Israel Journal of Entomology Vol. IV, 1969

THE BIOLOGY OF THE CAROB MOTH ECTOMYELOIS CEKATONIAE (ZELL.) IN ISRAEL

II. EFFECT OF FOOD, TEMPERATURE AND HUMIDITY ON DEVELOPMENT

by

Shmuel Gothilf

Volcani Institute of Agricultural Research*

S U M M A R Y

The development of the carob moth was studied under various conditions of tempe- rature, humidity and nutrition. Egg incubation period was optimal at 30°C and lasted 3 days . At 15° C development was arrested and at 45°C eggs died within 2 hours. At average temperature egg survival dropped considerably when exposed to humidities of 10% and lower.

Rate of larval growth varied in the different hosts studied. On unripe carob and acacia pods duration of larval together with pupal stage lasted, at 25 C, about 32 days, but on ripe, dry pods development was retarded and lasted for over 3 months. On almond development lasted 48 days and on grapefruit 66 days. Rapid growth of the insect on unripe pods was dependent on the presence of microorganisms in the food. Addition of water to ripe dry pods enhanced growth of microorganisms and thus improved larval growth; development was optimal under dixenic conditions, when the naturally occurring fungus Phomopsis sp. was present, Larvae went through five molts till pupation and growth appeared to be optimal at 30°C.

Adults emerged during the early hours of the night with males preceeding females. No sugary food was needed for normal longevity and oviposition, but water was important. Average lifespan of adults was 9 days. Oviposition began on the second night after emergence and the largest number of eggs was laid, during the same night. The number of eggs was affected by the larval diet; about 200 eggs were laid per female when larvae fed on acacia and carob.

Introduction

The carob moth is a significant pest of citrus, carob and almond fruits in Israel (Avidov & Gothilf, 1960; Bodenheimer, 1930; Gothilf, 1961), as well as in other Mediterranean countries (Balachowsky & Mesnil, 1935; Silvestri, 1951; Agenjo, 1959; Wood, 1963). The occurrence of the insect in other parts of the world and its taxonomic position was discussed by Heinrich (1956).

Although the carob moth has been known as a fruit pest for many years, no thorough study of its biology had been conducted until recently. With the realization of the severe damage caused by the pest to citrus fruits in the Mediterranean countries, the interest in the investigation of its biology increased. The present paper is one of a series of articles dealing with the biology of the carob moth.

Methods

Detailed procedure for obtaining eggs of the carob moth in the laboratory was described in a previous publication (Gothilf, 1968). In the present work eggs were laid on polyethylene instead of paper. Three-hour old eggs were used for studies on incubation period. The eggs were placed in closed 500 ml glass jars on a stand. The lower parts of the jars were filled with a NaOH solution at a concentration adjusted to maintain 80% R.H. (Madge, 1962). In tests where the effect of varying humidity on eggs was studied, 12-hour old eggs were placed in the glass jars and humidity was adjusted by saturated salt solutions (Winston & Bates, 1960). Each test was repeated three times. Temperature variations during incubation did not exceed one degree centigrade above or below the pre-adjusted temperature. Jars were checked twice a day.

For observing molts larvae were reared individually in small plastic cups, 1.5 cm. high by 3.5 cm o.d., and were fed on an artificial diet (Gothilf, 1968). The cups were checked daily. In other tests larvae were reared in groups of ten in 500 ml glass jars covered with cloth. At least 70 gm food was supplied in each jar. Jars were kept in incubators at various temperatures and a constant humidity of 75± 5% R.H.

Larvae were also reared individually on sterlized carob and acacia pods and, in some tests, these pods were inoculated with the fungus Phomopsis sp. In these experiments 25 ml flasks containing 20 gm chopped carob or acacia pods each, were heat-sterilized for 20 min. at 15 p.s.i.; some of these were then inoculated with Phomopsis sp., all common precautions being taken to prevent other contamination. A stock of Phomopsis sp., originating from carob pods, was maintained constantly in the laboratory by rearing the fungus on sterile soaked soy beans. Newly hatched larvae were transferred onto the food medium two days after it had been sterilized and inoculated with the fungus. By that time the colonies of Phomopsis sp. were large enough to support larval growth. To prevent undesired contamination in these experiments eggs were surface-sterilized and hatched in sterilized flasks from which the larvae were transferred to the media, while taking all usual bacteriological precautions. Eggs had been sterilized by dipping in a 0.1% $HgCl_2$ solution for 10 minutes, followed by three rinses in sterile water.

Adults were obtained from the stock culture routinely reared in the laboratory (Gothilf, 1968). When necessary, for experimental purposes, the artificial food in the rearing trays was replaced by chopped carobs or acacia, other rearing procedures being the same. Adults whose longevity was studied, were kept in groups of 50 in a screened cage measuring 53 x 40 x 45 cm. The fecundity of females was studied in females kept individually in small cups, 4.5 by 3.0 cm o.d. The cups were made of transparent plastic in which ventilation holes were punched. They were kept upside-down on a surface of rough paper so that eggs would be laid on the paper. Each day the cups with the females were moved to a new position on the paper surface, and the eggs laid were checked and counted. The females were fed with a 30% honey solution on cotton wool.

Experimental

Effect of temperature and humidity on incubation period and egg survival. Eggs kept at 15°C, or less, did not hatch, but in tests with other temperatures, the shortest incubation period and highest survival rate occurred at 30°C (Table 1).

Table 1

Incubation period at various temperatures and 80% R.H.

Temp. C°	No. of eggs	Percent hatched	Incubation days (min.-max.)
15	41	0	
20	41	80.5	8.3 (8.0-9.0)
25	42	88.1	4.0 (4.0-4.0)
27	42	90.5	3.5 (3.5-3.5)
30	66	95.5	3.0 (3.0-3.0)
34	34	85.3	3.1 (3.0-3.5)

Tests in which eggs were exposed to various combinations of humidity and temperature throughout their incubation period, revealed that eggs could stand low humidities; when relative humidity was lowered to 20% and the temperature did not exceed 34°C, survival ranged between 80-90%. Only at 10% R.H. (the lowest humidity tested) and at 20° to 30° C did the rate of survival drop to 50%. At the same humidity but at 34°C, survival dropped to 24%. At 45°C all eggs died within 2 hours, even if the humidity was high (see also Gothilf, 1964).

Larval instar. Out of 57 larvae reared individually, 51 went through 5 molts while 6 went through 6 molts. Size of larvae while at rest was as follows : first instar - 1.5 to 2.5 mm; second instar - 2.5 to 3.5 mm ; third instar- 3.5 to 6.5 mm; fourth instar - 5.5 to 10.5 mm; fifth instar - 9.0 to 15.0 and sixth instar - 13.0 to 16.0 mm. The period between the molts varied considerably from larvae to larvae (Table 2). Each of the first four stages lasted about 5 days while the last stage lasted 8 days. Three days before the last molt larvae stopped feeding and began webbing the cocoon. This took two days and on the third day, the last before molting. the larvae remained motionless.

Table 2

Duration of each larval stage

(23 larvae reared individually at 24 ± 1°C)

Larval instar	Average duration-days (min. -max.)
1st	5.7 (5 - 8)
2nd	4.3 (3 - 6)
3rd	5.0 (3 - 8)
4th	5.2 (4 - 7)
5th	8.1 (7 - 9)

Development of larvae on various hosts. In nature larvae feed mainly on pods of carob and acacia but almond nuts and grapefruit serve as hosts to the moth as well. Upon hatching, larvae penetrate their host, where they stay for the rest of their larval stage. Pupation takes place inside the same fruit. It had been observed that larvae cannot penetrate undamaged fruit except grapefruit, and in each case fruits had to be chopped. The significance of this findings, as a factor affecting the natural development of the carob moth population, will be discussed in a subsequent publication.

Larval development on green, unripe pods of carob and acacia was rapid, while on ripe, dry pods development was considerably slower (Table 3). Larvae feed on the pulp of carob pods, while in acacia the pulp is very thin and larvae feed mainly on the seeds. In ripe acacia pods these seeds are covered with a hard shell which the larvae cannot penetrate. In nature, seeds are damaged by bruchids which enable the carob moth larvae to penetrate them. Such damaged fruits were used in the present tests. The prolonged development period on ripe pods was shortened by the addition of water to the food. As shown later in this work, the addition of water affects larval growth indirectly through stimulation of development of microorganisms which renders the food more suitable to the larvae. A similar situation occurs in nature when dry pods become wet during the rainy season. Since the larvae pupate inside the food it was more convenient to record the duration of larval and pupal stages together. However, in some instances it was possible to record the time of pupation which lasted about 9 days at 25 ± 1°C.

Table 3

Survival and duration of larval and pupal stage on various natural foods at 25 ± 1°C

Food	No. of larvae	Survival to adulthood %	Larval+pupal duration days	(min. - max.)
Carobs, ripe	40	10	114.3	(97 - 127)
Carobs, ripe (water added [1])	40	35	45.4	(34 - 54)
Carobs, unripe	40	63	32.7	(29 - 43)
Acacia, ripe [2]	40	18	93.6	(71 - 121)
Acacia, ripe [2] (water added [1])	40	23	53.3	(33 - 80)
Acacia, unripe	40	33	31.9	(25 - 38)
Grapefruit	21	29	66.2	(46 - 105)
Almond (shelled)	20	50	48.5	(41 - 55)

1) 1:1 by weight

2) Seeds previously fed on by Bruchids.

As shown in Table 3, survival was low on ripe carob and acacia. Mortality occurred mainly among larvae and was much lower among pupae. The highest rate of survival was observed on unripe carob pods - a highly favorable natural food of the insect.

Effect of microorganisms in the food media on larval growth. As mentioned before, larval growth was enhanced by the addition of water to dried carob and acacia pods. Growth of molds and other microorganisms was conspicuous on the moist food. Molds also grew extensively on unripe pods on which the larvae developed well. Also in nature, larvae are found on carob pods infested with the fungus Phomopsis sp. and with other microorganisms. It was felt that larvae benefit from the presence of these microorganisms in the diet and this interrelation was subjected to the following test. Larvae were reared individually on a sterile medium of carob and acacia pods. Part of the sterile medium was previously inoculated with Phomopsis sp., and some of the sterile acacia was exposed to infestation by airborne microorganisms (mainly molds developed in the media). These tests (Table 4) clearly showed that larval development on carob pods depends on the presence of microorganisms in the food medium.

Table 4

Larval growth on sterilized carob and acacia pods with or without mold inoculation at 25 ± 1°C

	No. of larvae	Survival %	Average duration days	(min. - max.)
Carob				
Unripe pods	15	0		
Unripe pods, Phomopsis inoculated	14	9	27.9	(26 - 31)
Ripe pods	10	0		
Ripe pods, water added [1]	10	0		
Ripe pods, water added [2]	18	8	269.6	(240 - 315)
Ripe pods, water added [2] Phomopsis inoculated	20	19	33.1	(28 - 41)
Acacia				
Unripe pods	12	10	34.9	(26 - 44)
Unripe pods, Phomopsis inoculated	6	6	26.0	(24 - 28)
Unripe pods, inoculation by various molds	12	8	29.6	(25 - 42)

1) 1 part water to 2 parts carob by weight.

2) 3 parts water to 2 parts carob by weight.

While in nature Phomopsis and other microorganisms develop in unripe carob pods, no such development occurs in ripe pods as long as the pods are dry, and larval development, therefore, is slow. Once these pods are moistened by winter rains and microorganism development begins, larval growth improves. The presence of microorganisms seems to be less essential in acacia, where the carob moth can reach adulthood in axenic conditions.

It is not clear which are the factors enhancing larval growth on food medium inoculated with microorganisms. In one experiment pre-sterilized carob medium was inoculated with Phomopsis sp., again sterilized and only then infested with carob moth larvae. Thirty three larvae were used and reared individually; development was extremely slow and the test was discontinued after 300 days. By that time no pupation had occurred and 10 larvae were still alive. It is possible that heat-sensitive nutritional factors synthesized by the fungus were destroyed by the sterilization, but other factors dependent on the living fungus might be involved as well.

Effect of various temperatures on larval growth and survival. When studying the effect of varying temperatures on larval growth, conditions favorable to the development of microorganisms on the food medium were maintained. Water was added to the ripe dry carob pods, and food was kept at 70% R.H. to prevent drying. The results of these experiments are given in Table 5.

Table 5

Effect of various temperatures on survival and duration of larval + pupal stages on various foods. [1]

	Survival and average duration of larval + pupal stage on :							
	Carob-unripe [2]		Carob-unripe + Phomopsis		Carob-ripe [2]		Acacia-unripe [2]	
Temp. C°	Survival %	Duration days	Survival %	Duration days	Survival %	Duration days	Survival %	Duration days
15±1	0		0		0		0	
20±1			46	187	5	45	0	
25±1	63	33	90	32	15	36	33	32
30±1	70	22	80	26	30	26	35	23
34±1	65	22	80	24	25	31	15	25

1) Forty larvae were reared individually on each food and temperature combination; 13 larvae in the case of carobs inoculated with Phomopsis.

2) Food was naturally infested by microorganisms, mainly molds. Ripe carobs were previously mixed with 3 parts water to 2 parts carob by weight.

Larvae were reared in groups of ten (except on Phomopsis-inoculated carob where rearing was done by a different method), and the highest rate of survival was found on unripe carobs. Mortality, in all cases, occurred mainly among larvae and less in the pupal stage . Growth period was shortest on unripe carob and acacia; no growth took place at 15°C and only very few larvae survived and reached adulthood at 20°C. At 30°C growth was rapid and this temperature seems to be optimal for the development of the carob moth.

Adult Emergence. Adults are active during the night and stay motionless during the day. Emergence takes place during the early part of each night. Nightly emergence of 555 males and 508 females reared on acacia and 404 males and 352 females from carob was recorded, until no more adults emerged (Fig. 1). All breedings were started on the same day from one-day old larvae. The curves show clearly that males preceded females in emergence by one or two days. The largest number of males emerged on the fifth night and for 1 or 2 nights thereafter. The largest number of females emerged during the 7th night on acacia and one night later on carob. Except for a few moths that emerged at a later stage, emergence lasted about 2 weeks.

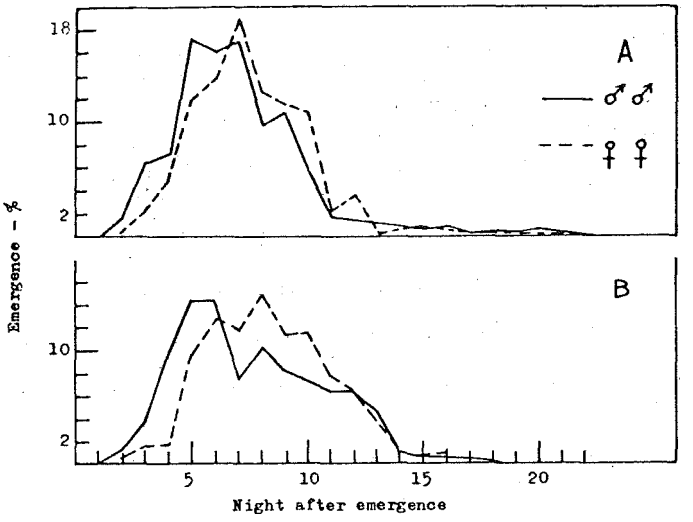

Figure 1. Nightly emergence of males and females from acacia (A) and carob pods (B). Reared at 25 ± 1°C and 75 ± 5% R.H.

Adult longevity. The curve of survival for males and females is given in Fig. 2; It shows that most adults died within a few days, starting from about the fifth day after emergence. Thereafter, only a few adults survived and remained alive for up to one month. No significant difference in longevity between males and females was noticed. Both male and female groups reached 50% survival on the 9th day. A honey solution of 30% in water served as diet, but in practice adults survive for the same length of time on water only. Without water longevity is shortened if adults are kept at a low humidity.

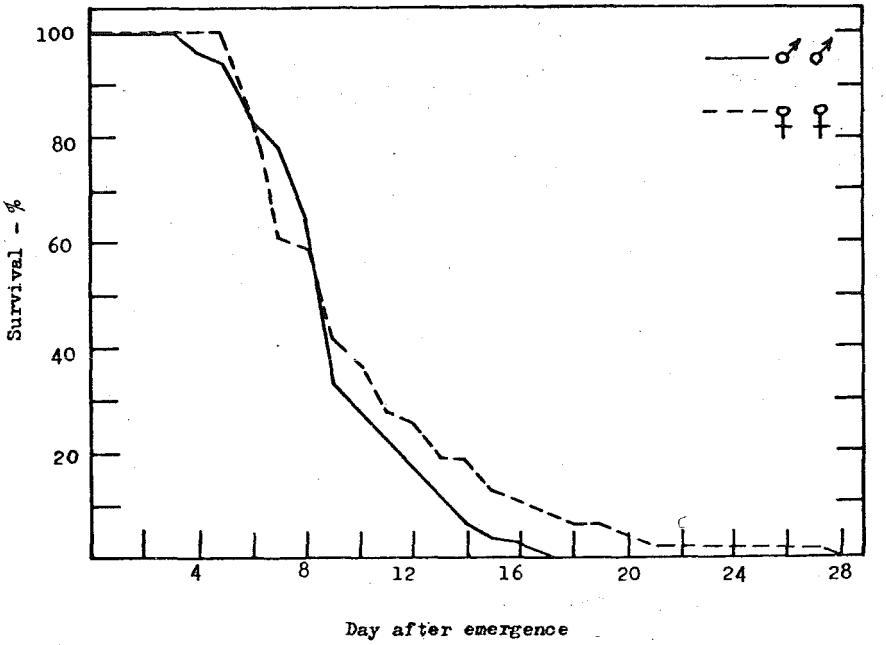

Figure 2. Daily survival of 50 males and 50 females at 25 ± 1°C and 75 ± 5% R.H.

Oviposition. The average number of eggs laid nightly by females is given in Fig. 3. Twenty five females were used in this experiment and were kept separated since emergence so that egg laying could be recorded as from the first night. On the first night very few females oviposited and the largest number of eggs was laid on the second night. The maximum for one female on the second night was 144 eggs. After the second night oviposition declined sharply. This trend was confirmed by additional tests carried out under outdoor conditions. In these tests no difference was found between the number of eggs laid by females feeding on honey solution and those feeding on water alone. The number of eggs laid was reduced when females were kept outdoors in the summer without water supply (Gothilf, 1964).

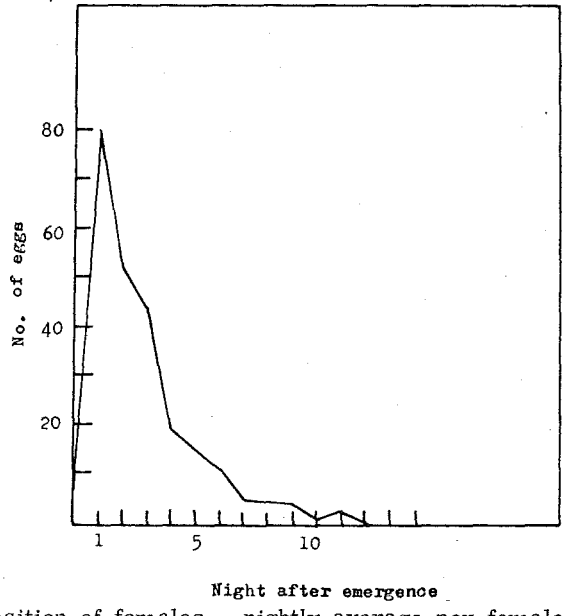

Figure 3. Oviposition of females - nightly average per female. $25 \pm 1^{o}C$ and $75 \pm 5\%$ R.H.

The effect of larval diet on female fecundity was discussed in a previous paper (Gothilf, 1968). It was shown that on the average, females reared from acacia fruit laid 217 eggs and females emerging from carob laid 201 eggs. On the other hand females reared on artificial diet laid 315 eggs. Fecundity was correlated to the female body-weight which differred significantly between females reared on artificial diet and those reared on carob and acacia.

Acknowledgements

The author wishes to express his thanks to Prof. Z. Avidov of the Hebrew University and to Dr. E. Swirski of the Volcani Institute of Agricultural Research, for their guidance and suggestions. This work is part of Ph.D. dissertation submitted to the Senate of the Hebrew University, Jerusalem and was supported by a grant from the Citrus Marketing Board of Israel.

References

Agenjo, R. 1959. La polilla de la garrofas, plaga actual de las naranjas (Lep. Phycit.). Graellsia (Madrid) 17(1-3), 7-17.

Avidov, Z. and S. Gothilf, 1960. Observations on the honeydew moth (Cryptoblabes gnidiella, Mill.). Israel J. Agric. Res., 10, 109-124.

Balachowsky, A. and L. Mesnil, 1935. Les Insectes Nuisibiles aux Plantes Cultivees. Vol. 1, Paul Lechevalier, Paris.

Bodenheimer, F.S. 1930. Die Schaedlingsfauna Palestinas. Paul Parey, Berlin.

Gothilf, S. 1961. Entomological factors responsible for grapefruit drop in Israel. In Report of the Entomologists' Expert Meeting on Citrus Fruits, Nicosia, Cyprus. Comité de liaison de l'agrumiculture Méditerranéene, Madrid.

Gothilf, S. 1964. Studies on the biology of the carob moth Ectomyelois ceratoniae (Zeller) in Israel. Nat. and Univ. Inst. Agr., Bul. 76 (Hebrew with Summary in English).

Gothilf, S. 1968. The biology of the carob moth (Ectomyelois ceratoniae (Zell.)) in Israel. I. Mass culture on artificial diet. Israel. J. Entomol. 3(2)109-118.

Heinrich C. 1956. American moths of the subfamily Phycitinae. Bull. U.S. Nat. Mus. No. 207.

Madge, D.S. 1962. The control of relative humidity with aqueous solutions of sodium hydroxide. Ent. Exp. et Appl. 4, 143-147.

Silvestri F. 1951. Compendio di Entomologia Aplicata. Vol. 2, Stabilimento Tipografico Guglielmo Genovese, Napoli.

Winston, P.W. and D.H. Bates. 1960. Saturated solutions for the control of humidity in biological research. Ecology, 41, 232-237.

Wood, B.J. 1963. Imported and indigenous natural enemies of citrus coccids and aphids in Cyrpus, and an assessment of their potential value in integrated control programmes. Entomophaga 7, 67-82.