

New method for rearing *Spodoptera frugiperda* in laboratory shows that larval cannibalism is not obligatory

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Abstract. New method for rearing *Spodoptera frugiperda* in laboratory shows that larval cannibalism is not obligatory. Here we show, for the first time, that larvae of the fall armyworm (FAW), *Spodoptera frugiperda* (Lepidoptera, Noctuidae), can be successfully reared in a cohort-based manner with virtually no cannibalism. FAW larvae were reared since the second instar to pupation in rectangular plastic containers containing 40 individuals with a surprisingly *ca.* 90% larval survivorship. Adult females from the cohort-based method showed fecundity similar to that already reported on literature for larvae reared individually, and fertility higher than 99%, with the advantage of combining economy of time, space and material resources. These findings suggest that the factors affecting cannibalism of FAW larvae in laboratory rearings need to be reevaluated, whilst the new technique also show potential to increase the efficiency of both small and mass FAW rearings.

KEYWORDS. Artificial diet; cannibalistic behavior; fall armyworm; mass production; rearing technique.

The fall armyworm (FAW), *Spodoptera frugiperda* (Smith, 1797) (Lepidoptera, Noctuidae), is a key pest of corn in Brazil as well as the greater Americas. For decades, researchers around the world have reared this species in laboratories for the purpose of conducting studies on its biology, behavior and also for using it as a host of natural enemies. Despite the several artificial diets suitable for FAW, its larval cannibalistic behavior is considered a challenge for most rearers. Cannibalism has been documented in field (Sarmiento *et al.* 2002; Farias *et al.* 2001; Chapman *et al.* 2000; Silva 1999) and indoor (Zennerde-Polania *et al.* 2009; Goussain *et al.* 2002; Chapman *et al.* 1999a,b; Mihm 1983;) conditions, encouraging one to rear the larvae in individual vials as a way to decrease the preimaginal mortality (Vilarinho *et al.* 2011; Busato *et al.* 2006; Chapman *et al.* 2000; Perkins 1979; Burton & Perkins 1972; Burton & Cox 1966). Although being a well-diffused method, to culture larvae of FAW individually is not practical and may be very time- and resource-consuming even in small rearings, suggesting the need to develop a more efficient technique. Here we describe a cohort-based method for rearing larvae of *S. frugiperda* and show, for the first time, that cannibalism is not necessarily an issue when FAW larvae are reared in cohorts instead of individually.

MATERIAL AND METHODS

In mid-October 2010 (early spring), corn plants (*ca.* 30 days after emergence) were collected from a plot (22°42' 11.5"S, 47°38' 1.1"W) infested with fall armyworm. In laboratory, caterpillars were picked from leaves and stem of plants and then reared individually on the artificial diet proposed

by Greene *et al.* (1976), according to the methods of Parra (2001) (24 ± 1°C, 70% RH, 14L:10D photoperiod). When molted to pupae, the insects were collected and placed in PVC cages (21 cm height x 10 cm diameter) until adult emergence, and then maintained as described by Parra (2001). Egg patches deposited by these adults were inserted in glass tubes (2 x 8 cm) and, as soon the caterpillars hatched, they were transferred to disposable plastic cups (200 mL) containing the artificial diet. When achieved the second-instar (*ca.* 4–5 day-old), forty caterpillars were introduced in rectangular containers (4 cm height x 15 cm width x 21 cm length) filled with 200 mL of the artificial diet (Figs. 1, 2). The containers were stacked closing each other and plastic lids were used to close the containers in the top of the piles (Fig. 3). In order to avoid excess of moisture on the container surface (Fig. 4) and the development of entomopathogenic microorganisms, we promoted a reduction of 11% of the water content proposed by Greene *et al.* (1976). After 14 days the pupae were collected and introduced in PVC cages as before, when the process was repeated, as already described, for several generations. In F₁₂, the larval survivorship rate, and the fecundity and fertility of adult female were measured. Larval survivorship in each container (N = 44) was evaluated by the formula: Number of healthy pupae/40*100. For measuring the fecundity, 20 FAW couples were introduced, as soon they emerged, in 20 PVC cages (10 cm height x 10 cm diameter), which were maintained in the shelves of a climatic chamber (24 ± 1°C, 70% RH, 14L:10D photoperiod). Egg patches were collected and counted daily. For evaluation of fertility, after egg counting, paper containing egg masses were maintained in the shelves of a second climatic chamber (24 ± 1°C,



Figs. 1–4. Cohort-based method for rearing larvae of *Spodoptera frugiperda*. 1. Top view of the plastic container (4 x 15 x 21 cm) with artificial diet (light brown) (Greene *et al.* 1976), frass (dark brown) and fifth instar larvae. 2. Bottom view of the container showing prepupae into their molt chambers. 3. Containers stacked aiming at saving space. 4. Water drops condensed on the inner surface of the container lid (this problem was addressed by reducing in 11% the amount of water suggested by Greene *et al.* (1976)).

70% RH, 14L:10D photoperiod) and the non-hatched eggs were counted after four days. To avoid an eventual cannibalism of newly-hatched larvae on non-hatched eggs we collected and killed the larvae twice a day.

RESULTS AND DISCUSSION

A mean of 36.3 ± 0.4 out of the 40 larvae of *S. frugiperda* were able to successfully develop and reach the pupal stage in each rearing container, which corresponded to $90.75 \pm 0.9\%$ of larval survivorship. Chapman *et al.* (1999a,b) found that cannibalistic behavior occurs in a frequency of about 43 to 80% when larvae are reared in groups and, in a further study, Chapman *et al.* (2000) reared FAW larvae individually. In Brazil, Nalim (1991) observed high rates of cannibalism when FAW larvae were fed on corn leaves, in contrast with the significantly lower rates found on artificial diet. The author explained these results suggesting that the cannibalism might be a way to get a meal of high nutritional value in an environment where only low nutritional value food, like the corn leaves, is available. Despite that, Brazilian researchers have reported exclusively the individually-cultured larvae when rearing FAW, always justifying it with the

occurrence of larval cannibalism (e.g. Goulart *et al.* 2011; Vilarinho *et al.* 2011; Bueno *et al.* 2010, 2008).

Our study showed that cannibalism is not a critical factor of mortality for larvae of FAW in laboratory rearings. Hence, individualizing the insects is not mandatory to avoid larval and pupal mortality due to such negative interaction. Our results and the discrepancies between the cannibalism rates reported by different authors suggest that the genetics of the FAW or even differences in the rearing methods play a role on the cannibalistic behavior. Thus, before the establishment of a FAW rearing, preliminary tests should be taken in order to check out its cannibalism rate and to adjust the most efficient method of rearing.

Each female of *S. frugiperda* was able to lay an average of $1,832.30 \pm 100.11$ eggs along their lifespan, whose $99.10 \pm 0.13\%$ were fertile. Busato *et al.* (2006), rearing a corn population of FAW also from Brazil in individualized vials and on the same substrate we used (Greene *et al.* 1976), found a fecundity of ca. 1,874 eggs/female, which is virtually the same reported by us. Such results point that the cohort-based rearing method here presented is not only efficient at ensuring a high larval survivorship, but is also suitable for promoting a female fecundity similar to the widespread method of culturing larvae individually.

The implications of a cohort-based method of FAW on the feasibility of its rearing can be easily measured. For example, four plastic containers can carry 160 larvae of *S. frugiperda* in an area of 1,260 cm² (42x30 cm). If we use exactly the same area to apply the individual method based on plastic cups (4x5 cm) (Vilarinho *et al.* 2006), we could rear roughly 40 larvae, which is only one-quarter of the cohort-based method here presented. Moreover, we should mention the economy in time promoted by the latter method. Although we did not take measurements, it is not difficult to have in mind that the time spent distributing cups in a tray and then filling them, one by one with artificial diet, one single larva, and a lid, will take substantially more time than doing the same steps for a group of 40 larvae at once. Indeed, considering that cannibalism was not shown to be a problem for FAW rearing, even larger containers might be used according to the availability of space. Finally, considering the amount of artificial diet often not consumed (out of the 200 mL available) by the larvae until the end of their development (Figs. 1, 2), new studies need to be performed in order to test the effects of decreasing the amount of diet offered, and of increasing the density of larvae per container.

A feasible method for rearing larvae of *S. frugiperda* in laboratory has been long demanded. Here were described a suitable technique for such need showing, for the first time, that the FAW larvae can be successfully reared under a cohort-based method with no negative effects of cannibalism on larval survivorship nor on fecundity and fertility of the adult females. These findings suggest that the factors affecting cannibalism of FAW larvae in laboratory rearings need to be re-evaluated, whilst the new technique also show potential to increase the efficiency of both small and mass FAW rearings.

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