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Study on oviposition behaviour of *Spodoptera frugiperda* (J.E. Smith), Noctuidae: Lepidoptera: The way ahead for ecological management in India

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Abstract
The selective ovipositional behaviour of Fall armyworm (FAW), *Spodoptera frugiperda* was studied at NIPHM under laboratory conditions through cage experiments to provide the basis for its oviposition preference on different crops to pave a pathway to find an ecological solution for its management. Oviposition preference of *Spodoptera frugiperda* was evaluated under multi-choice conditions with corn (*Zea mays* L.), sorghum (*Sorghum bicolor* [L.] Moench., pearl millet (*Pennisetum glaucum* (L.), Napier grass (*Pennisetum purpureum*) and *Desmodium* (non-specific). The number of eggs laid by the FAW on maize was high when compared to the pearl millet and sorghum indicating the oviposition preference towards maize over other crops. Eggs were laid by the fall armyworm on Napier where as *Desmodium* was not preferred for oviposition and thus experiment produced promising results that the Napier grass can pull the egg load of *S. frugiperda*. Based on our data Napier and Maize were highly preferred crops compared to others. The egg load was trapped by the Napier can be concluded by the nature of preference and the considerations observed in the laboratory host conditions.

Keywords: *Spodoptera frugiperda,* fall armyworm, *Desmodium,* Napier grass, oviposition

Introduction
The fall armyworm, *Spodoptera frugiperda* (FAW) is the most destructive pest of many economically important crops across the globe. FAW is native to the tropical and subtropical regions of America and it has invaded many countries of Africa and Asia. In India, FAW has been reported in 2018, since then causing massive outbreaks in maize (Sharanabasappa *et al.*, 2018) [7]. It affects the maize crop at different stages of growth from the early seedling stage and finally feed on developing kernels. It reduces yields through direct feeding and indirectly by exposing cobs to secondary infection leading to loss of grain quality.
The eggs *of Spodoptera frugiperda* are usually laid in masses of approximately 150-200 in two to four layers covered with the protective scales. Fecundity is 1000-1500 eggs/female and maturity takes 2-3 days at room temperature. On hatching, larvae are green with black lines and spots, and as they grow they either remain green or become buff-brown and have black dorsal and spiracular lines. Late stages larvae are characterized by an inverted Y-shape in yellow on the head and four black spots arranged in a square on 8th abdominal segment. The larval period is 16-20 days and pupation normally occurs in the soil, but could also occur in reproductive parts of plant such as mature maize ears. Duration of the pupal stage is about 8 to 9 days during normal maize season. Adult female after a pre-oviposition period of 3 to 4 days, deposits most of her eggs during the first 4 to 5 days but sometimes oviposition occurs for up to 3 weeks as it lives an average of 10 days, with a range of about 7 to 21 days (Prasanna *et al.*, 2018). Pilar Téllez-Rodríguez *et al.*, 2014 [8] have examined oviposition and survival of *S. frugiperda* across conventional and Bt maize and explored the impact of oviposition behaviour on the evolution of resistance and concluded that the damaged host plants have reduced attractiveness.

Materials and Methods
The experiment was conducted in the laboratory at a temperature of 25°C and 43% humidity. To maintain the culture, the *Spodoptera frugiperda* larvae were collected initially from maize fields of NIPHM. Larvae were separated instar wise and early instars were reared on tender

maize leaves/cobs in groups. From the third instar, each larva was placed in a separate Petri dish lined with a blotting paper and fed with fresh maize leaves obtained from the organic field of NIPHM. Larvae in the pre-pupation stage were shifted to pupal jars filled with the sand and the mouth of the jar was tied with a cotton cloth.

Fall armyworm rearing: After the emergence of the adults, pairs of adults were released into ovipositional jars, inside which a cotton swab dipped in 50% honey solution was placed on the side walls to facilitate the feeding of adults. Vitamin E-liquid was added to the honey solution to increase the fecundity levels of the female. After a pre-ovipositional period of 2 to 3 days inside the ovipositional jar, small twigs/leaves of maize were placed and eggs were collected daily. Fresh leaves were added every day. The eggs collected from ovipositional jars were put in a Petri dish lined with a moist blotting paper. In the laboratory eggs were incubated at the temperature of 25 °C and relative humidity was maintained at 43% to facilitate hatching. In 2-3 days the eggs were hatched and first instar larvae were allowed to feed on tender maize leaves and immature cobs in groups. From 3rd instar, larvae were separately reared. Thus obtained 2nd generation adults were used in experiments for the ovipositional studies.

Raising crops and preparing treatments: To study oviposition preference under multi-choice conditions on different crops, maize, sorghum, pearl millet, Napier grass, and *Desmodium* were raised without using synthetic fertilizers and pesticides in NIPHM organic field. The seeds of maize hybrid were procured from local market. The seeds of sorghum and pearl millet were procured from Indian Institute of Millet Research, Rajendranagar, Hyderabad. Napier grass cuttings were used from NIPHM field, Rajendranagar. *Desmodium* (Non-specific) seed was procured from Indian Grass land and Fodder Research Institute, Jhansi, UP.

To prepare maize, sorghum, pearl millet, Napier grass treatments, 5-6 tender leaves were collected and placed in a small glass bottle with moist cotton to sustain moisture levels. In the case of *Desmodium* small tender twigs were used instead of leaves. These treatments were then, placed in rearing cages measuring about 30X30X30 cm, made up of wooden frames, covered on three sides with plastic net and one side with a wooden door for easy operations. Cages were cleaned properly and dried under the sun for a day. Before placing the twigs inside the rearing cages, plastic net on three sides were covered with cotton cloth from inside, to facilitate oviposition and for easy collection of eggs. Twigs were then placed inside the rearing cage as per the treatment in three replications.

Treatments organized under multi choice crops:

Treatment No.1: Maize + Pearl millet + Sorghum + Napier Grass + *Desmodium*
Treatment No.2: Maize + Sorghum + Pearl millet
Treatment No.3: Maize + Pearl millet + Napier Grass
Treatment No.4: Maize + Sorghum + Napier Grass
Treatment No.5: Pearl millet + Napier Grass + *Desmodium*
Treatment No.6: Maize + Napier Grass + *Desmodium*
Treatment No.7: Sorghum + Napier Grass+ *Desmodium*

Releasing of adults into rearing cages: Tender twigs were placed inside rearing cages and 3 pairs of adult moths in 1:1 ratio of male and female, were released into each rearing cage. Male and female moths were distinguished with sexual dimorphism. Male is small with 1.6 cm body length and 3.7 cm wingspan. Forewing of the male is greyish brown with reniform indistinct spot, faintly outlined in black, with a small v-shaped mark, a light brown orbicular spot, and white patch at the apical margin of the wing. Female is big with 1.7 cm body length and 3.8 cm wingspan. The forewings of females are less distinctly marked, ranging from a uniform greyish brown to a fine mottling of grey and brown. Hind wings are straw-coloured with a dark-brown margin. Active and fresh emerged moths were collected and released. A cotton swab dipped in 50% honey solution was put in a shallow container and placed inside the rearing cage as feed for the adults. The container with food was replaced as and when needed. The females started ovipositioning after 2 days.

The number of egg masses in each treatment and the number of eggs in each mass were recorded daily. In each treatment, adults were allowed for oviposition until they were alive. The tender twigs placed inside the rearing cage were replaced with fresh farm obtained twigs every day. The container with food for the adults was replaced daily. If egg mass is found on the leaf, the leaf portion was cut and eggs were transferred in Petri dishes for incubation with moist filter paper lining. If eggs were observed on the inner cloth walls, a piece of cloth was cut and incubated. Eggs found on wooden walls were carefully collected on to filter paper with the help of a fine brush and incubated. The egg masses and number of eggs recorded during the entire period were added and the mean of 3 replications was calculated.

Relative ovipositional preference was calculated using the formula

$$\frac{\text{No. of eggs laid on test variety - No. of eggs laid on standard variety}}{\text{No of eggs laid on Test variety + No. of eggs laid on standard variety}} \times 100$$

Results and discussion

Table 1: Number of egg masses and number of eggs/mass laid on different crops by *Spodoptera furgiperda* under laboratory conditions, NIPHM 2018-19

Treatment	Maize		Pearl millet		Sorghum		Napier grass		*Desmodium*		Total	Total
	No. of egg masses	No. of eggs/mass	No. of egg masses	No. of eggs/mass	No. of egg masses	No. of eggs/mass	No. of egg masses	No. of eggs/mass	No. of egg masses	No. of eggs/mass	No. of egg masses	No. of eggs
T1	3	402	0	0	1	70	1	20	0	0	5	492
T2	5	178	2	126	3	58	NA	NA	NA	NA	10	362
T3	5	275	5	297	NA	NA	5	252	NA	NA	15	824
T4	0	0	NA	NA	2	70	1	10	NA	NA	3	80
T5	NA	NA	0	0	NA	NA	2	60	0	0	2	60
T6	2	160	NA	NA	NA	NA	6	720	0	0	8	880
T7	NA	NA	NA	NA	0	0	1	20	0	0	1	20
Total	15	1015	7	423	6	198	16	1082	0	0	44	2718

Table 2: Mean number of eggs/mass laid on different crops by *Spodoptera furgiperda* under laboratory conditions, NIPHM 2018-19

S. No	Crop	No. of treatments tested	Mean + SD	Rank
1	Maize	5	203+133.017	1
2	Pearl Millet	4	105.75+140.6565	3
3	Sorghum	4	66+33.48134	4
4	Napier	6	212.4+279.7439	2
5	*Desmodium*	4	0	5

Based on number of eggs per egg mass, egg masses were categorized into small (1-50), medium (51-200) and large (201 and above). Consistently large egg masses were found on maize, even though the Napier grass has almost equal number of egg masses. Egg masses were also observed on other parts of the cage. Egg masses on other parts of the cage were recorded and average of all treatments was calculated. On the sides of the cage 14.25 egg masses on wood and cloth, 1.2 at the bottom of the cage and 1.4 on feeding plate were recorded.

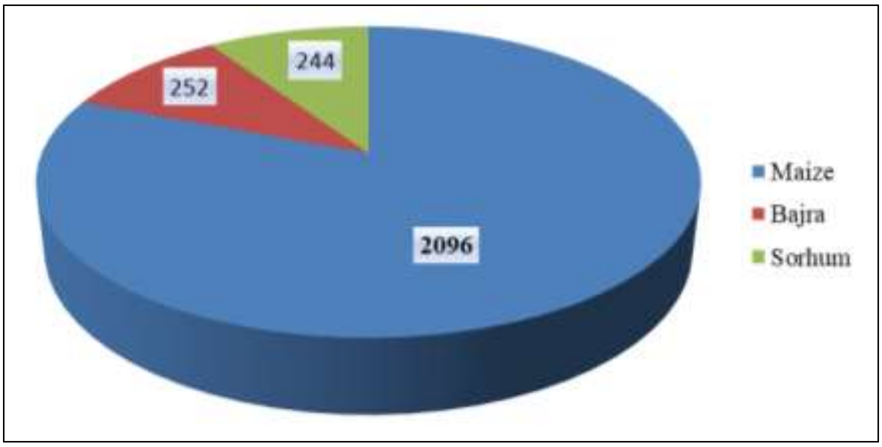

Fig 1: Total number of eggs laid by *Spodoptera furgiperda* on Maize in comparison to Sorghum and Pearl millet

The treatments T1 and T2 recorded total number of eggs 2096 on maize followed by 252 on sorghum and 244 on pearl millet showing its preference towards maize for ovipositon (Fig 1).

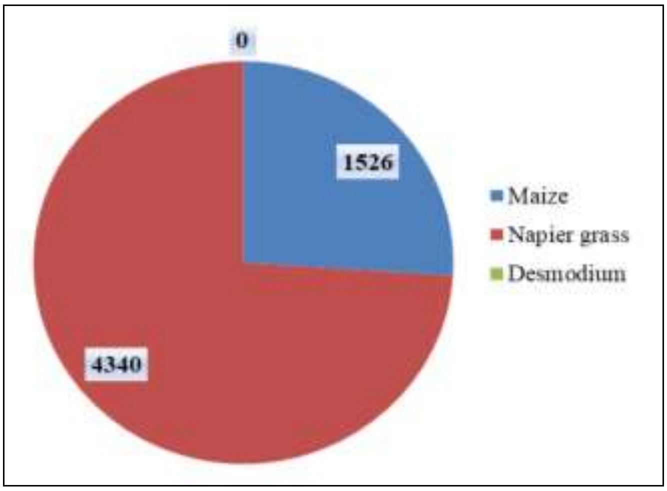

Fig 2: Total number of eggs laid by *Spodoptera furgiperda* on Maize in comparison to Napier grass and *Desmodium*

In the treatments T1 and T6, the total number of eggs laid by the FAW on Napier was high (4340) compared to the Maize (1526). The crop *Desmodium* was not preferred for oviposition in both the treatments (Fig 2).

When compared with maize as standard variety relative oviposition preference of pearl millet was -100% in T1, -55.9% in T2 and 3.9% in T3. Relative oviposition preference of sorghum was -89% in T1, 67.3% in T2 and 16.6% in T4 when compared to maize. Napier grass oviposition preference was -96.7 in T1, -4.3 in T3, -81.8 in T4 and 86.2% in T6. *Desmodium* was not preferred by FAW in all the treatments. Relative Ovipositional Preference of Pearl millet, Sorghum, Napier and *Desmodium* in comparison with standard variety of Maize showed the result that Napier grass has the maximum value followed by Pearl millet and the least ovipositional preference value was shown by sorghum.

The present results are similar with the findings of Nogueira *et al.*, 2019 [6] assayed oviposition preference using free and no-choice tests which suggests that maize is preferred for oviposition and even the resistance offered through non-preference for oviposition can be exploited for its management.

Eduardo M Barros *et al.*, 2010 [2] (a&b) have studied the host selection for oviposition by *S. frugiperda* (J.E. Smith) and its relationship with the biological characteristics under free and non-choice tests for oviposition among corn, millet, cotton and soybean, concluded that the non-selection of *S. frugiperda* may be due to the polyphagia of the species. The observation of oviposition in places outside the host as well as the high mobility of caterpillars supports the hypothesis of absence of host preference.

The egg masses found on other places of the cage surfaces in the current experimental result is also supported by the findings that neonates of *S. frugiperda* produce a silk thread by which they are hung to allow their dispersion to neighboring plants, reducing future competition and increasing the exploitation of resources in the vicinity of the oviposition site. Observation of egg masses on the walls of cages and feeding plates supports the observations of Rojas *et al*., 2003 [5]. Studied the oviposition behavior of *S. frugiperda*, and noted that tactile stimuli are more important than plant volatiles, and found more females ovipositing on corrugated surfaces rather than surfaces treated with host plant extracts as corn, tomato and cotton. Sparks, 1979 reported that, in high populations, *S. frugiperda* deposits its eggs in non-host plants and objects. Julio C. Rojas *et al* 2003 [5] investigated the role of the chemical and tactile cues in host–plant selection by *Spodoptera frugiperda* (Smith). Adult female of *S. frugiperda* seemed not to depend on plant volatiles for orientation to host plants. Tactile cues seemed to be highly important during oviposition and could neutralize the deterrent effect of the corn leaf extracts, females oviposited on grooved surfaces independently of the presence or absence of leaf extracts. In general, females oviposited more on control areas than on areas treated with leaf extracts of corn, cotton, and tomato. *S. frugiperda* females deposited more eggs on grooved or pitted surfaces than on smooth surfaces. The high number of egg masses on walls of the cages in the current study was in accordance with findings of Julio Rojas *et al.* 2003 [5] and Eduardo M Barros *et al.*, 2010 [2].

The cage experiments conducted to investigate the ovipositional preference of *S. frugiperda*, the adult moth exhibited wide host oviposition range showing oviposition on all crop plants except on *Desmodium*. Whitford *et al.*, 1988 [9] evaluated corn and rice host strains of the fall armyworm (FAW), *Spodoptera frugiperda* for oviposition preference, mating compatibility, and development on artificial diet and four plant species. Both strains oviposited a greater percentage of egg masses on corn (*Zea mays* L.), sorghum (*Sorghum bicolor* [L.] Moench., and Bermuda grass (*Cynodon dactylon* [L.] Pers. than on centipede grass (*Eremochola ophiuroides* [Munro] Hack). The corn strain oviposited preferentially on corn and sorghum, while the rice strain preferred Bermuda grass. Henry *et al.*, 1983 [3] evaluated egg-laying preference by the fall armyworm, *Spodoptera frugiperda* (J. E. Smith), ryegrass, wheat, corn, and sorghum were the most preferred, whereas cotton and soybean were the least preferred. The percentage of egg masses found on each crop plants as against the total no. of egg masses was 39% on Napier grass, 37% on Maize, 15% on Pearl millet, 7% on Sorghum and 0% on *Desmodium*. Napier and Maize were highly preferred crops compared to others. Napier proved to be equally preferred by the *S. frugiperda* for oviposition and 39% of the egg load was trapped by the Napier can be concluded by the nature of preference and the considerations observed in the laboratory host conditions.

Conclusion

The way ahead for ecological management in India

To reduce the FAW buildup, the suitable crop-diversity to be recommended with non- host crops as per local conditions. The experiments are to be conducted on the performance ecological engineering based demonstrations. Identifying suitable remunerative crops for strategic control/ trapping technology under Indian conditions is a researchable issue. Further studies may be done on the strategic use of *Desmodium* and Napier in maize fields to divert *S. frugiperda* population and to save farmers from its devastating crop damage. As the data is promising under laboratory conditions there is a need to be studied in field conditions. The cage experiments conducted to investigate the ovipositional preference of *S. frugiperda*, the adult moth exhibited wide host oviposition range showing oviposition on all crop plants except on *Desmodium*. Napier and Maize were highly preferred crops compared to others. The egg load was trapped by the Napier can be concluded by the nature of preference and the considerations observed in the laboratory host conditions.

Maize is a poor man's crop where the use of chemicals for pest management will be costly and lead to resurgence, resistance and residue problems. Growing maize hybrids having desirable agronomic characters like short duration and tight cob husk to avoid FAW attack along with farmer's friendly ecological engineering technologies can be easily adopted. Government should enhance/ put forth efforts on research as there is a need to fill the lacuna of research on this pest and elucidate the results and recommend strategic control measures and the suitable varieties of maize that ensures the yield so that its adoption will improve. Different maize hybrids and varieties available in India can also be tested for oviposition non-preference to recommend suitable varieties to the farmers.

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