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HOST PREFERENCE OF *XANTHOPIMPLA* SAUSSURE PARASITISING *SESAMIA INFERENS* WALKER

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ABSTRACT

Host selection sequence of two parasitoids of under the genus *Xanthopimpla* Saussure viz., *Xanthopimpla flavolineata* (Xf) Cameron [Xf] and *X. stemmator* (Thunberg) (Xs) and suitability of various pupal stages of *Sesamia inferens* as a factitious host for rearing was investigated. There was no significant difference in egg laying between the single and repeated attempts by both Xf and Xs. The probing time of Xf and Xs in the rice stem with concealed host (2.26± 0.10 and 1.60± 0.13 min) and a hollow stem without a host was found to be 1.19± 0.14 and 0.59± 0.15 min, respectively. However, Xs did not parasitize the naked pupa. The number of ovipositor insertions was 1.50± 0.17 in concealed pupae and 0.92± 0.15 in hollow stem without host. Among the stages of *S. inferens* pupae tested for acceptance by Xf and Xs, the prepupa was preferred the most followed by one- and two-day-old pupa, while five- and six-day-old pupae were not preferred.

Key words: Biological control, pupal parasitoid, ichneumonid wasps, *Xanthopimpla flavolineata, Xanthopimpla stemmator*, pink stem borer, factitious host, host selection, oviposition preference, oviposition sequence

Parasitoids are the most common natural enemies used in the biocontrol of insect pests (Orr and Suh, 1998). *Xanthopimpla* is one of the largest genera in the Ichneumonidae family, belonging to Pimplinae subfamily of the Hymenoptera (Dung et al., 2011) such as stem borer, pod borer, leaffolder, armyworm, bollworm, green stinkbug, but there are also several insect natural enemies. In the summer-autumn of 2009 and spring season 2010, 16 insect parasitoid species of several major insect pests were obtained in soybean production areas of Hanoi. Microplitis manilae Ashmead, Therophilus javanus (Bhat and Gupta. A total of 261 species of *Xanthopimpla* have been identified worldwide, with 149 species from the oriental region. *Xanthopimpla flavolineata* Cameron is a medium sized solitary endoparasitoid without black spots on the abdomen whereas *X. stemmator* (Thunberg) have a partially black in head and black marks in mesoscutum and abdominal segments (Akhtar et al., 2010; Pham, 2013; Kathirvelu et al., 2023a). Both the species were reported as pupal parasitoid of rice stemborers and distributed in a broad geographic area in Asia. These two species have a broad host range recorded from pyralid and noctuid pupae of numerous lepidopteran borers. An important factor defining the host range of parasites is the selection of specific microhabitats or niches within plant communities. Stem borer pupae are generally located in or near the terminus of tunnel excavated by latter instar larvae in the plant stem. The cues responsible for host finding are predicted to be associated with the cryptic habitat of the borer pupae rather than the parasitoid host species (Muturi et al., 2006; Yousuf et al., 2021). The host searching behaviour in insects is generally varied among individuals. A series of behavioural observations were made to highlight the importance of physiological state (i.e., number of mature eggs a female carries, amount of nutrient reserves, etc.) and learning state (i.e. prior host experience) on host approaching behaviour of parasitoids (Ueno and Ueno, 2005). A few species of ichneumonid wasps have been used in biological pest control, but the vast majority of them have yet to be used for such control methods. Their non-utilisation appears to be due to lack of understanding of their ethology (Dung et al., 2011) *Xanthopimpla stemmator* Thunberg, was imported into East Africa as a classical biological agent of lepidopteran cereal stem borers. Preference of *X. stemmator* females for four common borers of maize and sorghum; the crambid Chilo partellus (Swinhoe. For the successful colonization of any natural enemy for biological control, the parasite must follow the sequence of consecutive processes that includes habitat finding, host location, host acceptance, host suitability and host regulation (Vinson, 1998). Information on host selection process will be useful in the selection of release sites, and to provide insight into the establishment and efficiency of *Xanthopimpla* species viz., *X. flavolineata* (Xf) and *X. stemmator* (Xs). The aim of the present study was to investigate the behavioural responses of parasitoids in host selection sequence and suitability of various pupal stages of *S. inferens* as a factitious host in rearing the *Xanthopimpla* spp. This knowledge can be utilized to improve the mass rearing methods and employing these parasitoids as a candidate for biological control programmes to manage lepidopteran borer pests like *S. inferens* and *Scirpophaga incertulas* Walker in rice and other economically important agricultural pests.

MATERIALS AND METHODS

The experiment was conducted at the Department of Entomology, Faculty of Agriculture, Annamalai University, Annamalainagar, Tamil Nadu, India and the following methods were followed. One of the host insects of *Xanthopimpla* is rice pink stem borer, *S. inferens*. Its pupae were collected from rice fields of Annamalai University experimental farm premises of Cuddalore District, Tamil Nadu. The host insects were reared as described by Lingappa (1978); Kathirvelu et al. (2023b) and Xf and Xs parasitoids were reared using the obtained pupae of *S. inferens* under laboratory conditions were: temperature of 27± 2^{o}C, relative humidity of 65± 5% and a photoperiod of 16:8 h light: darkness. Newly emerged adults were fed with a small drop of honey and kept individually in vials (Diameter: 30 mm; Height: 15 cm) closed with nylon cloth at the top. The two species of parasitoids were used just once in the experiments. The host selection sequence of two *Xanthopimpla* species for oviposition was observed on the pupae of *S. inferens* kept inside the rice stem. In addition, the oviposition period and the oviposition punctures by parasitoid adults on host pupae were also observed. The female parasitoids were allowed inside the cages (15 x 15 x 15 cm) to lay eggs on *S. inferens* pupa concealed inside the rice stem under various situations viz., by single attempt and repeated attempts on host pupae. After the release of *Xanthoimpla* inside the cage, the behaviour of host selection was keenly noted and various actions done by them were photographed using Canon DSLR D1500 camera.

In single attempt, the pupae were transferred to plastic rearing containers (14 x 12 cm) immediately after single oviposition while in repeated attempts, the parasitoids were allowed to oviposit upto 30 minutes. The pupa thus exposed was dissected to count the total number of eggs laid per pupa per female. With the view, to gain knowledge on the host seeking stimulant, if any, present during host location. The experiment was conducted with a rice stem cut to size (15 cm) with and without host pupa and kept inside the cage (15 x 15 x 15 cm). Adult parasitoids of both the species were not exposed to any host previously was introduced into the cage and observed for ten minutes. The response of the parasitoid in terms of probing with antennal palpation and ovipositor insertion was counted. Various stages of pupa viz., prepupa, one, two, three, four, five and six day old pupae of *S. inferens* were arranged on a bed of cotton in a tray covered with filter paper. Mixture of frass materials and larval excreta were smeared against the top of each stage of host pupa. The preference of the *Xanthopimpla* spp. for oviposition was observed for 30 minutes. Stages of naked pupae viz., pre pupa, one, two, three, four, five and six day old pupae of *S. inferens* were exposed to *Xanthopimpla* spp. The preference and the host stage selected by the parasitoid for oviposition were studied for 30 minutes. This choice test was done inside a cage (15 x 15 x 15 cm) with the pupae kept on petri plates. Wherever single observation was made on growth factor or behaviour of the parasitoid, the standard error of mean (± SE) was calculated (Panse and Sukhatme, 1961). Least Significant Difference (LSD) was applied in rest of the analysis.

RESULTS AND DISCUSSION

The *Xanthopimpla* parasitoids released inside the cages for observing their behaviour on host seeking revealed that they used their fore legs to clean off antenna and head then to hold the substratum. The hind legs were used to clean off any dust from the abdomen including the ovipositor and then rubbed its hind legs together to get rid of the accumulated dusts. The host selection sequence of Xf and Xs are presented in Figs. 1 and 2. The adult female wandered rapidly up and down the rice stem till it located a possible host. The female used it's antennae by making vibrations on the surface of the host plant stem, which was used to locate a possible hidden host (Fig. 1a, 2a). After locating the hidden pupa, female parasitoids halted and bent their gaster to place the ovipositor tip at the right spot (Fig. 1b, 2b). The whole probing process including walking and antennation was recorded with 138.10± 7.52 sec in Xf and 99.45± 5.45 sec in Xs. The drilling was initiated by exposing the entire length of the ovipositor and bent their abdomen in an 'U' shape then inserted it in to the stem by thrusting it till it reaches the host (Fig. 1c, 2c, 2d). The drilling and oviposition time of Xf was 52.42± 4.24 sec and in Xs, it was 49.45± 3.00 sec (Table 1).

When the hidden host pupa gets detected, oviposition happens along with jerking movements of the abdomen, fanning of wings and vibration of the antennae (Fig. 1d, 2e). During drilling, the host pupa wriggled to escape but still the parasitoid did not leave the host free. In some cases, the pupa fell down during the process of wriggling if it was in partially concealed host plant. In such fallen cases, the adult female did not attempt again. The adult female prepared to remain head downwards on the host plant during oviposition even though few instances of head up positions were noticed. The oviposition period of Xf and Xs were recorded as 17.67± 2.39 and 22.13 ± 3.42 days, respectively (Table 1). The ovipositor insertion was found maximum in the stem with pupa than stem without pupa. The concealed pupa within the stem was easily identified by *Xanthopimpla* by echo-location through tapping the surface of the stem with its antennae (Fig. 3). Further, it preferred to oviposit through middle abdominal region of the host pupa (Fig. 4). Similarly, Bruce et al. (2021) also reported

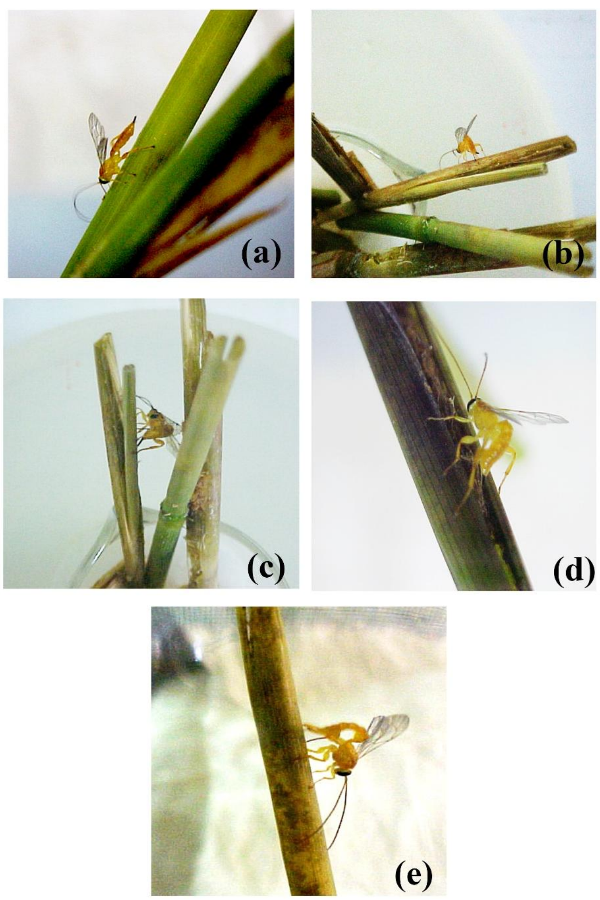

Fig. 1. Oviposition sequence of *X. flavolineata* on concealed *S. inferens* pupa within rice stem (a) walking and antennation; (b) probing the host pupa; (c) drilling; (d) oviposition inside concealed pupa and (e) withdrawing of ovipositor

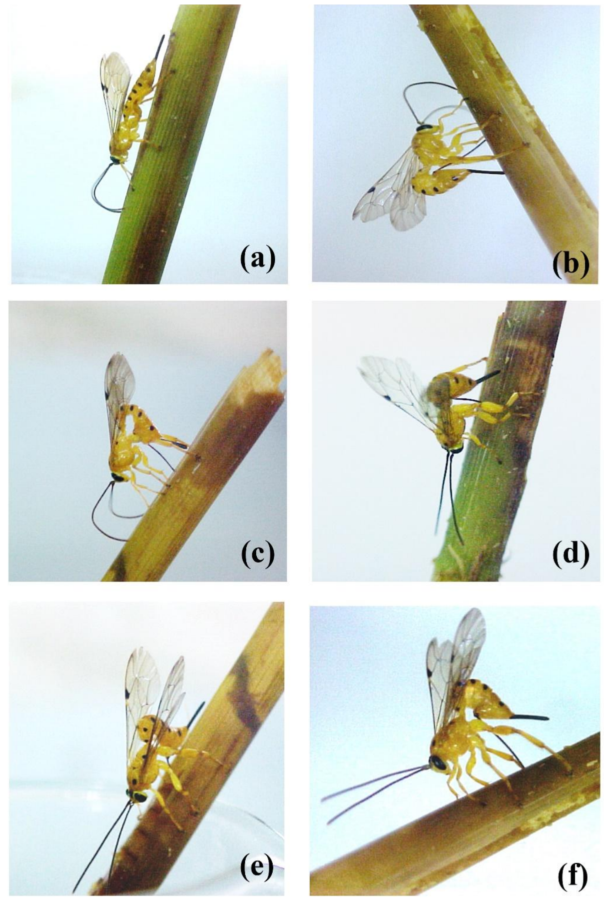

Fig. 2. Oviposition sequence of *X. stemmator* on concealed *S. inferens* pupa within rice stem (a) walking and antennation; (b) probing to confirm the host location; (c) placing ovipositor for drilling; (d) drilling to reach concealed host; (e) act of oviposition (entire length of ovipositor inserted) and (f) withdrawing of ovipositor

that the parasitoid directly alighted on mid-dorsal region of larvae, swiftly introducing an egg and quickly withdrawing the ovipositor. The time taken for drilling and oviposition was around one minute in both the species. The oviposition period was 17 days in Xf and 22 days in Xs. But the results are contrary to the report of Moore and Kfir (1996) who found that it was around 64 days in Xs. This might be because the synovigenic female produce eggs continuously throughout the life span but host unavailability might have caused the extension of oviposition period because the eggs might have been resorbed to increase the life span. Further, Gathalkar et al. (2017) reported that a gravid female of *Xanthopimpla* predator parasitizes the pupae of *A. mylitta* by depositing an egg, which produces a single offspring from the host.

Once the egg laying was over, the ovipositor was withdrawn by lifting the body straight back by

Table 1. Host selection of *X. flavolineata* and *X. stemmator*

Host selection sequence*	*X. flavolineata*	*X. stemmator*
Host probing time (Seconds)	138.10± 7.52	99.45± 5.45
Drilling and oviposition time (Seconds)	52.42± 4.24	49.45± 3.00
Oviposition period (Days)	17.67± 2.39	22.13± 3.42
Number of eggs laid at		
i. Single attempt	2.10 (1.59)[a]	2.30 (1.64)[a]
ii. Repeated attempts	3.10 (1.87)[a]	3.60 (1.99)[a]
SE.d	0.15	0.18
CD (p=0.05)	0.31	0.37
Preference of pupal stages		

Stage of pupa #	*X. flavolineata*		*X. stemmator*	
	% preference of concealed pupa	% preference of naked pupa	% preference of concealed pupa	% preference of naked pupa
Prepupa	53.33 (46.89)[a]	13.33 (21.39)[a]	62.33 (52.17)[a]	0.00
One day old	33.33 (35.24)[b]	13.33 (21.39)[a]	42.33 (40.55)[b]	0.00
Two day old	6.66 (14.89)[c]	13.33 (21.39)[a]	5.67 (13.73)[c]	0.00
Three day old	3.33 (10.47)[c]	10.00 (17.18)[b]	1.67 (7.33)[d]	0.00
Four day old	3.33 (10.47)[c]	6.66 (14.89)[b]	1.33 (6.53)[d]	0.00
Five day old	0.00 (0.00)[d]	0.00 (0.00)[c]	0.00 (0.00)[e]	0.00
Six day old	0.00 (0.00)[d]	0.00 (0.00)[c]	0.00 (0.00)[e]	0.00
SE.d	2.82	3.25	5.04	-
CD (p=0.05)	5.78	6.63	3.01	-

*Mean of 10 observations; Mean values followed by standard error (SE); Single female used per observation; Values in parentheses square root transformed; #Mean of three replications; Values in parentheses arc sine transformed; Ten females used (each observed for 30 min in sequence); Mean values followed by a common letter not significantly different (LSD, p= 0.05)

thrusting the legs against the substratum and the same was covered with ovipositor sheath (Fig. 1e, 2f). After drilling, oviposition and withdrawing of the ovipositor, dark brown oviposition punctures could be seen in the place of ovipositor insertion (Fig. 3). The sequence of steps observed in the process of host selection like walking, antennation, drilling, oviposition and withdrawing need not happen in all attempts by a female when encountering a host (Fig. 1, 2) as quoted by Fischer et al. (2004) and Cancino et al. (2021). It depends upon the suitability of the host (like parasitized

Fig. 3. Echo-location of concealed host by *X. stemmator*

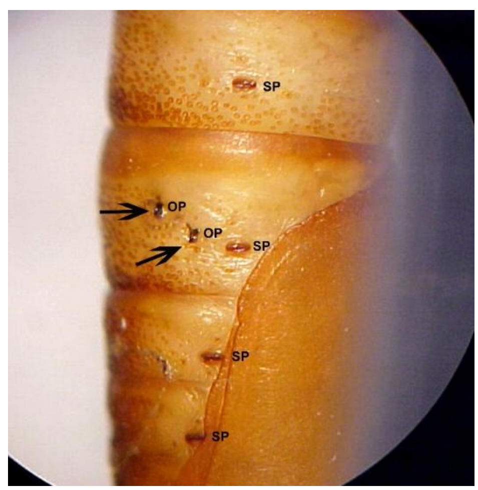

Fig. 4. Oviposition punctures of *Xanthopimpla* on *S. inferens* pupa; OP- oviposition punctures; SP- spiracles

or unparasitized). Parasitoids used many plant derived visual cues for initial host location. Such cues include discolourations, lesions, entrance holes and emergence windows (Vet, 2001; Cai et al., 2020). The process of host selection sequence started with probing of host by walking and antennation. During the above process, the parasitoid sends vibrating signal through its antennae via the substratum (stem) and receives echoing stimuli from inside of the stem through its middle and hind tarsi with which it ascertains the presence or absence of a concealed host. Chemicals produced by individual host insect play a major role in host location and host-parasitoid relationship as reported by Vinson (1998) and Fei et al. (2023). When the hidden host was detected, it started to drill by making up and down movement of the ovipositor after bending the gaster to place the ovipositor tip at the right spot at the same time fanning its stretched wings. The host selection process took around 140 sec in Xf and 100 sec in Xs. There was no significant difference in egg laying between the single attempt and repeated attempts by both Xf and Xs. The latter laid 3.60 eggs in repeated attempts and Xf laid 3.10 eggs whereas in single attempt, the same was 2.30 and 2.10, respectively (Table 1). The presence of ovariole was observed with matured eggs when the adult female was dissected out. Superparasitism was observed and laying more than one eggs per host was observed in both methods used. But only one parasitoid developed per host larvae irrespective of number of eggs laid.

The probing time of Xf in the rice stem with concealed host and a hollow stem without a host was found to be 2.26± 0.10 and 1.19± 0.14 min, respectively. The number of ovipositor insertion was 1.50± 0.17 in concealed pupae and 0.92± 0.15 in hollow stem without host. These results are in accordance with those of Gitau et al. (2005) as an endoparasitoid of lepidopteran stem borer pupae, imported into East Africa as a candidate biological control agent of gramineous stemborers. Suitability of *Busseola fusca* Fuller, *Chilo partellus* (Swinhoe) was studied and it was reported that volatile odours from different plants or feeding by products from different plants may influence the host searching and acceptance behavior. The probing time of Xs in the rice stem with concealed host and a hollow stem without a host was found to be 1.60± 0.13 and 0.59± 0.15 min, respectively. The number of ovipositor insertion was 0.82± 0.18 in concealed pupae and 0.21± 0.12 in hollow stem without host. The results are similar with the earlier reports of Pillai and Nair (1983) and Klopfstein (2022) who found that there was no ovipositor marks on naked pupae exposed to *Xanthopimpla nana nana* and other pimpline parasitoid wasps. As indicated by Sandlan (1982), Vinson (1998) and Josephrajkumar (2019), the parasitoid identified the host with the presence of lesion, frass and the cue emanated from the fed area. This is supported by the findings of Hailemichael et al. (1994), who observed that the presence of larval frass, host odour, and pupa movement influence the host seeking activity of Xs. Among the different stages of *S. inferens* pupae tested for acceptance by Xf and Xs, the prepupa was preferred the most (53.33 and 62.33%) followed by one- and two-day old pupa (33.33; 42.33% and 6.66; 5.67%), while five- and six-day old pupae were not preferred (Table 1) (Fig. 5). When different stages of naked pupae were exposed to the adult of Xf, it preferred to oviposit in the prepupa, one- and two-day old pupae (13.33% each) followed by three and four day old with 10.00 and 6.66%. The remaining 43.33% of adults did not prefer to oviposit in any stage of pupae. Five- and six-day old pupae were not at all preferred. Xs did not prefer any stage of the naked pupa (Table 1). Further, they preferred the prepupa for their oviposition over the other stages of the pupa exposed. The soft and the plumpy nature of this stage of pupa might be the main reason for preference. Next to prepupa, it preferred one- and two-day old pupa with about 33 and 6 % respectively. But it did not prefer five- and six-day old pupa. This might be again due to the comparative softness of pupa in one and two day old than five- and

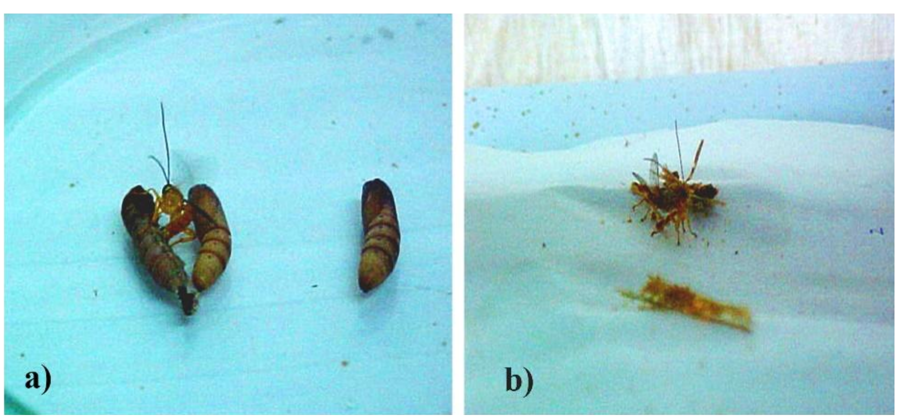

Fig. 5. Preference studies of *Xanthopimpla* spp. a) Naked host examination by *X. flavolineata*; b) Concealed pupae with frass detected by *X. flavolineata*

six-day old pupa. In addition, in the latter case the host attained an advanced stage of development. Xs preferring to oviposit two day old pupa of cereal stem borer was reported by Gitau et al. (2005). Irrespective of whether it is prepupa, one- or two-day old pupa, Xf parasitized only 13% if the host is offered naked (Table 1) (Fig. 5).

The host selection process of *Xanthopimpla* spp. involved walking and antennation, probing, drilling, oviposition and withdrawing and it laid one or two eggs inside the host pupa. There was no significant difference in egg laying between the single attempt and repeated attempts by both Xf and Xs. The two species of *Xanthopimpla* preferred to lay eggs in rice stem with host pupa than stem without pupa. Among the stages of host pupa were preferred prepupa and one day pupa than other stages. Therefore, parasitoids of concealed pupae might depend heavily on physical areas as indicators of their hosts where about. Hence, preference on host selection of Xf and Xs and suitability of the various pupal stages of *S. inferens* can be used as a factitious host in mass rearing so as to utilize these parasitic species effectively in the biological control of stem borers in graminaceous plants.

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AUTHOR CONTRIBUTION STATEMENT

All authors equally contributed.

CONFLICT OF INTEREST

No conflict of interest.

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