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Pupal duration, survival and occurrence of summer and winter diapause in *Helicoverpa armigera* (Hübner) under field conditions

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ABSTRACT

In two year study grown up *H. armigera* larvae were collected from cotton crop and other hosts, reared and allowed to pupate in the laboratory in the soil-filled plastic specimen tubes (10 x 4.5 cm). Such tubes were fixed in cotton field and kept under observation to record moth emergence, and pupal duration and survival. Based on the duration criterion, there was an occurrence of both summer and winter facultative diapause in this pest. During April-May, the duration of non–diapausing pupae was 12 days, while the diapausing pupae took duration of 57-70 days. During September to November, large variations in pupal duration were observed during both the years. During 1st year 64.43 per cent pupae took duration of 9-14 days and 0.52 per cent took 99-112 days. Similarly, during 2nd year 56.34 per cent pupae took 9-14 days, while 1.60 per cent took 99-126 days. The remaining pupae had an intermediate duration. This also indicated the presence of winter diapause. Pupal mortality during different weeks was quite variable. It was highest (>87%) during April-May mainly because of high summer temperature and low relative humidity. About 3 per cent mortality during this period was due to ants. Predation by ants was the major pupal mortality factor during 1st crop season causing 3.7-46.2 per cent mortality during September-early October, as compared to 2.94 per cent during 2nd. During October-December the major factors causing pupal mortality were blockage of emergence tunnels and unknown reasons. Pooled mean pupal mortality during the crop season was 26.79 and 39.45 per cent in 1st and 2nd, respectively.

The cotton bollworm, *Helicoverpa armigera* (Hübner) is an important pest of cotton in India. Development of an integrated management programme for this pest requires a detailed understanding of its population dynamics. An important factor affecting population dynamics of this pest is its ability to enter a facultative diapause in response to environmental factors (Wilson *et al.* 1979; Hackett and Gatehouse, 1982; Zhou *et al.* 2000; Liu *et al.* 2004; Saini and Olla 2004). A number of factors are reported to adversely affect the survival of *H. armigera* pupae. Young and Price (1968) reported up to 90.6 per cent mortality in irrigated fields as against 43 per cent mortality in non-irrigated fields. Murray and Zalucki (1990) reported 50 per cent mortality of *H. armigera* pupae when submerged in water for 35 hours.

However, it was not known how the pupal population survived through summer and what proportion of pupae formed during different months contributed towards population build-up during cotton growth season particularly in South Western Haryana. In this paper, observations on pupal survival, development and diapause of pupae in near natural conditions are presented.

MATERIALS AND METHODS

The experiment was conducted in the Research Farm of the Department of Entomology, CCS Haryana Agricultural University, Hisar (29° 10' N, 75° 46'E, 215.2 m m.s.l.), Haryana, India during cotton crop seasons of 2003 (1st year) and 2004 (2nd year). For recording pupal duration, pupal survival, and diapause, if any, during different months of cotton crop season, the larvae were allowed to pupate under near natural field conditions. For this purpose, grown up larvae were collected from cotton fields and from other hosts [gram (*Cicer arietinum*), sunflower (*Halianthus annus*) and tomato (*Lycopersicon esculentum*)] during off-season and further reared on the same host until full grown.

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These were released individually in perforated plastic specimen tubes (10x4.5cm) as described by Saini and Olla (2004). Such tubes were first filled with moist field soil up to the 3/4th capacity and one larva was placed over the soil in the tube. After the larva entered the soil for pupation, the tubes were numbered and fixed in the cotton field. These were kept under daily observation to record pupal duration and mortality. Pupal duration was taken as the interval between the date of pupation and date of moth emergence. Pupae from which moths did not emerge, including such cases wherein moths were found trapped in emergence tunnels, were considered as dead. There were three different sets of experiment i.e. one each during crop season of 2003 and 2004 and one during April to May, 2004. For pupae kept during crop seasons of 2003 and 2004 observations were taken till third week of March of the successive year after which the tubes were dug out from the field and emptied to record number of dead pupae. The live pupae were to be kept again in the tubes but none of the pupa was found live after this period. In the case of pupae placed in April-May, 2004, observations were recorded upto July. In all, 270 pupae were placed during different months in 2003 and 309 in 2004. Pupal duration was correlated with temperature, relative humidity and rainfall conditions during different months.

RESULTS AND DISCUSSION

Pupal duration and diapause (1st year)

The pupae placed in the field during different standard weeks took different periods for development. Mean pupal period in different weeks ranged from 9.14-10.33 and 11.56-22.83 days for pupae placed during September and October, respectively (Table 1). Pupal period increased as the temperature declined and showed a strong negative correlation with temperature (r = -0.86) and relative humidity (r = -0.60). The November formed pupae showed wide variations (29-103 days) in pupal duration. Frequency distribution of pupal duration indicated that out of 194 pupae that were able to survive, a large proportion (64.43%) took a pupal period of 9-14 days (Table 2). The remaining 35.57 per cent pupae exhibited large variations in pupal periods. Among these 4.12 per cent had a pupal period of 43-56 days while 0.52 per cent took pupal period as high as 103 days.

Pupal duration and diapause (2nd Year)

There were not much variations in the mean pupal periods for pupae placed between 14th and 18th standard weeks wherein this period ranged from 11-12 days while it was 38.16 days in 22nd week (Table 1). Pupal period was quite variable in pupae placed during 38th to 46th standard week. It ranged from 9-48 days for pupae placed during 38th week and 21-115 days for pupae formed during 46th week. Similar to that during 2003, pupal period showed a negative correlation with temperature during summer (r =-0.26) and crop season (r =-0.85). However, no clear relationship was observed with relative humidity. Two of the pupae formed in 46th week (second week of November) showed pupal period more than 100 days and yielded moths in March next year.

Out of 126 pupae that were able to survive, a large proportion (56.34%) took pupal period of 9-14 days (Table 2). The remaining 43.66 per cent population exhibited large variations in pupal period. Among these a small percentage (1.60%) exhibited pupal period as high as 99-126 days.

Both the years, a small proportion of pupae formed in November (0.52 and 1.60%, respectively), showed pupal period more than 100 days and was considered to be in a state of winter pupal diapause. In the May, 2004 formed pupae, 2.38 per cent had duration of 57-70 days, indicating the presence of summer pupal diapause.

Pupal duration of *H. armigera* exhibited considerable heterogeneity. During summer (April-May), the pupae normally took duration of 12 days. However, 2.38 per cent of the pupae formed during May took duration of 57-70 days. Based on duration criterion suggested by Hackett and Gatehouse (1982), these pupae were considered to be in facultative summer diapause. Similarly, Cullen and Browning (1978) suggested that in *Heliothis punctigera* (Wallengren) if emergence took longer than 20 days at 29°C or 50 days at 19°C, these were considered to have been in diapause. Percentage of pupae undergoing summer diapause seems to be quite significant. However, in view of the heavy pupal mortality during summer (discussed elsewhere), the net carry over of the population through summer might be quite low. Nevertheless this small population could help in early establishment of the pest on the crop. No information is available in the literature on

Table 1. Duration and survival of *H. armigera* pupae in relation to the prevailing weather conditions during different standard weeks.

Std Wk	Month	Mean Temp. (°C)		Mean R.H. (%)		Rainfall (mm)		No. of pupae kept		Pupal period (days) Mean		Range		No. of dead individuals		Per cent mortality		Cause of mortality	
		1st year	2nd year	1st year	2nd year	1st year	2nd year	1st year	2nd year	1st year	2nd year	1st year	2nd year	1st year	2nd year	1st year	2nd year	1st year	2nd year
14	Apr	-	27.7	-	43.0	-	0.0	-	19	-	12.00	-	9-12	-	17	-	89.47	-	A (10.52%) U (78.94%)
15		-	29.1	-	31.5	-	0.0	-	34	-	11.57	-	9-12	-	27	-	79.41	-	P (2.94%) F (5.88%) U (70.59%)
16		-	30.8	-	37.0	-	0.0	-	20	-	12.00	-	9-12	-	19	-	95.00	-	F (10.00%) A (10.00%) U (75.00%)
18	May	-	25.0	-	63.0	-	43.2	-	13	-	12.00	-	9-12	-	12	-	92.30	-	A (7.69%) U (84.61%)
22		-	31.4	-	49.0	-	9.7	-	22	-	38.16	-	57-70	-	19	-	86.36	-	U (86.36%)
Total									108						94		87.03**		A (4.62%) P (0.92%) F (3.70%) U (77.77%)
r (Pupal period)		-	-0.26	-	0.59														
37	Sep	28.7	-	76.0	-	2.2	-	13	-	9.14	-	9-10	-	6	-	46.15	-	P (46.15%)	-
38		29.8	28.1	69.5	70.5	0.0	0.0	30	30	9.29	13.21	9-12	9-48	6	6	20.00	20.00	P (10.00%) U (10.00%)	U (20.00%)
39		27.0	27.7	73.0	60.0	23.0	0.0	10	51	10.33	13.88	9-11	10-51	7	26	70.00	50.98	U (70.00%)	A (5.90%) U 45.10%)
40	°Ct	24.7	27.0	64.0	73.0	0.0	28.4	54	10	11.56	15.25	9-14	14-17	6	6	11.11	60.00	P (3.70%) A (1.85%) U (5.55%)	U (60.00%)
41		24.3	24.7	64.5	69.0	0.0	8.0	38	37	13.07	14.72	11-16	12-19	12	19	31.57	51.35	A (15.79%) U (15.79%)	U (51.35%)
42		22.9	23.5	58.0	59.0	0.0	0.0	55	10	15.44	17.90	12-19	16-24	5	0	9.10	0.00	A (7.27%) U (1.81%)	-
43		22.6	21.5	59.5	59.5	0.0	0.0	22	11	18.31	19.42	15-29	17-21	6	4	27.27	36.36	A (22.72%) U (4.54%)	U (36.36%)
44		21.2	-	60.0	-	0.0	-	11	-	22.83	-	10-34	-	5	-	45.45	-	A (36.36%) U (9.09%)	-
45	Nov	21.5	18.3	51.0	60.0	0.0	0.0	21	18	38.33	47.00	29-103	20-38	15	8	71.42	44.44	A (52.38%) U (19.04%)	A (5.55%) U (38.88%)
46		-	19.6	-	57.5	-	0.0	-	18	-	39.85	-	21-115	-	4	-	22.22	-	U (22.22%)
49	Dec	16.7	-	61.0	-	0.0	-	11	-	53.25	-	43-66	-	3	-	27.27	-	A (27.27%)	-
Total								265	185					71	73	26.79**	39.45**	P (4.15%) A (12.83%) U (9.81%)	A (2.16%) U (37.29%)
r (Pupal period)		-0.86	-0.85	-0.60	-0.27														

* P: Predation by ants; A: Adults trapped in tunnels; U: Unexplained mortality; F: Failure to pupate ** Pooled Mean

Table 2. Frequency distribution of pupal period of *H. armigera*.

Class interval of pupal period (Days)	Pupae falling in particular class interval			
	Number		Per cent	
	1st year	2nd year	1st year	2nd year
Upto 14	125	71	64.43	56.34
15–8	52	39	26.80	30.95
29–42	8	9	4.12	7.14
43–56	4	2	2.07	1.58
57–70	4	3*	2.07	2.38*
71–84	0	0	0.00	0.00
85–98	0	0	0.00	0.00
99–112	1	1	0.52	0.80
113–126	-	1	-	0.80
Total	194	126		

*These pupae were formed in last week of May

summer diapause in *H. armigera* under north Indian conditions. However, Bhatnagar and Davies (1978) reported that all *H. armigera* pupae entered summer diapause under agro-climatic conditions of Andhra Pradesh, India. Nibouche (1998) also reported high temperature induced diapause in *H. armigera*. Liu *et al.* (2004), in China, reported that 63.2 per cent of males and 10.9 per cent of females entered summer diapause when prepupae were exposed to alternative temperature of 33-39°C in the laboratory.

Likewise, winter diapause was recorded in *H. armigera* pupae formed during October-November. These findings corroborated the findings of earlier workers who observed that initiation of winter facultative diapause occurred in *H. armigera* during October and about 2/3rd of the pupae of *H. armigera* formed during December-February were in diapause in Haryana (Saini and Olla, 2004). Singh *et al.* (1990) inferred that under agro-climatic conditions of Punjab, India, facultative pupal diapause occurred in pupae formed at the end of October. Komarova (1959) reported that in Azerbaijan, the duration of pupae kept at 23-25°C ranged from 10 to over 200 days. Chaudhary and Sharma (1981) reported that in Haryana about 50 per cent pupae formed in January had an extended pupal period and suggested that such pupae entered diapause. In South India, the proportion of pupae which entered diapause was reported to vary from year to year, depending upon environmental conditions, while the insects were sensitive to diapause inducing cues (Souza *et al.* 1995). Fitt (1989) opined that populations from different geographical areas respond differently to prevailing climatic conditions and stated that winter diapause was more common in subtropical and temperate parts of the world. In the tropics populations breed continuously (Coaker, 1959), although a small proportion (2-4%) of the pupae may enter diapause (Reed 1965; Hackett and Gatehouse, 1982). On the other hand, in subtropical and temperate regions most, but not all, individuals enter diapause (Roome, 1979; Wilson *et al.* 1979; Kay, 1982).

Pupal duration exhibited negative correlation with temperature during September-October during both the years while no clear relationship was exhibited for relative humidity. Similar negative correlation of pupal period of *H. armigera* with temperature was reported by Olla (1998) and with relative humidity by Chaudhary and Sharma (1981).

Pupal survival (1st Year)

Pupal mortality varied during different weeks. It ranged from as low as 9.10 per cent for pupae placed during 42nd standard week (third week of October) to 71.42 per cent for pupae placed during 45th standard week (first week of November) (Table 1). Pupal mortality in different weeks was 20.0-70.0, 9.1-45.5, 71.4 and 27.3 per cent for pupae placed during September, October, November and December, respectively. The causes of such mortality were predation by ants, blockage of emergence tunnels and unknown factors. During September to early October, predation by ants was the important factor causing pupal mortality in the range of 3.7-46.2 per cent, although it accounted for only 4.15 per cent of the total pupal mortality. The blockage of emergence tunnels remained important factor during October to December and it led to 12.83 per cent of total pupal mortality. Mortality due to unknown factors accounted for 9.81 per cent and it was observed throughout the season from September to November.

Pupal survival (2nd Year)

During April to May, a high mean pupal mortality (87.03%) was observed which ranged from 79.41 per cent during 15th standard week (second week of April) to 95 per cent during 16th standard week (third week of April) (Table 1). A low level of predation by ants was observed in the second week of April resulting in 2.94 per cent pupal mortality. A small percentage of individuals 5.88 to 10.00 per cent failed to pupate during different standard weeks accounting for 3.7 per cent of total pupal mortality. Similarly, 7.69 to 10.52 per cent individuals failed to successfully emerge out leading to about 4.62 per cent of total pupal mortality. Unexplained mortality of the pupae formed during April-May remained quite high which ranged from 70.59 to 86.36 for pupae formed during different weeks. It was the leading cause of mortality during this period leading to 77.77 per cent of the total pupal mortality.

In the crop season, a variable trend in pupal mortality was observed. While there was no pupal mortality in the third week of October, it was as high as 60.00 per cent in the first week of October. About 37.29 per cent pupal mortality was ascribed to the unknown factors and 2.16 per cent to blockage of emergence tunnels.

Mortality factors for *H. armigera* pupae

During April-May, unexplained pupal mortality during different weeks was highest (73.97-85.71%) probably due to high temperature (mean: 27.7-31.4°C) coupled with low relative humidity (mean: 31.5-63.0%) (Table 3). The maximum mean temperature during this period ranged from 36.4 to 43.0°C and minimum relative humidity from (13.0-47.0%) (data not shown). Other pupal mortality factors recorded during this period were predation by ants (causing 1.36% mortality), blockage of emergence tunnels (2.85-5.47%) and failure of the pre-pupae to pupate successfully (5.47%).

In crop season 2003, predation by ants was observed in September and October accounting for 16.98 and 1.11 per cent pupal mortality, respectively. About 11.11-52.38 per cent moths failed to emerge out of emergence tunnels during October-December. Mortality due to unknown factors ranged from 6.66 to 19.04 per cent.

In 2004 crop season no predation of pupae by ants was observed during crop season. Per cent mortality due to blockage of emergence tunnels was 2.77-3.70. Higher mortality (30.55-42.64%) was observed due to unknown factors.

Thus, the overall trend of pupal mortality in 2003 and 2004 crop seasons due to various factors indicated that blockage of emergence tunnels and unknown reasons were the important mortality factors causing 2.24-27.27 and 16.53-29.10 per cent mortality, respectively. Mortality due to unknown reasons was as high as 85.71 per cent in May. Predation by ants was maximum in September (6.71%).

Wide variations in pupal mortality were observed during different months. Pooled mean mortality was 26.79 and 39.45 per cent during 2003 and 2004 crop seasons, respectively. Chaudhary and Sharma (1981) observed pupal mortality of *H. armigera* in the range of 7.4-29.4 during different months. However, during summer (i.e. April-May) pupal mortality was high i.e. more than 87 per cent. High pupal mortality was probably mainly due to high maximum temperature (36.4-43°C) and low relative humidity (13.0-47.0 per cent) prevailing during these months. Apart from high or low temperature or relative humidity, pupal mortality may also be caused by predation by ants and other agents, their drowning due to rains or irrigation water, disease and other unknown reasons. In the present studies significant ants' activity against *H. armigera* pupae was noticed during April and September only. Pupal mortality due to ants predation was relatively higher (3.70-46.15%) during 2003 as compared to 2004 (2.94 %). Hitchins (2000) reported maximum activity of ants in December in Australia. Pearson (1958) suggested that predation of prepupae and pupae by ants may be a significant mortality factor under tropical conditions.

Table 3. Mortality factors of *H. armigera* pupae as summarized in the form of partial ecological life table.

Month	Number of pupae placed in the field (lx)			Factor responsible for dx (dxF)	Number dying during x (dx)			qx (%) (qx=lx/dx*100)		
	1st year	2nd year	Total		1st year	2nd year	Total	1st year	2nd year	Total
April	-	73	73	Predation	-	1	1	-	1.36	1.36
				Adults trapped	-	4	4	-	5.47	5.47
				Failure to pupate	-	4	4	-	5.47	5.47
				Unexplained mortality	-	54	54	-	73.97	73.97
				Total	**-**	**63**	**63**	**-**	**86.30**	**86.30**
May	-	35	35	Predation	-	0	0	-	0.00	0.00
				Adults trapped	-	1	1	-	2.85	2.85
				Failure to pupate	-	0	0	-	0.00	0.00
				Unexplained mortality	-	30	30	-	85.71	85.71
				Total	**-**	**31**	**31**	**-**	**88.57**	**88.57**
September	53	81	134	Predation	9	0	9	16.98	0.00	6.71
				Adults trapped	0	3	3	0.00	3.70	2.24
				Failure to pupate	0	0	0	0.00	0.00	0.00
				Unexplained mortality	10	29	39	18.86	35.80	29.10
				Total	**19**	**32**	**51**	**35.84**	**39.50**	**38.06**
October	180	68	248	Predation	2	0	2	1.11	0.00	0.80
				Adults trapped	20	0	20	11.11	0.00	8.06
				Failure to pupate	0	0	0	0.00	0.00	0.00
				Unexplained mortality	12	29	41	6.66	42.64	16.53
				Total	**34**	**29**	**63**	**18.88**	**42.64**	**25.40**
November	21	36	57	Predation	0	0	0	0.00	0.00	0.00
				Adults trapped	11	1	12	52.38	2.77	21.05
				Failure to pupate	0	0	0	0.00	0.00	0.00
				Unexplained mortality	4	11	15	19.04	30.55	26.31
				Total	**15**	**12**	**27**	**71.42**	**33.33**	**47.36**
December	11	-	11	Predation	0	-	0	0.00	-	0.00
				Adults trapped	3	-	3	27.27	-	27.27
				Failure to pupate	0	-	0	0.00	-	0.00
				Unexplained mortality	0	-	0	0.00	-	0.00
				Total	**3**	**-**	**3**	**27.27**	**-**	**27.27**

- Pupae were not placed in the field during April-May of 1st year and December of 2nd year.

Since pupae were left undisturbed in the field for recording moth emergence till the end of experiment, the reasons for pupal mortality at different intervals could not be ascertained. However, pupal mortality in *H. armigera* due to flooding of fields has been reported by several workers (Young and Price, 1968; Murray and Zalucki, 1990; Singh, 2000).

Thus, from the present study it can be concluded that pupal duration increases as the temperature starts decreasing with the onset of winter and *Helicoverpa armigera* undergoes both summer as well as winter diapause under agro-climatic conditions of North Western Haryana. Various abiotic and biotic factors are responsible for pupal mortality in this region among which temperature, blockage of emergence tunnels and unknown factors are important ones. Also, results obtained in this study might provide useful information to *Helicoverpa* management such as pre-emergence cultivation of fields in March-April to expose diapausing pupae to the vagaries of nature.

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