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SHORT COMMUNICATION

LABORATORY STUDIES OF LARVAL CANNIBALISM IN SAME-AGE CONSPECIFICS OF FALL ARMYWORM, *Spodoptera frugiperda* (J.E. SMITH) (LEPIDOPTERA, NOCTUIDEA) IN MAIZE

Kasige RH[1], Dangalle CD[1*], Pallewatta N[1] and Perera MTMDR[2]

[1]Department of Zoology and Environment Sciences, Faculty of Science, University of Colombo, Sri Lanka

[2]Plant Quarantine Unit, Gannoruwa, Peradeniya, Sri Lanka

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Abstract

Fall Armyworm (*Spodoptera frugiperda*) (FAW) is an economically important pest worldwide and larval cannibalism is documented as one of its significant behaviors under field and laboratory conditions. The incidence of larval cannibalism amongst same-age larvae of Fall Armyworm was investigated under laboratory conditions using 73 laboratory trials with not less than five trials for each instar stage. Each instar stage was provided with their conspecifics along with sufficient food. Direct observations were carried out in 24 hour time laps to record the missing larvae which were presumed as cannibalized. The mean cannibalism proportion was calculated for each larval stage. All larval stages except the first and second instar stages displayed same-age conspecific cannibalism. An increasing trend of the mean cannibalism proportion was observed from the third (0.16±0.04) to the fifth instar stage (0.22±0.05) with the highest value recorded for the fifth larval instars. The trend dropped at the sixth instar stage. The study suggests that larval cannibalism is evident amongst same-age larval instars of *Spodoptera frugiperda*, which may affect ecological relationships between organisms of a system.

Keywords: Cannibalism, *Spodoptera frugiperda*, Same-age larval instars

INTRODUCTION

Cannibalism is a taxonomically widespread phenomenon (Chapman *et al.* 2000) documented for various animal species and commonly observed in arthropods, non-carnivorous phytophagous insects and larval lepidopterans (Chapman *et al.* 1999a). This feeding relationship is viewed as intraspecific predation between cannibal (predator) and the victim (prey), where killing and consumption of an individual (either fully or partly) of the same species take place. Cannibalism has an ecological and evolutionary significance which can directly influence the population dynamics and the community structure of a species as it may confer a direct fitness benefit by increased survival, developmental rate, and fecundity, or it may provide indirect benefits by removing potential competitors and predators (Richardson *et al.* 2010; Ren *et al.* 2020).

Cannibalism is a natural phenomenon in insects and has been documented in many insect orders including Odonata, Orthoptera, Thysanoptera, Hemiptera, Trichoptera, Diptera, Neuroptera, Coleoptera, Hymenoptera and Lepidoptera (Fernandez *et al.* 2020). It is known to occur in the cotton leafworm, *Spodoptera littoralis* and the black cutworm, *Agrotis ipsilon* when the availability of food is reduced; in damselfly larvae, *Lestes nympha*, in the presence of crowding; due to aggressive behavior of the corn ear-worm larvae, *Heliothis armigera*; in the Australian grasshopper, *Phaulacridium vittatum*, due to

Corresponding author: ddangalle@zoology.cmb.ac.lk

physiological and psychological stress; and in many other insects such as the freshwater backswimmer, *Notonecta hoffmanni*, Monarch butterfly larvae, *Danaus plexippus*, Queen butterfly larvae, *Danaus gilippus berenice*, milkweed bugs of the genus Lygaeus merely due to the availability of victims (Fox 1975).

Cannibalistic behavior has been documented for larval Fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), in both field and laboratory conditions (Raffa 1987; Chapman *et al*. 1999a; 1999b; 2000). It has been known to prevail in the presence of a viral disease amongst larval populations where infected larvae were consumed by the uninfected larvae (Williams and Hernandez 2006). According to Ren *et al*. (2020), cannibalism is common in Fall armyworm larvae as they feed primarily within the wrapped leaves of the whorl in young plants where frequent contact occurs. Cannibalism mainly occurs in mature larvae (4^{th} - 6^{th} instars) and can be seen amongst same -aged larvae or between larvae of different maturity ages (Ren *et al*. 2020). When co-occurring the older Fall armyworm larvae exhibits a cannibalistic behavior on younger larvae which is accentuated when food is limited and larvae are crowded (Assefa and Ayalew 2019). Paul and Deole (2020), further confirms the cannibalistic behavior in the fifth and sixth instar larvae of the Fall armyworm and reveal that the frequency of cannibalism among fifth and sixth instar larvae is significantly higher than among earlier instars. Furthermore, cannibalism seems to be an adaptive behavior for larger larvae and is often seen in pairs of different instar classes in which smaller larva are frequently cannibalized by larger larvae (Bentivenha *et al*. 2017). Cannibalism amongst same-aged larvae, especially in the immature instar stages have not been studied in detail which is unfortunate as the basic premise of pest control is to control the pest before it becomes injurious and the first to third instar larvae should be the focus of the pest management practices (Ren *et al*. 2020).

From a pest management perspective, cannibals risk injury or death from defensive responses of conspecifics and may suffer an extra cost by consuming conspecifics infected with pathogens or parasites. Further, intraspecific competition may cause a reduction in inclusive fitness, in which pest management programs may take advantage (Ren *et al*. 2020).

However, cannibalism is known to hinder biological control methods in pest management by reducing (limiting) resources for the natural enemy, reducing the quality of resources (harmed prey) and thus accounting for the loss of fitness in the natural enemy (Prasad and Prasad 2018).

Fall Armyworm is a serious insect pest in Sri Lanka affecting corn (Perera *et al*. 2019), sugarcane (Wanasinghe *et al*. 2019), and has the potential of harming several other important economic crops in the country. It was first recorded in Sri Lanka in August 2018, especially from locations in the Uva, Eastern and North-Central provinces of the country (Perera *et al*. 2019). Currently, it is spreading to many other locations in Badulla and Mahiyanganaya.

In Sri Lanka, biological control measures for fall armyworms on sugarcane have been practiced using egg parasitoids of Hymenoptera and larval predators of Coleoptera (Wanasinghe *et al*. 2019). Biological control agents have also been used against the pest on corn cultivated in Ratnapura (Perera *et al*. 2019). However, for the success of these control measures, recognizing all the relationships within the pest system including cannibalism is of fundamental importance.

The present study was conducted to determine cannibalistic behavior amongst same-age conspecifics of 1- 6 instar stages of larval Fall armyworm under laboratory conditions. As rearing conditions in the laboratory usually prevent dispersal and affect cannibalistic behavior, subsequent field trials are suggested.

MATERIALS AND METHODS

Collection and separation of Fall armyworm larvae into different instar stages

Fall armyworm larvae were collected from four locations of Sri Lanka from January to October 2019. The locations were corn-fields that were highly infested with Fall armyworms – Mahailuppallama, Anuradhapura district, North-Central province; Polpithigama, Kurunegala district, North-Western province; Meegahakiula, Badulla district, Uva province; Rideemaliyadde, Badulla district, Uva province (Figure 1). Sampling was employed along five strip transects within the interior of the field and larvae were collected by handpicking. The collected larvae were separated into the six instar stages according to the following features.

First instar stage

Small larvae with a body length ranging from 0.9 – 1.8 mm and a head capsule width ranging from 0.35 – 0.39 mm (n=50). Body light-green in colour. Head large, conspicuous and black. Visible black spots on thoracic and abdominal segments of the body from which black setae arise. Thoracic legs are large and black, while the abdominal legs are small and light-green (Figure 2a).

Second instar stage

Larger than the first instar with a body length ranging from 2.0 – 4.3 mm and a head capsule width ranging from 0.45 – 0.90 mm (n=50). Body green in colour. Black spots on the body are less visible than in the first instar stage. A dark coloured band is visible in the first thoracic segment (Figure 2b).

Third instar stage

The third instar stage is larger than the second instar stage with a body length ranging from 5.1 – 7.8 mm and a head capsule width ranging from 1.0 – 1.3 mm (n=140). The dorsal surface of the body is brownish-green with the appearance of white lines extending along the body (Figure 2c).

Figure 1: Agricultural lands (Maize cultivations) from which *Spodoptera frugiperda* larvae were collected. (A) Mahailuppallama (B) Polpithigama (C) Rideemaliyadde (D) Meegahakiula

Figure 2: The six larval instar stages of *Spodoptera frugiperda*. a – First instar; b – Second instar; c – Third instar; d – Fourth instar; e – Fifth instar; f – Sixth instar

Fourth instar stage
Body large with a body length ranging from 9.0 – 15.7 mm and a head capsule width ranging from 1.5 – 2.0 mm (n=240). Body brown in colour with a reddish-brown head mottled with white (Figure 2d).

Fifth instar stage
Body length ranges from 17.1 – 24.0 mm and head capsule width from 1.8 – 2.4 mm (n=200). Body brownish-green with a reddish -brown head mottled with white. A prominent inverted "Y" mark can be seen on the head between the eyes. Mouthparts are well developed (Figure 2e).

Sixth instar stage
Largest instar stage with a body length ranging from 23.5 – 36.0 mm and head capsule width ranging from 2.6 – 3.1 mm (n=50). Body brownish-green in colour. A mid-line arises from the base of the inverted "Y" mark on the head and extends towards the last abdominal segment. Pronotal shield consists of three white stripes. Four large black dorsal spots arranged in a square occur on the body segment previous to the last segment (Figure 2f).

The larval instars were translocated to the insectary facilities of the University of Colombo and kept under a temperature of 28

± 2°C and 80 ± 10% relative humidity, with a 12 h:12 h light: dark natural photoperiod.

Experiment to record cannibalism amongst larval conspecifics

Cannibalism was tested amongst larvae of each instar stage separately under laboratory conditions. Ten larvae of a particular instar stage were housed within a rectangular glass cage of 4 cm height x 15 cm width x 21 cm length and maintained on a diet of maize leaves. The feeding rate and foliage consumption of larvae were assessed before to the experiment to determine the sufficient food requirement of each larval stage. Feeding rate and foliage consumption of larval *S. frugiperda* was determined by quantifying the area of maize leaf ingested by individual larvae of a particular instar stage on each day. Leaf segments were photocopied before and after a feeding period of 24 hours using 1 mm^2 graph paper (Chapman *et al.* 1999a). A sufficient amount of fresh maize leaves were provided every 24 hours during the experimental period. After 24 hours observations were taken and the remaining larvae were recorded and missing larvae were presumed as cannibalized. The test was repeated (trials) for each instar stage. However, the number of trials conducted differed according to the instar stage and a higher number of trials was completed for instar stages in which cannibalism was known to be evident: first instar stage = 05 trials, second instar stage = 05 trials, third instar stage = 14 trials, fourth instar stage = 24 trials, fifth instar stage = 20 trials, sixth instar stage = 05 trials.

The mean cannibalism proportion was calculated for each larval instar stage by dividing the number of larvae cannibalized by the number of larvae that were initially present.

RESULTS AND DISCUSSION

Cannibalism during the larval stage of development may confer direct fitness benefits due to removal of potential competitors, reduction in the probability of attracting predators, and thus increased survival, development rate and fecundity (Williams and Hernandez 2006). However, it will affect the ecological relationships between organisms within a system and may adversely affect natural enemy populations (Fernandez *et al*. 2020). Thus, biological control systems where various natural enemy organisms are used as predators, parasitoids and entomopathogens may be affected negatively. Biological control is a highly desirable management practice in controlling *Spodoptera frugiperda* (Koffi *et al*. 2020). However, for the method to be successful, it is important to recognize all effects of ecological relationships of the system such as cannibalism.

The present study confirmed the presence of cannibalistic behavior amongst larvae of *Spodoptera frugiperda* which has also been reported by numerous other studies going way back to more than two decades ago. Chapman *et al*. (1999a) reported that cannibalism was frequent in *Spodoptera frugiperda*, given the opportunity that all larvae predated on at least one younger conspecific, and that the frequency of cannibalism was not affected by the sex of cannibal or by the availability of alternative food. Further studies revealed that cannibalism was more evident in the fourth instar stage (Chapman *et al*. 2000), and according to Goussain *et al*. (2002), groups of Fall armyworm display increased cannibalism at the end of the second instar stage. Ren *et al*. (2020) records cannibalism in same-aged Fall armyworm larvae with the fifth and sixth instar stages experiencing a higher incidence of cannibalism, which, however, is opposed by Andow *et al*. (2015) which states that cannibalism occurs in equal-aged larvae in the first and second instar stages. The present study addressed these conflicting revelations by providing information that displayed cannibalism in same-aged Fall armyworm larvae of the latter instar stages. The first and second instar stages did not reveal cannibalism and the proportion of cannibalism increased from the third to the fifth instar stages, with a decline in the sixth instar stage (Figure 3).

High rates of cannibalism in mature larval stages of insects when compared to immature

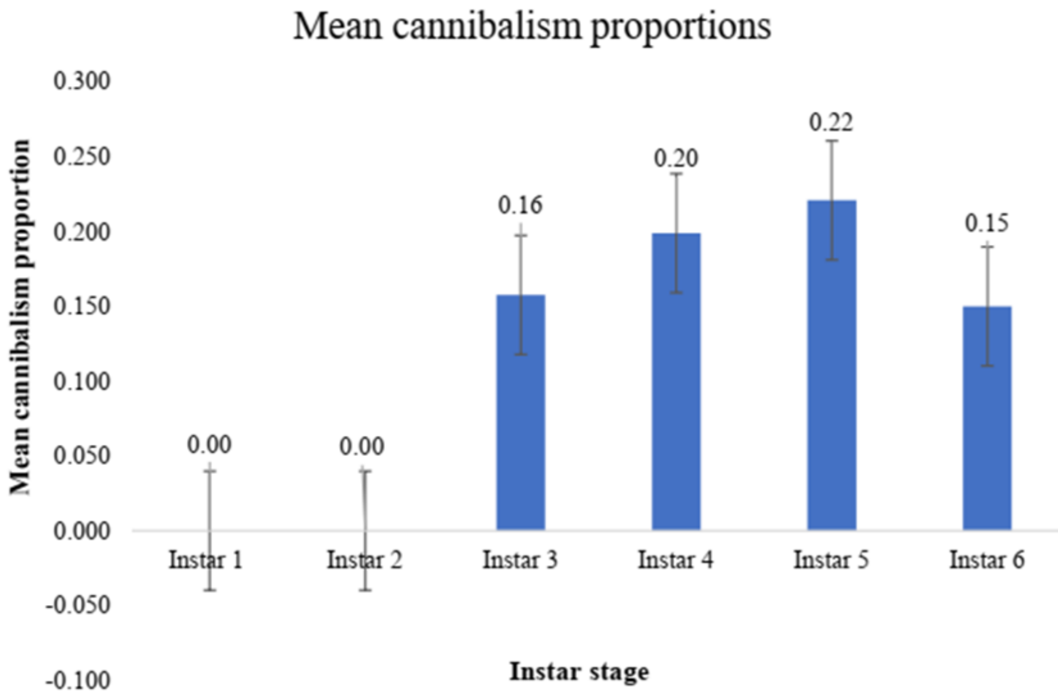

Figure 3: Mean cannibalism proportions of the different instar stages of *Spodoptera frugiperda*

larval stages have also been recorded by Zago -Braga and Zucoloto (2004) and the observation has been attributed to the higher mobility and higher predation power provided by the stronger mandible physical structure in the mature larva (Zago-Braga and Zucoloto, 2004). During sample collection in the present study, it was observed that many infested maize plants contained a single larva which at most of the time was either an instar of 4th or 5th instar stages. Although an aggregation of larvae is hatched from a cluster of eggs, the plants only carried a single larva after a few days. The missing larvae can be accounted for the cannibalistic behaviour among larvae. The surviving larvae have been associated with larger body weights and survival rates (Porretta *et al*. 2016), which may be more injurious to crops that are harmed by these insects. In *Spodoptera frugiperda* the immature larval instars harm the leaves of the plant, while the older larvae are more destructive and harm the plant as a whole (Luginbill, 1928). Higher survival of mature instars and larger body sizes may exert more damages to plants harmed by *Spodoptera frugiperda* and produce serious implications.

However, in the present study cannibalism decreased amongst the larvae of the sixth instar stage revealing a decline in food consumption. This may be due to the fact that occasionally, cannibalism amongst sixth instar larvae has not been observed as they become less active due to getting ready for pupation (Ren *et al*. 2020).

Further, fall armyworm larvae are known to release volatile exudates, especially when they are injured that may attract natural enemies (Chapman *et al*. 2000). Low population densities resulting due to cannibalism produces less amounts of exudates reducing the attraction of natural enemies. This may significantly affect the biological control practices of *Spodoptera frugiperda*.

However, if larval cannibalism is viewed in the perspective of a self-regulating measure of populations, this natural phenomenon can limit population size while suppressing population outbreaks. Most importantly larval cannibalism can be then used as a natural biological control of economically important larval pests. Therefore, further work is required to elucidate various other facets of larval cannibalism and the significance of cannibalism in population dynamics of fall armyworm under field conditions.

CONCLUSION

The present study confirmed cannibalistic behavior amongst same-age larvae of *Spodoptera frugiperda*. The behavior was associated with the developmental stage of the larvae and immature larvae (first and second instars) did not display cannibalism while it was evident in the mature larval stages (fourth to sixth instars). The mean cannibalism proportion steadily increased from the third to the fifth instar stage of larvae indicating a relationship between cannibalism and growth of larvae. However, a decline in the mean cannibalism proportion was observed amongst the sixth larval instars possibly due to less activity prior to pupation. Cannibalistic behavior affects ecological relationships between organisms of a system, especially between hosts and natural enemies. Therefore, the behavior must be better understood when initiating biological control measures for serious insect pests such as *Spodoptera frugiperda*.

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AUTHOR CONTRIBUTION

CDD, NP and MTMDRP designed the study. RHK conducted the experiments. RHK, CDD and NP analysed and interpreted the data. All authors contributed in discussing the results and preparation of the manuscript.

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