting season (0.5 and 1 ha).

Table 1. ANOVA results of scouting quadrats related to the number of FAW egg masses, and the log-transformed number of larvae and infested plants during first and second maize growing seasons.

Source of variation	**First maize growing season**			**Second maize growing season**		
	Egg masses	**Larvae**	**Infested plants**	**Egg masses**	**Larvae**	**Infested plants**
Quadrat¶	$F_{24,770}$= 1.094 P= 0.343	$F_{24,772}$= 0.875 P= 0.638	$F_{24,770}$= 1.047 P= 0.401	$F_{24,4173}$= 2.195 P= 0.0006	$F_{24,4173}$= 2.058 P= 0.001	$F_{24,4125}$= 2.759 P= $9.07*10^{-6}$
Field¶¶	$F_{1,770}$= 35.106 P= $4.70*10^{-9}$	$F_{1,772}$= 9.671 P= 0.001	$F_{1,770}$= 30.049 P= $5.71*10^{-8}$	-	-	-

Period¶¶¶	$F_{2,770}$= 93.684 P= $2.2*10^{-16}$	$F_{2,772}$= 105.014 P= $2.0*10^{-16}$	$F_{2,770}$= 440.007 P= $2.2*10^{-16}$	$F_{2,4173}$= 15.473 P= $2.01*10^{-7}$	$F_{2,4173}$= 576.491 P= $2.2*10^{-16}$	$F_{2,4125}$= 727.613 P= $2.2*10^{-16}$
Quadrat x Period	-	-	-	-	-	$F_{48,4125}$= 1.573 P= 0.007
Field x Period	$F_{2,770}$= 4.723 P= 0.009	-	$F_{2,770}$= 6.593 P= 0.001	-	-	-

¶ Scouting quadrat (25 plot quadrats) ︱ ¶¶ Field size (0.5 and 1 ha) ︱ ¶¶¶ Maize growth period [early whorl stage (0-21 days), late whorl stage (22-49 days) and beyond tasseling (> 50 days)]. Non-significant interactions were excluded from full models and they were not considered in final reduced models.

Table 2. ANOVA results of scouting patterns related to the number of FAW egg masses, and the log-transformed number of larvae and infested plants during first and second maize growing seasons.

Source of variation	**First maize growing season**			**Second maize growing season**		
	Egg masses	**Larvae**	**Infested plants**	**Egg masses**	**Larvae**	**Infested plants**
Pattern¶	$F_{6,1108}$= 0.378 P= 0.892	$F_{6,1108}$= 0.487 P= 0.817	$F_{6,1108}$= 0.401 P= 0.878	$F_{6,5871}$= 0.442 P= 0.850	$F_{6,5871}$= 2.152 P= 0.044	$F_{6,5871}$= 2.532 P= 0.018
Field¶¶	$F_{1,1108}$= 55.183 P= $2.18*10^{-13}$	$F_{1,1108}$= 25.413 P= $5.40*10^{-7}$	$F_{1,1108}$= 62.525 P= $6.32*10^{-15}$	-	-	-
Period¶¶¶	$F_{2,1108}$= 139.126 P= $2.2*10^{-16}$	$F_{2,1108}$= 160.224 P= $2.2*10^{-16}$	$F_{2,1108}$= 636.135 P= $2.2*10^{-16}$	$F_{2,5871}$= 25.837 P= $6.72*10^{-12}$	$F_{2,5871}$= 851.085 P= $2.0*10^{-16}$	$F_{2,5871}$= 953.761 P= $2.0*10^{-16}$
Pattern x Period	-	-	-	-	-	-
Field x Period	$F_{2,1108}$= 7.211 P= 0.0007	$F_{2,1108}$= 6.378 P= 0.001	$F_{2,1108}$= 14.265 P= $7.63*10^{-7}$	-	-	-

¶ Scouting pattern (7 pattern design) ︱ ¶¶ Field size (0.5 and 1 ha) ︱ ¶¶¶ Maize growth period [early whorl stage (0-21 days), late whorl stage (22-49 days) and beyond tasseling (> 50 days)]. Non-significant interactions were excluded from full models and they were not considered in final reduced models.

3.2. Parasitoid oviposition and scouting patterns

The egg parasitoid *Telenomus remus* (Nixon) (Hymenoptera: Platygastridae) was recorded in highest numbers compared to the egg-larval parasitoid *Chelonus* sp. (Hymenoptera: Braconidae) during the first maize growing season (Figure 3). The highest oviposition of *T. remus* occurred during the late maize whorl stage (22-49 days). That of *Chelonus* sp. was clearly bimodal and covered the first two maize growth periods with the parasitoid oviposition peaks, on the 13th and 24th days post emergence of maize plants on 1 ha maize field, and on the 6th, 13th and 34th days post emergence of maize plants on 0.5 ha maize plot. *Telenomus remus* oviposition was statistically higher during the late maize whorl stage (22-49 days), while *Chelonus* sp. parasitism occurred mostly during the early maize whorl stage (0-21 days) (Tables 3 & 4). Both parasitoid adult emergence data aggregated show similar trend of *T. remus* (Figure 3c). Neither quadrat nor scouting pattern effects proved significant in any case for both parasitoid species adult emergence (Tables 3 & 4).

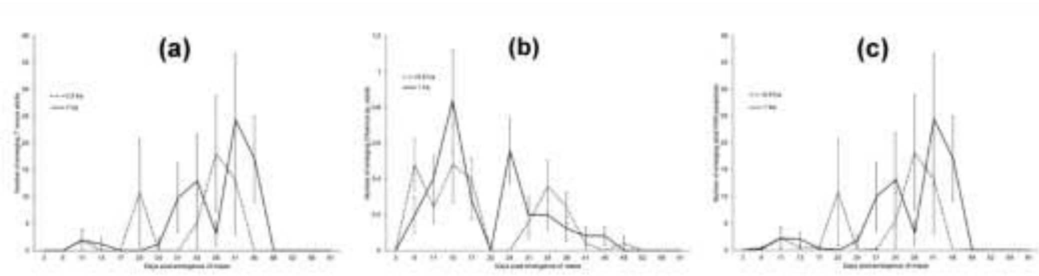

Figure 3. Average FAW parasitoid oviposition fluctuation on all scouting quadrats, (a) *Telenomus remus*, (b) *Chelonus* sp. and (c) both parasitoid species, during the first maize growing season.

Table 3. ANOVA results of scouting quadrats related to the log-transformed number of emerging adult parasitoids during first maize growing season.

Source of variation	*T. remus*	*Chelonus* sp.	Both parasitoids
Quadrat¶	$F_{24,772}$= 0.584 P= 0.944	$F_{24,772}$= 1.127 P= 0.305	$F_{24,772}$= 0.623 P= 0.919
Field¶¶	$F_{1,772}$= 3.358 P= 0.067	$F_{1,772}$= 0.685 P= 0.408	$F_{1,772}$= 3.896 P= 0.048
Period¶¶¶	$F_{2,772}$= 14.951 P= $4.25*10^{-7}$	$F_{2,772}$= 27.046 P= $4.43*10^{-12}$	$F_{2,772}$= 16.245 P= $1.22*10^{-7}$
Quadrat x Period	-	-	-
Field x Period	-	-	-

¶ Scouting quadrat (25 plot quadrats) ǀ ¶¶ Field size (0.5 and 1 ha) ǀ ¶¶¶ Maize growth period [early whorl stage (0-21 days), late whorl stage (22-49 days) and beyond tasseling (> 50 days)]. Non-significant interactions were excluded from full models and they were not considered in final reduced models.

Table 4. ANOVA results of scouting patterns related to the log-transformed number of emerging adult parasitoids during first maize growing season.

Source of variation	*T. remus*	*Chelonus* sp.	Both parasitoids
Pattern¶	$F_{6,1110}$= 0.975 P= 0.440	$F_{6,1110}$= 1.558 P= 0.156	$F_{6,1110}$= 0.706 P= 0.644
Field¶¶	$F_{1,1110}$= 0.377 P= 0.561	$F_{1,1110}$= 2.347 P= 0.125	$F_{1,1110}$= 1.094 P= 0.295
Period¶¶¶	$F_{2,1110}$= 22.286 P= $3.24*10^{-10}$	$F_{2,1110}$= 31.309 P= $5.91*10^{-14}$	$F_{2,1110}$= 27.166 P= $3.02*10^{-12}$
Pattern x Period	-	-	-
Field x Period	-	-	-

¶ Scouting pattern (7 pattern design) ǀ ¶¶ Field size (0.5 and 1 ha) ǀ ¶¶¶ Maize growth period [early whorl stage (0-21 days), late whorl stage (22-49 days) and beyond tasseling (> 50 days)]. Non-significant interactions were excluded from full models and they were not considered in final reduced models.

4. Discussion

4.1. FAW oviposition and scouting patterns

High FAW infestation during first season compared to second season supports earlier preliminary observations (Figure 2). The most plausible explanation for this occurrence is the increased natural enemy populations by the end of the first growing season, ready to colonize second season maize fields and hence having a substantial impact on FAW incidence. Another explanation might be less suitable bioclimatic factors regulating FAW population dynamics during the second planting season. The highest FAW oviposition during the early maize whorl stage (0-21 days) (Figure 2) can be attributed to the preference of the pest for fresh plant organs. The chemical ecology of higher young maize plants attractiveness to FAW for female oviposition also deserves further attention [9]. Silica accumulation in mature and stronger maize plants might explain the decrease of female FAW oviposition and the increase of host tolerance during post-tasseling stage (Figure 2) [10]. The highest oviposition rate recorded on the larger plot (1 ha) compared to 0.5 ha during the first growing season cannot be explained only by a preference to larger fields since the scouting quadrat size was bigger on the 1 ha plot (10 x 10 m) and smaller on the 0.5 ha field (6.5 x 5.5 m). Our data did not support clear statistical differences of the tested scouting patterns meaning that all seven studied scouting approaches are equally relevant.

The FIA scouting algorithm is constructed on a "Diagonals" shape. This pattern did not differ statistically from the commonly recommended "W" and "Ladder" models. This finding has tremendous practical implications in the sense that any of the scouting pattern is accurate enough to explain all FAW oviposition-related variabilities in a field. We anticipate that the "Diagonals" scouting shape is one of the easiest implementable approach particularly for low-literate farmers. Therefore, scouting data collected by farmers using such simple methods can be equally accurate with those generated in a more complex and sophisticated manner.

4.2. Parasitoid oviposition and scouting patterns

The egg parasitoid *T. remus* is well-known to be highly prolific with capabilities to parasitize FAW eggs concealed inside egg masses [11]. However, the highest parasitism rate of the egg parasitoid occurring in the late maize whorl stage during our experiments raises some concerns about the delayed potential for natural control of FAW by the wasp. In effect, FAW oviposition was higher during early maize crop phenology and biocontrol using *T. remus* can only be enhanced by inoculative releases in this utmost susceptible maize growth period. The egg-larval parasitoid *Chelonus* sp. appeared with relatively abundant populations earlier but with very low parasitism rates. Both parasitoids can provide complementary benefits in FAW and other closer species control [12,13].

Neither quadrat nor scouting pattern effects proved significant in any case for both parasitoid species adult emergence (Tables 3 & 4). This is another finding that all seven studied scouting patterns are equally relevant for the two naturally occurring parasitoid species.

5. Conclusions

This is the first demonstration of non-significant differences in seven scouting patterns to assess FAW and parasitoids oviposition. FAW scouting is of paramount importance in pest surveillance and monitoring systems. Limited resources and several other constraints often lead to weak and insufficient governmental agencies-led pest reports. Farmer access to simple tools and approaches can contribute covering the gap. The current work is a pilot demonstration of opportunity to engage farmers in massive surveillance and monitoring efforts using simple approaches and by deploying appropriate tools. A range of scouting patterns can be implemented by professionals, the simplest scouting model within those tested in this endeavor may be used by small-scale farmers supporting that FIA is well fitted and user-friendly.

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