Spodoptera frugiperda egg mass scale thickness modulates Trichogramma parasitoid performance

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With 5 figures

Abstract: The fall armyworm (FAW) Spodoptera frugiperda (Smith) is a major agricultural pest in Americas, Africa and Asia. Egg masses are covered with scale layers and the various scale layer thicknesses of these masses can affect parasitoids efficiency. The present study aimed to determine dynamics of the scale thickness on egg masses and its effect on Trichogramma parasitoid performance. The scale thickness ranged from 0 μm to 400 μm and can be graded in three levels. Level I was the naked egg masses without or covered with a thin scale, and the thickness was below 80 μm. Level II was medium covered, with scales where 20%–80% eggs could be seen; the thickness was between 80 μm to 180 μm. Level III was fully-covered with scales and the thickness was above 180 μm (up to 400 μm). The egg mass scale thickness decreased with increasing age of egg laying FAW females; the proportion at level I increased during female aging, while proportion of levels II and III decreased during oviposition period. During FAW female lifetime, the level I showed the highest proportion (51.9%) while the level III showed the lowest (9.9%). The parasitism rate of FAW eggs by Trichogramma dendrolimi varied according to scale thickness, with higher parasitism on eggs and egg masses at level I (31.6%, 78.3%, respectively) and lowest parasitism on level III (eggs: 1.9%; egg masses: 23.1%). We documented factors modulating parasitism effectiveness on FAW and we suggested that timely parasitoid releases targeting egg masses at Level I scale thickness could enable maximizing biocontrol service provided by Trichogramma on FAW.

Keywords: fall armyworm; physical defense; oviposition; parasitism; biological control; Trichogramma dendrolimi

1 Introduction

Lepidopterans have evolved a variety of defense mechanisms against parasites or predators, such as physical defense, evasive and aggression behaviors (Gross 1993; Luna et al. 2016). The defense mechanisms vary according to life stage, e.g. adult females can protect egg masses using scales (acting as a physical defense) deposited around and/or over eggs at the time of oviposition (Powell & Common 1985; Gross 1993; Fukuda et al. 2007). The scales often originate from an anal tuft at the tip of the female abdomen, but some could fall off from hind margin of the wing (Powell & Common 1985). The scales could act through various mechanisms (i) as physical barriers or (ii) aposematic warning against various natural enemies, and (iii) for decreasing risk of eggs being washed away by rain (Floater 1998).

The thickness of scales on egg masses varies largely among species, with egg masses showing no moth hairs or scales (with eggs easily visible) or egg masses being fully covered with scales (with the structure of egg masses completely invisible) (Fukuda et al. 2007; Dong et al. 2021). Female adults can oviposit several layers of egg masses showing different scale thickness (lowering predation or parasitism) and denser scales on top of egg masses, maximizing the protection of eggs. For example, Trichogramma embrophagum (Hbg.) can parasitize only eggs of the pine processionary Thaumetopoea pityocampa (Den. & Schiff.) that were not covered by scales and that were located at the top of egg masses (Tsankov 1990). Efficiency of spinosad was slower and weaker on scale-covered egg masses of Spodoptera littoralis (Boisd.) than on naked egg masses (Temerak 2006). Therefore, it is key to assess potential
effects of scale thickness on efficiency of biocontrol agents when developing biological control programs.

The fall armyworm (FAW) Spodoptera frugiperda (Smith) is a major pest on key crops such as rice, maize, wheat and vegetable crops in Americas, Africa and Asia (Brevault et al. 2018; Montezano et al. 2018; Parra et al. 2022; Toepfer et al. 2021; Wu et al. 2021a, b). Due to the risks posed by insecticides, notably their high toxicity to the environment, human, and non-target organisms (Weisenburger 1993, Desneux et al. 2007) and potential insecticide resistances in targeted pests (Gul et al. 2019; Richardson et al. 2020; Wan et al. 2021), the releases of egg parasitoids for controlling FAW is increasingly considered as potential key management strategy (Dasilva et al. 2015; Laminou et al. 2020; Zang et al. 2021). However, effective use of egg parasitoids is facing issues; FAW females oviposit egg masses (with 30 to 300 eggs) (Boiça Júnior et al. 2013) that are pasted on the substrate by a substance from females accessory glands, but are also covered with one or more layers of abdominal hairs or scales (Beserra & Parra 2005; Dong et al. 2021). The scales provide a strong barrier against oviposition behavior of some parasitoids (Beserra & Parra 2005; Sun et al. 2020; Tian et al. 2020).

In the present study, the scale layer on FAW egg masses was systematically studied by measuring the scale thickness and making a classification standard. Using the FAW adult female emergence time as timeline, the dynamics and the variations of scale thickness on egg masses were measured. In addition, we tested Trichogramma dendrolimi Matsumura parasitism rates with scale layer at different levels to assess the importance of the scale thickness according to their classification standard. The parasitoid species was chosen as it is used for wide-scale biological control programs with cost-effective mass-rearing at industrialized scale in China (Huang et al. 2020; Zang et al. 2021), and exhibits better parasitism on FAW eggs than other trichogrammatidae species (Sun et al. 2020).

2 Material and methods

2.1 Insects

Egg masses of FAW were collected in a maize field at Anlong County, Guizhou Province, China (25°05’N, 105°41’E). The population was maintained for three generations under laboratory conditions before the experiments started. FAW larvae were reared individually using 6-well cell culture plate and fed with an artificial diet (Sun et al. 2020) until pupation. Pupae were transferred to petri dishes with wet cotton and were checked daily, until FAW adult emergence. One-day-old males and females were paired in rearing cages (square, 35cm side) and provided with absorbent cotton soaked in 10% honey solution as food for the adults. Each rearing cage were covered with plastic film for oviposition. All developmental stages of FAW were maintained in a climate chamber with a constant temperature of 25 ± 1 °C, 70 ± 5% RH and photoperiod of 16:8h L:D.

A population of T. dendrolimi showing higher parasitism on FAW than other Trichogramma species (Sun et al. 2020) was selected as parasitoid to be studied. The parasitoids were collected using sentinel Chilo suppressalis Walker egg masses deployed in a rice field in Changchun (43°48’N, 125°23’E), Jilin province, China in 2011, and identified by male genital capsules using micrographs and further confirmed by rDNA-ITS2 sequences (GenBank: FR750279) (Pinto 1992). Trichogramma dendrolimi was maintained and reared using Corcyra cephalonica (Stainton) eggs under laboratory conditions (26 ± 1 °C, 70 ± 5% RH, 14:10h L:D). Every five generations, the population was supplemented with eggs of C. suppressalis. Voucher specimens were deposited in the Institute of Biological Control, Jilin Agricultural University, China. Newly emerged T. dendrolim adult females were mated within 8 h and used for the experiments.

2.2 Collection of egg masses of Spodoptera frugiperda and oviposition dynamics

A pair of newly emerged female and male adults of FAW were introduced in an insect rearing cage. The plastic film was renewed daily and newly laid egg masses of each female adult were checked and collected; egg masses collected were placed in a separate petri dish. We recorded the number of egg masses, eggs and egg layers laid by female adult per day and the thickness of the scale layer on FAW egg masses. Egg masses produced throughout the whole life cycle by at least 40 female adults were observed.

2.3 Evaluation of scale thickness of Spodoptera frugiperda egg masses

The thickness of the scale layer on FAW egg masses were measured by a depth-of-field microscope (VHX-2000, Keyence, Keynes LTD, Japan). The egg masses were placed under the microscope lens of Z100:x200. Three dimension images of egg masses were generated using deep synthesis function of depth-of-field microscope. The distance between egg surface and scale layer surface were measured using 3D measurement pattern, which were considered as thickness of the scale layer on measuring position. Five points were randomly selected for the measurement of thickness of the scale layer of every egg mass, mean values of which were considered the thickness of the scale layer of the egg mass. In addition, the scale thickness was graded according to the thickness data measured under depth-of-field microscope and the visibility of eggs under scale layer with naked-eye observation. The proportions of egg masses covered with scale thickness belonging to each level was calculated as:

$$\text{Level } N (\%) = 100 \times \left( \frac{\text{number of egg masses at the level } N}{\text{total number of FAW egg masses}} \right)$$
2.4 Parasitism of *Spodoptera frugiperda* egg masses according to scale thickness levels

FAW egg masses at each of the three levels (see results section) were offered to *T. dendrolimi*. Mated parasitoid females were introduced individually into clear glass tubes (4 cm diameter, 12 cm length) with only one newly laid FAW egg masses of the different scale thickness levels. Each tube contained a cotton wool with 15% honey solution. After 24h exposure time, each *T. dendrolimi* female was checked for vitality and removed. If the females died, the replication was discarded. The experiment was replicated 25 times for each scale thickness level. To avoid getting parasitized eggs fed on by FAW larvae, newly hatched FAW larvae were gently removed from egg mass using a brush every 12h. Six days later, the egg masses were examined under a stereoscopic microscope; the numbers of parasitized eggs (black) were recorded (Pizzol et al. 2010). The experiment was conducted under laboratory conditions (26 ± 1 °C, 70 ± 5% RH, 14:10h L:D cycle).

2.5 Data analysis

The number of eggs, egg masses, egg layer, the thickness of the scale layer, proportions on FAW egg masses in the different scale thickness levels on the days after adult emergence and parasitism rate by *T. dendrolimi* (with scale layers at different thickness levels) were analyzed using one-way ANOVA, and means were separated using Tukey’s HSD posthoc test. The datasets of the number of eggs, egg masses, egg layer of FAW were subjected to the Shapiro-Wilk test for normality prior to ANOVA analysis. Proportions of FAW egg masses with scale layers at different thickness levels and parasitism rate by *T. dendrolimi* with scale layers at different thickness levels were transformed to arcsine square root to stabilize variances before being subjected to ANOVA. The correlation between the thickness of the scale layer on FAW egg masses and days after adult emergence (square of Pearson correlation coefficient) was analyzed using the Bartlett χ² statistic and a log-linear model.

3 Results

3.1 Classification criteria of scale thickness of *Spodoptera frugiperda* egg masses

The egg masses and scale on the top of egg masses of FAW taken by camera and depth-of-field microscope were shown in Fig. 1. The thickness of the scale layer on FAW egg masses ranged from 0 μm to 400 μm (depth-of-field microscope). The thickness of the scale layer on egg masses were graded into three levels by the visibility of egg under scale layer with naked-eye observation. Level I was the naked egg masses without or a few scale or hair covered on the surface. The scales were barely visible and only a thin layer could

Fig. 1. Levels of scales on the top of FAW egg masses. Level I: naked egg masses without (A) and a thin scale (B) (< 80 μm). Level II: egg masses covered with scales with thickness ranging between 80 μm and 180 μm (C). Level III: egg masses fully covered with scales, the thickness being >180 μm (D).
be observed. All eggs under scale or hair could be clearly observed, the thickness of scale on the top measured up to 80 μm. Level II was the egg masses medium-covered with scales or hairs. A thin layer of scales or hairs covered the egg masses which was visible to the naked eyes. 20–80% eggs could be seen, the thickness of scale on the top of eggs was between 80 μm to 180 μm. Level III was the egg masses fully-covered with scales or hairs. A thick layer of scales or hairs cover the egg masses which is visible to the naked eyes. The egg mass totally covered and hidden by scale layer, the thickness of scale on the top of eggs ranged from 180 μm to 400 μm.

3.2 Egg mass layers and scale thickness throughout Spodoptera frugiperda lifetime

Egg masses contained 150–230 eggs (ranging from 5 to 750 eggs, in few cases). After a short pre-oviposition period (2–3 days), mated females began to lay eggs, with daily oviposition peaking on the 4th and 5th Day after Adult Emergence (DAE) \((F_{11, 434} = 14.545, P < 0.001)\). The average numbers of eggs per day were 400 eggs and max number were 750 eggs on the 4th and 5th DAE (Fig. 2A). There was a significant decrease in number of eggs laid per day from the 6th day (with average number of eggs laid per day being 50 on the 10th DAE, Fig. 2A). The number of layers in eggs masses varied from 1 to 4, with most of egg masses showing 2 layers. The number of egg layers did not vary with time throughout FAW female lifetime (Fig. 2B, \(F_{9, 295} = 2.377, P = 0.0718\)).

The thickness of scale layer on FAW egg masses decreased significantly with female age (DAE) \((\chi^2 = 7.49, P < 0.001; \text{coefficient } \pm \text{SE: } -0.067 \pm 0.0065; t = 7.81, P < 0.001)\). The maximal average scale thicknesses were observed on the 3rd (153.93 μm) and 4th (157.11μm) DAE \((F_{9, 670} = 215.23, P < 0.001)\) with highest value recorded at 4th DAE (413.2 μm). Significantly thinner average scale thicknesses were observed on the egg masses laid on the 5th (109.96 μm) and 6th (80.49 μm) DAE. By contrast, the eggs masses laid after the 9th day DAE were covered only by thin scale layers (less than 60 μm), which were barely visible.

There was no significant difference between the proportions of FAW egg masses at level II and level I on the 4th and 5th DAE, and level III were the lowest proportions of egg masses \((F_{2, 264} = 32.135, P < 0.001; 5\text{th DAE: } F_{2, 154} = 26.441, P < 0.001)\). However, the proportion of FAW egg masses at level I was the highest (>50%) from the 6th DAE \((6\text{th: } F_{2, 101} = 35.237, P < 0.001; 7\text{th } F_{2, 75} = 30.114, P < 0.001; 8\text{th: } F_{2, 42} = 26.116, P < 0.001; 9\text{th } F_{2, 19} = 16.348, P < 0.001)\). The proportion of FAW egg masses at level I significantly increased \((F_{9, 353} = 36.149, P < 0.001)\) while proportions of levels II and III significantly decreased with female adult age (level II: \(F_{9, 271} = 35.237, P < 0.001; \text{level III: } F_{9, 57} = 14.23, P < 0.001\)). The proportion of egg masses at level I was the lowest in the 4th and 5th DAE during whole oviposition period of FAW females and from 10th DAE, all egg masses were from level I. The proportion at level II in the 4th and 5th DAE were significantly higher than other oviposition days \((F_{9, 271} = 35.237, P < 0.001)\). The proportion at level III were the highest in the 4th DAE during oviposition period of FAW females and no egg mass of level III was observed from the 9th day.

3.3 Parasitism of Spodoptera frugiperda eggs and egg masses with various scale thickness

There were significant differences in the proportions of FAW egg masses parasitized by T. dendrolimi when considering thickness levels \((F_{2, 40} = 38.658, P < 0.001)\). The proportion of level I eggs masses parasitized (78.3%) was significantly higher than level II (48.2%), and with level III
showing lowest parasitism (23.1%). Furthermore, parasitism rates by *T. dendrolimi* varied significantly among FAW eggs from egg masses showing different thickness levels (Fig. 5B, eggs: $F_{2,63} = 27.113, P < 0.001$; egg masses: $F_{2,63} = 31.54, P < 0.001$). The parasitism rate on level I eggs (31.6%) was significantly higher than on level II eggs (12.6%), with the significantly lowest parasitism rate being observed on level III eggs (1.9%).

### 4 Discussion

In some Lepidoptera species adult females cover their eggs with scales at the time they oviposit, such scale acting as a physical defense (Gross 1993; Carneiro et al. 2012). The scales provide a strong barrier against oviposition behavior of some parasitoids (Beserra & Parra 2005; Sun et al. 2020; Tian et al. 2020). We demonstrated that the thickness of scale layer of FAW egg masses varied significantly with age of ovipositing female. The various scale thickness on egg masses drastically affected parasitism success in *T. dendrolimi* and complemented previous studies carried out on parasitism of FAW by egg parasitoids. For example, Sun et al. (2020) and Tian et al. (2020) reported *T. dendrolimi* had strong parasitic capacity on FAW eggs, with an average number of parasitized eggs of 20 in their experimental conditions. However, FAW egg masses covered with scales are not readily parasitized by *T. dendrolimi*, with an average parasitism number of parasitized eggs being 14% with scale and 26% without scale (Dong et al. 2021). Higher parasitism rate in presence of scale was reported for *T. pretiosum* (20%) and *Telenomus remus* (78%) (Dong et al. 2021) while very low parasitism rate was reported for *T. chilonis* (7%) (Tian et al. 2020). The obvious difference in parasitism rate by *Trichogramma* spp.
may be due to thickness of scales and layers of the selected FAW egg masses selected during the assays. The above phenomenon caused a heated debate on the biological control potential of *Trichogramma* spp. Therefore, it is important to determine the thickness of scale layers on egg masses of targeted pests for successfully using biological control agents such as *Trichogramma* spp.

In previous studies, the scale layer on egg masses were graded on the basis of with or without scales layer (Dong et al. 2021), but not on the thickness of the scale layer on egg masses. This is the first report on the thickness of the scale layers of FAW egg masses and its categorization. The thickness of the scale layer on egg masses varied significantly, ranging from 0 μm to 400 μm. The thickness of the scale layer showed a decreasing trend during FAW female aging. The proportion of FAW egg masses at level I increased continuously during FAW oviposition period while proportions of levels II and III decreased accordingly. Various reasons may explain this variability scale thickness in Lepidopterans, including weather conditions, natural enemies, and fecundity of moths (summarized by Temerak 2006). Most egg masses belong to levels I and II at each day during oviposition period of FAW females. The present results may contribute towards reconsidering the choice of parasitoids for biocontrol of FAW. Grading of the scales on the FAW egg masses and measuring the proportions of egg mass covered with scales of different thickness should be taken into important consideration in designing protocols for the release of parasitoids. Previous studies focused on selecting a parasitoid that could effectively control all egg masses which were fully covered with scales (Dasilva et al. 2015). In fact, considering the mass rearing cost of parasitoids and control efficiency, a variety of parasitoids can be combined to control FAW according to the proportions of egg mass covered with scales of different thickness.

Previous research suggested that *Trichogramma* parasitoids can hardly parasitize FAW eggs (reporting parasitism rates below 15%) because of the thick scales on egg masses (e.g. Dong et al. 2021). These results actually only reflected partially the possible parasitism as the studies failed encompassing all type of egg masses that could be parasitized by *Trichogramma* species. The parasitism rate of *T. dendrolimi* on naked FAW egg masses (i.e. level I, without or with a few scale or hair cover on egg surface) was about 40%, but only 9% of the egg masses fully-covered with scales or hairs (level III) was parasitized by *T. dendrolimi*. These results indicated that thicker scales on egg masses exhibited stronger physical defense against parasitoids, providing an explanation for the varied parasitism rates of *Trichogramma* spp. in previous different studies. Although the parasitism of FAW eggs by *T. dendrolimi* was not quite high, the mass rearing of this parasitoid is highly cost effective at industrial scale (Zang et al. 2021), notably when compared to the mass rearing of other *Trichogramma* species (Hou et al. 2018; Wang et al. 2020; Zang et al. 2021; Zhang et al. 2021). In addition, entomopathogens can be carried by *Trichogramma* parasitoids to pest eggs, contaminating unparasitized eggs or hatched larvae (Potrich et al. 2015; Wang et al. 2021). Considering the proportion of FAW egg masses at different scale thickness levels during female aging, *Trichogramma* parasitoids may be recommended for biocontrol of this key pest.

In addition, our results revealed that most FAW egg masses had more than two layers, with only 9.2% showing a single layer. The number of egg layers is an important factor affecting the biological control efficiency of FAW using parasitoids (Beserra & Parra 2005). Previous studies have shown that some *Trichogramma* species cannot parasitize effectively the layered eggs of FAW because *Trichogramma* females can only reach the eggs in the surface layer of the egg mass, whereas eggs at the internal layer were hard to parasitize (Tsankov 1990; Beserra & Parra 2005). For example, when *T. atopovirilia* parasitized FAW eggs, the parasitism on single-layer eggs was higher than those with two or three egg layers (Beserra & Parra 2005). The number of egg layers in FAW egg masses may be a factor explaining some field reports of failures in control of FAW when using *Trichogramma*.

Our study provides a comprehensive picture on biological characteristics of FAW oviposition and on egg mass scale thickness dynamic in FAW, providing thickness grades for the first time. More specifically, the impact of host scale thickness on *T. dendrolimi* performance when parasitizing eggs of FAW was studied. Considering the production cost of the parasitoids to be released, biocontrol efficiency and the proportions of egg masses covered with scales of different thickness, the release strategy of multiple natural enemy species, including *Trichogramma* sp., may be feasible to control FAW. The potential roles of FAW egg mass scales on parasitism efficiency may be integrated when designing strategies that include release(s) of parasitoids for biocontrol of FAW, and will need additional works in the future research.

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References


