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Evaluation of Developmental and Reproductive Potential of *Lysiphebus ambiguus* (Braconidae: Hymenoptera)

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Abstract: This study was conducted to find out the developmental and reproductive potential of *Lysiphebus ambiguus*. None of the aphid species supported complete development of *L. ambiguus* despite oviposition by the parasitoid except *sipha maydis*. Maximum number of aphids were parasitized on the consecutive days. Percent emergence from *S. maydis* parasitized was decreasing from first towards the last day. Being a very specific strain with restricted host range, a stable sex ratio provides significant advantages in survival under less favorable conditions.

Key words: Reproductive, developmental potential, *Lysiphebus ambiguus*

Introduction

Cereals production is very important in different agro-ecological regions of Pakistan and its average production exceeds 12 million hectares per annum and yearly production is in exceed of 20.9 million tones (Anonymous, 1994). *Shipha maydis* is an important pest of cereals (Hamid, 1983; 1984, 1987). *Sipah flava* commonly known as yellow sugar-cane aphids a new pest of sugar-cane in North America, (especially Hawaii) Caribbean and Central and South America. Its host plants are mainly graminaceous plants and also some members of plant family Cyperaceae (Blackman and Eastop, 1994). No natural enemy has been recorded from this aphid species in the USA. Introduction of natural enemy was considered desirable as several native pests in the world have been controlled by introducing natural enemies from related species (Pimental, 1963; Carl, 1982). Previous attempt to establish a coccinellid Coleophthora inequalities had failed. (Simmonds, 1969). In 1991-93 *Adialytus ambiguus* from Europe was introduced which also failed to establish (Mahmood *et al.*, 1998). Therefore, the Department of Agriculture, Hawaii decided to use promising parasitoids of *Sipha maydis* from Pakistan. *Sipha maydis* is attached by three parasite species but *Lysiphlebus ambiguus* has proved to be a promising one in Italy (Stary, 1981) and is very efficiency in keeping it's host population under control in Pakistan (Mahmood *et al.*, 1988). Sands (1997) reported development on non-target organisms and adaptation of *L. ambiguus* to new habitat and its subsequent impact on bio-diversity.
The main objective of this study is to determine developmental and reproductive potential of *L. ambiguus*.

Materials and Methods

This study was carried out to determine host range of Pakistan strain of *L. ambiguus* reared from *Sipha maydis* from Quetta and Parachinar at CABI Bioscience Pakistan Center Rawalpindi during 1997-98. The culture of *L. ambiguus* was maintained in laboratory using *Sipha maydis* as principal host. Different host plants of *Sipha maydis* were tried but barley produced the best results. Plants were grown in small jars. When reached at two leaf stage aphids were released on these. When sufficient number of aphids established and formed colonies, field collected *L. ambiguus* was released on plants and caged. Dried leaves were constantly removed and aphids on these were transferred to fresh barley leaves with camels hairbrush. On complete drying, dried plants were replaced with fresh plants. Aphid mummies on development were regularly removed from the plants and stored in gelatin capsules for emergence of the parasitoid.

Host Stage Preference by *L. ambiguus* tapping and ovipositional response: In this experiment, different instars of *S. maydis* were exposed to *L. ambiguus* to check the tapping and ovipositional response. Different instars of *S. maydis* were separated by visual observation based on their relative size. Twenty five individuals of each stage were transferred on barley leaves measuring about 10 x 3 cm^2 and placed in a petridish of 10 cm diameter for thirty minutes. Observations were made for tapping and ovipositional attempts made by *L. ambiguus* in different instars of *S. maydis*

Development of *L. ambiguus* on different instars of *S. maydis*: Different instars of *S. maydis* were separated by visual difference in the size and fifteen aphids were transferred on piece of barley leaf. All the stages were exposed simultaneously to freshly emerged, well fed, mated female for one hour in a petridish. Aphids were then transferred on barley plant separately and caged till all the aphids became mummified or died.

Hatching period of eggs of *L. ambiguus* in *S. maydis*: For this study hundred aphids were kept with 15-20 adult female parasitoids which were already fed and mated. After one hour exposure in a test tube, all the aphids were transferred on a barley plant for future dissection. Dissections of the aphids were started after 24 hours and subsequent dissections were carried out after each twelve hours till 84 hours. Observations were recorded on the number of aphids in which parasitoids egg had hatched and first instars larva of *L. ambiguus* was found.

Development period of *L. ambiguus in S. maydis*: Parasitizing fifteen aphids of the second and third instars of *S. maydis* studied developmental period of *L. ambiguus*. After parasitism, aphids were transferred on to a barley plant. These aphid were allowed to complete the development. Daily observations were made for any change in shape. Development of mummies was considered an indication of start of pupal period and end of larval period. On development each mummy was separated and kept in transparent gelatine capsule individually for emergence of parasitoid. Mummification and emergence period was noted separately.

Progeny of *L. ambiguus* at different densities of *S. maydis*: To determine progeny of *L. ambiguus* 50, 100, 150 aphids were exposed for 24 hours to one mated and well fed female parasitoids on different dates in a glass test tube for parasites. The exposed aphids were transferred on separate plants. Record of total recoveries of mummies, total emergence and sex ratio was kept. Data analyzed by using generalized modeling Technique (Mc Cullagh and Nelder, 1983) in the statistical package GLIM 3.77 (1985, Numerical Algorithms Group, Oxford, UK).

Results and Discussion

This study was conducted to find out the developmental and reproductive potential of *Lysiphebus ambiguus*. None of the aphid species supported complete development of *L. ambigus* despite oviposition by the parositoid expect *S. maydis* maximum number of aphids were parasitized on the consecutive days. Percent emergence from *S. maydis* parasitized was decreasing from first towards the last day. Being a very specific strain with restricted host range, a stable sex ratio provides significant advantages in survival under less favorable conditions.

Development of *L. ambiguus* in aphid Spp: None of the aphid species listed during these experiments consecutive days. Percent emergence from *S. maydis* parasitized was decreasing from first towards the last day. Being a very specific strain with restricted host range, a stable sex ratio provides significant advantages in survival under less favorable conditions. Development of *L. ambiguus* in aphid Spp. None of the aphid species listed during these experiments supported complete development of *L. ambiguus* despite oviposition by the parasitoid except *Sipha maydis*. Single ovipositing female of *L. ambiguus* parasitized about 28.8 $\pm$ 2.9 *Sipha maydis*. Some of

Table 1: Parasitism preference on five stages of *S. maydis* by *L. ambiguus*

Stages	Aphids in which parasitoid complete development		Unparasitised aphids		Dead/ missing aphids	
Instar	No.	%	No.	%	No.	%
1	23	30.66	20	26.66	32	42.66
2	50	66.66	14	18.66	13	17.33
3	45	60.00	20	26.66	10	13.30
4	39	52.00	27	36.00	9	12.00
Adult	20	26.66	48	64.00	7	9.33

No. of aphids exposed, 65

Table 2: Mean number of mummies of *L. ambiguus* developed at different densities of *S. maydis*

No. of aphids/day	Days 1 X	S.D	2 X	S.D	3 X	S.D
50	18.75	1.26	10.70	51.71	6.33	0.58
110	17.25	2.99	11.75	3.20	6.5.0	2.12
150	18.25	3.4	11.00	1.15	8.00	8.00

Table 3: Mean number of mummies of L. *ambiguus* emerged at different densities of *S. maydis*

No. of aphids/day	Days 1 X	S.D	2 X	S.D	3 X	S.D
50	15.75	0.5	9.75	2.22	4.3	31.53
110	15.00	5.58	9.75	2.06	5.5	2.12
150	16.25	3.2	8.25	0.96	6.5	0.71

Appendix 1: Analysis of variance for number of dead aphids of various instars of *S. maydis* after being parasitized to check development of *L. ambiguus*.

Source of variation	d.f	Sum of squares	Means square	F-value	Pr>F
Model	8	90.32	11.29	4.88	0.0035
Replication	4	7.36	1.84	0.79	0.545
Host stage	4	82.96	20.74	8.96	0.0005
Error	16	37.04	2.315		
Corrected total	24	124.36			

Appendix 2: Analysis of variance for number of mummies of *L. ambiguus* developed at different days after emergence at different densities of *S. maydis*.

Source of variation	d.f	Sum of squares	Means square	F-value	Pr>F
Model	11	2498.29	227.17	1.28	0.3092
Replication	3	310.38	103.46	0.58	0.6349
No. of aphids exposed	2	123.01	61.50	0.35	0.7100
Life of parasite (days)	2	89.50	44.75	0.25	0.7800
No. of aphids x life of parasites in days	4	19.76	494.00	2.77	0.0500

the aphid species which were previously recorded as hosts, such as *Aphis gossypii* on cotton and citrus (Mentzelos, 1964; Rosen, 1967; Argyrious 1970) and *Aphis donacis* on *Arundo donax* (Sharma, 1966) when cultured on the reported host did not support development of *L. ambiguus*. These result may be due to the conditioning effects of the host plant. Laing (1937) also reported strong influence exerted by plant species and stated that parasitoids may ignore suitable hosts on plants to which they are not attracted. Lack of development of *L. ambiguus* on reported host may be indicative of a new bio type of the parasitoid and resultantly a change in host range (Mayer and Yeyer, 1946).

Host Stage Preference by *L. ambiguus*: Tapping response of *L. ambiguus* on four instars of *S. maydis*: *L. ambiguus* showed no taping preference for different instars of *S. maydis* (F = 0.21; Pr > F = 0.88). Slightly higher number of tapping attempts were made on 2nd instar (X = 4.5 ± 5.01) followed by 3rd 1st and 4th instar being X = 4.62±4.52, 3.5 ±3.4 and 3.13 ± 3.01 respectively. Although higher proportion of 2nd instar (X = 0.19) and 3rd instar (X = 0.18) were tapped by *L. ambiguus* as compared to 4th instar (X 0.13) but the difference were not significant (F = 0.21; Pr > F=.88). These results show a random search pattern adapted by the parasitoid females and support the similar finding reported by Hildebrands and vidals (1997).

Oviposition by *L. ambigus* on four instars of *S. maydis*: Analysis of variance to test ovipositional preference by *L. ambiguus* to different instars of *S. maydis*. There were no significant difference for oviposition (F = 0.32, Pr > F = 0.8076) to different instars. Mean ovipositional attempts in thirty minutes observation time, on 2nd and 3rd instars were 3.87 ± 4.28 followed by 1st and 4th instars X = 2.5 ± 3.35 and X = 2.25 ± 2.2, respectively. Proportion of instars ovi-posited also proved to be non-significant (F = 0.32; Pr > F = 0.80). Relatively higher proportion of 2nd and 3rd instars were ovi-posited (X = 0.15 each followed by 1st instar and 4th instars X = 0.10 and X = 0.09, respectively). But this difference was not significant. The linear regression equation explain antennal tapping and ovipositional response indicated that oviposition was significantly influenced by tapping attempts. There were more ovi-position attempts by *L. ambiguus* on 2nd and 3rd instars which received higher antennal tapping.

Development of *L. ambiguus* in different instars of *S. maydis*: Different instars of *S. maydis* were parasitized to determine the most suitable instars for parasitoid development. Number of aphids on which parasitoid completed development was highly different fro different instars ($F = 6.84$; $Pr > F = 0.0021$). Aphid parasitized when in second instars were the most suitable stage as development of *L. ambiguus* was completed on 66 % of the exposed aphids followed by 3rd instar on which development of the parastoid was completed on 60 % of the exposed aphid. Significant differences ($F = 8.96$; $Pr > F = 0.0005$) were also observed in mortality after parasitism in different instars of *S. maydis*. Analysis of variance is presented in Appendix 1 ($F = 8.96$; $Pr > = F = 0.0005$). The highest mortality 32 % was observed in 1st instar followed by 13 % in 2nd instars (Table 1). Significant differences ($F = 4.19$; $Pr > F = 0.016$) were also observed for the number of individuals left un-parasitized. The highest number escaped from being parasitized were adults i.e., 64 % followed by 4th instar 36%. These results indicated that 2nd and 3rd instars of *S. maydis* were more suitable for parasitism whereas adults were either able to escape from being parasitized or parasitoid stayed away from them. Similarly 1st instar aphids may be prone to injuries due to oviposition and hence died more as compared with 2nd or 3rd instars. Suitability of 2nd and 3rd instar of *S. maydis* may also be due to the fact that at this stage sufficient time had passed for aphids to settle on the host plant, their body is able to withstand ovipositor injuries and body size is suitable to support complete development. Adults of *S. maydis* were less likely to support complete development of parasitoid. On the other hand hard exoskeleton of the adult may hinder oviposition by the parasitized aphids (Rosen, 1967).

Developmental period of *L. ambiguus* on *S. maydis*: After oviposition, eggs of *L. ambiguus* started hatching after 36 hours and continued till 72 hours. Mean hatching period was 65 hours. Eggs and Larval development completed in about $X = 7.8 \pm 1.25$ days. Mummification period (pupal) period was about $X = 5.69 \pm 1.14$ and total development completed in 13.5 ± 18 days.

Reproductive potential of *L. ambiguus* at different densities in *S. maydis*: To determine the reproductive potential of *L. ambiguus* and assess the impact of host density, mated and well fed female of *L. ambigus* were released singly in glass tubes containing 50, 100, 150 aphids of mixed nymph instars. After every 24 hours, exposed aphids were transferred on the host plant for completion of development and female parasitoid was supplied another sets of aphids and this was repeated for three days as parasitoid female survived at the maximum for 3 days. The results of the experiment are presented in Table 2. Single female *L. ambiguus* parasitized 27.41 aphids, with an average $33 + 4$ per female. There was significant difference ($F + 55.30$; $Pr > = 0.0001$) in the number of aphids parasitized on the first, second and third day after emergence from the mummies (Table 3). Maximum number of aphids wee parasitized on the first day ($X = 18.08$) followed by second day ($X = 11.12$) and the third day ($X = 6.85$). Percent emergence from *S. maydis* paritised on the first, second and third days was 86.75 , 83.45 and 77.07 %, respectively. Sex ratio was not significantly effected by aphid densities ($F = 0.35$; $P > F=0.71$) or by age of parasitizing mated *L. ambiguus* females ($F = 0.25$; $P > F = 0.78$). Analysis of variance is presented in Appendix 2. The over all sex ratio was determined to be (M: F = 1: 0. 9). Being a very specific strain with restricted host range, a stable sex ratio provides significant advantages Ambiguus adult survived for 3 days in our study and the Host range studies have indicated *L. ambiguus* being very specific to *Sipha maydis* and hence may be a different strain than one reported by Hamid (1983) and host densities of 50, 100, 150 aphids of 4 nymph per 24 hours to single mated female for 3 days produced no significant difference ($F = 0.24$; $Pr > 0.789$).

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