



Effect of *Plutella xylostella* (Lepidoptera: Plutellidae), gut bacteria on host plant utilization behavior for its survival and fitness

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ABSTRACT: This study aims to exploring the potential relationship between the gut endosymbionts and plant utilization for the survival of insects. The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) is a destructive pest of cruciferous crops. Here, we investigated the symbiotic and aposymbiotic *P. xylostella* fitness on various host plants. Developmental period of symbiotic and aposymbiotic *P. xylostella* were vary between the hosts. Fecundity was greatly influenced by different host plants. Maximum egg production was observed on symbiotic *P. xylostella* when reared on cauliflower (100.2 eggs). Whereas aposymbiotic population it was minimum (51.2 eggs). Our study suggests that endosymbiotic bacteria are the most important factor for *P. xylostella* survival. Result of fecundity directly related with the insect fitness and survival.

Keywords: *Plutella xylostella*, host utilization, fecundity, gut bacteria.

INTRODUCTION

Mediators of direct and indirect interactions between herbivorous insects and their host plants include attraction or deterrence by volatile chemical cues, differences in nutritional quality, differences in physical and chemical defences (Renwick 2001; Schoonhoven *et al.* 2005) and associations with bacterial endosymbionts. However, the degree of insect ecological specialization and reproductive isolation on different host plants will be dependent on the genetic control of fitness traits and mating influencing traits or any trade-offs between these traits (Via and Hawthorne 2002). The bacterial endosymbionts occur in a diverse array of insects which provide a source of genetic and functional variation in insect herbivores. There are two groups of endosymbionts are present in the insect such as, primary endosymbionts and secondary endosymbionts.

The primary endosymbionts are obligatory and mutualistic to the host, as they play a prominent role in insect nutritional ecology by providing essential nutrients that are limited or lacking in the diet or aid in digestion and detoxification of food. For example, almost all aphids carry an obligate bacterial endosymbiont *Buchnera* that synthesizes certain essential amino acids and hence allows the aphid to feed on a pure phloem diet. Only in the presence of *Buchnera*, aphid survival is achieved

or else it would not survive. Gibson, C.M. and Hunter, M.S. (2010), Feldhaar, H. (2011). The presence of titer and functioning of bacterial endosymbionts can have profound effects on insect fitness. It directly states that bacterial endosymbionts could influence the colonizing behavior of insects in different plants.

Functional role of bacterial symbionts in Lepidoptera has been investigated for a long time, and several studies showed that many symbionts play a nutritional role. On the other word, loss of gut bacteria often results in the detrimental fitness costs for the hosts, such as growth impairment, shortened life spans, and sterility. Gut microbes contribute to insect morphogenesis, digestion, nutrition, protection against toxic substances, pheromone production and reproduction

The diamondback moth (DBM) *P. xylostella* (Lepidoptera: Plutellidae), a well-known insect pest that infests broccoli, Brussel sprouts, cauliflower, collard, mustard, radish, rapeseed and turnip. *P. xylostella* has developed resistance to the most of the plant protection chemicals (Insecticides and biopesticides). Several tactics are used to delay the resistance development but all are void.

The reason behind the *P. xylostella* survival and fitness on different host is achieved by the presence

of gut bacterial consortia. It will be interesting to see whether symbionts are important in allowing their hosts to exploit novel ecological niches such as non-native crops, as was found in the stinkbug. In the view of above discussed points, *P. xylostella* gut bacterium was removed by antibiotics and the biology of symbiotic and aposymbiotic *P. xylostella* were studied on different hosts.

MATERIALS AND METHODS

For evaluating the role endosymbiotic bacteria on plant utilization pattern, Symbiotic *P. xylostella* (without modification of gut bacterial community i.e field collected population) and Aposymbiotic *P. xylostella* (modification of gut bacterial community i.e. Antibiotic treatment) was maintained in the Department of Entomology, TNAU, Coimbatore.

a. Collection and rearing of symbiotic *P. xylostella*

The larvae of *P. xylostella* were collected from cauliflower fields from Odanchatram, Tamil Nadu and brought to the laboratory. The culture of *P. xylostella* was maintained on cabbage leaves for a generation.

b. Rearing of aposymbiotic *P. xylostella*

Using Next Generation Sequencing *P. xylostella* gut bacterial diversity was analyzed (Unpublished Data). Based on the metagenomic results different antibiotics were selected for screening of effective antibiotic, to develop aposymbiotic *P. xylostella*. From the results of antibiotic screening Cefixine 5mg/ml selected, for developing the aposymbiotic populations.

P. xylostella larvae were exposed to Cefixine via leaf dip method. Antibiotic exposed *P. xylostella* was reared on cabbage leaves. The larvae were reared at room temperature on cabbage until pupation. Newly emerged adults were transferred in to wooden cages (30 x 30 x 45 cm) for mating and egg laying on cabbage leaves. The cabbage leaves were provided for eggs,

which were replaced daily. To keep the leaves turgid, wet cotton wool was wrapped around the petiole of the leaves. Adults were fed on cotton wool impregnated with 10% honey solution. *P. xylostella* were rearing was continued for getting a steady supply of larvae for different experiments.

c. Development and survival of immature *P. xylostella*

To test the effect of host plants on the duration of developmental time, *P. xylostella* were taken from the symbiotic and aposymbiotic population. Leaf discs (9 cm diameter) of cauliflower, cabbage, radish and broccoli respectively were cut and placed individually in plastic container, each containing one leaf disc and one moistened filter paper. On each leaf disc, five neonate larvae were released. Larval food was changed every day and the duration of development of different larval instars, total larval development period, pupal period, adult longevity fecundity were recorded. The experiment was conducted in a Completely Randomized design (CRD) with five replications.

d. Reproduction of *P. xylostella* on different host plants

Ten pairs of adults from each host plant were kept in separate cages with a leaf as described above and fed on cotton wool saturated 10% (w/v) honey solution. The leaves were changed daily and the numbers of eggs laid were counted. The eggs were kept at room temperature. Hatching usually occurred within 3 - 4 days but the eggs were given up to 7 days to hatch before the number of unhatched eggs was counted. The data on all biological parameters were analyzed statistically using Agres the differences between means were compared using LSD ($P=0.05$).



Plate1. Bouquet of different host



Plate 2. Experimental set up



Plate 3. Eggs of *P. xylostella* on cabbage



Plate 4. Eggs of *P. xylostella* on broccoli

RESULTS

a. Egg period

There was no significant difference observed among the symbiotic and aposymbiotic *P. xylostella* egg period when, reared on all the five host plants.

b. Larval period

Maximum larval period was recorded in symbiotic *P. xylostella* when reared on broccoli with 6.84 days which was on par with 6.44 days radish. Minimum larval period of 3.76, and 3.44 days was observed on cabbage, cauliflower respectively.

There was significant difference in larval period of aposymbiotic *P. xylostella*, when reared on different host. Aposymbiotic *P. xylostella* larval period was 6.88 days on cauliflower. 6.72, 5.56, and 3.12 days were recorded, when reared on radish and broccoli, cabbage respectively.

c. Pupal period

There were no significant differences in the symbiotic and aposymbiotic pupal duration. Duration of pupal period of symbiotic *P. xylostella* was 3.52, 6.24, 3.96, 4.08 days on cauliflower, broccoli, cabbage, and radish respectively.

d. Female longevity

There was no significant difference in female

longevity of symbiotic *P. xylostella*, when reared on different hosts. Duration of female longevity of aposymbiotic *P. xylostella* was recorded as 10.08, 10.68, 12.6, 10.04 days on broccoli, cauliflower, cabbage, and radish respectively.

e. Male longevity

There was significant difference in female longevity of symbiotic and aposymbiotic *P. xylostella*, when reared on different hosts. Duration of male longevity of symbiotic *P. xylostella* was 9.56, 9, 13.28, and 10.12 on cauliflower, broccoli, cabbage, and radish respectively. Duration of male longevity on broccoli, cabbage, and radish were on par with each other. Whereas, aposymbiotic *P. xylostella* male longevity of were on par with each other when reared on broccoli, cauliflower and radish.

f. Fecundity

Fecundity was greatly influenced by host plant in both the population. Maximum egg production was observed on cauliflower host plant (100.2 eggs) female eggs. Whereas, in the case of aposymbiotic *P. xylostella* fecundity was maximum on cauliflower (51.2 eggs) as a host. Aposymbiotic *P. xylostella* fecundity was reduced when reared on broccoli (12.6 eggs). Fecundity of broccoli and radish were on par with each other in symbiotic and aposymbiotic *P. xylostella*. Aposymbiotic *P. xylostella* has reduced fecundity, it indicate that the survival of *P. xylostella* was not achieved in the Aposymbiotic population.

Table 1. Biology of symbiotic *Plutella xylostella* on different hosts

Parameter	Different hosts				Sig	SEd	C D at 5%
	Cauliflower	Broccoli	Cabbage	Radish			
Egg period	3	3.2	3.4	3.2		0.0537	0.1120
Larval period	3.44c	6.84a	3.76bc	6.44a	**	0.1047	0.2184
Pupal period	3.52	6.24	3.96	4.08		0.2520	0.5257
Adult female longevity	13.12	13.8	11.6	14.2		0.1577	0.3290
Adult male longevity	9.56a	9bc	13.28c	10.12bc	**	0.1228	0.2561
Fecundity	100.2c	39a	75.6bc	36.4a	**	0.3631	0.7574

The values are means of five replications. Means followed by common alphabets in each row are not significantly different

Table 2. Biology of Aposymbiotic *Plutella xylostella* on different hosts

Parameter	Different hosts				Sig	SEd	C D at 5%
	Cauliflower	Broccoli	Cabbage	Radish			
Egg period	3.1	3.1	3.2	3.1		0.0511	0.1066
Larval period	6.88a	5.56ab	3.12b	6.72a	**	0.1407	0.2936
Pupal period	5.76	4.32	4.28	4.96		0.2417	0.5042
Adult female longevity	10.68	10.08	12.6	10.04		0.1494	0.3117
Adult male longevity	8.43b	10.2b	9.72a	11.88b	**	0.1421	0.2964
Fecundity	51.2a	12.6d	29.2bc	16.4cd	**	0.6556	1.3676

The values are means of five replications. Means followed by common alphabets in each row are not significantly different.

DISCUSSION

Insect mutualists may be more important ‘hidden players’ in insect-plant interactions. A few studies indicated that the role of endosymbionts on their food plant utilization. The result of this study revealed that *P. xylostella* food plant utilization was based on host and gut endosymbionts.

Although the host utilization of symbiotic and Aposymbiotic *P. xylostella* in corroboration with the results of few people. Aposymbiotic *P. xylostella* has reduced fecundity than the symbiotic *P. xylostella* this result are more comparable with the following example, a pest stinkbug species, *Megacopta punctatissima*, performed well on crop legumes, while a closely related non-pest species, *Megacopta cribraria*, suffered low egg hatch rate on the plants. When their obligate gut symbiotic bacteria were experimentally exchanged between the species, their performance on the crop legumes was, strikingly, completely reversed: the pest species suffered low egg hatch rate, whereas the non-pest species restored normal egg hatch rate and showed good performance. It provides a clear example of insect symbiotic involvement in host plant use.

The result of the present investigations corroborate with the findings of Gibson, C.M. and Hunter, M.S. (2010), who reported that the survival of aphid was depends on presence of *Buchnera*.

CONCLUSION

As insect symbionts are extremely common (Buchner 1965; Douglas 1989), we suggest that symbiont-induced changes in herbivore life-history patterns may have played a key role in the evolution of herbivore–host plant specialization in many species. Symbionts may promote specialization, and perhaps speciation, by changing countless aspects of a host organism’s life-

history, including altering host phenotypic expression. Manipulating symbionts may be exploited to improve pest control and finding out more about insects and their microbial associates will be both fascinating and useful. Understanding these factors may give us insights into ecological significances of endosymbiont infection, the short and long-term evolutionary tracks they mediate, the ecological differentiation and adaptation to host, the responses to environmental alteration, and species extinction risk.

REFERENCES

- Buchner, P. 1965. Endosymbiosis of animals with plant microorganisms. New York, NY: Wiley.
- Douglas, A. E. 1989. Mycetocyte symbiosis in insects. *Biol. Rev. Camb. Phil. Soc.*, 64, 409–434.
- Feldhaar, H. 2011. Bacterial symbionts as mediators of ecologically important traits of insect *Ecological Entomology.*, 36, 533–543.
- Gibson, C. M. and Hunter, M. S. 2010. Extraordinarily widespread and fantastically complex: comparative biology of endosymbiotic bacterial and fungal mutualists of insects. *Ecology Letters.*, 13, 223–234.
- Renwick, J. A. 2001. Variable diets and changing the taste in plant-insect relationships. *Journal of Chemical Ecology.*, 27:1063–1076
- Schoonhoven, L. M. Van Loon, J. J. A. and Dicke, M. 2005. Insect-plant biology. Oxford University Press, Oxford.
- Via, S. and Hawthorne, D. J. 2002. The genetic architecture of ecological specialization: correlated gene effects on host use and habitat choice in pea aphids. *American Naturalist.*, 159: 76– 88.

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