

Biology of *Phthorimaea absoluta* (Meyrick) (Lepidoptera : Gelechiidae) on Different Solanaceous Host Plants

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ABSTRACT

The tomato leaf miner, *Phthorimaea absoluta* (Meyrick) a lepidopteran pest in the Gelechiidae family, is an invasive pest on tomato crop in India and widespread globally on many other solanaceous crops. However, studying the host preference and biology with its solanaceous crop and weed host range could give a way to understand and prevent the dispersion behaviour of this pest in the crop ecosystem and to develop appropriate control strategies. A host preference study was carried on six host crops (tomato, potato, chilli, capsicum, brinjal and tobacco) and three weed hosts (black night shade, datura, tropical soda apple) to test the adult oviposition preference. Tomato followed by potato was most preferred host for oviposition. The morphometrics of different life stages revealed that *P. absoluta* on tomato exhibited significantly higher values of egg length and width, larval head capsule width, larval length and width, pupal and adult measurements. Adult characteristics such as mating period, pre-oviposition period, oviposition period and post-oviposition were substantially higher when the larvae were reared on tomato.

Keywords : Tomato, *Phthorimaea absoluta*, Morphometrics, Incubation period, Head capsule

Phthorimaea absoluta (Meyrick), a lepidopteran leaf miner also known as the pin borer, is a significant economic pest. It is originated in Peru (South America) and has since spread to many other countries in South America, Europe, Africa and Asia, becoming one of the most damaging pests in the tomato industry since its introduction. Its invasion raged on, invading Afro-Eurasia, Europe, Serbia and Turkey, as well as the majority of Asian countries (Desneux *et al.*, 2011; Tosevski *et al.*, 2011 and Han *et al.*, 2018). *P. absoluta* is a significant threat to Australia, Northern Europe, New Zealand, Russia and the United States of America, according to the CLIMEX prediction model (Tonnang *et al.*, 2015).

In India, the pest was first detected and identified in October 2014 in Pune, Maharashtra and later spread to other parts of the country (Sridhar *et al.*, 2014 and Anithakumari *et al.*, 2015) including Karnataka (Kalleshwaraswamy *et al.*, 2015). Abiotic and biotic stresses are among the major constraints to enhance production and productivity of tomato (Nandan *et al.*, 2021) and cryptic species of *Bemisia tabaci* have been

developed (Sujatha *et al.*, 2021) on tomato demonstrating the importance of a holistic approach to pest management. Previous research indicates that *P. absoluta* can complete 10-12 generations per year, with a life cycle of 30-35 days per generation and a high reproductive potential. Adults are nocturnal and can over winter as eggs, pupae or adults depending on the environment. Adults are distinguished by filiform antennae (bead-like antennae), silverfish-grey scales, and distinctive black spots on the anterior wing. From the second to fourth instars, the larvae's appearance changes from greenish to light pink.

The trophic relationship between *P. absoluta* and its potential host plants other than tomato and the pest's choice of host plant within the same group is influenced by the plant's physiology, morphology, chemical and physical defences, which in turn affects the insect's life history traits. The choice of host plant is important for Lepidoptera fitness; classically, females are attracted to odour blends released by plants, which signal oviposition site and thus larval food sources. As a result, insect management strategies based on host

plant preference and performance include the development of cultivars with enhanced direct or indirect defence mechanisms, as well as the extraction of plant volatiles to develop products that lure insects.

In addition, Biocontrol and microbial agents can successfully control *P. absoluta* in closed greenhouses (Chailleux *et al.*, 2013; Galdino *et al.*, 2014; Sanchez *et al.*, 2014 and Zappala *et al.*, 2013), but do not adequately protect against *P. absoluta* infestations in open field production. Continuous tomato cultivation, both in a greenhouse and in an open field, promotes its growth and may spread to other crops. So, the objective of the study was to investigate the host preference and biology of *P. absoluta* on solanaceous species.

MATERIAL AND METHODS

Rearing of *P. absoluta*

Experiments were carried out at laboratory and net house facilities of ICAR-NBAIR, Yelahanka Campus, Bengaluru. Tomato was used for mass culture of *P. absoluta* under nethouse conditions. The larvae were reared on fresh tomato plants in insect rearing cages. After pupation, the pupae were collected and kept in specimen tubes for adult emergence. The newly emerged moths were released (1:1 sex ratio) in separate cages provided with fresh tomato plants for mating and egg laying. The cotton wads soaked in sugar solution (0.2%) or honey (0.5%) was provided as food for the moths. The plant parts containing the freshly laid eggs were removed daily and kept for hatching. Newly hatched larvae were transferred to specimen jars with different host plants. Rearing was continued till the emergence of the adults. Eggs laid on tomato leaves were kept in a climate chamber ($25\pm 1^\circ\text{C}$, $65\pm 5\%$ RH; photoperiod: 16L:8D).

Host Preference of *P. absoluta*

Choice Oviposition Assay of *P. absoluta*

A choice oviposition assay was performed on nine solanaceous host plants to assess egg laying preference of *P. absoluta*. The experiment was carried out by placing each potted plant of six host crops (Tomato,

Potato, Chilli, Capsicum, Brinjal and Tobacco) and three weed hosts (Black night shade, Datura, Tropical soda apple) in a net cage (80 x 80 cm) with a spacing of 20 x 20 cm between and within the pots. Eighteen pairs of newly emerged adults were transferred into cages having seedlings of each host and cotton wads soaked in 0.5 per cent honey solution was provided *ad libitum* to serve as adult food. *P. absoluta* females were removed from the cage after two days of oviposition and recorded the number of eggs deposited on each plant, number of eggs hatched, per cent egg hatching and number of blotches. Five replications were maintained to evaluate host preference under choice oviposition assay.

Biology of *P. absoluta* under Laboratory Conditions

Incubation Period

Two pairs of newly emerged adults were transferred into cages having seedlings of each host which were raised under similar conditions. The adult moths were allowed to mate and oviposit on seedlings for about ten days to successfully oviposit the host plants. Leaves containing eggs were separated out daily by examining under microscope. Four replications were maintained for each host. Incubation period was recorded as the duration of time taken from egg laying to hatching which was recognized by emergence of the larva through the egg chorion.

Larval Period

The duration of larval period was recorded as the number of days taken from hatching of egg till pupation. To determine the number of larval instars and the duration of each instar, leaves with eggs laid on the same day were separated and placed in petri plates. From these, leaves with the eggs hatching on the same day were separated and pooled. Every day, they were carefully dissected and the stage (instar) of larva present inside the leaf mine was determined based on the width of head capsule. The observation continued till pupation. The sex of individual was determined at the pupae stage.

Morphometric Measurements

In the laboratory, the body measurements were made for all the stages by using light microscope supported with image analysis. Length and breadth of egg, larva, pupa and adults for 20 samples were measured. The head capsule (length and breadth) measurements were also recorded. The recorded observations were computed and expressed in mean.

Dyar's law was used to differentiate instars of immature insects to predict size of instars. Head capsule width of larvae follow a geometric progression.

Geometric progression: $y = ab^x$

Where, y = measure of size

x = instar number

a and b = constants

Adult Longevity

To study the pre mating and mating period ten pairs of adults were released individually in test tubes and observed and to know the pre oviposition and oviposition period the mated females were taken and kept individually in different cages along with the seedlings of each host and data was recorded. Fecundity was recorded by counting the number of

eggs laid during the oviposition period for each female and adult longevity was recorded by enclosing male and female adults in a glass test tubes with food. Twenty such vials were maintained for recording the adult longevity.

Statistical Analysis

Data on biological parameters of *P. absoluta* were subjected to one factorial analysis of variance (ANOVA), followed by Tukey's honest significant difference (HSD) test at $p=0.05$.

RESULTS AND DISCUSSION

Choice Oviposition Assay of *P. absoluta*

P. absoluta adults preferred to lay more eggs (125.5 eggs/female) on tomato over the other host plants. Among the solanaceous plants, chilli was the least preferred host, whereas there was no oviposition on *S. viarum*, capsicum and Datura (Table 1). In terms of egg hatchability, the maximum egg hatch was observed in tomato, which were found to be significantly superior to other solanaceous plants and brinjal had the lowest hatchability of the eggs (Table 1). Previous studies suggests that solanaceous species were the major host plants of *P. absoluta*, with tomato, potato and European black night shade

TABLE 1
Choice oviposition assay of *P. absoluta*

Hosts	Number of eggs laid /female	Number of eggs hatched	Per cent egg hatching	Number of blotches /50 neonates
Tomato	125.5 (11.21) ^a	64.25 (8.04) ^a	51.43	42.00 (6.51) ^a
Potato	42.25 (6.52) ^c	24.50 (4.99) ^b	57.99	25.25 (5.03) ^b
Brinjal	18.25 (4.32) ^d	10.50 (3.33) ^d	57.53	14.75 (3.90) ^c
Black night shade (<i>S. nigrum</i>)	47.25 (6.89) ^b	15.75 (4.02) ^c	39.68	7.50 (2.81) ^d
Chilli	2.50 (1.69) ^e	0.00 (0.70) ^e	0	0.00 (0.70) ^e
<i>S. viarum</i>	0.00 (0.70) ^f	0.00 (0.70) ^e	0	0.00 (0.70) ^e
Capsicum	0.00 (0.70) ^f	0.00 (0.70) ^e	0	0.00 (0.70) ^e
Tobacco	0.00 (0.70) ^f	0.00 (0.70) ^e	0	0.00 (0.70) ^e
Datura	0.00 (0.70) ^f	0.00 (0.70) ^e	0	0.00 (0.70) ^e
SE(m)	0.301	0.352		0.388
CD	0.866	1.014		1.117
CV	2.568	6.162		8.718

Parenthesis followed by different letters are significantly different according to Tukey's HSD test at $p=0.05$

TABLE 2
Head capsule width of larval stages of *P. absoluta* on different hosts

Hosts	I instar (mm)	II instar	III instar	IV instar
Tomato	0.158 (0.811) ^a	0.281 (0.882) ^a	0.411 (0.954) ^a	0.722 (1.027) ^a
Potato	0.156 (0.810) ^a	0.246 (0.863) ^b	0.387 (0.941) ^b	0.7013 (1.014) ^{ab}
Brinjal	0.156 (0.809) ^a	0.246 (0.863) ^b	0.371 (0.933) ^c	0.688 (1.019) ^b
<i>Solanum nigrum</i>	0.145 (0.803) ^b	0.235 (0.857) ^b	0.375 (0.935) ^{ab}	0.712 (1.012) ^c
SE(m)	0.00022	0.000678	0.000431	0.000325
CD	0.000615	0.001894	0.001205	0.000909
CV	0.301651	0.291359	0.177876	0.129394

Parenthesis followed by different letters are significantly different according to Tukey's HSD test at $p=0.05$

(*Solanum nigrum*) being the most suitable, it may however, oviposit and develop on various plants of the Amaranthaceae, Convolvulaceae, Fabaceae and Malvaceae families (Bawin *et al.*, 2016). Females employ plant volatiles to guide themselves towards host plants and leaf touch is an important factor in triggering oviposition (Proffit *et al.*, 2011). The host preference of *P. absoluta* towards tomato is a result of evolutionary adaptation of the pest to tomato toxins and female's ability to select sites most favourable for the survival of their offspring and this behavioural strategy of *P. absoluta* facilitate improvement in their performance after acquiring better resources, which helps reduce their mortality by preventing the stimulation of plant defence compounds (Galdino *et al.*, 2015).

Morphometric Measurements

The mean egg length on tomato and potato was significantly longer than in brinjal and other hosts (Table 3). Similarly, the mean egg width on tomato was significantly greater than brinjal and other hosts (Table 4). In the first instar, tomato, potato and brinjal larvae had statistically similar head capsule widths, but in the second, third and fourth instars, tomato reared larvae had significantly wider mean head capsule widths (Table 2). The results for larval length and width showed that tomato had significantly higher larval length and width than other hosts.

Pupa morphometrics revealed that pupal length was significantly greater on tomato and potato than on brinjal and *S. nigrum* (Table 3). Adults were measured

TABLE 3
Morphometric data of length of *P. absoluta* on different hosts

Hosts	Egg (mm)	I instar (mm)	II instar	III instar	IV instar	Pupa	Male adult	Female adult
Tomato	0.462 (0.980) ^a	1.562 (1.436) ^a	2.656 (1.775) ^a	4.194 (2.164) ^a	7.4.4 (2.804) ^a	4.515 (2.238) ^a	10.170 (3.261) ^a	10.44 (3.02) ^b
Potato	0.464 (0.982) ^a	1.360 (1.363) ^b	2.508 (1.733) ^b	3.829 (2.079) ^b	6.883 (2.707) ^b	4.450 (2.223) ^a	9.935 (3.224) ^a	10.12 (3.25) ^a
Brinjal	0.444 (0.971) ^b	1.285 (1.335) ^c	2.390 (1.699) ^c	3.533 (2.011) ^c	6.408 (2.622) ^c	4.211 (2.169) ^b	9.095 (3.091) ^b	10.09 (3.24) ^a
<i>Solanum nigrum</i>	0.451 (0.974) ^{ab}	1.251 (1.322) ^c	2.318 (1.677) ^c	3.470 (1.989) ^c	6.291 (2.601) ^c	4.486 (2.232) ^a	1.170 (3.261) ^a	10.37 (3.29) ^a
SE(m)	0.000481	0.001085	0.001613	0.002523	0.00477	0.00163	0.004821	0.005101
CD	0.001343	0.003029	0.004506	0.007047	0.013323	0.004553	0.013466	0.014247
CV	0.194522	0.371422	0.491874	0.703021	1.164609	0.438026	1.076411	1.127573

Parenthesis followed by different letters are significantly different according to Tukey's HSD test at $p=0.05$

TABLE 4
Morphometric data of width of *P. absoluta* on different hosts

Hosts	Egg	I instar	II instar	III instar	IV instar
Tomato	0.286 (0.886) ^a	0.193 (0.832) ^a	0.393 (0.944) ^a	0.665 (1.078) ^a	1.110 (1.265) ^a
Potato	0.276 (0.882) ^{ab}	0.180 (0.824) ^{ab}	0.362 (0.928) ^{bc}	0.591 (1.043) ^b	1.021 (1.230) ^{ab}
Brinjal	0.267 (0.176) ^b	0.170 (0.818) ^b	0.341 (0.916) ^c	0.577 (1.036) ^b	0.945 (1.198) ^b
<i>Solanum nigrum</i>	0.279 (0.882) ^{ab}	0.170 (0.818) ^b	0.371 (0.933) ^{ab}	0.582 ¹ (1.039) ^b	.021 (1.231) ^{ab}
SE(m)	0.000375	0.000655	0.000684	0.001204	0.002174
CD	0.001047	0.001829	0.001911	0.003362	0.006072
CV	0.159643	0.288717	0.283712	0.470064	0.783614

Paranthesis followed by different letters are significantly different according to Tukey's HSD test at $p=0.05$

for wing expansion length and the results showed that male adults' wing expansion length was significantly greater in adults obtained from tomato, potato and *S. nigrum* than in adults obtained from brinjal and when the larvae were grown on tomato, potato or *S. nigrum*, there was no significant difference in male adult size (Table 3). When female adults were fed tomato, potato, brinjal or *S. nigrum* there was no significant difference in wing expansion length (Table 3). The morphometric measurements of 0.15 mm, 0.29 mm, 0.39 and 0.75 mm width of first, second, third and fourth instar larva, respectively confirms the findings of Pervin *et al.* (2014).

Duration of different Immature Stages

When compared to tomato and potato, the incubation period in *S. nigrum* and brinjal was significantly longer (Table 5 and Fig. 1). The tomato results corroborate the findings of Pervin *et al.* (2014), who found that the average length of the egg period was 4.10 days and Kanle *et al.* (2019) found egg durations of 6.44 ± 0.31 , 5.10 ± 0.16 and 3.10 ± 0.1 days in brinjal, potato and tomato, respectively. *S. nigrum* had significantly longer larval duration across all instars than tomato, potato and brinjal (Table 5), Pervin *et al.* (2014) and silva *et al.* (2015) reported similar results for *P. absoluta* larval duration on tomato. The obtained results were consistent with the observations of Gharekhani *et al.* (2014), who observed that the larval

duration on tomato was 10.11 days and Kanle *et al.* (2019) also reported different developmental times of larval instars on brinjal, potato and tomato.

When larvae were grown on *S. nigrum*, the pupal period was significantly longer and when larvae were grown on potato and brinjal, the pupal period was statistically similar (Table 5 & Fig. 1). The obtained results do not agree with the findings of Silva *et al.* (2015), who found that *P. absoluta* pupa takes 7.14 days to pupate on the tomato Bravo line and 6.14 days on the Tex 317 line, but they do agree with Pervin *et al.* (2014) observations, these differences could be attributed to the presence of nutrients and biochemical constituents in different hosts.

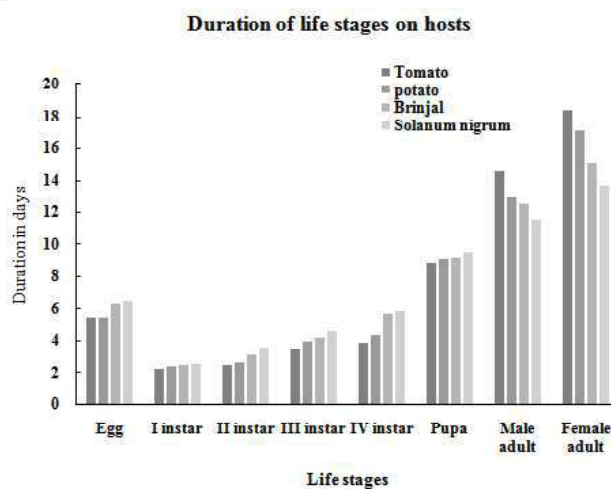


Fig. 1 : Duration of life stages of *P. absoluta* on different hosts

TABLE 5
Duration of *P. absoluta* stages on different host plants

Hosts	Egg (days)	I instar	II instar	III instar	IV instar	Pupa
Tomato	5.42 (2.42) ^b	2.30 (1.66) ^b	2.50 (1.72) ^c	3.50 (1.99) ^c	3.90 (2.08) ^c	8.90 (3.06) ^a
Potato	5.45 (2.42) ^b	2.42 (1.70) ^{ab}	2.72 (1.79) ^c	4.00 (2.99) ^b	4.35 (2.18) ^b	9.10 (3.09) ^{ab}
Brinjal	6.37 (2.61) ^a	2.50 (1.72) ^{ab}	3.17 (1.91) ^b	4.25 (2.17) ^b	5.67 (2.48) ^a	9.20 (3.10) ^{ab}
<i>Solanum nigrum</i>	6.50 (2.63) ^a	2.60 (1.75) ^a	3.57 (2.01) ^a	4.60 (2.25) ^a	5.85 (2.51) ^a	9.55 (3.16) ^a
SE(m)	0.005453	0.003564	0.003681	0.003743	0.003669	0.004324
CD	0.015229	0.009954	0.010282	0.010455	0.010246	0.012076
CV	1.372256	1.089069	1.079598	1.02482	0.963384	0.98104

Parenthesis followed by different letters are significantly different according to Tukey's HSD test at $p=0.05$

Adult Longevity

Adult parameters such as mating period, pre-oviposition period, oviposition period, and post-oviposition period were significantly higher when larvae were raised on tomato versus brinjal (Table 6). Marina *et al.* (2014) reported similar mating period results, and Silva *et al.* (2015) reported a pre-oviposition period of 1.07 days on tomato Bravo line, which supports the results obtained. The results obtained for oviposition

period differ from those of Pervin *et al.* (2014), who reported 7.88 days, as well as those of Silva *et al.* (2015), who reported 10.87 days on the Bravo line but supported by 16.00 days on the Tex 317 line on tomato. Kanle *et al.* (2019) found that the oviposition period for brinjal, potato and tomato was 7.11 ± 0.45 , 9.70 ± 0.33 and 12.58 ± 0.54 days, respectively and Silva *et al.* (2015) reported similar results for post-oviposition period on Bravo line with 3.60 days on tomato.

TABLE 6
Adult longevity and fecundity of *P. absoluta* on different hosts

Hosts	Mating period (hrs)	Pre-oviposition period (d)	Oviposition period (d)	Post oviposition period (d)	Fecundity	Male adult longevity (d)	Female adult longevity (d)
Tomato	4.400 (2.209) ^a	1.100 (1.249) ^a	14.90 (3.912) ^a	3.050 (1.868) ^a	135.88 (11.67) ^a	14.57 (3.87) ^a	18.40 (4.34) ^a
Potato	4.200 (2.160) ^b	0.937 (1.178) ^{ab}	12.150 (3.549) ^b	2.650 (1.758) ^{ab}	102.00 (10.11) ^b	12.90 (3.65) ^{ab}	17.10 (4.18) ^b
Brinjal	3.825 (2.072) ^c	0.675 (1.073) ^b	7.800 (2.872) ^c	2.250 (1.643) ^b	37.13 (6.07) ^d	12.50 (3.59) ^{ab}	15.02 (3.92) ^c
<i>Solanum nigrum</i>	4.312 (2.187) ^b	0.650 (1.064) ^b	10.600 (3.324) ^b	2.850 (1.814) ^a	66.75 (8.19) ^c	11.57 (3.46) ^b	13.67 (3.75) ^d
SE(m)	0.004168	0.004502	0.006253	0.006003	0.075155	0.005749	0.006573
CD	0.011642	0.012574	0.017465	0.016768	0.217683	0.016056	0.01836
CV	1.135073	1.685443	1.353534	1.804382	2.002752	1.203788	1.305952

Parenthesis followed by different letters are significantly different according to Tukey's HSD test at $p=0.05$

The number of eggs laid per female was significantly higher in tomato, followed by potato, *S. nigrum* and brinjal (Table 6). The obtained results differ from those of Pervin *et al.* (2014), who reported 141.16 eggs/female and also from those of Silva *et al.* (2015), who reported 172 eggs/female on the tomato Bravo line and 200 eggs/female on the Tex 317 line. The number of eggs laid by each adult on different hosts varied and the number of eggs laid by females on leaf surfaces may vary due to the repellent activity of volatile compounds, which causes irritability to the females during oviposition. In contrast, Rostami *et al.* (2016) reported 56.19 eggs per female, while Kanle *et al.* (2019) reported the fecundity (eggs/female) of 18.11 ± 1.20 , 55.2 ± 2.42 and 86.92 ± 2.70 eggs on brinjal, potato and tomato, respectively.

Adult longevity was measured for both male and female moths; male moths lived significantly longer on tomato than *S. nigrum*, but longevity on potato and brinjal was similar (Table 6 & Figure 1). The results obtained do not agree with the findings of Silva *et al.* (2015), who found that male *P. absoluta* took 16.47 days to complete on the tomato Bravo line and 21.07 days on the Tex 317 line. The results obtained by Pervin *et al.* (2014) supported the results with 15.8 days on tomato. Female moths outlived male moths on tomato, followed by potato, brinjal and *S. nigrum* (Table 6 and Fig. 1). The obtained results do not agree with the findings of Silva *et al.* (2015), who found that *P. absoluta* female took 15.53 days to complete on the tomato Bravo line and 21.53 days on the Tex 317 line. The obtained results were supported by the findings of Pervin *et al.* (2014), who reported 18.6 days on tomato and Rostami *et al.* (2016), who reported 17.97 days of adult longevity in the grandella tomato variety. Kanle *et al.* (2019) reported total female longevity in brinjal, potato and tomato of 45.44 ± 1.09 , 40.9 ± 0.46 and 42.08 ± 0.47 days, respectively.

In this study, we demonstrated that different *Solanum* species could serve as potential hosts for *P. absoluta* and that the feeding nature and performance of this pest on hosts other than tomato pose a threat to the pest management in solanaceous crops.

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